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Impact of Engineered Nanomaterials on Health: Considerations for Benefit–Risk Assessment

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Picture: Tin nanoparticles synthesized and photographed by JRC-IHCP.

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Foreword

From the JRC Director General and the EASAC President

This policy report is the result of the first strategic liaison between the JRC and EASAC and provides independent, cross-referenced, science-based analysis of the impact of nanomaterials on human health. Our report is directed at European and national policy-makers and citizens. Nanomaterials have the potential to play a major role in European innovation, economic growth and industrial competitiveness. In order to capitalise on this technology and reap the promised benefits, the EU must ensure the appropriate framework for its success. A key element in this regard concerns a harmonised assessment of the safety of nanomaterials and this requires a strengthened dialogue between policy-makers and scientists.

The joint initiative of EASAC and the JRC also contributes to the collective EU targets and supports integrated efforts for nanotechnology innovation, as well as public debate on the future of nanomaterials. Based on the experience of this initiative, and the existing synergies between the activities of the two organisations, a more structured cooperation will be developed. The cooperation will address other scientific topics relevant to the key priorities of the EU and serve to create closer links between EU national science academies and the policy-making processes in the EU.

Dominique Ristori
JRC Director General

Sir Brian Heap
EASAC President

From the Chairs of the Expert Group

A first meeting took place in Lugano, Switzerland, on 17th August 2009 between EASAC¹ and EC-JRC², where it was decided to draft a joint report on the impact of manufactured nanomaterials on human health. Because the agreed focus of the report was to be on manufactured nanoparticles and the legal and societal implications related to their potential risks and benefits, the report was entitled: 'Considerations on Benefit–Risk Assessment of Engineered Nanomaterials'.

A representative group of 13 experts (listed in the Annex) across the EU was selected on to a panel to address these issues. The panel included expert representatives from both EASAC and EC-JRC. This group met for the first time in February 2010 in Ispra (Italy) and thereafter in Zurich (Switzerland) in July and in November 2010.

The health and environmental effects of engineered (by humankind) nanomaterials are not yet clearly understood, although they are already used in a variety of applications. However, health effects of nanoparticles present unintentionally in the environment, for example deriving from combustion processes (even in a simple process such as lighting a candle), have been studied extensively, since the time of Leonardo da Vinci.

The goal of the present report is to highlight the state-of-the-art knowledge on safety aspects of engineered nanomaterials and to identify needs for further scientific investigations.

The exploitation potential of nanotechnology has only just begun to be tapped, and the associated economic and technological gains are likely to be considerable for those who are able to capitalise from the technology from an early point. In this regard, Europe should not be disadvantaged. It is the intent of this report to point to the ways in which Europe can best reap the promised rewards without compromising appropriate and due consideration of the necessary health and environment safeguards, especially concerning nanomaterials, which otherwise may ultimately counteract any preliminary gains.

We wish all readers of this report a stimulating reflection on the primary issues pertinent to accomplishing this goal.

Elke Anklam
Director of JRC–IHCP

Peter Gehr
President of SC NRP 64

1 Represented by Prof. Dr. Denis Monard, President of the Swiss Academy of Sciences, and Prof. Dr. Peter Gehr, President of the Steering Committee of the National Research Programme 64 'Opportunities and Risks of Nanomaterials' (SC NRP 64) of the Swiss National Science Foundation.

2 Represented by Prof. Dr. Elke Anklam, Director of the Institute for Health and Consumer Protection (IHCP), and Dr. Hermann Stamm, IHCP Head of Unit responsible for nanotechnology.

Executive Summary

Nanotechnology and its importance for Europe

Nanotechnology encompasses the design, characterisation, production and application of materials and systems by controlling shape and size at the nanoscale (nanometres). Nanomaterials may differ from other materials because of their relatively large specific surface area, such that surface properties become particularly important.

There has been rapid growth in investment in nanotechnology by both the public and private sectors worldwide. In the EU, nanotechnology is expected to become an important strategic contributor to achieving economic gain and societal and individual benefits. Although there is continuing scientific uncertainty and controversy about the safety of nanomaterials, there is only a limited amount of scientific evidence about nanomaterials and human health risks.

It is important to ensure that timely policy development takes these issues into consideration. Uncertainty about safety may lead to polarised public debate and to business unwillingness to invest further in nanotechnology.

A clear regulatory framework to address potential health and environmental impacts, within the wider context of evaluating and communicating the benefit–risk balance, must be a core part of Europe’s integrated efforts for nanotechnology innovation.

Purpose of the present report

Although several studies have examined the effect of environmental nanoparticles, for example from combustion processes, on human health, there is as yet no generally acceptable paradigm for safety assessment of nanomaterials in consumer and other products. Therefore, a working group was established to consider issues for the possible impact of nanomaterials on human health focussing specifically on engineered nanomaterials. This represents the first joint initiative between EASAC and the JRC. The working group was given the remit to describe the state of the art of benefits and potential risks, current methods for safety assessment, and to evaluate their relevance, identify knowledge gaps in studying the safety of currently used nanomaterials, and recommend priorities for nanomaterial research and the regulatory framework.

This report focuses on key principles and issues, cross-referencing other sources for detailed information, rather than attempting a comprehensive account of the science.

The focus is on human health although environmental effects are also discussed when directly relevant to human health.

Benefits and safety of nanomaterials

The term ‘nanotechnology’ covers a very broad range of entities and industrial applications. It is expected that many of the applications will help to improve human health and quality of life. The medical application of nanotechnology is probably one of the fastest growing fields, with developments in therapeutic, diagnostic and imaging uses (e.g. in cancer).

Applications in food include objectives to enhance flavour and texture and encapsulate micronutrients to prolong their stability, augmented by packaging applications to prolong shelf life and avoid bacterial contamination. Potential applications in other sectors include environmental remediation to detect and eliminate toxic substances, energy generation and storage plus multiple other commercial uses of novel materials.

Several inventories of consumer nanoproducts exist, some containing more than 1,000 items; however, inclusion was based on the producer’s claim rather than a standard definition, and this could create challenges for a coherent and systematic safety assessment. The rapid increase in the use of nanomaterials in industry and consumer products is causing concerns about the potential effects on human health and on the environment. It is generally accepted that many areas of nanotechnology do not present new hazards so that current regulatory frameworks are adequate. But it is also possible that new forms of engineered nanomaterials may require existing regulations to be modified or even new specific regulations to cover the lifecycle of production, use and disposal.

We emphasise that the regulatory framework for the safety assessment of nanomaterials should follow the same principles and sector-specific requirements as for other products: risk is a function of hazard and exposure. Direct exposure depends on the intended application; indirect exposure arises from involvement in manufacturing processes and from the environment more generally.

We conclude that it is essential to invest significantly in research for safety assessment while seeking to expedite regulatory review of the products emanating from that science.

Current state of knowledge and priorities for filling the gaps

Although there are many research projects worldwide assessing the potential hazards and risks, there have been concerns about their quality and relevance. The lack of standardised materials and methodologies makes it difficult to compare results from different researchers and different nanoproducts.

Nanosafety assessment has its origins in research on ultra-fine dust and lung damage; the study of particle–lung interactions remains a major research topic with the objective to clarify biological processes and potential consequences for disease. Beyond the lung, there are many gaps in knowledge about the health impacts that may occur after inhalation, in relation to uptake, distribution, accumulation and biological effects in secondary organs, although it is known, for example, that some nanoparticles can cross the blood–brain barrier. Other routes of entry that may be intended for some nanomaterial applications, such as oral ingestion, and in particular exposure to the skin and intravenous administration, are less well-characterised.

As a generalisation, it can be said that the same properties that are desirable in some applications, such as the ability to cross biological barriers and the manifestation of high surface reactivity, are also the properties that may give rise to toxicity (the so-called ‘nanomaterials paradox’). This paradox is not unique to nanomaterials or nanomedicine as the principle applies also to pharmaceuticals.

Among the key issues for hazard assessment are the following:

- **Dose** – testing should aim to identify potential hazards by establishing dose response relationships, over the long-term when necessary, but many reported studies are short-term, have used very high doses and their relevance to likely exposure can be questioned. Furthermore, dose must be quantified based on a detailed understanding of the physico-chemical properties of the nanomaterial, but this was not always possible in the earlier studies.
- **Standardisation** – it is critically important to use validated, standardised assays so that (1) results from different researchers can be compared and (2) assays *in vitro* or animal studies can reasonably be expected to predict an effect in humans. Again, this has not always yet been possible.
- **Differences in individual susceptibility** – relatively little progress has been made in exploring factors that may influence the response of individuals, for example ageing, genetic predisposition and epigenetics.
- **Studies at cellular level** – there is still little knowledge about how nanoparticles interact with the cell membrane, how they are transported into cells, into

lysosomes, mitochondria and the nucleus, and the consequences of these interactions.

- **Studies at organ and system levels** – among the priorities there is need to study toxicokinetics for extra-pulmonary translocation and transport after other routes of entry, particularly for effects on foetal development and cardiovascular, nervous, hepatic, immune and endocrine systems and organ–organ interplay (activation of a response to nanoparticles in one organ with effects elsewhere).

It is equally important to do much more in assessing exposure, whether intended or unintended. Few studies describe workplace, consumer or environmental exposure or relate exposure to real-life conditions. There is very little information on workplace exposure in smaller companies or lower-technology sectors downstream from the point of manufacture. More must be done to assess inhalation exposure in occupational settings where the greatest exposure is anticipated (and where there is the potential for environmental release) and to raise awareness in other settings. It is also important to assess exposure by other routes, whether or not intended for the application. One key issue is the choice of practical metrics to quantify the concentration of nanoparticles. Measurement of total surface area or particle number is likely to be more meaningful than total mass concentration. Consensus on methodologies is needed to construct integrated datasets and provide the reference point for particle characterisation in terms of morphology and stability.

Regulatory and governance framework

The working group raised no new ethical issues for nanotechnologies beyond those already established for other technologies. Indeed, nanomaterials can be viewed as possessing intrinsic societal value in the context of sparing resources and contributing to social, economic and environmental sustainability. It is acknowledged that public concerns have been voiced about nanotechnology and such concerns are prone to amplification in sensationalist media accounts.

It is important for the European Commission and European national authorities to encourage social science research exploring public attitudes. We observe that public engagement is likely to be more effective if there is shared understanding about the boundaries of nanotechnology and the appropriate balance of benefits and risks.

Regulation is challenging today because of uncertainties in definition and behaviour of nanomaterials, because of their application in many different industrial sectors and the lack of appropriate standards and validated testing procedures. The legislative framework can be distinguished as sector-specific (e.g. for cosmetics, medicines, foods) or horizontal (e.g. for chemicals, worker, consumer, environmental protection).

The latter regulations at EU and national levels comprise a variety of initiatives, including legislation on chemicals (e.g. Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)) and on occupational health and safety protection. There will be additional product-specific (e.g. food and cosmetic products) regulatory challenges. Further, into the future, it is important to understand how nanoparticle-based systems combining disease imaging and therapeutic delivery should be regulated—as a drug or medical device?

Agreeing codes of conduct and other voluntary measures is vital in advance of regulatory reform. In 2008, the European Commission adopted a Code of Conduct for nanoscience and nanotechnology—it is important to monitor its implementation and revise as appropriate. Manufacturing initiatives are also welcome in developing risk management systems based on best practice with reporting schemes that collect information on the characteristics and use of nanomaterials.

Our recommendations: measures needed to understand and manage benefits versus risks of engineered nanomaterials

To manage benefits versus risks of engineered nanomaterials, the EU needs a coherent strategy in nanotechnology that has the flexibility to respond to future developments. This strategy must be multidisciplinary and multi-sectoral, requiring new effort in data collection, new infrastructure and new training initiatives, involving academia, industry, policy-makers and others in society.

The current safety assessment approach for nanomaterials is to start from a precautionary perspective and refine the strategy once sufficient knowledge is available to understand hazards, exposure potential and the means to protect workers, consumers and the environment from unwanted levels of contact. This is analogous to the European Chemicals Legislation (REACH) process. The challenge is to accomplish this assessment when the number and extent of industrial applications is growing rapidly, and to ensure that benefit–risk is judged rather than risk alone. If nanotechnology is to realise its potential, it is vital to empower the research and regulatory community to apply the precautionary principle in a focused and cost-effective manner. Risk assessment and management requires intelligent and case-specific consideration guided by potential exposure scenarios. It is also relevant to note that new knowledge will help to engineer safer nanomaterials.

We emphasise some cardinal points for the European Community:

- Safety research is an essential part of the innovation of nanomaterials and has to take place during the innovation process ('safety by design').

- Research planning/management and product regulation must be sufficiently flexible to cope with future developments.
- Over-regulation should be avoided as it can slow down an improvement of the total benefit–risk balance in case regulation prevents industries from adopting novel techniques that would lower traditional risks. It may therefore act as an obstacle to innovation and research, and may prevent the translation into products that, when used in a safe manner, can contribute to EU societal objectives.

The main conclusions and recommendations in our report cover priorities for the following:

- **Research and its translation into applications** – there is scope to do more to integrate safety assessment into projects dealing with the development of new materials. The pharmaceutical sector can be considered as a relevant model where hazards and risks are addressed at an early stage in research. The research communities across nanomedical, nanoengineering and nanosafety are not yet well-linked and cross-talk should be encouraged in addition to teaching basic understanding about related fields. Funding strategies of the EU and its Member States need orchestration, identifying the strategic research questions and methodological developments to be pursued. It is important to avoid duplication in setting up new safety research centres. The long-term use of simple engineered nanomaterials like metal oxides over the past three to four decades suggests that the newly emerging nanomaterials of higher sophistication may also find use in diverse applications, resulting in sustained public exposure. Measurements and monitoring of these nanomaterials is currently extremely tedious or cannot be done at all. Risk assessment cannot be confined to studying short-term effects after acute exposure but must also examine the potential for chronic effects arising from cumulative exposure. It is vital both to support basic safety research to fill knowledge gaps and to translate that knowledge more effectively. There are new opportunities, provided for example by meta-analysis and modelling, to maximise the value of the knowledge already available.
- **Connecting science and regulation** – the European Commission together with the European scientific community should strengthen efforts to define and implement a common terminology and identify common needs for data collection for safety endpoints that enable comparison of results from disparate groups and nanomaterials. The European Commission and European Agencies should continue to review the regulatory landscape and develop the evidence base to respond to queries from the European Parliament on whether the law

specific to nanomaterials is adequate. Introduction of a definition of nanomaterial in the Cosmetic Product Regulation has created a precedent for adopting a definition in other product areas and renders urgent harmonisation of a definition.

A definition or any other standardising intent must be science based, unambiguous and enforceable if it is to facilitate progress and be successfully implemented.

- **Public engagement** – it is also important that the European Commission together with the scientific community should make provision of accessible and accurate information about nanomaterials, emphasising that their risks are assessed according to the same principles applied in the assessment of other products. This communication activity must deliver balanced description in lay language and must describe both the potential societal benefits of scientific advances and the societal protection afforded by proportionate, sector-specific regulation.
- **Nanospecific training** – modules can be included in EU research programmes. There is also broader

need for training toxicologists, material scientists and production engineers in the risk assessment procedures for developing new materials. Training should be incorporated into both Master's and PhD-level activities, building on current best practice, for example the Marie Curie PhD training programme in nanotechnology safety.

The development of new generations of nanomaterials requires a new generation of interdisciplinary scientists. New training initiatives are essential to confer this interdisciplinarity and secure the future of nanotechnology.

In conclusion, we reiterate that there is only a limited amount of scientific evidence to suggest that nanomaterials present a risk for human health and we advise that the principles of risk assessment procedures should conform to the same procedures as any other new material, paying due respect to new phenomena that may occur due to new properties related to the nanoscale.

Successful innovation, if it is to encompass both regulatory and consumer approval, must incorporate safety by design.

1 Introduction

1.1 Development and use of nanotechnology

Nanotechnology is an enabling technology that has the potential to bring benefits to multiple areas of research and application and to enrich our lives in many ways. It is attracting rapidly increasing investment from governments and businesses around the world. Currently, relevant industrial sectors include those associated with, for example, information technologies, electronics, energy generation and storage, material sciences, bio-physico-chemical processing and catalysis, food and feed refinement, environmental remediation, security, transport and space, diagnostic and therapeutic applications in medicine.

It is becoming clear from the scientific perspective that advances in the handling of atoms and molecules will increasingly allow manipulations in a targeted way, making use of structure-dependent molecule-to-molecule interaction and processing. These 'bottom-up' scientific and technological principles and practices will be complemented by 'top-down' industrial strategies enabling the general introduction of engineered nanomaterials or nanoparticles of smaller and smaller sizes in many different applications.

