RESEARCH PRIORITISATION TO DELIVER AN INTELLIGENT TESTING STRATEGY FOR THE HUMAN AND ENVIRONMENTAL SAFETY OF NANOMATERIALS
# Content

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXECUTIVE SUMMARY</td>
<td>5</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>9</td>
</tr>
<tr>
<td>Vision</td>
<td>9</td>
</tr>
<tr>
<td>Ambition</td>
<td>9</td>
</tr>
<tr>
<td>Document Structure</td>
<td>10</td>
</tr>
<tr>
<td>Process</td>
<td>11</td>
</tr>
<tr>
<td>1 PHYSICOCHEMICAL IDENTITY</td>
<td>13</td>
</tr>
<tr>
<td>Ambition</td>
<td>13</td>
</tr>
<tr>
<td>Definition</td>
<td>13</td>
</tr>
<tr>
<td>Introduction</td>
<td>13</td>
</tr>
<tr>
<td>Future research emphasis and prioritisation</td>
<td>15</td>
</tr>
<tr>
<td>Final recommendations</td>
<td>22</td>
</tr>
<tr>
<td>2 EXPOSURE IDENTITY</td>
<td>24</td>
</tr>
<tr>
<td>Ambition</td>
<td>24</td>
</tr>
<tr>
<td>Definition</td>
<td>24</td>
</tr>
<tr>
<td>Introduction</td>
<td>24</td>
</tr>
<tr>
<td>Future research emphasis and prioritisation</td>
<td>25</td>
</tr>
<tr>
<td>Final recommendations</td>
<td>36</td>
</tr>
<tr>
<td>3 HAZARD IDENTITY</td>
<td>37</td>
</tr>
<tr>
<td>Ambition</td>
<td>37</td>
</tr>
<tr>
<td>Definition</td>
<td>37</td>
</tr>
<tr>
<td>Introduction</td>
<td>37</td>
</tr>
<tr>
<td>Future research emphasis and prioritisation</td>
<td>39</td>
</tr>
<tr>
<td>Final Recommendations</td>
<td>52</td>
</tr>
<tr>
<td>4 GROUPING/RANKING</td>
<td>53</td>
</tr>
<tr>
<td>Ambition</td>
<td>53</td>
</tr>
<tr>
<td>Definition</td>
<td>53</td>
</tr>
<tr>
<td>Introduction</td>
<td>53</td>
</tr>
<tr>
<td>Future research emphasis and prioritisation</td>
<td>55</td>
</tr>
<tr>
<td>Final Recommendations</td>
<td>62</td>
</tr>
<tr>
<td>5 PRACTICES FOR IMPLEMENTATION INTO THE RISK ASSESSMENT FRAMEWORK</td>
<td>63</td>
</tr>
<tr>
<td>Ambition</td>
<td>63</td>
</tr>
<tr>
<td>Definition</td>
<td>63</td>
</tr>
<tr>
<td>Introduction</td>
<td>63</td>
</tr>
<tr>
<td>Future research emphasis and prioritisation</td>
<td>68</td>
</tr>
<tr>
<td>Final Recommendations</td>
<td>77</td>
</tr>
<tr>
<td>Annexes</td>
<td>85</td>
</tr>
</tbody>
</table>
EXECUTIVE SUMMARY

The rapid introduction of engineered Nanomaterials (NMs) to the market raises several challenges to the traditional risk assessment approach. The problems include a lack of appropriate tools for effective NM risk decision-making and insufficient capacity (time and money) to fully assess or evaluate risks associated with all NMs. The challenges are, therefore, to develop both appropriate and optimal approaches to screen NMs and evaluate associated risks. To tackle these challenges, the European Commission funded the ITS-NANO project to develop a research strategy for the next 20 years (Grant Agreement no: 290589).

The vision is a way forward in which there is a knowledge-based sustainable development of engineered NMs.

The vision forms the basis for a safe, sustainable but accelerated knowledge-based development of engineered NMs. This should be achieved through formulating robust procedures for more effective management of existing NMs and the future development of novel materials with inherent characteristics that emphasise safety or sustainability.