As the particle size shrinks, the proportion of molecules and/or atoms on the surface increases, leaving lesser proportions located within the inner volume of nanomaterials and enhancing and altering surface reactivity, modulated by the surface curvature and structure. Nanotechnological tools are increasingly available to allow such manipulations under greater control. Realistic opportunities for application are appearing on the horizon but, at the same time, there is need to guard against hyperbole expressed about both benefits and risks.

It is important to achieve a common understanding of what is a nanomaterial. The boundary between nano- and other materials is not yet entirely clear and it is evident that nanomaterials cover a very broad range of entities and industrial applications. An overview on definitions of nanotechnology and nanomaterials is provided in Box 1.1 and developments in this area have been informed by other important broader EU initiatives, for example the SCENIHR Scientific Committee on Emerging and Newly Identified Health Risks (whose activities are described in section 2.2.2).

Box 1.1

Terms and definitions relating to nanotechnology and nanomaterials

Nanotechnology is a broad term, referring to the deliberate creation, manipulation and application of structures with one or more dimensions in the 'nanoscale'. The nanoscale is often taken to refer to the size range from 1 nanometre (nm) to 100 nm³, although these limits are not accepted by all involved in the field, and indeed there are several particulate 'nanomaterials' that fall outside this range that are usually taken as being products of nanotechnology.

A JRC Reference Report of 2010 on the subject of defining the term 'nanomaterial' stated that the term usually refers to 'materials with external dimensions, or an internal structure, measured in nanometres that exhibit additional or different properties and behaviour compared with coarser materials with the same chemical composition'.⁴ The European Commission will soon make a recommendation for a more precise regulatory definition, including specific limits for the relevant size range (see Chapter 2). The JRC report includes a non-exhaustive summary of definitions related to this issue and to nanotechnology in general.

Several publications suggest that 'nanomaterials' should not be exclusively defined in terms of a size range (e.g. between 1 and 100 nm), but that specific new effects that the material exhibits below a size threshold should also be taken into account⁵. For many effects (e.g. quantum effects) this threshold is considerably less than 100 nm. In this regard it is important to note that some particles below a certain size threshold interact with living cells in a different way than their larger counterparts (see Chapter 3). This biological threshold has been shown⁶ to be larger than 100 nm.

An understanding of the size distribution within a nanomaterial is also essential.

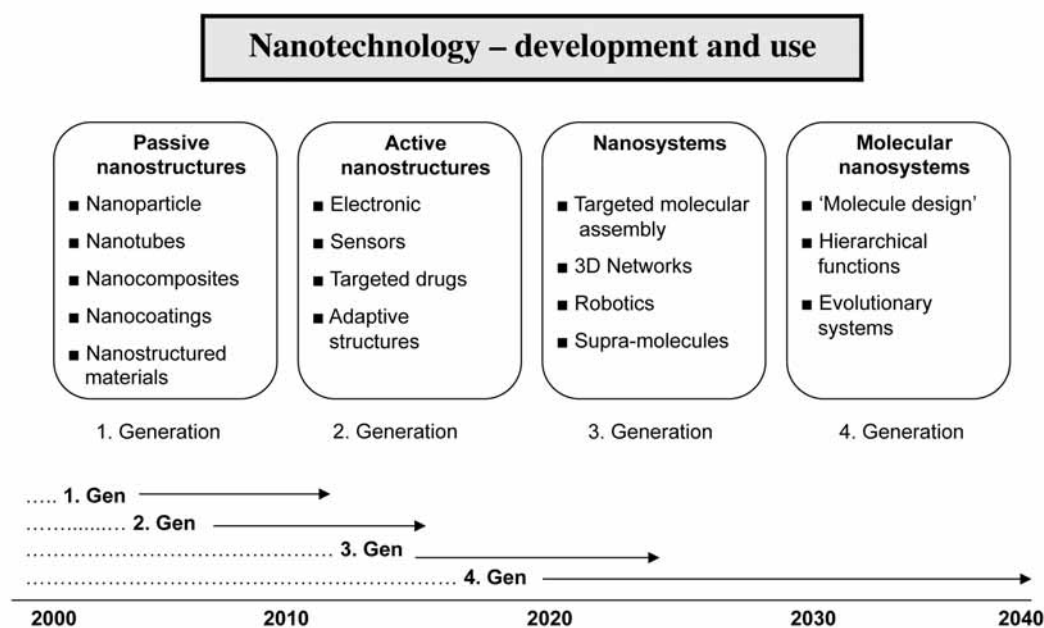
There is a potential evolution of nanotechnologies – ranging from a first generation of rather simple passive nanostructures through to a fourth generation of highly functionalised molecular systems (see Figure 1); more details on prospective benefits for certain applications are discussed in Chapter 3. Our report focuses on the impact of engineered nanomaterials on human health. In this respect, the term 'engineered' corresponds also to 'synthetic', 'man-made' or 'manufactured'. But it should be noted that nanomaterials are also naturally present in the environment as nanoparticles in consequence, for example, of combustion processes.

3 International Organization for Standardization, ISO/TR 8004-1.

4 Loevestam, G. et al., European Commission, Joint Research Centre, EUR 24403 EN (2010) and papers cited therein.

5 Auffan, M. et al., *Nature Nanotechnology* **4**, 634 (2009); Brune, H. et al., *Ethics of Science and Technology Assessment* **27**, 11 (2006).

6 Shvedova, A.A. et al., *Annual Review of Pharmacology and Toxicology* **50**, 65 (2010); Rothen-Rutishauser, B.M. et al., *Environmental Science & Technology* **40**, 4353 (2006); Wick, P. et al., *Environmental Health Perspectives* **118**, 432 (2010).

Figure 1: Evolution of nanotechnologies

Modified after Roco, M.C., *American Institute of Chemical Engineers Journal* 50, 890 (2004)

1.2 Safety considerations

New technologies have potential to bring benefits as well as disadvantages in the exposure of humans to new materials. There are three main contexts for considering safety: environmental pollution, unintentional human exposure (e.g. because of pollution or exposure in the workplace) and purposeful human exposure (the intended applications). The exposure of populations in the workplace and consumers and of the environment to nanomaterials is likely to rise significantly.

The complexity of the consequences for the benefit–risk balance is illustrated in the application of nanomaterials in the clinical field, where the very same properties that are desirable, such as the ability to cross biological barriers and the high degree of surface reactivity, may also give rise to unexpected and adverse effects.

Although there is already considerable knowledge on the impact of those environmental nanoparticles produced unintentionally (e.g. through combustion processes), on human health primarily mediated through respiratory pathways, there are still deficits in the appropriate risk assessment methodology to evaluate the safety of engineered nanomaterials. In common with every other new technology, research and development (R&D) of nanomaterial products needs to be accompanied by safety assessment, including risk assessment and risk-management. Risk assessment and management requires intelligent and case-specific consideration guided by potential exposure scenarios.

In very many applications, nanomaterials are embedded in large structures (e.g. electronics, information technology; see Box 1.2).

Box 1.2

Free versus embedded nanomaterials

There are different physical states, in particular free and embedded, that have a major effect on exposure. However, embedded forms may become free, for example by manipulations or erosion. Therefore, it is critically important to take into perspective lifecycle analysis. One prominent example may be carbon nanotubes, which are mainly embedded in composites and, therefore, exposure during manufacturing and potentially during subsequent manipulation such as recycling may be the principal concern⁷.

In these cases they are likely to pose low risk because of minimal consumer exposure and environmental release, at least during their lifetime, before disposal and/or recycling. However, it is important to take a whole lifecycle approach (from manufacturing to use to waste management) in considering impact.

By comparison, other engineered nanoparticulate materials, for example in food and nutritional ingredients, paints and coatings, cosmetics and healthcare products, nanomedicinal drug applications, and textiles, might lead to substantial direct exposure and may, therefore, pose a potential risk to humans and the environment.

⁷ Poland, C.A. et al., *Nature Nanotechnology* 3, 423 (2008).

These cases require careful risk assessment and management based on realistic exposure scenarios of well-identified groups within the European population. Furthermore, experience accruing from the long-term use of simple nanomaterials such as metal oxides or carbon black over the past three to four decades suggests that (if the analogy applies) the newly emerging materials of higher sophistication may also find their way into many diverse applications, leading to long-lasting exposures of the public.

The wide variety of consumer products within categories such as health and fitness, home and garden, electronics and computers makes it difficult to devise and verify a generic exposure assessment and risk management for nanoproducts as a class; the principles of sector-specific regulatory practice introduced for other products should also be applied to nanomaterials. Chapter 3 gives an extensive overview on the current knowledge concerning the impact of nanotechnology on human health and will demonstrate how proportionate risk assessment methodologies can be applied.

1.3 Response by public and private organisations: the current situation

As noted above, a potential impact of nanomaterials on human health is often anticipated but in reality often not quantified. This relative lack of evidence has led both to articulation of public concern and commitment by public policy-makers to strengthen the regulatory environment. However, it can also be said that the scientific community is relatively well prepared by comparison with the situation that has often characterised the advance of other enabling technologies. In the rapidly growing field of nanotechnology, questions about potential risk have been posed early on—and probably much earlier than in other technological advances. There are strong expectations that this attention to possible risks will be an additional and innovative driver to guide nanotechnologies into a safe and sustainable future for human health and environmental protection.

Research projects that include safety assessment are performed worldwide. Therefore, it is of vital importance to ensure that comparable results are obtained in order to create coherent science- and evidence-based risk assessment and management. This comparability objective requires harmonisation and standardisation of test methods, test materials and data, as discussed in Chapters 2, 3 and 4. Despite the growing use of nanomaterials in consumer products and innovative technological applications, there is at present no widely accepted definition of the term 'nanomaterial' that is

suitable as a basis for legislation on their safe use (see Box 1.1 for background). Any definition in legal terms will have implications according to the context in which it is used and may need adaptation for specific European regulations or directives. It is, however, of the utmost importance to have a definition available for it has a significant impact on research for safety assessment (see Chapter 2 for further discussion).

It is important to appreciate – especially in terms of exposure assessment – how many products containing nanomaterials are already available to consumers. Since 2006, there has been a voluntary database (Woodrow Wilson inventory⁸) accessible to consumers. In 2006, the inventory contained about 200 different products, increasing to nearly 600 products in 2007, with product number 1,000 added in 2009. The Woodrow Wilson inventory is indeed a valuable source of information about commercially available nano-products, but it should be noted that inclusion in the database is made on the producer's claim that the product is a 'nano-product'. Thus, the actual nanomaterials used in the consumer products are not always known⁹. To overcome these uncertainties, a mandatory European Register of those nanoproducts on the market has been requested by a growing number of EU Member States and by the European Parliament. The European Commission is currently assessing the needs and requirement for such a database.

1.4 Aim of the present report

As mentioned previously, several studies have examined the effect of environmental nanoparticles, for example generated from combustion processes, on human health. However, there is no generally applicable paradigm for safety assessment of consumer and other products containing nanomaterials.

This deficit is widely acknowledged, not only by the research organisations studying effects and interactions of nanomaterials at the cellular level and developing risk assessment methodologies, but also by policy-makers and their advisory bodies. A report released by the European Parliament¹⁰ and another by the German NanoCommission¹¹ are recent examples where decision-makers are calling for more advice.

The objective of the present report is not just to review the current state-of-the-art concerning research on nanotechnology safety but, in addition, to identify the gaps for further research, its translation and the related actions necessary to achieve the goal of a science-based evaluation of the impact of nanomaterials on human health. There is already a large literature and we have

8 Woodrow Wilson Center: www.nanotechproject.org/inventories/consumer.

9 Hansen, S.F. et al., *Ecotoxicology* **17**, 438 (2008).

10 European Parliament, Science and Technology Options Assessment (STOA), PE 446.050 (2010).

11 German Federal Government, NanoKommission: Verantwortlicher Umgang mit Nanotechnologien (2011).

cited key publications to exemplify issues to guide further discussion. It is not the purpose of this report to provide formal guidelines (e.g. on definition), for which more discussions are needed within the scientific community, as it is important to recognise that research on nanomaterials is rapidly advancing.

Different levels of controls may be needed for different categories of nanomaterials and the likelihood that they can be dispersed during use and coming into contact with human beings (see Box 2.1). Therefore, the main focus of this report is on free nanomaterials.

Both organisations involved in this report, the JRC and EASAC provide independent science-based evidence and advice to European policy-makers and citizens. Whereas the JRC has in-house expertise and laboratories in nanotechnology research, EASAC has access to first-class research through its Academy members and their academic networks. A strategic liaison between our two organisations was agreed to generate a first joint policy report in order to achieve the strongest possible impact for our advice on this very important topic for Europe.

A working group was established in 2009 to consider issues for the possible impact of nanomaterials on human health, focusing specifically on engineered nanomaterials.

The working group was given the remit to (1) describe the state-of-the-art in regard to benefits and potential risks; (2) review current methods for safety assessment and evaluate their relevance; (3) identify knowledge gaps in studying the safety of nanomaterials; and (4) recommend priorities for nanomaterial research and the regulatory framework.

This report focuses on key principles and issues, cross-referencing other sources for detailed information, rather than attempting a comprehensive account of the science. The focus is on human health although environmental effects are also discussed when directly relevant to human health. Our aim is that this joint initiative between EASAC and the JRC will lead to wide dissemination of our recommendations to audiences in academia, industry, the policy-making community and other stakeholders, to support the collective goal of safe nanotechnology-based products on the European market.

2 Legal and Societal Implications of Nanosafety: Regulations and Governance

2.1 Societal issues and risks perceptions

Advances in nanotechnology raise questions about how to deal with uncertainty when there is insufficient knowledge regarding health impacts. Questions emerge at different 'levels' and range from the following:

- very specific scientific queries on how to understand the interaction of nanomaterials with the human body, to
- concerns of consumers on the safety of products and the general benefits for the use of nanotechnology, and finally to
- policy questions on how to address safety issues and concerns from the regulatory side and how to develop appropriate governance systems to cope with the novelties of nanotechnology.

These questions have, therefore, also to be addressed on different levels by using appropriate approaches. These include use of research funding to provide new information, regulatory actions, self-regulation, governance structures to assure transparency and comprehensive information, stakeholder involvement, as well as public engagement and dialogue, as discussed subsequently in this and the following chapters. It should be emphasised that the issues for nanotechnology are not different in principle to any other emerging technology, where appropriate tools and practices for dealing with potential risks must be developed. However, ethical issues and societal concerns are not always clear-cut and may become more pressing with the increasing uses or potential misuses of nanotechnologies. Public engagement to address concerns may require different policy instruments from those technical tools applied to deal with the regulation of safety risks.

As described in Chapter 3, nanotechnology holds considerable promise in many different technological areas. To realise progress, it is necessary to adopt an '*integrated, safe and responsible*' strategy as already laid down in the first Action Plan of the EC¹².

Innovation through the development of nanotechnology-based products must ensure a high level of human health, worker safety and environmental protection in order to obtain and secure consumer confidence and workers' trust. For this reason an integrated approach must foster both innovation and safety by addressing all safety issues while enabling industry to enhance its competitiveness.

Activities on the governance of nanotechnologies should encompass all issues related to environment, health and safety (EHS) and take due account of ethical, legal and social aspects (ELSA). This requires the use of appropriate instruments¹³ including the following:

- Knowledge gathering.
- Self-regulation and voluntary measures.
- Regulation by adaptation of the existing regulatory framework.
- Transnational collaboration.

These instruments are applied according to the different timescales for which actions are desirable and progress can be achieved: for example, information gathering and funding of research programmes as immediate action; adoption of voluntary measures and self-regulation by involvement of relevant stakeholders in the short to medium term; the implementation of regulation, taking into account specific nanotechnology issues, in the medium/longer term. These activities are discussed in further detail in the following sections.

At the present time, concerns about nanotechnology products and their perceived risks relate mainly to materials that are in a particulate form at the nanoscale, and which are mobile in their immediate environments. For this reason, current activities about regulation and governance of nanotechnology concentrate on 'free' engineered nanoparticles and their applications as part of the focus on first-generation, passive nanomaterials (see Figure 1 in Chapter 1), but as described previously (Box 1.2) it is also important to take account of possible changes during the product lifecycle, for example when a composite product is manipulated.

An urgent need for appropriate legislation to manage the potential risks of nanomaterials was communicated in a non-binding resolution adopted in April 2009 by the European Parliament¹⁴. In this resolution, it was questioned whether current EU legislation is adequate to deal with the potential health, environment and safety hazards of nanomaterials and the Commission was requested to review all relevant legislation by 2011.

The Parliament considered it particularly important to address nanomaterials explicitly, at least within the scope of legislation on chemicals, food, waste, air and water, and worker protection. The Parliament's opinion also included several specific requests to the Commission, about certain

¹² European Commission: http://ftp.cordis.europa.eu/pub/nanotechnology/docs/action_plan_brochure.pdf.

¹³ Observatory Nano Project: <http://www.observatory-nano.eu>.