This vision has been converted in a series of ambitions to prepare foundations for well-structured, prioritised future research concerning the interactions of NMs with biological systems in order to: (i) intelligently design nanosafety evaluation and risk assessment strategies, including rapid screening, and computational models, (ii) identify high risk materials, and (iii) implement effective strategies to counter the risks. The presented prioritisation for NM research follows a risk evaluation paradigm based on the approach currently used for chemicals, but adapted where necessary to take account of NM-specific factors. This paradigm therefore requires the systematic acquisition and implementation of key information related to exposures, hazards and physicochemical characteristics (IDs) of the materials. We offer conclusions of differing scope for four timeframes:

- In the short-term (<5 years) needs include development of an understanding of the connections between NMs’ physicochemical, exposure and hazard characteristics (IDs). This will enable the grouping/ranking required for efficient screening of materials to identify needs for further quantified RA.
- In the mid-term (5-10 years) the ambition includes an understanding of the relationship between faster and less comprehensive techniques (e.g. high throughput system and in vitro models) with more comprehensive and complex techniques (detailed methods and in vivo models), in order to enable in future a faster evaluation of risk.
- In the longer-term (10-15 years) the development of modelling approaches for risk assessment with a continual reduction of in vivo and in vitro hazard testing is required.
- In the distant future (>15 years) RA can be based on modelling and extrapolations, and only if additional information is required with focused physicochemical, exposure and hazard testing.

To provide substantiated background knowledge for the strategy, the ITS-NANO project partners reviewed the current literature to identify knowledge gaps (Annex II) and supplemented the acquired information with data from responses to an online questionnaire distributed to participants in relevant ongoing EU projects (Annex III). In addition, the ITS-NANO partners (see data sheet), stakeholders from academia, industry, regulators, funding bodies and non-governmental organis-
tions (NGO’s) (see data sheet) contributed to the gap analysis and subsequent strategy formulation in two face-to-face workshops.

A traditional risk assessment approach includes assessment of hazard and exposure. The Intelligent Testing Strategy (ITS) presented here also uses exposure and hazard information, but increases emphasis on physicochemical (PC) characterisation of NMs. All three aspects are defined and the definitions are used to identify the research needed to deliver the tools and information required for robust RA. The ITS will require increased reliance on computational approaches and decreased reliance on testing with time, thus this prioritisation document identifies the research required to enable grouping/ranking of NMs to optimise the risk decision process in the short term, and modelling approaches in the longer term. Strategies for identifying PC, exposure and hazards of relevant materials could be developed independently to some extent, but highly integrated research is required in order to advance as rapidly as possible. Finally, a process for implementing research findings into current and future risk assessment frameworks has been established.

The major recommendations derived from the individual research areas and cross-cutting research priorities, including a perspective on future time-frames, are presented below. Specific, detailed recommendations for each aspect of the strategy are presented in focused chapters of the report, but as emphasised an integrative research effort is required. It is proposed that the topics deemed highest priority should be addressed (or begin to be addressed) in the short term, while lower priority areas should be addressed in the longer term (or if and when they become higher priority according to new research) based on when information is needed. This does not mean that the lower priority areas are less important, rather that they will be easier to address in the longer term when more relevant information becomes available. Some work on the longer term goals needs to start now in order to frame the short-term research required.

Physicochemical identification (PC ID) refers to a set of physicochemical characteristics of NMs, which change during their life cycle and can be used for RA and associated decision-making. In the short term, provision of stakeholder-tailored standard/reference materials, validated instruments and standard protocols is recommended in order to maximise the cost-effectiveness of physicochemical characterisation. In the mid-term standard/reference materials, validated instruments and standard protocols will contribute to effective characterisation of materials at different life cycle stages (when they may be transformed by various processes) and in complex matrices. The development, validation and implementation of novel physicochemical descriptors, techniques and instrumentation will be further goals for PC ID. In the long-term, the goal is to develop standardised protocols for monitoring and characterising NMs according to their PC properties throughout their life cycle, in complex matrices and both in vitro and in vivo models, using flexible, tailored or tiered approaches. In the distant future, high-quality data for in vitro, in vivo and in silico approaches for exposure assessment and hazard identification will be required.

For assessing exposure, standardisation of methods for discriminating NMs from background particles in complex matrices, throughout their life cycles, is of paramount importance. In the short term, for assessing human exposure attention should focus on inhalation in occupational settings and ingestion, with parallel definition of internal doses. For assessing environmental exposure, the focus should be on identification of long-term accumulation and concentration hot-spots in soils and sediments. The development of standardised protocols must remain a continuous priority over time. In the mid-term, concentrations in differ-
ent matrices should be linked to actual exposure. In addition, robust strategies for sampling and determining concentrations in appropriate indicator organisms and/or potentially sensitive environmental compartments need to be formulated and thoroughly validated. In the long term, data should be acquired and disseminated regarding low priority exposure routes, such as dermal, air and water exposure. This approach will facilitate grouping of NMs in informative exposure-classes (exposure IDs) and modelling their exposure, bioaccumulation and fate throughout their life cycles. In the distant future the development of standardised protocols for multi-metric and innovative detection tools is essential.