¹⁴ European Parliament, A6-0255/2009 (2009).

aspects of regulation, the definition of nanomaterials, labelling, ethics, the involvement of stakeholders, fact-finding, research and co-ordination.

Box 2.1

Summary of findings from the European Parliament, Science and Technology Options Assessment (STOA) report on nano-safety – risk governance of manufactured nanoparticles

The STOA report gives a brief review of regulatory activities about manufactured particulate nanomaterials at the European level, discusses advantages and limitations of selected regulatory instruments and presents first ideas for options for parliamentary action.

The report concludes that risk assessment needs further information on hazard and exposure. Nanoscale particulate material implies novel material properties which may lead to novel health and environmental risks. Certain nanomaterials may induce pathologic conditions at high dose (hazard) and/or over a long period of time (exposure).

Data available provide a basis for further investigations on fate and behaviour in the environment and on toxicity, including clarification of underlying mechanisms. But there are only limited data available on the hazard and fewer on exposure. Therefore, information is needed on acute and chronic exposure, appropriate instruments to assess exposure and hazards, toxicity studies of particulate material on a case by case basis, investigation of the biological relevance and dose dependence.

The STOA report suggests various actions, to foster research activities and their co-ordination internationally, standardisation of methods, publication of no-effect data, the independent systematic review of information and the multidisciplinary training of young scientists.

Recently, two reports were published considering scientific knowledge, risk aspects and scientific governance, which are relevant to our present report: the Nanosafety report (interim report phase II) on manufactured nanoparticles by the European Parliament¹⁴ (the STOA Report; see Box 2.1) and the EASAC Policy Report Number 13 on Synthetic Biology¹⁵ (see Box 2.2). The STOA Report will be of particular importance in informing and stimulating further discussion with politicians at the European level but, of course, there is also need to build engagement with politicians at the national level and with policy-makers in the other European institutions.

The previous EASAC Report covers various specific issues for safety, security, public involvement and product regulation for synthetic biology that are also very relevant for nanomaterials. Taken together, it is hoped that these two reports with our present report and other relevant outputs (for example, from the recent Nanomedicine Roundtable and other initiatives in public engagement (see Chapter 4), will provide a very useful collective resource for communicating strong evidence-based

messages. Relevant issues outlined in the two boxes (2.1 and 2.2) will also be discussed in further detail in the following chapters.

Of course, the issues are not confined to Europe. Transnational collaboration is an important aspect of nanotechnology governance, because all countries face in principle the same ethical, legal and societal implications and related problems, the degree depending on their involvement through industrial activities, public research funding and regulatory assessment. In the EU, Member States follow closely the developments on Community level, several countries with strong involvement in nanotechnology development, for example Germany, UK, France and the Netherlands, have launched activities at a national level (see section 2.2.1). Furthermore, international collaboration with the USA, Canada and Australia and Asian countries, in particular, Japan, China, India and Taiwan, is being intensified at various levels regarding governance, research and regulatory activities, as well as attending to standardisation and harmonisation issues.

Box 2.2

Summary of findings of the EASAC Policy Report 13: “Realising European potential in synthetic biology: scientific opportunities and good governance”

Synthetic biology is the engineering of biology; it is deliberately designing and constructing novel biological systems to perform new functions. The report is derived from activities by national academies of science together with analysis and advice from an EASAC expert Working Group. It recommends identifying features that distinguish synthetic biology from systems biology and other technologies, exploring what contribution synthetic biology might make to tackle EU societal needs and economic growth, assessing what is needed to create an appropriate regulatory environment and clarifying the implications for EU policy-making priorities.

Synthetic biology will lead to a better understanding of natural biological systems. Scientific advances in methodology, where EASAC identifies continuing opportunities for European research include, minimal genomes, i.e. the smallest number of parts needed for life (basis for engineering minimal cell factories for new functions), orthogonal biosynthesis (engineering the cells to expand the genetic code), regulatory circuits (artificial networks to provide new functions in cells and organisms), metabolic engineering (new levels of complexity), protocells (synthetic cells) and bio-nanoscience (molecular-scale motors for cell-based machines or cell-free devices to perform complex new tasks). Some research directions in synthetic biology overlap with nanotechnology: issues for biosafety may also be relevant for nanotechnology.

Synthetic biology offers the potential to engineer new levels of safety into the applications. It is concluded, that existing legislation is adequate as long as synthetic biology remains an extension of recombinant DNA technology and the scientific community commits to developing voluntary codes of conduct.

¹⁵ EASAC Policy Report 13, EASAC, Halle (2010).

2.2 Regulation and governance

2.2.1 Regulatory landscape

As will be indicated in Chapters 3 and 4, there are still significant gaps in the understanding of nanomaterials and their impact on health and the environment. Moreover, the absence of an accepted definition of nanomaterials (Chapter 1), their application in many different industrial sectors, and the lack of appropriate standards and testing procedures, imposes obstacles to progress on regulation that must now be overcome. So far, nanomaterials and related products are dealt with under existing broader regulatory schemes and worldwide there are only very few examples where nanospecific regulation has been put in place. Recently, an overview of the worldwide regulation landscape has been compiled within the Framework Programme 7 project ObservatoryNano and some findings are outlined below.

In general, regulatory authorities in Europe, the USA, Canada and Australia have become more proactive in recent years to cope with the complex issues for the regulation of nanomaterials and products. The European Commission, Canada and Australia, in particular, have adopted an approach that provides guidance and adapts regulation for nanotechnologies. Asian countries such as China, Japan, India and Taiwan are looking to Europe (and the USA) for information in developing their legislation dealing with nanomaterials and products thereof.

With respect to relevant EU legislation, a distinction can be made between horizontal legislation (e.g. chemicals legislation, worker protection and environmental legislation) and sector-specific legislation (e.g. cosmetic products, food legislation, biocidal products, medicinal products, medical devices, electrical and electronic equipment). In general, current EU legislation applies to nanomaterials without specifically addressing them. However, the Commission acknowledges that regulatory changes may be needed, based on new scientific findings. Discussions about legislative initiatives on nanomaterials are also taking place at national level in some EU Member States, in particular in France, Germany, the Netherlands, UK, Austria, as well as in other countries, in particular Switzerland and Norway.

Though on regulatory matters the European Member States tend to follow the inputs from the EC, several countries have activities at the national level of their own. Most of the other European countries have also started activities on nanotechnology regulation mainly with respect to REACH and occupational and health safety aspects of nanomaterials.

2.2.2 Nanospecific adaptations of EU regulations

As noted in the previous section, initiatives for the adaptation of regulation to take into account the specific attributes of nanomaterials are proceeding worldwide with different emphasis according to the national involvement in nanotechnology developments and traditions in legislation and regulation.

In Europe, issues about regulation of nanomaterials were discussed in different Scientific Opinions of working groups and technical committees of the European Commission and EU Agencies¹⁶. Based on an internal regulatory review, the European Commission adopted the Communication 'Regulatory Aspects of Nanomaterials' in June 2008, concluding that the existing EU regulatory framework 'covers in principle the potential health, safety and environmental risks in relation to nanomaterials'¹⁷. It is, however, acknowledged that regulatory changes may be needed in the light of new information becoming available. In its resolution, adopted in April 2009, the European Parliament¹⁶ queries this position and, in addition to the request for a regulatory review by 2011, it states that the current EU legislation is inadequate and that nanomaterials should be explicitly addressed.

At present, the regulations governing the areas of chemicals and materials, medical devices, cosmetics, pharmaceuticals, foods, as well as horizontal regulation related to occupational health and worker safety, and environmental safety are scrutinised for nanospecific provisions. Moreover, following the requests of the European Parliament, horizontal aspects are being discussed by the European Commission to cover the definition of nanomaterial, labelling of nanomaterials in products, and the establishment of a nanomaterial inventory at Commission level.

2.2.2.1 Chemicals: REACH

The most comprehensive horizontal piece of legislation relevant to nanomaterials is the EU chemicals legislation, REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals)¹⁸. REACH applies to chemical 'substances' on their own, in mixtures or in products. Although there are no provisions in REACH referring specifically to nanomaterials, the scope of REACH includes chemical substances, in whatever size, shape or physical state (CA/59/2008 rev.1)¹⁹.

Substances at the nanoscale are therefore covered by REACH and its provisions apply. The same consideration applies to other legal instruments that use the same

16 SCENIHR Opinion, 10.03.2006; SCENIHR Opinion, 29.11.2007; Scientific Committee on Consumer Products (SCCP) Opinion, 18.12.2007; European Food Safety Authority (EFSA) Opinion, 10.02.2009.

17 European Commission, COM(2008) 366 final 17 June 2008.

18 European Commission, Regulation (EC) No 1907/2006 – OJ L 136, 29.5.2007, p. 3.

19 Nanomaterials in REACH (CA/59/2008 rev.1).

(Biocidal Products Directive²⁰ and Cosmetic Products Regulation²¹) or a similar (Plant Protection Products Directive²²) substance definition as REACH.

Concerns that have been expressed about REACH relate to its applicability for the chemical safety assessment of nanomaterials because of the lack of knowledge about their physico-chemical features and effects on human health and the environment. Moreover, REACH registration and chemical safety assessment requirements depend on the volume of the chemical substance manufactured or imported on an annual basis (currently 1 tonne/year threshold level for registration and 10 tonnes/year for conducting a chemical safety assessment, although discussion is continuing as to whether these are the appropriate thresholds). These limits may put some nanomaterials manufactured or imported in low volumes outside the requirements of registration. However, it should be noted that other provisions in REACH, such as requirements for classification and labelling, provisions of Safety Data Sheets, as well as the Authorisation and Restriction procedures apply without a threshold level.

To exchange views on existing and future implementation issues and other matters related to nanomaterials under REACH, the REACH Competent Authorities Sub Group on Nanomaterials (CASG Nano) was created in March 2008. The group will provide recommendations to the REACH Competent Authorities and advise the Commission taking into account stakeholder views. In support of the Group, the JRC Institute for Health and Consumer Protection has performed and co-ordinated three REACH Implementation Projects on Nanomaterials dealing with (1) the substance identification of nanomaterials, (2) information requirements on intrinsic properties of nanomaterials and (3) exposure assessments and hazard and risk characterisation of nanomaterials.

2.2.2.2 Medical devices and medicinal products

Medical devices and medicinal products are subject to a detailed authorisation procedure and the existing provisions are generally considered adequate for products containing nanomaterials. The evaluation and authorisation procedures of such products should properly take into account specific properties of nanomaterials in the various applications. A particular problem for the application of nanomaterials in this field arises with possible complex mechanisms of action causing a blurring of borderlines between different regulatory and classification systems (e.g. those pertaining to therapeutic, diagnostic and imaging products). For both medical devices and medicinal products there are activities continuing at the European level, discussing

the consequences of advances in nanomedicine for risk assessment and the development of guidance.

2.2.2.3 Cosmetics

The European Parliament and the Council have adopted the new Cosmetic Products Regulation, which will enter into force in July 2013 and which introduces various provisions specific to nanomaterials. These provisions include a notification obligation for manufacturers about the presence of nanomaterials in cosmetics not subject to prior authorisation; a possibility for the Commission to request a safety assessment for such materials by the Scientific Committee for Consumer Safety; and a labelling requirement for nanomaterial ingredients. This means that in the list of ingredients the names of such substances shall be followed by the word 'nano' in brackets.

The Cosmetic Products Regulation also introduces a definition of nanomaterials: '*nanomaterial*' means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm'. The Regulation also includes a review clause, which states that the definition shall be adapted to technical and scientific progress. Other Regulations/Directives with such provisions might follow.

2.2.2.4 Definition of nanomaterial

The introduction of provisions specific to nanomaterials requires the adoption of a definition. Consequently, the regulation on cosmetics and the future regulation on the provision of 'Food Information to Consumers' introduced a definition (more details below). However, in view of the various definitions of nanomaterials published by different bodies, and the constant technical and scientific developments in the field (Box 1.1, Chapter 1 and see later in this chapter), the Commission is given a mandate to adjust these definitions in the light of technical and scientific progress, and to align them with definitions subsequently agreed at international level. This raises the question as to whether an overarching, 'harmonised' definition of nanomaterial across the different regulatory areas would be appropriate²³.

An advantage of different definitions would be that the definition could be tailored to the needs of specific legislative instruments. However, a chemical substance might be used in different industrial sectors and areas of application. Thus, different definitions would lead to the situation, that the same substance could be regarded as a nanomaterial under one legal instrument, but not under another. To avoid such confusion, a common definition

20 European Commission, Directive 98/8/EC concerning – OJ L 123, 24.4.1998, p. 41.

21 European Commission, Regulation (EC) No 1223/2009 – OJ L 342, 22.12.2009, p. 59.

22 European Commission, Directive 91/414/EEC – OJ L 230, 19.8.1991.

23 Maynard, A.D., *Nature* **475**, 31 (2011).

would be desirable if it could be made broadly applicable in EU legislation and policies, for example in chemicals legislation, worker protection legislation, and legislation on air and water quality or waste. The implicit condition – even if difficult to achieve – is that such a common definition is suited for all the regulations and policies it intends to serve.

For legal instruments it is required that the definition is clear and unambiguous so that it is easy to implement. In addition, compliance checks must be possible, for example in order to assess whether the envisaged future labelling requirements for certain products could be fulfilled. Thus, the definition must be enforceable and this implies that appropriate measurement techniques are available and methods and procedures for their application are agreed. In recent reports considerations on elements for a definition for regulatory purposes⁶ and proposals for such a definition²⁴ have been published.

The Commission had drafted a recommendation for a generally applicable definition which was subject to public consultation in 2010. A final decision on a recommendation taking into account opinions of stakeholders is still pending. To preserve the integrity of the EU internal market, it is necessary to ensure that such a definition is accepted by all EU Member States, thus avoiding claims of additional national regulatory needs. Furthermore, in light of the global market, a European definition should be in line with international initiatives, e.g. Organisation for Economic Co-operation and Development (OECD) and the standardisation bodies, the International Organization for Standardization (ISO). It should also be emphasised that each definition has implications within the context in which it is used. Therefore, any definition will also involve policy choices, and accordingly will inevitably entail political decisions.

2.2.2.5 Labelling

The resolution of the European Parliament calls *'for the provision of information to consumers on the use of nanomaterials in consumer products'* requesting that *'all ingredients present in the form of nanomaterials in substances, mixtures or articles should be clearly indicated in the labelling of the product'*.

As previously discussed, this stipulation has already been included in the European Regulation on Cosmetic Products²² where it is stated that all ingredients present in the form of nanomaterials shall be clearly indicated in the

list of ingredients. The EP request has also been taken into account in a recent resolution of the European Parliament on the provision of 'Food Information to Consumers'²⁵. Amendments proposed for the Restriction of Hazardous Substances Directive, which places restrictions on the use of certain hazardous substances in electrical and electronic equipment, also include the request for notification of the use of nanomaterials, a standard for the identification and detection of nanomaterials, and harmonised labelling.

2.2.3 Self-regulation and voluntary measures

In the current phase characterised by the simultaneous rapid accumulation of scientific knowledge on nanosafety together with progressive penetration of the market by innovative nanotechnology products, where regulation faces difficulty to keep pace with the new developments, a culture of responsibility is necessary to maintain trust. As part of this responsibility, adopting voluntary measures for risk management systems and codes of conduct can have an important role in dealing with current uncertainties about the impact of nanotechnologies.

In February 2008 the Commission adopted a recommendation for a 'Code of Conduct for responsible nanosciences and nanotechnologies research'²⁶. It contains a series of principles and guidelines that Member States and ultimately all stakeholders in the field of research are invited to adopt and promote. The objectives are far-reaching and among the principles that must be respected, (1) sustainability, (2) precaution, (3) inclusiveness, and (4) accountability, are of particular relevance. The Code of Conduct is voluntary and complementary to existing regulations. The Code will be monitored and revised every two years by the Commission to take into account new developments in nanotechnology.

In addition, because of the current uncertainty in the regulatory situation, some stakeholders, mainly at industrial level, have developed (or are developing) their own risk management systems, defining best practices and procedures for safety control and handling of nanomaterials in occupational settings.

The DuPont/Environmental Defence Nano Risk Framework²⁷ and the CENARIOS risk management and monitoring system²⁸, are two examples of such an approach. Other voluntary measures involve the development of reporting schemes²⁹. These instruments are used by regulatory authorities, for example Voluntary Reporting Scheme, DEFRA (UK)³⁰ and the Nanoscale

24 SCENIHR Final Opinion 08.12.2010.

25 European Parliament in Document P7_TA(2011)0324

<http://www.europarl.europa.eu/sides/getDoc.do?type=TA&reference=P7-TA-2011-0324&language=EN&ring=A7-2011-0177>.

26 European Commission, Commission Recommendation of 07/02/2008.

27 Environmental Defense - DuPont Partnership June 2007, Nano Risk Framework, <http://www.nanoriskframework.com>.

28 CENARIOS - Certifiable risk management and monitoring system, TÜV SÜD Industrie Service GmbH and The Innovation Society.

29 Linkov, I. et al., *Nanotechnology Law & Business* 6, 203 (2009).

30 DEFRA, UK Voluntary Reporting Scheme for engineered nanoscale materials (2006).

Materials Stewardship Program, (US Environmental Protection Agency)³¹, to collect information from industry about the manufacturing, production and use of nanomaterials³². Information required includes material specifications, production volumes, risk assessment and risk management data, and methods to provide firmer evidence for regulatory and policy decisions.

One other activity relevant in this context is exemplified by the Swiss Action Plan for Synthetic Nanomaterials³³ (see Box 2.3) which was the basis for the development of a precautionary matrix³⁴ for products and applications that involve engineered nanomaterials. The matrix provides a structured method to assess the 'nanospecific precautionary need' of workers, consumers and the environment arising from the production and use of synthetic nanomaterials. The matrix is a tool to support trade and industry to meet their obligations of care and self-monitoring. It helps them to recognise applications that might entail risk and to take precautionary measures to protect human health and the environment. In the case of new developments, the matrix can contribute to the innovation of safer products. It enables users to conduct an initial analysis on the basis of currently available knowledge and indicates when further investigations are necessary.

Box 2.3

An example of a voluntary approach

The Swiss Action Plan on Synthetic Nanomaterials, based on a detailed report³⁵ focuses on the following priority actions:

- Creating the scientific and methodological preconditions to recognise and prevent the possible harmful impacts of synthetic nanoparticles on health and the environment.
- Creating the regulatory framework for responsible handling of synthetic nanomaterials.
- Promoting public dialogue about the opportunities and risks of nanotechnology.
- Using better existing promotional instruments for the development and market launch of sustainable applications of nanotechnology.

This action plan aims to develop a precautionary matrix for products and applications that involve engineered nanomaterials as the core measure for empowering industry, commerce and trade to take greater responsibility in this area and to apply the precautionary principle in a targeted and cost-effective manner. This was the background for developing the Swiss precautionary matrix for synthetic nanomaterials, which is intended as a screening tool for trade and industry to follow a structured approach to recognising the risk potential when dealing with engineered nanomaterials.

The matrix described in Box 2.3 is already in use by a broad circle of applicants in Switzerland and elsewhere. It will be further developed in close co-operation with trade, industry and science as well as with consumer and environmental organisations. Despite this initiative and other voluntary actions and registries across Europe, there is also an ongoing debate on the need for mandatory registration. Some EU Member States are currently discussing the creation of an appropriate database, and this may result in the development of a European-wide registry with implications for labelling.

2.3 Standardisation and harmonisation of test methods

Internationally harmonised standards and methods are indispensable for the evaluation of environmental, health and safety risks. The OECD and the standardisation bodies, the ISO and the European Committee for Standardisation (CEN) have established working groups and technical committees that play a key role in the development of measurement standards and formally recognised test methods and guidelines for nanomaterials. In the USA, there has been substantial work by the US National Nanotechnology Characterisation Laboratory, for example relating to nanomaterials for cancer therapies and diagnostics³⁶. In Europe most test guidelines applicable under EU regulations are based on the work of the OECD. In 2006 the OECD established the Working Party on Manufactured Nanomaterials (WPMN), to promote international co-operation in the health, safety and environmental issues of manufactured nanomaterials. It is the main forum for international co-operation in this area for the development of test methods needed for the proper implementation of regulation.

Box 2.4

Actions of OECD Working Party on manufactured nanomaterials

- Development of a database on human health and environmental safety research.
- 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.

31 US EPA, Concept Paper for the Nanoscale Materials Stewardship Program under TSCA (2007).

32 Fiedeler, U. et al., Institute of Technology Assessment of the Austrian Academy of Sciences, Nanotrast-Dossiers No 16 (2010).

33 Swiss Federal Council, Action Plan Synthetic Nanomaterials (2008).

34 Swiss Federal Office of Public Health FOPH, Swiss Federal Office for the Environment FOEN, Guidelines on the precautionary matrix for synthetic nanomaterials (2010).

35 Swiss Federal Office for the Environment FOEN, Federal Office of Public Health FOPH, Grundlagenbericht zum Aktionsplan (2007).

36 See, e.g. <http://ncl.cancer.gov>.

The Working Party is implementing its work through specific projects to further develop appropriate methods and strategies as shown in Box 2.4.

A flagship activity is the so-called 'sponsorship programme'³⁷ launched in November 2007. This pools resources from all of the OECD Member countries and industries in an effort to perform tests on an agreed priority list of 13 commercially relevant nanomaterials³⁸. The outcome of this programme is expected in the next years and will serve as a very significant resource to support the implementation of enhanced safety requirements.

A key condition is to make tests comparable, given the involvement of many independent research institutions. For nanomaterials this is a significant challenge because reference nanomaterials, measurement and dosimetry are still in continuing development.

There is a close collaboration with the standardisation work of ISO and CEN. Standardisation activities for nanotechnology with relevance for European legislation are driven by ISO and CEN in the Technical Committees CEN/TC 352 and ISO/TC 229, both initiated in 2005. Several EU national bodies contribute to the ISO work; industry associations, consumer organisations as well as the EC have become liaison members.

To avoid duplication, and because of the global relevance of harmonised standards, EU members have expressed their preference for the development of standards at the ISO level. For topics of mutual interest to both ISO and CEN, work is performed under the Vienna Agreement³⁹, with an ISO or CEN lead.

The contribution to the development of ISO standards by CEN is, incidentally, one way of involving in the nanotechnologies standardisation process those EU countries that do not have the means to participate at ISO level.

Forty different ISO documents are currently being developed in ISO/TC 229 in the fields of terminology and nomenclature (JWG1); measurement and characterisation (JWG2); health, safety and environmental aspects of nanotechnologies (WG3); and materials specifications (WG4). Harmonisation of methods requires also other quality assurance tools such as standardised (reference) materials.

The EC-JRC is supporting the activities of standardisation by maintaining a repository of currently 25 reference nanomaterials⁴⁰ (including many of the OECD priority materials) which can be used for a harmonised safety assessment in research institutions.

37 OECD-WPMN Sponsorship Programme: <http://www.oecd.org>.

38 OECD: [http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono\(2010\)46&doclanguage=en](http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono(2010)46&doclanguage=en).

39 International Organization for Standardization: <http://www.iso.org>.

40 European Commission, JRC, IHCP: <http://ihcp.jrc.ec.europa.eu>.

3 Opportunities and Safety Considerations

A prerequisite of success for any new product on the market, whether or not containing nanomaterials, is to gain acceptance by consumers. As there is no such thing as zero risk, safety aspects need to be carefully examined to understand whether the expected benefits of new products outweigh the potential risks. Consumers accept risks more readily if there are clear benefits and if the risks can be controlled. As information on benefits is provided by many other published sources, our focus in this chapter, as in the report overall, is on the safety considerations. At the outset, we note that there is greater knowledge about the hazard assessment of engineered nanomaterials than there is about human exposure to such materials.

3.1 Applications and characteristics

Nanotechnology has already demonstrated its great potential, as described in Chapter 1, and nanomaterials are increasingly used in innovative applications and products. Every-day consumer products may be made lighter, stronger, cleaner, less expensive, more effective and efficient, more precise, or more aesthetic. Products containing nanomaterials may improve our quality of life through more efficacious, targeted, pharmaceuticals, improved medical diagnosis tools, faster computers, clean water and cleaner energy production, to mention just some of the impending applications. It is also worth mentioning that nanomaterials can be used as research tools for investigation in laboratory settings and these are not intended for wider dissemination. The present report focuses on products to which consumers may be exposed.

Nanomaterials often display different chemical, physical and biological characteristics when compared with larger-sized materials and thus behave differently, even when the elemental or molecular composition is the same. Some of their properties can be extrapolated from the macroscale, whereas other attributes change significantly below a certain size. Nanomaterials have a much larger specific surface or interface area, i.e. a larger area to mass ratio, than coarser materials. Furthermore, there are intrinsic nanoscale properties that result from the confinement of atoms and electrons within boundaries of a few nanometres. These effects are dominant at sizes below a few tens of nanometres and they can change fundamental physical material characteristics such as the optical, electrical and magnetic properties of the nanomaterial. For safety assessment it is important to take into account, as for any other chemical, potentially important other properties that may include surface charge, penetration

ability, adhesion, solubility, immunogenicity, aggregation, shape, hardness, degradability, biopersistence, reactivity and other specific toxicities. Complexity of surface, multifunctionality of nanomaterials and covalent or adsorbed surface coatings all play a role in the determination of risk. Thus, it is important to address nanomaterial-specific considerations in the context of the general nanosafety objectives (environmental, occupational exposure and purposeful use).

In the following sections, we do not attempt a comprehensive description of all areas of application but focus primarily on consumer products and medical applications, two of the principal areas currently of interest to researchers and regulators.

3.1.1 Consumer products

Several of the consumer end-products available today that utilise nanomaterials have been developed from existing products, for example by the incorporation of nanomaterials into solid, viscous or liquid matrices. Examples of applications with end-products containing nanomaterials are listed in Box 3.1.

Box 3.1

Examples of consumer products that may contain nanomaterials

- Cosmetics and personal care products
- Paints and coatings
- Household products, e.g. for cleaning
- Catalysts and lubricants
- Sports products
- Textiles
- Food and nutrients
- Food packaging and kitchenware
- Consumer electronics

According to the Woodrow Wilson Nanotechnology Consumer Products Inventory (see Chapter 1), about one-third of these products are sunscreen lotions or cosmetics such as skin-care and colorant products.

For sunscreens, titanium dioxide and zinc oxide nanoparticles are used because they absorb and reflect ultraviolet rays but are still transparent to visible light: the resulting sunscreen becomes both more appealing to the consumer and is claimed to be more effective. Uncertainties about which cosmetic products already on the market actually contain nanomaterials led to questions about their safety⁴¹.

41 Nohynek, G.J. et al., *Skin Pharmacology and Physiology* **21**, 136 (2008).

In the food product sector, although there is not much knowledge about the occurrence of manufactured nanomaterials, a beneficial effect has been shown for food contact materials⁴². Further information is provided in the European Food Safety Authority (EFSA) report⁴³. Nano-silver has many applications in many consumer products such as, for example, textiles making use of established antibacterial properties. A recent study has comprehensively investigated the challenges associated with human health risk assessment of nano-silver⁴⁴.

The diversity of materials and products renders it a difficult task to ascertain how many 'nano-products' are on the market today. As discussed in Chapter 1, it is relevant to observe that the inclusion of products in the Woodrow Wilson database has been made on the producer's claim that the product is a 'nanoproduct'. Thus, the actual nanomaterials used within consumer products are not always known and, for the purpose of quantifying exposure assessment, it is also important to realise that information on the concentration of the nanomaterials in individual consumer products is generally not available¹¹. If neither the identity nor the concentration of the nanomaterials used is known, it is not possible to take the safety aspects of nanomaterials into account.

3.1.2 Medicine

Nanotechnology in medicine plays an important role in novel diagnostic and therapeutic approaches, drug delivery systems and tissue engineering. The use of nanoparticles in medicine is estimated to be the most rapidly expanding nanomaterial field of research⁴⁵. This field is very broad: nanoparticles of many sizes, shapes, materials and structures with many core physico-chemical properties, and in many combinations with multiply structured coatings, are being investigated for diagnostic or therapeutic use. Intended routes of exposure include, for example, oral, intravenous, intranasal, vaginal, buccal and dermal. To cite just one example: an advanced area is the application of nanoparticles in cancer therapy, using gold nanorods, magnetic nanoparticles and carbon nanotubes to generate heat upon electromagnetic or infrared stimulation after direct injection into tumours or accumulation in tumours after systemic administration. Nanoparticle-mediated thermal therapy is a new and

minimally invasive tool as a treatment of cancers⁴⁶. This nanoparticle platform for thermal ablation of tumours can be combined with magnetic resonance imaging contrast agents to enhance simultaneous imaging modalities. Moreover, single-walled carbon nanotubes can be used as a novel contrast agent for non-invasive photo-acoustic imaging of tumours⁴⁷.

Another active area of research is leading to the development of drug delivery systems for cell-specific therapy by receptor-targeted nanocontainers. Of particular interest are injectable nanovehicles that are programmable towards specific targets, able to evade the immune defence, and sufficiently versatile to be suited as carriers of complex functionality.

Rather than attempting here to provide comprehensive discussion of the agents currently being tested in pre-clinical and clinical research, the opportunities and challenges in the science and the issues for the regulatory framework in nanomedicine, key documents and activities are cited from previous European initiatives⁴⁸; the work of the US National Nanotechnology Characterisation Laboratory cited in Chapter 2 is also highly relevant.

Box 3.2

The nanomaterials paradox: desired effects versus unexpected hazardous impact on health

The introduction of nanomaterials into clinical and other applications highlights the so-called 'nanomaterial paradox': the very same properties that are desirable and potentially useful from a technological or biomedical perspective, such as the ability to cross biological barriers and the high degree of surface reactivity, are also the properties that may give rise to unexpected and hazardous toxicities. It can be noted, however, that the nanomaterials paradox is not unique to nanomaterials or indeed to nanomedicine; for example the principle applies also to pharmaceuticals.

As a more general point, the nanomaterials paradox (Box 3.2) is pertinent in raising issues for safety assessment of nanomaterials in consumer products.

All medical applications require careful evaluation of the biodistribution, biopersistence and biocompatibility of the administered nanomaterial. However, the fundamental challenge in nanomedicine as, indeed, in other applications

42 Chaudhry, Q. et al., *Food Additives & Contaminants A* **25**, 241 (2008).

43 European Food Safety Authority, EFSA Scientific Opinion, *The potential risks arising from nanoscience and nanotechnologies on food and feed safety*, (2009).

44 Christensen, F. et al., *Nanotoxicology* **4**, 284 (2010); Nowack, B. et al., *Environmental Science & Technology* **45**, 1177 (2011).

45 Ben-Haim, N. et al., *Nano Letters* **8**, 1368 (2008); Seigneuric, R. et al., *Current Molecular Medicine* **10**, 640 (2010).

46 Krishnan, S. et al., *International Journal of Hyperthermia* **26**, 775 (2010).

47 De la Zerda, A. et al., *Nature Nanotechnology* **3**, 557 (2008).

48 European Science Foundation Forward Look on Nanomedicine 2005, followed by a series of research conferences and training events, accessible on www.esf.org; JRC, Nanomedicine: Drivers for development and impact, 2006; European Technology Platform on Nanomedicine, accessible on <http://cordis.europa.eu/nanotechnology/nanomedicine.htm>; European Commission Safety for Success Dialogue annual meetings, accessible on http://ec.europa.eu/health/nanotechnology/events/ev_20110329_en.htm; EMA first Intern. Workshop on Nanomedicine Reg.: http://www.ema.europa.eu/docs/en_GB/document_library/Agenda/2010/03/WC500075789.pdf; Duncan, R. and Gaspar, R., Nanomedicines under the microscope, 2011, accessible on <http://www.ruthduncan.co.uk/#/2011-nanomedicines-review/4551969069>.

is how to modify nanomaterials so that the toxic effects are mitigated while preserving the unique and highly desirable properties of these materials. Of course, this challenge for developing novel healthcare approaches is not specific to nanomaterials and the regulatory framework for novel therapeutics routinely includes the study of side-effects in order to assure appropriate product safety. Furthermore, there is extensive experience in the pharmaceutical and biotechnology sectors for optimising the benefit–risk balance by assessing structure–activity relationships for series of chemically or biologically related candidates. The ‘quality by design’ approach with an integrated assessment of quality (including specification of product and reproducibility in manufacturing), safety and benefit, which is standard practice in the medical sector, provides an important model more generally for ‘safety by design’ for nanomaterials for other applications.

3.2 Safety aspects: overview on risk assessment methodologies and current results

As observed in Chapter 2, there is always a possibility that adverse effects will accompany the introduction of new technologies. Therefore the development and introduction of new materials and products must be accompanied by an appropriate risk-evaluation and risk-management process. For nanomaterials as for other materials, risk is a function of hazard and exposure; the general risk assessment paradigm^{7,49} comprises the following:

- Hazard identification, to understand which adverse effects are elicited.
- Hazard characterisation, to determine the amount of nanomaterial needed to provoke a response (the dose–response function).
- Exposure assessment, to understand the amount of the material to which consumers or the environment are exposed.