For Hazard ID generation, key short-term priorities are to develop dose metrics that allow determinations of NMs’ mode of action, bioavailability and toxicokinetics. In the mid-term validated in vitro and in vivo models should be developed, including long-term or chronic models, and reliable biomarkers (in vitro and in vivo) should be identified. The in vivo models will be needed to determine time courses of responses allowing distinction between short and long term effects, rapid and delayed onset, and reversible and irreversible effects. There will be a need to generate more relevant multi-tissue in vitro models. In the long-term, knowledge of NMs’ population-level effects, bioaccumulation and biomagnification will be required. Studies will generally require robust, appropriate in vitro and in vivo models of susceptibility to focus on vulnerable individuals or populations. To accelerate in vitro testing high throughput (HTP) screening will be essential, while modelling approaches will be required to reduce the burden of testing. In the distant future in vitro HTP screening will allow focused hazard testing.

Grouping and ranking of NMs are considered to be key steps towards the development of modelling approaches that will be core features of a future ITS. Informative grouping/ranking requires precise, accurate Physicochemical-, Hazard-, and Exposure-ID inputs. Further, sufficient targeted experimental data are required to provide the weight of evidence needed to eliminate uncertainty and robust foundations for grouping, ranking and modelling. Understanding NMs’ modes of action and their relations to a rigorously defined set of physicochemical characteristics is also crucial. Thus, research into new approaches for grouping, ranking and numerical extrapolation/interpolation of results between species/models and between materials is required.

Future outputs generated from application of the ITS-NANO research prioritisation will provide information on NMs’ physicochemical characteristics, hazards and exposure, including data obtained from in vitro tests, read-across/grouping/ranking, in silico models and validated exposure models. Thus, they will provide secure, evidence-based foundations for formulating and implementing “best practices” for RA and data management (DM) of NMs. Alternative and non-testing methods are already encouraged in current RA frameworks, provided they are validated or scientifically justified. The acquisition and use of high quality data are continuous priorities, while training and additional guidance will be required for interpretation and integration of these data and their regulatory acceptance. Mid- to long-term issues that are foreseen include the potential need to adapt the current regulatory framework to accommodate novel quantitative tools and probabilistic approaches.

The diagram below illustrates the connections between the identified research priorities, and the implementation of the subsequent acquired knowledge and methods in the risk evaluation process. Each hexagon represents a priority research need, and each interface a logical relationship; with black hexagons representing NMs around the margins and the ITS modelling tools in the centre.
Between the three priority research areas (Physicochemical, Exposure and Hazard ID) and the central ITS are the grouping/ranking approaches (bold hexagons) needed to streamline the data requirements. The outputs of the ITS feed into the risk assessment frameworks.

NB: Larger version available at end of document (p83).
INTRODUCTION

A key phenomenon that is exploited in nanotechnology is that properties of materials change as their dimensions diminish to the nanometre range. These changes may cause adverse (eco)-toxicological effects. However, effects of exposure to nanomaterials (NMs) have been less widely studied than effects of chemicals. This is of concern because, for example, it has been established in recent decades that toxicity is profoundly affected by, factors such as the crystalline phase and physical dimensions of some materials. With the development of nanotechnology it has become increasingly clear that even seemingly subtle changes of properties such as surface chemistry or chemical composition can significantly change the toxicity of NMs. Further, the distributions of NMs dispersed as particles in the environment and biological organisms may differ markedly from those of chemicals (molecules).

The rapid introduction of engineered NMs to the market raises several challenges for current RA approaches. The problems include a lack of appropriate tools for effective NM risk assessment in various regulatory frameworks (see Chapter 5) and insufficient capacity to fully assess or evaluate risks associated with all NMs. The key challenges are, therefore, to develop appropriate, optimised approaches for screening and NMs and evaluating associated risks. Hence, there is a requirement to develop better tools for NM evaluation in general, for grouping or ranking NMs for RA purposes, and identifying (and prioritising) materials requiring full risk analysis. The ultimate goal is to acquire the tools and databases required to base risk decisions on streamlined physicochemical data, exposure information and modelling with focused testing. The aim of this report is to clearly identify the research areas that should be prioritised in order to reach this goal rapidly, thus facilitating robust, efficient evaluation of the risks associated with NMs throughout their entire life-cycles.

This prioritisation should help to focus research efforts to deliver an intelligent testing strategy (ITS) for nanomaterials. In this context, an ITS is a process that allows the risks of NMs to be assessed accurately, effectively (i.e. yields rational conclusions) and efficiently.

Vision

The vision is a way forward in which there is a knowledge-based sustainable development of engineered NMs.

The vision forms the basis for a safe, sustainable but accelerated knowledge-based development of engineered NMs. This should be achieved through formulating robust procedures for more effective management of existing NMs and the future development of novel materials with inherent characteristics that emphasise safety or sustainability.