Hazard identification for nanomaterials is covered by the discipline of nanotoxicology; the toxicological aspects of nanomaterials are summarised in numerous publications, of which we cite only a few of the more recent⁵⁰. Toxicity testing of engineered nanomaterials using *in vitro* or *in vivo* assays should aim to characterise a potential hazard by establishing the dose–response relationship. However, as the risk of adverse effects is a function of hazard and exposure, the assessment of the extent of possible exposure is essential if meaningful conclusions are to be generated. In this context it is important to appreciate that

any nanomaterial administered at high enough doses will induce a significant ‘toxic’ effect. Therefore to be relevant, toxicity testing must identify which assays should be used, at which doses effects occur, and how realistic are these doses compared with human exposure conditions. Use of the dose–response approach also allows comparison of different nanomaterials and their comparison to reference substances of known properties. Well conducted dose–response studies with the observation of no effect levels may give (with proper use of uncertainty/safety factors) some indication on exposure levels resulting in low or negligible harm. Even relatively harmless titanium dioxide nanoparticles given at high enough and repeated doses through inhalation have been demonstrated to induce lung tumours in rats due to lung overload⁵¹. More details can be found in Box 3.3.

Box 3.3

Problems concerning unrealistic exposure doses

Overload conditions especially in the lung (macrophages) have been described for 20 years. It is now well known that overloading the lung with dust particles will severely influence (reduce) the clearance process, thereby prolonging dramatically the biological lifetime of particles within the lung. Eventually, this leads to persistent inflammatory effects with all the characteristics of lung diseases which often end in tumour formation. Therefore, it is recommended that overload conditions should be avoided both in animal studies and for *in vitro* experiments otherwise excessive doses will generate false-positive results.

This sense of realism is equally warranted for intended applications where engineered nanomaterials are targeted to the individual in relatively precise amounts. Moreover, testing genotoxicity with overloading concentrations (often cytotoxic concentrations) is also unrealistic as dying cells (apoptosis as well as necrosis) cleave their own DNA, resulting again in false-positive effects⁵². If non-overload conditions are chosen, no carcinogenic effects for such dust particles are found⁵³.

Most of the discussion of risk in this chapter refers to the risk of specific harm to human health. But there is another relevant aspect to the consideration of risk: the risk of not generating robust and relevant data, such that decision-making is poorly informed. Addressing both of these dimensions of risk requires the use of high quality procedures, for collecting data and for making regulatory judgements.

Performing risk assessment for engineered nanomaterials is a challenging task, not only because of scientific uncertainty and lack of data⁵⁴, but also because of the

49 Walker, N.J. and Bucher, J.R., *Toxicological Sciences* **110**, 251 (2009).

50 Shatkin, J.A. et al., *Risk Analysis* **30**, 1680 (2010); Sharma, M., *Indian Journal of Occupational and Environmental Medicine* **14**, 3 (2010); Clift, M.J.D. et al., *Archives of Toxicology*, Epub ahead of print (2010); Krug, H. and Wick, P., *Angewandte Chemie International Edition in English* **50**, 1260 (2011).

51 Bellmann, B. et al., *Environmental Health Perspectives* **97**, 189 (1992); Benn, T. and Westerhoff, P., *Environmental Science & Technology* **42**, 4133 (2008); Heinrich, U. et al., *Inhalation Toxicology* **7**, 533 (1995); Ferin, J., et al., *American Journal of Respiratory Cell and Molecular Biology* **6**, 535 (1992).

52 Roller, M., *Inhalation Toxicology* **21**, Suppl. 1, 144 (2009).

53 Yokohira, M. et al., *Toxicologic Pathology* **36**, 620 (2009).

54 Grieger, K.D. et al., *Nanotoxicology* **3**, 1 (2009).

necessity of taking into account the wide range of different materials, their functional properties and applications⁵⁵. Among the limitations often mentioned is the time-lag for an effect, and the associated costs to generate meaningful, quantitative results for risk assessments⁵⁶. But again, this situation is not necessarily unique to nanomaterials, and the general limitations to chemical risk assessment procedures have been noted⁵⁷.

In the following sections, we provide an overview on exposure and hazard identification and characterisation for risk assessment of nanomaterials. We start by discussing the prerequisite for assessing exposure and potential hazard: the possible entry of nanomaterials into the human body and an understanding of interactions of nanomaterials with tissues and cells.

Much of the research discussed in the following sections relates to the lung. This partly reflects the history of the field with its origins in a study of risk assessment for nanoparticles generated by combustion processes (ultrafine particles) but also takes account of the importance of the lung as portal of entry.

3.2.1 Entry of nanomaterials into the human body and interaction with cells

3.2.1.1. Biological barriers

There are three portals of entry for nanoparticles in the human body: the skin, the gastro-intestinal tract and the lung although, of course, these portals can be circumvented by direct injection or implantation of a substance. The lung is a major portal of entry and has been relatively well-studied: analysis of this knowledge base provides guidance on the types of study that may be needed to characterise other portals of entry (see also Chapter 4). Over the huge alveolar surface area of 150 m² the deposited particles are separated from the capillary blood by a tissue barrier. This barrier is less than 1 micrometre between the deposited particles and capillary blood⁵⁸. Therefore, they may penetrate through the very thin tissue barrier into the blood with which they will be transported into secondary organs⁵⁹. Nanoparticles may move through the tissue barriers in organs. Three critical structural parameters of the tissue barriers which

are important for human health have to be taken into consideration: (1) the distance of blood capillaries; (2) the distance of sensitive cells, in particular cells of the defence system, from the location of deposition of the nanoparticles; and (3) the character and property of the barrier structures. In the lungs there is a particularly thin air–blood tissue barrier. The nanoparticles deposited on the alveolar surface of the lung come into closest vicinity with the blood and they have been shown to be able to cross the air–blood tissue barrier and to penetrate into the blood capillaries. When within the blood stream they can translocate to any other organ of the body⁶⁰. Micrometre-sized particles have never been observed crossing the air–blood tissue barrier and nothing is known yet about the mechanism as to how nanoparticles can cross this tissue barrier.

The blood–brain tissue barrier, although rather thin, has so far usually been considered impermeable except to some drugs. However, it has now been shown that nanosized gold particles have promising applications for therapeutic and diagnostic purposes and if their surface is appropriately modified they may penetrate from the blood into the brain⁶¹. This ability of certain nanoparticles to cross the blood–brain barrier could enable the delivery of therapeutic compounds to the brain⁶² but it also demonstrates the potential for side-effects.

Another inner tissue barrier reached through the blood stream is the placental barrier⁶³. It has recently been shown in an *ex vivo* study that nanosized fluorescent polystyrene particles were able to cross the placenta, i.e. to move from the maternal blood circulation into the foetal blood circulation. The blood–blood tissue barrier is rather thin and there is still a lot of research needed for the study of the nanotoxicological translocation from the mother to the child (see Chapter 4).

Many nanoparticles may enter the body and the blood circulation through the gastro-intestinal system. The tissue barrier between the surface and the blood capillaries is rather thick, 10–100 times thicker than the air–blood tissue barrier. Nevertheless, this is a common route of exposure for nanoparticulate matter which can be delivered in high concentrations over a surface area even larger than the lung, and nanoparticles may reach the capillaries in the connective tissue under the epithelial layer covering

55 Maynard, A.D. et al., *Nature* **444**, 267 (2006); Owen, R. and Handy, R., *Environmental Science & Technology* **41**, 5582 (2007); see footnote 26.

56 Choi, J., *Environmental Science & Technology* **43**, 3030 (2009); Hansen, S.F., PhD thesis, Technical University of Denmark, 2009; Grieger, K. J., *Nanoparticle Research* **12**, 383 (2010).

57 Briggs, D.J., *Environmental Health* **7**, 61 (2008); Kapustka, L., *Integrated Environmental Assessment and Management* **4**, 290 (2008).

58 Gehr, P. et al., *Respiratory Physiology* **32**, 121 (1978).

59 Kreyling, W. et al., *Journal of Toxicology and Environmental Health* **65**, 513 (2004).

60 Geiser, M. et al., *Environmental Health Perspectives* **113**, 1555 (2005); Kreyling, W.G. et al., *Journal of Toxicology and Environmental Health* **65**, 1513 (2002); Rothen-Rutishauser, B., et al., *Interaction of particles with membranes*. In: *The Toxicology of Particles* (eds K. Donaldson and P. Borm), pp. 139–160, Taylor & Francis Group, CRC Press, Boca Raton, 2007; Rothen-Rutishauser, B. et al., *American Journal of Respiratory Cell and Molecular Biology* **32**, 281 (2005); Semmler-Behnke, M. et al., *Small* **4**, 2108 (2008).

61 Guerrero, S. et al., *Nanomedicine* **5**, 897 (2010).

62 Karatas, H. et al., *Journal of Neuroscience* **29**, 13761 (2009).

63 Wick, P. et al., *Environmental Health Perspectives* **118**, 432 (2010).

the intestines which also leads to translocation into secondary organs. Some consider the gastro-intestinal tract as important for the uptake of nanoparticles as the lung and, of course, it plays an important role in the fate of nanocarriers after oral delivery⁶⁴. The outer tissue barrier of the human organism, the skin, when intact, appears to be a rather tight barrier for all nanoparticulate material. Its surface area is rather small, about 1.5–2 m², which is only about 1% of the alveolar surface area.

Most of this surface area is rather impermeable for nanoparticles; there are only the hair follicles and the openings of the sweat glands available for particle penetration, but even these 'weak' locations are hardly crossed. It is suggested that titanium dioxide nanoparticles neither penetrate into viable cell layers nor cause any cellular changes⁶⁵. The rather impermeable healthy and intact skin barrier may, however, be overcome by a combination of nanoparticulate drug carriers with protein drugs⁶⁶; other issues for additional research, including the study of damaged skin, are discussed in Chapter 4.

3.2.1.2 Interaction with tissue and cells

The interaction of nanomaterials with cells can be regarded as a first step in the induction of possible health effects although it must also be appreciated that nanomaterials do not always have to be taken up by cells to exert effects. There are different hypotheses as to how nanomaterials are taken up by cells or how they enter cells⁶⁷ but events always begin with the interaction of the nanomaterial with the cell membrane.

Artificial organelles or nanoparticles may enter cells by a Trojan-horse-type mechanism⁶⁸ and tailoring the lipoplex composition to the lipid composition of the cell membrane may enhance this Trojan-horse-like entry into cells⁶⁹. Moreover, only biopersistent engineered nanoparticles (ENP) maintain their ENP properties and according biological responses over retention time which are clearly distinct from readily soluble or moderately soluble ENP. Those ENP dissolving within days or a few weeks will lose their ENP properties such that the toxicologically relevant interactions of the dissolved metabolic constituents of the previous ENP determine the biological response. The kinetics of dissolution, for example in extracellular body fluids or intracellular compartments after endocytosis, is determined by an ENP-material-dependent dissolution rate constant and the specific surface area of

the ENP and the biochemical properties of the dissolution solvent under the thermodynamic conditions of the living organism.

In summary, information on the multiple biological issues covered in this section 3.2.1 is crucial for a sound risk assessment of nanomaterials⁷⁰. Moreover, to enable better comparison of studies performed in different laboratories, we emphasise that the concentrations used should be comparable and realistic, and materials must be well defined (see also Chapter 4). In particular, characterisation in terms of the mass concentration in a given experiment is often not adequate, as this does not reflect the real particle-to-cell ratio (see later sections and Box 3.5).

3.2.2 Exposure assessment

3.2.2.1 Exposure identification

Human exposure to nanomaterials can arise directly from the intended targeted delivery of product to individuals (for example in food, cosmetic and medical applications) and, indirectly, from the unintended exposure of workers during manufacturing and downstream use, and – for the general public – from nanomaterial accumulating in the environment.

Exposure assessments must aim to summarise, both quantitatively and qualitatively, information on the duration, frequency, concentration and material of exposure of humans or the environment. Box 3.4 lists the categories of likeliness of consumer exposure in terms of product composition. However, it must also be emphasised that the products for which consumer exposure might be expected most frequently are in the category of products for which no information on the nanomaterial involved is available.

The first step in an environmental exposure assessment is the identification of exposure (see also Box 3.4).

This means exploring the following:

- Are there engineered nanomaterials present in the direct vicinity of people?
- Can those be discriminated from the natural and anthropogenic background nanomaterials?
- Can they be linked to a specific source or product?

64 Roger, E. et al., *Nanomedicine* **5**, 287 (2010).

65 Adachi, K. et al., *Nanotoxicology* **4**, 296 (2010); Butz, T. et al., Final Report of the project NANODERM (2007).

66 Huang, Y. et al., *Biomaterials* **31**, 9086 (2010).

67 Krug, H. and Wick, P., *Angewandte Chemie International Edition in English* **50**, 1260 (2011). Geiser, M. et al., see footnote 60; Rothen-Rutishauser, B. et al., see footnote 60; Brandenberger, C. et al., *Small* **6**, 1669 (2011); Blank, F. et al., *American Journal of Respiratory Cell and Molecular Biology* **36**, 669 (2007).

68 Limbach, L.K. et al., *Environmental Science & Technology* **41**, 4158 (2010).

69 Marchini, C., et al., *Langmuir* **26**, 13867 (2010).

70 Borm P.J. et al., *Particle Fibre Toxicology* **3**, (2006); Krug, H. (Ed) 'Nanotechnology – Volume 2: Environmental Aspects', Wiley-VCH, Weinheim, 2008, pp. 328; Abbott, L.C. and Maynard, A.D., *Risk Analysis* **30**, 1634 (2010).

Box 3.4**Categories for consumer exposure through products**

Using knowledge on the location of the nanomaterial within the products, they may be divided into three categories, depending on the physical state (see Box 1.2):

- Expected to cause consumer exposure (relevant for categories 'nanoparticles suspended in liquids' and 'airborne nanoparticles').
- May cause consumer exposure (relevant for the category 'surface-bound nanoparticles').
- No expected exposure to the consumer (relevant for category 'nanoparticles suspended in solids').

As discussed previously (Chapter 1), it must also be emphasised that unintended exposures might arise from the manipulation of previously embedded nanomaterials (e.g. in composite products).

Exposure studies at work places or relating to specific consumer products may be easier to conduct than those aiming to identify exposure for the general public. In the former cases the type of nanomaterial, its chemistry and morphology, is mostly known and the concentration can be expected to be elevated above background. In the latter case, possible exposure of the population, concentrations can be expected to be significantly lower and the matrix in which the nanomaterial is embedded or attached to may be more complex. Furthermore, nanomaterials released into the environment usually undergo changes during their transmission, making it barely impossible to differentiate them from non-engineered materials of the same size. Therefore, at workplaces specific exposure identification strategies are recommended to be applied and pursued on a regular basis. But although these may represent feasible strategies for workplaces, they cannot be applied in the same way to indicate exposure for the public. Here, new strategies have to be developed and deployed.

3.2.2.2 Exposure routes

Most studies so far have focused on nanoparticle exposure through inhalation or through the skin, as these are the two main exposure routes in the occupational setting (see preceding section). Although the latter has been shown to be a good barrier for nanomaterials, uptake of airborne nanomaterials by inhalation is quite likely. In addition, systemic administration will be evaluated for nanoparticles that are intended for clinical use and application of nanomaterials in the food sector including food packaging materials. The latter may lead to exposure through the gastro-intestinal tract.

71 Oberdörster, E., *Environmental Health Perspectives* **112**, 1058 (2004).

72 Brouwer, D., *Toxicology* **269**, 120 (2010).

73 Azong-Wara, N. et al., *Journal of Nanoparticle Research* **11**, 1611 (2009); Kuhlbusch, T.A.J. and Fissan, H., *Journal of Occupational and Environmental Hygiene* **3**, 558 (2006); Kuhlbusch, T.A.J. et al., *Journal of Occupational and Environmental Hygiene* **1**, 660 (2004); Vorbau, M. et al., *Journal of Aerosol Science* **40**, 209 (2009).

Quantitative information on the uptake through the gastro-intestinal tract is still sparse.

As described previously, once nanomaterials have overcome the biological barriers associated with the portals of entry, it is important to consider that the particles may travel to other distant organs. Moreover, in addition to the major route of transportation in the blood, studies in animal models have shown that inhalation of certain nanoparticles may result in uptake through the olfactory nerve in the area of the olfactory epithelium with subsequent translocation of nanoparticles into the central nervous system through the olfactory nerves⁷¹. It is crucial to take into account particle-specific phenomena when assessing the hazard of engineered nanomaterials.

3.2.2.3 Exposure characterisation

The characterisation of exposure may also include studies related to nanomaterial release and the behaviour and mobility in the environment since this significantly influences the probability of exposure. While workplace exposure data become more common⁷², very few published studies describe consumer or environmental exposures to nanomaterials, even for the most abundant particle types. Most current studies for exposure assessment are either stationary measurements allowing detailed analysis of nanoscale particles in the environment or using explorative personal samplers which cannot discriminate between engineered, by-products or natural nanoscale particles.

3.2.2.4 Exposure assessment

Among the key questions related to exposure assessment are the following:

- How can we effectively discriminate ambient from engineered nanoscale particles/ objects?
- How reliable are current measurement results?
- What metric shall be used for exposure assessment (see also Box 3.5)?
- Is standardisation needed? If yes, what exactly shall be standardised?
- How can particle morphology and other alternative information be assessed?

For the future, it is vital to integrate detailed knowledge from personal samplers for exposure assessment, work place monitors and tests of release probabilities from consumer products, into a comprehensive usable exposure assessment and safety design⁷³.

Box 3.5**Dose-metrics for *in vivo* and *in vitro* studies**

One further obstacle to overcome in the near future is the choice of preferred metrics of exposure and treatment concentrations. Mass concentrations are currently seen as too insensitive a metric and possibly not really relevant for nanospecific health effects. Other metrics currently discussed for exposure concentrations are particle surface area and particle number concentrations⁷⁴. It is important that proposed measurement techniques are feasible in widespread practice.

Once agreement on the best exposure metric is reached, a congruent dataset relating the exposure to health effects will have to be developed. The same challenge applies to experimental studies *in vivo* and *in vitro*. So far there is no consensus on metrics, but the specific surface area was suggested as the best choice for *in vivo* experiments⁷⁵. For *in vitro* experiments it was demonstrated that mass concentrations given as mass per volume are correlated poorly with the outcome, and as experimental conditions are often different (cell number per dish and surface area; amount of medium above cells) the effects differ strongly even when the same mass concentrations has been used. Thus, it was suggested to calculate results in terms of more than one dose or concentration metric for each of these experiments, which could include (1) mass per volume, (2) mass per surface area, (3) number of particles per cell and (4) mass per cell⁷⁶.

sources other than those associated with the handling of nanomaterials may significantly contribute to measured particle concentrations is now common knowledge. Still, clear identification of exposures and the sources is currently a difficult task.

Consumers

As mentioned above, the assessment of consumer exposure to nanomaterials is difficult and complex, and at a stage of early development. Even though consumers may be directly exposed to nanomaterials through consumer products, especially cosmetics, drugs and other applications, there are considerable uncertainties about the exposure dose for some of these applications (cosmetics) although perhaps not others (medicines). For those products containing nanomaterials that are already on the market, there are no indications so far that these materials cause specific health problems but it is generally agreed that more work needs to be done⁷⁹. Owing to the (current) difficulties in direct measurements and monitoring of consumers, a focus should be set on investigations studying release of nanomaterials from products. To reiterate a previous point, it is also critically important to consider the benefit–risk balance: toxicity issues may be regarded rather differently if the product is delivering substantial benefit, for example in cancer thermo- and chemotherapy.

3.2.2.5 Exposure scenariosWorkers

For workers, an exposure to nanomaterials may occur if these materials are released to the environment, for example by combustion and or shear forces in the solid, liquid or gas phase (e.g. by spraying). The study of agglomerate stability in the airborne phase⁷⁷ is particularly important. The different methods used in these studies cover a wide range of the shear forces to which nanomaterials may be exposed and the results can be used for ranking of particle release for different nanomaterials as well as functioning as an input parameter for different workplace processes. The first workplace-related study differentiating ambient nanoscale particles from engineered nanoparticles was performed in the carbon black production industry⁷⁸. The finding that

Environment

The number of ecotoxicological studies of engineered nanomaterials has rapidly increased in the past few years⁸⁰. However, the data obtained so far are somewhat inconsistent and not sufficiently systematic to allow an overview of the potential environmental hazards relating to engineered nanomaterials. The increasing use and production of nanomaterials leads to multiple potential points of entry resulting in environmental exposure, including the traditional exposure routes for assessment of conventional chemicals, for example production wastes (liquid, solid, airborne), release from products during the product life, and during the waste cycle. In terms of exposure to the aquatic environment, the widespread and diverse use of consumer products

74 Abbott, L.C. and Maynard, A.D., *Risk Analysis* **30**, 1634 (2010); Brouwer, D. et al., *Journal of Nanoparticle Research* **11**, 1867 (2009); Fissan, H.S. et al., *Journal of Nanoparticle Research* **9**, 53 (2007); Oberdörster, G., *Philosophical Transactions of the Royal Society of London* **A 358**, 2719 (2000).

75 Rushton, E.K. et al., *Journal of Toxicology and Environmental Health* **A 73**, 445 (2010).

76 Krug, H. and Wick, P., *Angewandte Chemie International Edition in English* **50**, 1260 (2011).

77 Bach, S. and Schmidt, E., *Annals of Occupational and Environmental Hygiene* **52**, 717 (2008); Kuhlbusch, T. et al. (eds), 'NanoCare: health related aspects of nanomaterials', DECHEMA e.V. (2009); Schneider, T. and Jensen, K.A., *Annals of Occupational and Environmental Hygiene* **52**, 23 (2008); Stahlmecke, B. et al., *Journal of Nanoparticle Research* **11**, 1625 (2009).

78 Kuhlbusch, T.A.J. et al., *J. Occupational and Environmental Hygiene* **1**, 660 (2004); See Kuhlbusch and Fissan in footnote 73.

79 Borm, P.J. et al., *International Journal of Cancer* **110**, 3 (2004); Fryzek, J.P. et al., *Journal of Occupational & Environmental Medicine* **45**, 400 (2003); Gardiner, K. et al., *Annals of Occupational Hygiene* **36**, 477 (1992); Hext, P.M. et al., *Annals of Occupational Hygiene* **49**, 461 (2005); Merget, R. et al., *Archives of Toxicology* **75**, 625 (2002).

80 Baun, A. et al., *J. Environ. Monit* **11**, 1774 (2009); Farré, M. et al., *Environmental Science & Technology* **43**, 9216 (2009); Gottschalk, F. et al., *Environmental Toxicology and Chemistry* **29**, 1036 (2010); Kahru, A. and Dubourguier, H.C., *Toxicology* **269**, 105 (2010); Klaine, S.J. et al., *Environmental Toxicology and Chemistry* **27**, 1825 (2008); Mueller, N.C. and Nowack, B., *Environmental Science & Technology* **42**, 4447 (2008); Stone, V. et al., Final Report of FP7 Coordination and Support Action. Grant Agreement number: 218433; Stone, V. et al., *Science of the Total Environment* **408**, 1745 (2010).

containing engineered nanomaterials makes municipal wastewater an area of specific concern⁸¹, because several studies have demonstrated a release of engineered nanomaterials incorporated in textiles and paints to the aquatic environment⁸². A significant fraction of engineered nanoparticles were found to escape the clearing system of the wastewater plant, demonstrating the complex interactions between dissolved species and nanoparticles within the continuously changing environment of the clearing sludge⁸³.

Bioaccumulation of nanomaterials in environmental species may, in theory, be a route for human exposure similar to that previously found for numerous persistent organic pollutants and metals. However, it remains unknown whether this is indeed a significant exposure route for nanomaterials because the number of studies dealing with this issue is still too limited.

3.2.3 Hazard assessment

Epidemiological studies have revealed that pollution by ambient particulates is associated with respiratory and cardiovascular diseases, particularly in the elderly and in patients with pre-existing cardiopulmonary diseases. Moreover, there is evidence that ambient particulate matter can act as an adjuvant for allergic sensitisation, which raises the possibility that long-term particulate matter exposure may lead to increased prevalence of asthma⁸⁴.

As this is often dependent on the mixture of substances and materials comprising the particulate matter, a detailed understanding of the physico-chemical properties of engineered nanomaterials is required to predict their possible interaction and/or interference with biological systems.

This lesson has been ingrained into researchers in the field of nanosafety over the past few years and it is important to use well-characterised and described nanomaterials in toxicology studies. An overview of the knowledge gained by studies on particulate matters from the environment is given in Box 3.6.

Box 3.6

Historical case studies on combustion-derived particulate matter

Stimulated by ongoing discussion about the fine dust problem within the environment, and the health-related consequences of ultrafine particles released by fossil fuel and wood combustion, it was a logical extension to cover synthetic or engineered nanoparticles. Many of the experts in the topic of nanotoxicology started their research on ultrafine dust and lung damage. Early studies, in the 1990s compared the toxic effects of environmentally relevant ultrafine particles with ultrafine titanium dioxide⁸⁵. In 1990 a first paper was published on synthetic particles and their effects on primary lung macrophages⁸⁶ with, some years later, a publication about fullerene toxicity⁸⁷.

For the first time a multicellular three-dimensional *in vitro* model was developed for the lung which was used for nanoparticle research and optimised during the past 5 years⁸⁸. The research on particle–lung interactions started as early as the 1980s with one of the first papers on so-called submicron-particles⁸⁹.

Thus, the possible health effects of ultrafine particles have been investigated long before the consideration of the possible risks of nanoparticles, and the methodological advances that were achieved are relevant for the assessment of engineered nanomaterials. It is important to fill the knowledge gaps for the specific health aspects related to the uptake, distribution, possible accumulation and biological effects in secondary organs induced by nanoparticles (see Chapter 4). It is also important to appreciate, however, as discussed elsewhere in this report, that the deliberate targeting of new generations of well-defined engineered materials in specific amounts – direct exposure – will stimulate new thinking on those aspects of risk assessment and risk management that, hitherto, have been based on experience of indirect exposure.

3.2.3.1 Hazard identification

Hazard identification aims to identify causality between inherent physical and chemical properties and observed adverse effects. This structure–toxicity type of approach has much to commend it but is proving as difficult in

81 Biswas, P. and Wu, C.Y., *Journal of Air & Waste Management* **55**, 708 (2005); Boxall, A.B.A. et al., *Nanomedicine* **2**, 919 (2007); Kuhlbusch, T.A.J. et al., see footnote 78.

82 Benn, T. and Westerhoff P., *Environmental Science & Technology* **42**, 4133 (2008); Kaegi, R. et al., *Environmental Pollution* **156**, 233 (2008).

83 Limbach, L.K. et al., *Environmental Science & Technology* **42**, 5828 (2008).

84 Li, N. et al., *Free Radical Biology and Medicine* **4**, 1689 (2008).

85 Ferin, J. et al., *American Journal of Respiratory Cell and Molecular Biology* **6**, 535 (1992); Oberdörster, G. et al., *Environmental Health Perspectives* **97**, 192 (1992); Oberdörster, G. et al., *Environmental Health Perspectives* **102**, 173 (1994); Oberdörster, G. et al., *Inhalation Toxicology* **8**, 73 (1996); Osier, M. and Oberdörster, G., *Fundamental and Applied Toxicology* **40**, 220 (1997); Kreyling, W.G. et al., *Experimental Lung Research* **19**, 445 (1993); Donaldson, K. et al., *Toxicology Letters* **88**, 293 (1996).

86 Wilczek, F. et al., *Journal of Aerosol Science* **21**, S513 (1990).

87 Adelman, M.D. et al., In: Toxic and carcinogenic effects of solid particles in the respiratory tract. ILSI Press, Washington, DC, 1994, pp. 405.

88 Mögel, I. et al., *Toxicology Letters* **96**, 25 (1998); Wotrich, R. et al., *International Journal of Hygiene and Environmental Health* **207**, 353 (2004); Geiser et al., see footnote 60; Rothen-Rutishauser et al., see footnote 60; Blank et al., see footnote 67; Mühlfeld, C. et al., *American Journal of Physiology – Lung Cellular and Molecular Physiology* **294**, 817 (2008); Brandenberger, C. et al., *Toxicology and Applied Pharmacology* **292**, 56 (2009); Lehmann, A. et al., *European Journal of Pharmaceutics and Biopharmaceutics* **77**, 396 (2010).

89 Brain et al., *Experimental Lung Research* **6**, 115 (1984); Gehr, P. et al., *Nature* **302**, 336 (1983).

90 Hansen, S.F. et al., *Nanotoxicology* **3**, 243(2007).

nanotoxicology as in conventional particle toxicology. It is useful to discriminate between different hazards⁹⁰:

- hazard at sites of deposition;
- hazard resulting from translocation (which then gives rise to a hazard at the new site of deposition where translocation deposits the particles) or from transmission of a response from one target site to another (e.g. mediated by cytokines).

Hazard identification for effects at sites of deposition

The lung shows very clear size-dependent pro-inflammatory effects of low toxicity nanomaterials. It is important to understand that some nanoparticles may have high surface reactivity and the factors that induce surface high reactivity include charge, free radical activity and general chemical reactivity. Quartz is a good example of a particle that has high surface reactivity in the form of reactive groups and free radicals⁹¹. It was suggested that surface free radical activity measured in several assay systems *in vitro* is an attribute that predicts inflammatory potential⁷⁵. If surface area and surface reactivity together drive the inflammatory response to insoluble particles, then it seems clear that any nanomaterials possessing high surface reactivity would be highly inflammatory because the total area of reactive surface would be high.

Shape may also be an important characteristic in the case of long thin or fibrous nanoparticles, otherwise defined as high aspect ratio nanoparticles. The case of asbestos has drawn attention to the extra hazard of the pleura from long, biopersistent fibres, and various types of nanofibre are available (e.g. carbon nanotubes) that appear to show some of the properties of harmful asbestos⁹².

Translocation hazard

Translocation away from site of deposition has been considered to be one of the defining properties of engineered nanomaterials, a consequence of their small size. Although it may seem intuitively likely that small size would be associated with the property of translocation from, for example, the lungs, there is no evidence for this in humans, although there is in rodents⁹³. In rats, it has been shown that translocation away from the lung is more efficient for smaller nanomaterials in the case of radioactive iridium (20 nm compared with 80 nm)⁹⁴. Care must be taken in the interpretation of translocation studies

for soluble nanomaterials, because soluble ions might be the translocated entity. Although small nanomaterials might be more readily translocated it is, as in all toxicology, the extent of the translocated dose that will determine any effects at targets remote from the portal of entry.

There are usually insufficient data available from toxicokinetics to ascertain whether there may be sufficient dose reaching the extra-pulmonary organs to elicit a response. What is clear is that there can be effects at extra-pulmonary sites as a consequence of inflammatory signals emanating from the lungs⁹⁵.

In summary, there is good hazard identification for the relationship between structure and toxicity for low-toxicity engineered nanomaterials: namely surface area is the driver of inflammation at sites of deposition. However, surface reactivity and solubility and the other characteristics of nanomaterials, listed in section 3.1, are not well understood in their capacity as modifiers of small size in the translocation dose–response curve.

3.2.3.2. Hazard characterisation

Taking the data on the lung, because it is a well-characterised organ, it can be summarised that hazard characterisation of nanomaterials draws on various sources:

- Literature on ultrafine particles/particulate matters epidemiology/toxicology (as listed in Box 3.6).
- Literature on rat lung overload e.g. using ultrafine titanium dioxide, carbon black (as listed in Box 3.3 and 3.5).
- Literature on animal studies with nanomaterials, although there are not many of these: carbon nanotubes have been examined extensively and cause rapid inflammation and fibrosis at low to moderate lung burdens⁹⁶. Other studies suggest that some metallic engineered nanomaterials can cause immunopathology by delayed hypersensitivity mechanism but this is by instillation⁹⁷.
- Literature on human exposures to nanomaterials⁹⁸.
- Literature on conventional particle toxicology (see also Box 3.7): the occupational exposure paradigm is high levels of exposure in relative healthy males producing fibrosis (e.g. quartz) and cancer (e.g. asbestos).

91 Duffin, R., *Inhalation Toxicology* **19**, 849 (2007).

92 Donaldson, K. et al., *Particle Fibre Toxicology* **7**, 5 (2010).

93 Nemmar, A. et al., *Circulation* **105**, 411 (2002); Mills, N.L. et al., *American Journal of Respiratory and Critical Care Medicine* **173**, 426 (2006).

94 Kreyling, W.G., *Inhalation Toxicology* **21**, 55 (2009).

95 Van Eeden, S.F. and Hogg, J.C., *Journal of Toxicology and Environmental Health A* **65**, 1597 (2002); Kagan, V.E. et al., *Nature Nanotechnology* **5**, 354 (2010).

96 Shvedova A.A. et al., *American Journal of Physiology - Lung Cellular and Molecular Physiology* **295**, L552 (2008); Pauluhn, J., *Toxicological Sciences* **113**, 226 (2010); Ma-Hock L. et al., *Toxicological Sciences* **112**, 468 (2009); De la Zerda, A. et al., *Nature Nanotechnology* **3**, 557 (2008); Park, J.-H. et al., *Nature Materials* **8**, 331 (2009).

97 Cho, W.S. et al., *Environmental Health Perspectives* **118**, 1699 (2010); Yokohira, M. et al., *Journal of Toxicologic Pathology* **22**, 1 (2009).

98 Davis, M.E. et al., *Nature* **464**, 1067 (2010); Powell, J.J. et al., *Journal of Autoimmunity* **34**, J226 (2010).