Ambition

The ambition is to outline research priorities for the next 20 years that will allow the foundation to realise the vision: structured research concerning the interactions of NMs with biological systems in order to intelligently design risk assessment and management strategies. This includes the development and validation of rapid screening techniques, better models, identification of high risk materials, and implementation of strategies to counter identified risks.
The formulation of such risk evaluation procedures will require general tools, protocols and instruments that facilitate the development of clear rules to prioritise NMs for risk assessment. In the short term, the general rules will need to be supplemented by screening and modelling, either individually or in combination, to group or rank NMs. Ideally, in the longer run the developed models should be stochastic, predictive and capable of taking into consideration known uncertainties.

More specifically:
• In the short-term (<5 years) needs include development of an understanding of the connections between NMs’ physicochemical, exposure and hazard characteristics (IDs). This will enable the grouping/ranking required for efficient screening of materials to identify needs for further quantified RA.
• In the mid-term (5-10 years) the ambition includes an understanding of the relationship between faster and less comprehensive techniques (e.g. high throughput system and in vitro models) with more comprehensive and complex techniques (detailed methods and in vivo models), in order to enable in future a faster evaluation of risk.
• In the longer-term (10-15 years) the development of modelling approaches for risk assessment with a continual reduction of in vivo and in vitro hazard testing is required.
• In the distant future (>15 years) RA can be based on modelling and extrapolations, and only if additional information is required with focused physicochemical, exposure and hazard testing.

The prioritisation for NM research follows a risk evaluation paradigm based upon the approach currently used for chemicals, but adapted where necessary to take into account NM-specific factors. This paradigm therefore requires the systematic acquisition and implementation of key information related to exposures, hazards and physicochemical characteristics (IDs) of the materials.

The future research emphasis has been framed in the context of the gaps in current knowledge (see Annex II), taking into account both published information and anticipated knowledge gains such as information expected to be delivered by current research projects (see Annex III).

Document Structure

The document can be read and used in many ways, but users are recommended to either read the entire document or the Introduction followed by pertinent chapters (which can be read independently, although they are cross-referenced).

The following chapters include definitions of the focal topic, a brief introduction and a summary of the identified knowledge gaps (addressed in more detail in Annexes II and III), followed by a presentation of identified future research needs and discussion of requirements to meet the needs.

The chapters entitled Physicochemical identity (ID), Exposure ID, Hazard ID and Grouping/Ranking IDs outline the research and knowledge required to generate the information needed to formulate a robust ITS and hence efficient risk decision processes. Within each chapter, the research needed to deliver the knowledge and tools required is outlined, with prioritisation for each research step summarised in hexagon diagrams. The hexagon diagrams show that for each issue (hexagon) there is more than one way to progress. The hexagon diagram design has been chosen, since a strictly consecutive approach is considered inappropriate. The hexagon shape reflects the potential for each component to interact with multiple components, thus the organisation could be shuffled according to the
specific research question addressed or as knowledge accumulates. Each chapter relates to a key component of the NM risk assessment paradigm. When reading individual chapters it should be noted that the keywords used in the hexagon figures are directly linked to both the related text and associated recommendations. The implementation chapter highlights where the proposed research strategy can be applied in current research. The final chapter integrates recommendations from the full document, with strong cross-referencing to the preceding chapters. It should be noted that the optimal route through the hexagons is heavily influenced by the specific research objective, and the methods, protocols, models and approaches shown in some of the hexagons may not be required to address some questions.

It should also be noted that while the hexagon diagrams indicate research prioritisation, research issues situated on the right hand sides (long term and distant future priorities) of each prioritisation diagram need to be considered at an early stage to ensure that any short-term research generates outputs that will be useful for developing longer-term priorities. Without this consideration, the information generated may lack an appropriate focus and could lead to gaps in knowledge that retard achievement of the longer-term priorities.

Although each component of the paradigm/strategy is addressed in individual chapters, it is imperative that the future research strategy has a cross-cutting perspective, integrating several research areas. Thus, it is vital to continuously work towards goals such as the development and implementation of: (i) a common language (i.e. shared ontology, terminology and nomenclature); (ii) comprehensive, user-friendly information-sharing tools (e.g. databases); (iii) synergistically applicable advanced techniques (by providing, for instance, an efficient research framework and facilitating access to advance analytical equipment); and (iv) in-depth RA methodologies. This work has already been initiated to a large extent through various EU nano-projects and the Nanosafety cluster.