These sources vary in the amount of relevant information they offer for hazard identification of engineered nanomaterials in the lung. Taken together, the literature suggests that potential hazards of nanomaterials may include several pathobiological processes such as cytotoxicity, oxidative stress, inflammation, genotoxicity and sensitisation that might lead to diseases such as fibrosis, cancer, bronchitis, alveolitis, and immunopathology (e.g. asthma). In addition, the hazard associated with translocation from the pulmonary portal of entry to the blood, or the systemic consequences of inflammation/oxidative stress in the lungs, could in theory result in neurophysiological effects, cardiovascular effects (atherothrombosis, cardiotoxicity) and foetal damage/abnormality among other consequences (see Box 3.7).

As described in Chapter 4, it is important to develop a comparable knowledge base for other routes of exposure, using the knowledge base on the lung as a model for what can be achieved.

3.2.3.3. Hazard assessment

For the testing of biological effects by nanomaterials, an international working group of the International Life Sciences Institute suggested a tiered approach⁹⁹:

- physico-chemical characterisation;
- cell-free assays (solubility; reactive oxygen species-generating potential; chemical reactivity; agglomeration/aggregation; zeta potential; other properties);
- *in vitro* assays (primary cells; cell-lines; primary and secondary organs; co-cultures);
- *in vivo* assays (generally rodents; diverse methodologies: respiratory tract, skin, gastrointestinal tract).

A primary question to be answered is whether any of the *in vitro* tests used would be able to predict *in vivo* toxicity. However, this question again is not specific for studies with nanomaterials. Some studies were designed to determine

whether *in vitro* assays are predictive for *in vivo* effects¹⁰⁰. Further results are available from the NanoCare project funded by the German Government¹⁰¹. This consortium demonstrated that there is good correlation between the results obtained from experiments with primary lung macrophages and *in vivo* studies. For the future, it can be expected that there will be rapid advances in new testing procedures that can be applied to nanomaterials-drawing on substantial progress in introducing platform technologies for high-throughput safety screening, use of proteomics, metabolomics and other 'omic'-based measurements and the use of 'humanised' cell systems. There will also be increasing opportunities to feedback information, for example on relevant biomarkers as clinical correlates, from studies in humans and experimental animals, to improve predictive tests. One other lesson that must be learnt from clinical medicine is that safety responses can be influenced by individual variation in genotype and phenotype.

In summary, testing of nanomaterials is far from being standardised and many previous studies are not capable of being directly compared, although differences can be informative if investigated in sufficient detail. Furthermore, exposure data are only rarely available and there are many remaining questions about the risk assessment of engineered nanomaterials. We recognise that much of the research reviewed in this chapter relates to lung exposure; this reflects the twin origins of the nanosafety field in exploring unintended exposure through inhalation to environmental pollution and in the occupational health agenda for employee protection. We emphasise that research priorities are rapidly emerging for the broader field of enquiry that covers the safety of intended exposure to engineered nanomaterials by various routes. In Chapter 4 we identify the priorities for filling the gaps in knowledge that will begin to answer many of the questions. However, it is worth also emphasising again that the safety assessment procedures used for nanomaterials in established product sectors such as medicine can be expected to be very similar to the procedures already developed and validated by those sectors for assessing innovation from any other source.

Box 3.7 Aspects of conventional particle toxicology	
Pathobiological processes:	Cytotoxicity, oxidative stress, inflammation, genotoxicity, sensitisation, immunotoxicity
Diseases at lung portal of entry:	Fibrosis, cancer, bronchitis, alveolitis, immuno-pathology, mesothelioma (other pleural effects)
Extra-pulmonary effects following lung exposure:	Atherogenesis, plaque rupture, cardiotoxicity, foetal effects, brain neurotoxicity

99 Oberdörster, G. et al., *Particle Fibre Toxicology* **2**, 8 (2005).

100 Seagrave, J. et al., *Journal of Toxicology and Environmental Health* **66**, 1113 (2002); Sayes, C.M. et al., *Nano Letters* **7**, 2399 (2007).

101 Krug, H. (Ed), *Nanotechnology: Environmental Aspects*, Wiley-VCH, Weinheim, 2008, pp. 328.

4 Filling the Gaps

In the previous chapters, examples were selected from current knowledge as the basis for identifying what types of further effort will be required to understand the health impacts of nanomaterials. This extra research effort has to span the continuum from basic, through translational, to applied research. It must be multidisciplinary and connected by validation, standardisation, regulation and innovation, to industry and other users of research, in pursuit of societal objectives.

Clearly, any successful application of the risk assessment within the broader context of establishing the benefit–risk balance requires better information about which nanospecific features need to be taken into account (see also section 3.1). Although a modification of the dose–response relationship might reasonably be expected in association with decreasing particle size, it could be interpreted, variously, in terms of biological distribution, surface effects, increased solubility or other attributes, as discussed in Chapter 3. The current evidence is rarely sufficient to distinguish between the different possible explanations. Nonetheless, in the absence of this definitive evidence, a precautionary approach to nanomaterials¹⁰², consistent with the procedures involved in REACH regulation and adopted in the action plans of governments and industrial associations, seems to have been successful so far in preventing major problems although it should be recognised that in some cases there may not have been sufficient exposure over longer time periods to allow chronic effects to be ruled out.

Can this successful governance continue into a future where the number of applications and extent of industrial production rapidly grow? We judge that it can, but it requires the joint efforts of academia, industry and public policy-makers to incorporate safety-by-design, to understand both the acute and longer-term biological responses, and to ensure that production and downstream handling entail good working practices.

In the following sections, we provide detail on our recommendations for the support both of basic research to fill gaps in fundamental knowledge, and the translation of that knowledge more effectively in product and policy development.

In the space available we cannot provide detail on the entire research agenda. Rather, we again provide examples to represent and illustrate the different types of investigation required.

We believe that it is also increasingly possible to maximise the value of that knowledge already available by capitalising on new opportunities for meta-analysis and modelling.

4.1 Basic science

We advise that the funding strategies of the EU and its Member States should be better co-ordinated in order to identify and resolve the strategic research questions and methodological developments. We recommend particular attention to the following topics.

4.1.1 Relevant dose

To reiterate our previous point, research projects often study safety responses at very high doses and this may not always be relevant to likely exposure. There is much more to be done to establish realistic dose–responses, over long-term dosing where necessary, using well-defined materials. Consideration of appropriate dose is also particularly important in devising animal experiments in order to limit animal use, although studies *in vitro* can allow expansion of doses considered. *In vitro* models are currently particularly useful for screening and mechanistic studies but, in many cases, need further validation before being usable for risk assessment.

There are additional challenges in assessing dose–responses relating to (1) the ability of some nanoparticles to deliver high concentrations at the local, cellular, level because of dissolution or expression of surface reactivity, and (2) consideration of potential differences in individual susceptibility arising from, for example, genetic disposition, age and disease. The importance of standardisation in experiments, both to generate valid data and to allow the more systematic aggregation of findings from different laboratories, has been emphasised in Chapter 3; in this context it is also vital to pay attention to potential differences in quality between different production batches of nanomaterials.

4.1.2 Cellular level

There are many gaps in the understanding of how nanoparticles interact with cell membranes, are transported and behave inside cells. Among the

102 Hoeck, J. et al., Instructions concernant l'usage d'une grille de précaution pour les nanomatériaux synthétiques, Office Fédéral de la Santé Publique (OFSP) et Office Fédéral de l'Environnement (OFEV), Berne 2010.

fundamental questions to be answered by further research are the following.

- How do nanoparticles enter mitochondria and interact with the respiratory chain?
- In what circumstances do nanoparticles enter the nucleus and interact directly or indirectly with DNA? Might genetic variation or epigenetic changes¹⁰³ modify the response? Are nanoparticles more genotoxic than larger particles of the same material¹⁰⁴?
- How do nanoparticles interact with other cellular organelles such as lysosomes and could this interfere with lysosomal chemistry and function? Initial studies¹⁰⁵ suggest that some nanoparticles localise in lysosomes, triggering lysosomal cell death. Production of cytokines and chemokines may be triggered. Oxidative stress and pro-inflammatory reactions may follow. Also, the inflammasome may be activated¹⁰⁶. It should be stressed, however, that these reactions and pathways are not unique to nanoparticles. Responses may also depend on the type and amount of impurities potentially present in the particles.
- To what extent can nanoparticles induce indirect effects on cells, across a biological barrier, for example through transmission of purine nucleotides and intracellular signalling through gap junctions¹⁰⁷?
- What are the nanoparticle properties that mediate cellular responses? Smallness, surface reactivity and solubility could all be determinants of nanoparticle translocation and dose-dependent toxicity (see Chapter 3). More research is required to identify the specific physico-chemical features that are associated with biological behaviour of nanoparticles at the cellular level. There are concomitant questions for fundamental research on the consequences of these features: for example, could interference with protein folding¹⁰⁸ be a problem?

4.1.3 Organ level

As described in Chapter 3, nanosafety assessment has its origins in the study of particle–lung interactions and this remains a significant research topic. Beyond the lung,

there are many gaps in knowledge about the health impacts relating to uptake, distribution, accumulation and biological consequences in secondary organs after inhalation. The responses to the other routes of administration intended for some nanomaterial applications are also relatively poorly characterised. It is uncertain which nanoparticles can cross the blood–brain barrier although current evidence suggests that any such transfer is small¹⁰⁹. It is also not clear if some nanoparticles can cross the barrier between blood and testes or ovary, or between the blood and the thymus tissue, for example: if they can, there will be a significant research agenda for reproductive toxicology and immune toxicology. The maternal–foetal translocation of nanoparticles is possible¹¹⁰ (see also Chapter 3), which raises the possibility of developmental effects.

It is conceivable that effects demonstrated in one organ will have implications elsewhere but there are still many knowledge gaps. For example, in air pollution research, deposited reactive particles have been proposed to cause oxidative stress in the lungs (Chapter 3) and the resultant pro-inflammatory response in the lung endothelium might be transmitted to organs susceptible to the effects of thrombosis and atherosclerosis¹¹¹.

Nanoparticles might also be deposited directly in atherosclerotic plaques but their propensity to contribute locally to inflammation is unknown. This knowledge gap might be filled by collaboration with the manufacturers of radiological contrast material that consists of nanoparticles to visualise organ systems.

4.1.4 Immune system

The immune system is designed to protect against all forms of foreign intrusion, not only micro-organisms but also particles¹¹². Recent research, including the Framework Programme 7 project Nanommune¹¹³, is beginning to provide a comprehensive evaluation of the influence of nanoparticles on the immune system¹¹⁴. However, more research is warranted in models of disease susceptibility to understand if an increased risk is posed in certain populations, for example those with infection or asthma.

103 Riediker, M. et al., In: *Towards Predicting Nano-Biointeractions: An International Assessment of Nanotechnology Environment, Health and Safety Research Needs*, Vol. 4. Rice University, Houston, Texas, 2008 pp. 2–62.

104 Papageorgiou, I. et al., *Biomaterials* **28**, 2946 (2007).

105 Shaper, K. et al., *Molecular BioSystems* **7**, 371 (2011).

106 Tschopp, J. et al., *Nature Reviews Immunology* **10**, 210 (2010).

107 Bhabra, G. et al., *Nature Nanotechnology* **4**, 876 (2009).

108 Linse, S. et al., *Proceedings of the National Academy of Sciences of the USA* **104**, 8691 (2007).

109 Oberdörster, G. et al., *Inhalation Toxicology* **16**, 437 (2004).

110 Yamashita, K. et al., *Nature Nanotechnology* **6**, 321 (2011); Myllynen, P.K. et al., *Reproductive Toxicology* **26**, 130 (2008).

111 Brook, R.D. et al., *Circulation* **121**, 2331 (2010).

112 Shvedova A.A. et al., see footnote 96.

113 FP 7 Project Nanommune: www.nanommune.eu.

114 Konduru N.V. et al., *PLoS One* **4**, e4398 (2009).

An important concept may be emerging from the potential for accretion of a biomolecular corona around nanoparticles: the addition of a coat of protein or lipids in the biological environment¹¹⁵. This surface coating may determine interaction and biological effects. Does the immune system respond to the naked nanoparticle or the corona? Could the nano-bio-interface be modified to mitigate any toxicity response, while retaining the desired properties of nanoparticles?

4.2 Exposure

Several peer-reviewed studies related to workplace measurements and possible nanomaterial exposure can be found in literature⁷⁵. The European NANEX project¹¹⁶ found rather little published information on acute or chronic exposure. Both investigations clearly showed the inadequate comparability of the studies and their results. Owing to the limit of validated and comparable information, exposure scenarios and risk assessments can only be set up with relative high uncertainty. In the few comprehensive databases, all built on country-level (e.g. Switzerland and USA¹¹⁷), data on occupational exposure were collected, but the potential for consumer exposure was not addressed.

As noted earlier in our report, in many applications, nanomaterials are closely embedded in macro-structures, for example in the electronics, IT, transport and space sectors. In such cases, engineered nanomaterials are likely to pose low risk for consumer exposure and environmental risk during their lifetime embedded in the larger structures but then there is need to take account in lifecycle analysis of the potential later consequences of manipulating the embedded product (see Chapters 1 and 3). In contrast, nanomaterials in food applications, paints and coatings, cosmetics, textiles and healthcare products may lead to substantial early exposure.

Risk management requires assessment based on realistic exposure scenarios in well identified groups. In our view, the major gaps that need to be filled in assessing exposure are the following.

4.2.1 Use, production and disposal

In the country survey mentioned above, about 1% of Swiss companies reported the presence of nanoparticles but none of these companies was a primary producer. Thus, in addition to clarifying exposure during primary production, there is a need to generate further data on the downstream use of nanoparticles and on consequences for consumers and the environment.

¹¹⁵ Nel, A.E. et al., *Nature Materials* **8**, 543 (2009).

¹¹⁶ FP 7 Project Nanex: www.nanex-project.eu.

¹¹⁷ Schmid, K. et al., *Journal of Occupational and Environmental Hygiene* **7**, 224 (2010); Methner, M. et al., *Journal of Occupational and Environmental Hygiene* **7**, 163 (2010).

4.2.2 Release processes

Exposure to nanoparticles during manufacturing and use (including disposal and recycling) depends on particle size distribution and other material characteristics (see Chapter 3 for further discussion). Besides the usual parameters such as geometry and flow rate, net release is influenced by agglomeration and other forms of loss. Future research will hence need to establish what factors – nanomaterial attributes or operational conditions or their combination – are the principal determinants of release in a range of settings to allow minimising emission and control possible exposure.

4.2.3 Routes of entry and modelling exposure

Most research has focused on the inhalation route, examining airborne particles. Much more research to evaluate dermal and especially ingestion routes is necessary, and this requires development of appropriate methods to quantify exposure.

There is often poor correlation when data from models for inhalation exposure are compared with real-life measurements. The existing models describing exposure to nanoparticles are inadequate in incorporating nanospecific features such as agglomeration. Similar problems are likely to arise in attempting to develop validated models for other routes of exposure. Progress in modelling requires much more detailed analysis of the factors that influence routes of nanoparticle disposition such as agglomeration and aggregation.

Given the current state of knowledge about exposure to nanomaterials, it is likely that in the short term it will be necessary to continue to rely on precautionary risk management measures. In the longer term, if the gaps can begin to be filled by detailed, harmonised or even standardised research approaches then more specific risk assessments can be anticipated, as described in the NANEX report. This certainly requires better funded and well co-ordinated approaches.

4.3 Transfer of knowledge to practice

The outputs from the various research studies described in the previous sections are vitally important for multiple purposes: (1) for developing, refining, standardising and harmonising methods of safety assessment; (2) for the definition of objectives for engineers and manufacturers in designing safety; and (3) for the formulation of regulatory safety guidelines and legislation. Feedback from the users of this research knowledge will also be

crucial in suggesting new directions for research and new opportunities to build multidisciplinary.

We note that the research communities across nanomedicine, nanoengineering and nanosafety are not always well linked. We suggest to the research funding and higher education bodies that cross-talk should be encouraged in addition to the teaching of basic understanding about related fields.

Among the translation gaps that need to be filled for the multiple transfers of knowledge into practice are the following.

4.3.1 Harmonisation and standardisation

The harmonisation of scientific effort is important because it allows comparison of results and pooling of data between groups. In addition, it facilitates the considerable effort involved in transferring laboratory-scale innovative science into routine testing schemes appropriate for industrial and regulatory processes internationally, such as those developed by the OECD (Chapter 2).

An important initial step is the harmonisation of protocols and reporting so that results can be interpreted adequately. European harmonisation necessitates better sharing of the scientific and technological perspectives from the various partners across academia, industry and policy in pursuit of agreed, common goals. It would also be helped by new incentives for the transfer of knowledge, given that such transfer tends to be neglected in most schemes for researcher funding and recognition.

4.3.2 Safety-by-design

The early objective in the design of additional safety is to provide engineers and manufacturers with robust benchmarks for new materials and products. Although there is nothing necessarily special about nanomaterials in the concept of safety-by-design, the particular challenge lies in the many current knowledge gaps and the multidisciplinary approaches involved.

There is considerable uncertainty about which characteristics best explain the hazards and exposure potential of nanomaterials. Nonetheless, progress is being made. While a refined risk assessment and safe design approach may not yet be possible, there are already simplified toolkits available for evaluating nanospecific risks, compatible with the precautionary approach

reflected in REACH regulation (see Chapter 2 for further details).

4.3.3 Early attention to safety in research

A recent survey¹¹⁸ disclosed that many nanoscientists are unaware of safety measures in their own laboratories. Consequently, more effort is highly desirable to ensure the safety not only of workers during manufacturing and downstream use but also of researchers in laboratories. The introduction of a code of conduct for researchers (Chapter 2) should be helpful in raising awareness and improving working practices: we suggest that the European Commission should continue to encourage use of this code of conduct and monitor the impact of implementation. Although very valuable in the research environment, a voluntary code may have increasing limitations during subsequent product development steps where a formalised system of sector-specific regulation needs to be applied, as described elsewhere in this report.

In the experience of our working group participants, there has been a large global increase in research spending in both the public and private sectors on health and safety aspects relative to nanomaterials¹¹⁹. However, there is room to do more to integrate safety objectives into research. For example, the Austrian NanoInitiative¹²⁰ conducts a dual review of submitted research proposals, for the engineering elements and for the adequacy of health, safety and environmental measures. This approach can be commended for wider application because it requires safety issues to be discussed at an early stage when the project still has the greatest flexibility to change.

We suggest that integration of safety assessment into projects dealing with the discovery and development of new materials might build on the relevant model from the pharmaceutical sector, where hazards and risks are customarily addressed at an early stage in research.

4.3.4 Research, regulation and public engagement

As discussed in Chapters 1 and 2, we recommend that the European Commission together with the scientific community strengthen efforts to define a common terminology and common criteria for data collection for safety endpoints. We also advise that there is a corresponding collective responsibility for policy-makers and researchers to provide accurate and accessible information to the public about nanomaterials.

118 Balas F. et al., *Nature Nanotechnology* **5**, 93 (2010).

119 Riediker M. and Katalagarianakis, G. (Eds.), 'Compendium of Projects in the European NanoSafety Cluster', Lausanne, Switzerland: Institute for Work and Health, 2010.

120 Austrian Nano Forum: www.nanoinitiative.at.

Various national activities have shared scientific findings with the general public, for example, the UK Royal Society dialogue¹²¹, the TA-Swiss PubliFocus¹²², German NanoCare⁸¹ and French nanodebate¹²³.

These initiatives demonstrated consistently that the public are interested in the topic once they are informed. There have also been attempts to involve non-governmental organisations associated with environmental and consumer protection in stakeholder discussion, for example in NanoImpactNet¹²⁴ and the Swiss Action Plan on Nanotechnology (Chapter 2).

The Framework Programme 7 project Framingnano¹²⁵ explored issues with non-governmental organisations for the governance of risks and might provide one model for discussing future challenges. Although the Framework Programme 7 project Nanomedicine Round Table¹²⁶ reported patients as uncertain whether nanomedicine raises specific safety concerns, they did not view it as inherently unsafe. This project called for better co-ordination and harmonisation of existing regulatory procedures, to facilitate data collection and improve regulatory clarity, a recommendation that we endorse. A particular priority was seen to be the clarification of the regulatory pathways for 'combination products' which may span medical, food and cosmetic product sectors.

In the recent Eurobarometer survey on biotechnology¹²⁷, only 45% of Europeans say that they have heard of nanotechnology, described in the survey in the context of consumer products. Sixty per cent of those who expressed an opinion support such applications of nanotechnology. Safety is identified as the main concern, but those who are most actively interested in nanotechnology tend to be much more inclined to perceive it as safe and beneficial compared with those for whom nanotechnology is unfamiliar, reinforcing the importance of additional effort to communicate and inform.

4.4 Professional education and training

Generally, there are too few European universities offering training in toxicology and in occupational and environmental health and safety. Without wider commitment to safety science, it will be difficult to develop expertise in specialised areas. In terms of nanospecific training, the priorities include the following:

- Training of researchers in hazard exposure and risk assessment to understand what may be distinctive about the nanoscale. Such training could be included as modules within EU and other research programmes.
- Training of scientists and engineers developing new nanomaterials and production technologies, to incorporate safety into design.
- Joint training on interdisciplinary collaboration for all: researchers, material scientists and production engineers. One particularly interesting example of such training is the new TWIN Institute in Tokyo, a merger between the Engineering School at Waseda University and the Medical School at Tokyo Women's Medical University, with emphasis on both basic and applied research relevant to nanobiotechnology training.

Examples of current European programmes are described in Box 4.1 and we suggest that these can serve as models for future expansion of training.

Box 4.1

Examples of current training initiatives

- MSc level: QNano¹²⁸ infrastructure, knowledge and training hub builds on foundations laid by NanoImpactNet and uses expertise of established groups in toxicology, modelling, nanobiology, occupational exposure and ethics. It will function as virtual training for users of European infrastructure and includes a repository of lifelong learning materials and expert panel resource to tackle educational outreach issues. Several national and regional Master's initiatives (for example NanoConnect Scandinavia, a joint venture between Universities of Copenhagen, Denmark, and Lund, Sweden) in nanoscience and nanotechnology also provide models for adoption more widely.
- PhD level: One major example is the EU Marie Curie training programme NanoTOES (Nanotechnology: Training of Experts in Safety¹²⁹), which provides interdisciplinary training to both students and experienced researchers in a network of projects associated with the refinement and standardisation of existing methods and development of new assays for analysing the biological effects of nanomaterials. Training in communication, verification and practical application of results are important parts of the programme and the highly interdisciplinary objectives are a good model for future European PhD training schemes.

121 The Royal Society and The Royal Academy of Engineering, 'Nanoscience and nanotechnologies: opportunities and uncertainties', London, 2004.

122 TA-Swiss: www.ta-swiss.ch/en/publifocus-nanotechnology.

123 <http://www.developpement-durable.gouv.fr>.

124 FP 7 NanoImpactNet Project: www.nanoimpactnet.eu.

125 FP 7 Framingnano Project: www.framingnano.eu.

126 FP 7 Nanomedicine Round Table Project: www.nanomedroundtable.org.

127 Gaskell G. et al., European Commission, EUR 24537 EN (2010).

128 FP7 QNano Research Infrastructure Project: www.qnano-ri.eu.

129 University of Salzburg: www.uni-salzburg.at.

We are convinced that the development of new generations of nanomaterials requires a new generation of interdisciplinary scientists.

New training initiatives are essential to confer the interdisciplinarity and other attributes that will secure the future of nanosafety.

4.5 Summary of main messages

Our main recommendations from this Chapter on 'Filling the Gaps' are as follows:

- The necessary basic and translational (transfer from basic science to application) research framework encompasses the development of innovative methodologies to assess nanospecific hazards, validated biological models (*in vitro* and *in vivo*), and use of meta-analysis and modelling techniques to maximise the value of the information gained.
- Particular research priorities, using well-characterised materials, include better definition of relevant dose–response relationships, exploration of effects at the cellular level, individual organ level and organ–organ interaction, and on the immune system.
- The scope of exposure science must be clarified according to the key issues appertaining for nanomaterial primary producers, downstream users, consumers and the environment. Current priorities include evaluation of the determinants of nanomaterial release processes, comprehensive assessment of all major biological routes of exposure, and the modelling of alternative exposure scenarios.
- Transfer of knowledge to practice requires new efforts in harmonisation and standardisation of methodologies; sharing best practice on attributes for safety-by-design; raising awareness of safety issues in the research setting as well as in manufacturing, with implementation of codes of conduct; and ensuring connectivity between the research and regulatory communities.
- Researchers and policy-makers can benefit from working together to support public information and engagement, capitalising on recent findings indicating that more knowledgeable consumers are more confident about nanosafety.
- New initiatives in nanospecific training at Master's and PhD levels are important to support interdisciplinarity and to provide the next generation of researchers with the skills to assess the next generations of nanomaterials.

5 Conclusions

Our Working Group discussions confirmed that the regulatory framework for safety assessment of nanomaterials should adopt the same principles and sector-specific requirements applied in other product development. A clear scientific and regulatory framework to address potential health and environmental impacts of nanomaterials must be central to Europe's integrated efforts in nanotechnology innovation. Even if many areas of nanotechnology do not create new hazards, it is important to evaluate whether new forms of engineered nanomaterials may require modification of existing regulations.

A recent publication from policy-makers in the European Commission, OECD and US Environmental Protection Agency¹³⁰ emphasised a continuing requirement for high-quality data on the toxicology of nanomaterials, both to support specific regulatory decisions and to generate the wider knowledge base needed to determine how data can be extrapolated for other nanomaterials that have not been studied to the same level of detail. Current test methods must be optimised and standardised while also evaluating alternative testing strategies and providing robust measurements of exposure. Given the magnitude of the task, there is also the need for screening approaches that inform decision makers how to set priorities for testing in more depth of different nanomaterials.

This is consistent with the issues identified, and recommendations offered, in the preceding chapters of our report. We propose the following:

- Regulators and researchers should work together in identifying priorities for gaining new knowledge.
- Scientifically sound approaches should be used for managing nanomaterial risks in the absence of sufficient specific data.
- Opportunities to minimise risk by 'safety-by-design' (Chapter 4) can be identified before nanomaterials enter into use.

The recent review by the policy-makers also observed that the first widely read report to evaluate the benefits and risks of nanotechnology was published by the Royal Society and the Royal Academy of Engineering in 2004 (see footnote 121). As generation of new knowledge must keep pace with the future development of nanomaterials, the Academies of Science from across Europe, together with the JRC, have aimed to extend that earlier analysis and interpretation to reduce the many uncertainties

about the potential impacts of nanomaterials on health, safety and the environment.

We advise that it is important to do the following:

- Use as precise as possible a definition of what is meant by nanomaterials: researchers and regulators may need to develop a more differentiated approach to assessment and regulation¹³¹.
- Distinguish between embedded and free nanomaterials (while also recognising that the status of the nanomaterial may vary during its lifecycle) as part of any differentiated risk assessment.
- Differentiate more precisely between the unintended exposure to environmental nanoscale particles from combustion or other natural processes, and the unintended and intended exposure to engineered nanomaterials.
- Build on the increasingly well understood principles and standards that are now part of the broader field of toxicology, in consequence of advances in other areas, for example in the safety assessment of chemicals and pharmaceuticals.

Recommendations for the responsible development of nanomaterials

Specific recommendations have been discussed in the previous chapters, where we have tried to clarify 'what we currently know' and 'what we need to know'.

The improved assessment of the potential risks of engineered nanomaterials requires significant effort to promote, extend and co-ordinate basic and applied research, and to translate research outputs into products and into informed policy decisions. A coherent, well-orchestrated strategy for the EU must be multidisciplinary and multi-sectoral, requiring new efforts in data collection, new infrastructure and new training, involving academia, industry, policy-makers and other stakeholders.

New knowledge on safety assessment must be considered within the overall objective of establishing the benefit–risk balance. In this context, we identify three key messages:

- Safety research is an essential part of the innovation of nanomaterials ('safety-by-design' principle).
- Research governance and product regulation must be sufficiently flexible to cope with future developments.

¹³⁰ Morris, J. et al., *Nature Nanotechnology* **6**, 73 (2011).

¹³¹ Drezek, R.A. and Tour, J.M., *Nature Nanotechnology* **5**, 168 (2010).

- Inappropriate or over-regulation can act as an obstacle to fundamental research and its translation into products which, when used in a safe manner, can contribute to justified societal objectives.

Some of the key action areas to be addressed in order to improve understanding of the current applications and prepare for future applications of nanomaterials are as follows:

- **Research capacity.** Increasing volume and quality of research on safety aspects are required to progress further standardisation of methods; to widen laboratory networks across the EU and beyond; to build upon existing and develop new centres of excellence, avoiding unnecessary duplication; and to promote linkage between disciplines. We advise that it is essential to use well-characterised materials, to study chronic as well as acute effects, to assess toxicokinetics in systemic studies and to use realistic dose levels, considering exposure throughout the nanomaterial's lifecycle.
- **Training capacity.** Support for younger scientists – and the European Commission has a pivotal role in its funding programmes – is greatly needed. Multidisciplinary training and integrated teaching of nanotechnology at all levels from undergraduate to post-doctoral have to be introduced.
- **Research governance and integrity.** Awareness and responsibility have to be developed at the individual, research institution and company levels, building on current codes of conduct while exploring the options for other governance frameworks.
- **Product regulation.** Generally, the approval of novel products emanating from nanotechnology should be subject to the same regulatory principles and practices as exist for products derived from other sources. As in other regulation, there are issues to be considered for cost–benefit balance as well as risk–benefit balance. Product regulation should include consideration of the issues, where appropriate, for sustainable disposal, degradation and recycling of engineered nanomaterials. Approaches to product regulation and to research governance within the EU should be integrated, where possible, with international initiatives to ensure complementarity and minimise duplication of effort; further duplication should be avoided by assigning responsibility to specialist national or European institutions; to offer test certificates to companies producing nanomaterials or consumer products containing them.
- **Societal engagement.** Scientific and policy-making communities must work together in providing accurate and relevant information to support public

dialogue on hopes and concerns and to address alarmist assertions that sometimes appear in media reports of nanotechnology. This dialogue should include ethical as well as legal issues. The rapid advances in technology present challenges both for policy-makers and for public understanding and will induce many uncertainties. A recent EASAC report¹³² discusses in further detail some of these issues for synthetic biology as an emerging technology and the recommendations in that report for public engagement are also relevant for nanomaterials. Common terminology and consensus definition of nanomaterials should be developed for effective public engagement, as well as for the objectives of standardising research methodologies and developing an optimised regulatory framework.

- **EU competitiveness.** Nanotechnology has the potential to play a major role in European innovation and economic growth. There are significant implications for small and large companies.
- **Worldwide harmonisation.** Ethical, legal and societal issues as well as environmental, health and safety issues of nanomaterials allow the building of bridges beside the worldwide competitiveness. This facilitates worldwide trade, with significant implications for business.

In conclusion, the scientific community has a continuing responsibility to advise the European Commission and European Parliament about the opportunities now coming within range. It is often difficult to estimate the timeframe for the development of specific engineered nanomaterials and their launch as novel products. Therefore, it is vitally important to create the appropriate supportive environment for innovation and flexibility in risk management (e.g. mandatory product register, labelling) to prepare for the envisaged longer term as well as for the shorter term encompassing the current and next generation of products. To this end, it is essential to invest in the science of safety assessment while, at the same time, seeking to expedite the regulatory review of the products emerging from that science. The stringency of the controls should match the potential of exposure.

We reiterate that there is only a limited amount of scientific evidence to suggest that nanomaterials present a risk for human health and we advise that the principles of risk assessment procedures should conform to the same procedures as any other new material, paying due respect to new phenomena that may occur due to new properties related to the nanoscale. Successful innovation, if it is to encompass both regulatory and consumer approval, must incorporate safety by design.

¹³² EASAC, *Realising European potential in synthetic biology: scientific opportunities and good governance*, 2010; accessible on www.easac.eu.

6 Annex

6.1 List of abbreviations

CA	Competent Authority
CASG Nano	Competent Authorities Sub Group on Nanomaterials (REACH)
CEN	European Committee for Standardisation
DEFRA	Department for Environment, Food and Rural Affairs (UK)
DNA	Deoxyribonucleic acid
EASAC	European Academies Science Advisory Council
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EFSA	European Food Safety Authority
EHS	European Health and Safety
EPA	Environmental Protection Agency (USA)
EU	European Union
FP7	Seventh Framework Programme
ISO	International Organisation for Standardisation
JRC	Joint Research Centre
JWG	Joint Working Group
MSc	Master of science
NanoTOES	Nanotechnology: Training of Experts Safety
NT	Nanotechnology
OECD	Organisation for Economic Co-operation and Development
PM	Particulate matter
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCCP	Scientific Committee on Consumer Products
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
STOA	Science and Technology Options Assessment (European Parliament)
TC	Technical Committee
TSCA	Toxic Substances Control Act
WG	Working Group
WPMN	Working Party on Manufactured Nanomaterials

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Abstract

This report presents an independent and scientifically-based evaluation on the impact of engineered nanomaterials on human health taking into account state-of-the-art knowledge of the Research Community in this field. It is suggested that principles of risk assessment procedures should conform to the same procedures as any other new material paying due respect to new phenomena that may occur due to new properties related to the nano-scale. Successful innovation, if it is to encompass both regulatory and consumer approval, must incorporate safety by design.

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