Process

The strategy document was coordinated by participants in the EU FP7 project ITS-NANO (Grant Agreement no: 290589) in collaboration with stakeholders (see Annex I) invited to contribute during two stakeholder meetings. To provide substantiated background knowledge for the strategy, the ITS-NANO project partners reviewed the current literature to identify knowledge gaps (Annex II) and supplemented the acquired information with data acquired from responses to an online questionnaire distributed to participants in relevant ongoing EU projects (Annex III).

The framework of the strategy was then developed and discussed within the ITS-NANO group and during a stakeholder meeting organised by the ITS-NANO consortium in Edinburgh, September 2012. Inputs from the stakeholders were then incorporated into the research prioritisation, and another stakeholder meeting was held in Venice, March 2013. Stakeholders from the EU and US attended the meeting, including representatives of academia, industry, regulators, funding bodies and non-government organisations (NGOs). The stakeholders were invited to provide
feedback on the entire document, focusing during the meeting on the aims, definitions, recommendations and prioritisation of research for each major aspect of NM risk evaluation (as reported in the following chapters). After the second meeting, the stakeholders’ inputs (oral and written) were discussed internally by the ITS-NANO partners and incorporated in the final research prioritisation document.

The interaction with stakeholders and the consultation with the ongoing project participants was all done within the relatively short project duration, thus the priority document should be clearly considered to be the basis for a continuing process – providing firm guidance for all stakeholders, but also a source of inspiration for further development.

Finally, the ITS-NANO document is a result of the joint collaboration between the ITS-NANO group and the invited stakeholders, the latter representing all major stakeholder groups.
1 PHYSICOCHEMICAL IDENTITY

Ambition

The ambition is to define research priorities to develop a focused set of physical and chemical descriptors (Physicochemical ID) to identify NMs unequivocally at each life cycle stage, thereby facilitating the development of effective risk assessment and management practices. This knowledge will provide different stakeholders with sets of standard/reference materials and standard protocols for tailored approaches to maximise the cost-effectiveness of physicochemical characterisation. The Physicochemical IDs should allow categorisation and grouping/ranking of NMs in relation to Exposure ID and Hazard ID for humans and environmental species. Prioritisation of research needs to meet this challenge are outlined below.

Definition

A Physicochemical Identity (Physicochemical ID) is defined as the dynamic pattern of physical and chemical characteristics (identified using appropriate analytical techniques) associated with one or several specified NMs during their life cycle. The physicochemical descriptors should describe key inherent features of the NMs (i.e. what they are), their influence on biological and environmental fates (i.e. where they go) and their inherent activity (i.e. what they do).

Introduction

The physicochemical descriptors required for sound risk assessment are considered to be the fundamental factors determining NMs’ specific interactions with the environment (fate) and biological systems (exposures/hazards).

The identification of key physicochemical descriptors for NMs must contribute to informed interpretation of exposure and toxicity data (enabling predictions), considering their intended use. This will allow the grouping of NMs based on specific Physicochemical IDs, either alone or in combination with induced biological effect or exposure data. Hence, there is a need to identify appropriate sets of parameters (or dimensions) for efficiently determining such IDs.

Therefore, a paramount requirement for defining future Physicochemical IDs is the availability of technical equipment and protocols allowing the estimation of key NM-related characters and properties (including well-established properties and new physicochemical endpoints), through both the optimisation of existing methods and the development of new tools. Diverse techniques have been or are being developed for this purpose, but most of them lack one or more of the key requirements, i.e. ideally they should be fully quantitative, provide accurate, precise and sensitive measurements of similar properties of diverse materials, enable determinations of NMs in complex media, and be widely available, convenient, rapid, cost effective, high throughput (preferably enabling measurement of multiple properties) and thoroughly validated. Moreover, they should provide information on at least the minimum number of descriptors for clear description of the NMs during their life cycles. The use of standardised analytical procedures, methods and instrumentation, and the quantification of the selected physicochemical descriptors will require standard or reference materials for NMs. Such standard/reference materials may also be useful for assessing human health and environmental risks associated with the NMs.
In order to address the above issues for prioritising future research efforts, we need to define requirements for a Physicochemical ID. This can be done by breaking the requirements into broad categories or Physicochemical ID questions (Figure 1.1):

- Can definitions for NM be derived?
- How can we identify physicochemical properties (Which properties? What techniques are available?), including characterisation of transformation processes in NMs’ life cycles?
- What new equipment must be developed?
- What new endpoints must be recognised?
- Can standard and reference materials be generated for each of the purposes (instrument calibration, quantitative measurement of NMs at a time before the exposure scenario, detection and monitoring of NMs in complex matrices, etc.)?
- Can tailored approaches for Physicochemical ID be defined for different purposes, e.g. regulatory, research and industrial applications?
- Can we apply in silico tools (e.g. grouping or advanced modelling approaches) to Physicochemical ID and relate them to NMs’ fate and toxicity?
- Does the implementation of developments require additional training?

PC IDs should be generated in synergy with the generation of exposure IDs (to harmonise definitions of standards and methods for characterising NMs throughout the transformation processes of their life cycles) and Hazard IDs (to determine a set of PC IDs related to their toxicity to enable predictions of their biological/en-
Future research emphasis and prioritisation

To obtain a rationale for grouping NMs based on their physicochemical identities, the following aspects should be emphasised, both for pristine and in situ NMs: (i) identification of research (in a broad context) that should be initiated, (ii) standardisation of tools and protocols to ensure that the results are useful in wider ITS and RA contexts, (iii) procedures to ensure that standardised protocols are appropriately and efficiently implemented, (iv) development of high-throughput systems to deliver fast evaluations, and finally (v) the development and improvement of models to extrapolate acquired information across NMs and media/biological endpoints.

As mentioned in the Introduction, future research targets must be founded on present knowledge and knowledge gaps. Hence, the future research emphasis, summarised below (Figure 1.2), has been framed in the context of the identified gaps in current knowledge (see Annexes II and III). The references e.g. "B.3" refer to corresponding points in the Knowledge Gaps and Research Priorities document (Annex II). Each gap is followed by approaches to address it.

Figure 1.2. Proposed research prioritisation for generating an effective PC ID system to inform an Intelligent Testing Strategy. The requirements within a column are related (emphasised by the tone of blue), some of which can be met using techniques that are available now or under development in current projects, while others are models for the future. The order of priority is graded across the diagram, with hexagons to the left being of short term-priority (<5 years) stretching to longer term priorities on the right (>15 years). Grey hexagons represent modelling components that will lead to the ITS. As mentioned in the Introduction, the short-term priorities need to be considered in the context of the long-term priorities to ensure that they generate the information needed to provide robust foundations for developing the longer-term priorities.
Short-term priorities (achievable in 5 years)

Identified gaps: A critical obstruction to furthering knowledge and research is the lack of standard and reference materials and protocols [B.3, B.5].

Future research emphasis

Reference and standard materials/validating instrument and methods
Reference and standard materials are crucial for both validating well-established techniques and developing new ones. These materials must serve as references for the single physicochemical characteristics required for further development of techniques, and/or validating instrument and methods already in use or under development for determining NMs’ PC characteristics and exposure assessments (see also the following chapter). They are also required for the development of new exposure assessment techniques, which must be capable of detecting NMs, discriminating them from background particles, characterising them and extracting them from diverse media (see the following chapter). Moreover, the development of standard/reference materials is essential for establishing foundations for modelling (see the next modelling chapter and longer-term priorities). They will assist researchers to design and compile libraries of NM with specific chemical motifs or tunable physicochemical characteristics (such as controlled levels of aggregation, charge, solubility, dissolution, hydrophobicity, solubility). The possibility for quantitative comparison with standards or related reference materials could be highly valuable for the identification of particular structural properties or motifs that may be predictive of toxicity and exposure (see also Exposure and Hazard chapters).

Development of standardised methods/certified protocols
Standardisation of protocols is another key issue. It is crucial for both harmonisation of the quality of results obtained during physicochemical characterisation and the development of novel techniques and related validation (see also mid-term priorities). Indeed, there should be an emphasis on development of standard materials (for instrument calibration and NM measurement), reference materials and characterisation protocols to identify acceptable ranges of values to ensure that all techniques applied provide sufficiently high quality data for the intended purposes. These values should be defined in relation to the developed techniques. Some important standardisation efforts have been already been made by the international standard organisations, who have issued some guidelines and protocols for the standardisation of available processes and techniques, especially for characterisation of the size, shape and chemical composition of nanoparticles, e.g. by the US National Institute of Standards and Technology. However, further rigorous definition of methods, techniques and quantitative criteria is required to harmonise tolerance acceptance criteria (TAC) for reference models and quantitative validation. Standardisation should not only help regulators and industry, but should also guide researchers in both the use of techniques and setting milestone for developing new techniques affording narrower acceptance levels. Further, it may lead to the setting of a new range of quantification criteria for a given property, which may be related to the specific techniques used for the measurements, as addressed by ISO TC24/SC4.

Inter-laboratory comparisons/Data validation
Inter-laboratory comparison and data validation are already being addressed in many ongoing projects (e.g. Q-Nano, the new project NANOReg and Marina). However the procedures will need further refinements and harmonisation through centralised systems for NM delivery (for instance, on-line NM databases, harmonised sub-sampling and on-line ordering).

1 See glossary for definitions
Ongoing research projects: These aspects are key foci of several projects, including those mentioned above, NANOHETER, NanoValid and NanoSolutions.

Mid-term priorities (achievable in 5-10 years)

Identified gaps: Research is needed to improve understanding of NMs’ characteristics that are most strongly related to their toxicity, to establish conditions in which these characteristics should be assessed, to identify strategies to group NMs based upon these characteristics (B.4), and to develop standard/reference methods and materials to validate the results (B.3, B.5).

Future research emphasis

Characterisation of NM (transformation processes throughout NM life cycle/in different matrices)

A key first step for assessing risks associated with NMs is their unambiguous identification both as primary NMs (as produced) and during their life cycles (when they may be transformed by various processes). Therefore, there is a need to develop new analytical techniques, or implement those already available, to allow extensive qualitative and quantitative physicochemical characterisation of NMs during their life cycles and in relevant complex matrices for determining Exposure and Hazard IDs. Scientists involved in NM fabrication can contribute to these efforts, as their expertise may facilitate the identification of innovative parameters (new physicochemical endpoints) that are not presently considered in routine physicochemical characterisation (e.g. surface roughness). Such parameters may also be essential for thorough description of NMs in relation to their interactions with the environment, bio-fluids and living organisms. However, any new characterisation techniques, methods and endpoints must be carefully evaluated to maximise their cost-effectiveness, open to further refinement in order to be widely distributed and have a high-throughput element, while remaining reliable and relevant. These criteria should also be considered when defining priorities for NM characterisation, related techniques and evaluations of the relevance of physicochemical characteristics to health and environmental impacts. All aspects of PC characterisation must be adjustable in accordance with technological developments and advancements in understanding of NMs.

Innovative PC endpoints

The determination of physicochemical descriptors of NMs by standardised, quantitative approaches is fundamental for progress in risk assessment as it allows unequivocal description of their physicochemical nature at particular moments in time, most importantly at the moment of contact with the environment, or biological system of potential concern. Such sets of PC descriptors are mainly related to structural and functional properties of NMs (what they are and what they do; Figure 1.3). Extension of the standardisation approaches and harmonisation of data to the physicochemical interface properties of NMs is of high importance. Indeed, the interfacial properties (generally defining clusters of NMs on the basis of “Where they go”) may change markedly during the NMs’ life cycles, and these changes may be responsible for experimental variability and changes in biological responses (Figure 1.3). Given the changes in NMs that occur during their life cycles, it is important to develop models that can predict the impact of environmental stressors on their physicochemical characteristics. Further, standardised protocols would be helpful for reliable characterisation of NMs (and the related PC ID) when present in complex bio-fluids or as product components throughout their life cycles. This may be helpful in determining clusters of properties or set of PCs influencing their bioaccumulation and fate, effective internal doses, and fate during their life cycles (which may directly link PC IDs to Exposure and Hazard IDs).
Finally, the definition and quantitative measurement (by reference materials and acceptance values, using validated methods and techniques) of key descriptors could help grouping, i.e. applying specific paradigms based on likeness principles (for example, grouping NMs with similar or dissimilar physicochemical properties corresponding to groups of NM with similar or dissimilar biological effects due to their similar or effects on the effective dose). These steps are required for standardisation of protocols, and together with appropriate quantification, are essential for the regulation of NMs as they provide the means to set quality requirements that must be met in commercial and accredited laboratories.

Ongoing research projects: As stated above, NanoSolutions, NanoValid and NANOHETER are focused on the development of standard materials and protocols. NanoSolutions and nanOxiMet are focused on elucidating characteristics of NMs that are most strongly related to their toxicity, the conditions in which these characteristics should be assessed, and the identification of strategies to group NMs based upon these characteristics.

Identified gaps: Characterisation instrumentation should be developed and optimised to provide “standard protocols” for detecting, characterising NMs and extracting them from pertinent media (B.3).

Future research emphasis
Instrument development (throughput and multimeitrics)
New techniques are expected to be developed that: i) increase the scope for high throughput and high content implementation, ii) provide new information on novel physicochemical endpoints, and iii) serve as baseline (validation) techniques for simpler, more rapid, robust relevant techniques. This will reduce costs of both the instrumentation and research staff involved in future routine measurements. It is expected that scientists working in engineering and materials sciences will be strongly involved in this task and that the development of suitable HTP will lead to the optimisation of protocols for NM characterisation. However, any new instrumentation must be rigorously investigated, tested and validated using suitable reference materials.

Fast, efficient analysis of the minimal sets of physicochemical descriptors of NMs (see definition paragraph above), could be achieved by using novel hyphenated separation and detection techniques, e.g. coupled chromatographic and spectral methods [1]. Current examples of hyphenated systems include flow fractionation instruments coupled to mass spectrometers, various kinds of microscopes (TEM, SEM) coupled to elemental analysers (e.g. energy dispersive X-ray spectrometers), and high pressure chromatographs coupled to various detectors (UV, IR, Diode Array, or sophisticated analytical centrifuges equipped with multiple detectors). The hyphenation of high content systems (enabling, for instance, sizing or analysis of NMs’ elemental composition, reactivity or stability) could be highly valuable for simultaneously monitoring multiple properties of NMs and related kinetics. Several nanodevices for characterising NM properties have been developed in the EU FP7 NANODEVICE project recently, and these or other nanodevices and nanosensors may also be useful for characterising novel properties of NM in solution (new endpoints). A further novel concept for hyphenated techniques is to link physicochemical measurement and hazard identification techniques, preferably in a HTP format, as this would allow on-line characterisation during toxicity testing.

Ongoing research projects: A substantial part of the focus of NANOGEN, NANOHETER, and NanoValid is on the development of innovative methods for characterising NMs in different media.
Long-term priorities (achievable in 10-15 years)

Identified gaps: Characterisation instrumentation should be developed and optimised to provide standard protocols for detecting and characterising NMs, and extracting them from pertinent media (B.3).

Future research emphasis
Development of protocols for measuring NM PCs (throughout NM life cycle/in different matrices)

Once a suitable set of physicochemical properties that are quantitatively related to Exposure and Hazard IDs has been identified, standardisation of general protocols would be helpful for reliable characterisation of NMs (and related PC IDs) when present in complex bio-fluids or as components of products during their life cycles. This may be helpful for harmonising data acquisition, checking the quality of the acquired data, and facilitating interpretation of relationships of properties and/or set of PCs with NMs’ bioaccumulation and fate, effective internal doses, and fates during their life cycles (all of which would directly link PC IDs to Exposure and Hazard IDs.

Ongoing research projects: A substantial part of the focus of NANOREG, NANOHETER, and NanoValid is on the development of innovative methods for characterising NMs in different media.

Identified gaps: There is a requirement to minimise the efforts involved in physicochemical characterisation in the future (B.1), as there may be several lists of characterisation requirements (e.g. a tiered approach), depending on various scenarios (B.2).

Future research emphasis
Tailored approaches for PC ID for specific requirements e.g. regulatory, industrial and research

Any strategy for using physicochemical properties to group NMs must be flexible and reflective to accommodate increasing understanding of NMs’ effects as research progresses.

Progress in grouping NMs according to physicochemical characteristics, and their reactivity may lead to the identification of critical groups of NMs, whose further development may raise environmental health and safety (EHS) issues. A preliminary set of tailored approaches can be defined, considering the depths of information needed:

• Industrial R&D testing: Development of techniques and protocols for grouping NMs according to their physicochemical characteristics, and possibly their activities, should lead to the identification of critical groups of NMs with severe expected EHS issues (see the Grouping/Ranking chapter). Consequently, appropriate analytical techniques tailored to the classification of NMs within and/or outside these groups (in terms, for instance, of their dissolution, solubility, aggregation in bio-fluids and/or photocatalytic activity) should support this tailored approach, thereby providing industries with quick and cost effective tools to decide whether or not to proceed with further development of such NMs (applying a substitution/replacement approach when possible and there are unacceptable risks). The aim should be to develop procedures that provide the best definitions and maximal flexibility within time, current knowledge and cost constraints.

• Regulatory testing: A set of physicochemical characteristics to unambiguously characterise NMs for their registration is required. The Joint Research Centre (JRC) has recently submitted a list of 13 endpoints to the Organisation for Economic Co-operation and Development (OECD) to be added to the next release of the International Uniform Chemical Information Database (IUCLID).
which should be characterised for this purpose. Specific testing is expected to elucidate whether such endpoints can be substituted with novel endpoints: for instance, the octanol/water partition coefficient is not considered by members of the consortium and project stakeholders to be fully applicable to NMs, therefore tests to identify more pertinent hydrophobicity/hydophilicity parameters is encouraged, as well as specific research into solubility kinetics and related standard operating procedures (SOPs). Moreover, the European Chemicals Agency (ECHA) and Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations currently recognise that characterising a set of physical and chemical properties of nanoforms is likely to require a multi-method approach, which will vary for different types of NM. Reference and best practice guidance are reported on the official websites of the cited agencies. In particular, the OECD guidance on sample preparation and dosimetry is not intended to be fixed and definitive, but continuously updated as knowledge accumulates. Similarly, the tunable approach of ITS-NANO is intended to be flexibly tunable in accordance with advances (and requirements).

- Research: At least five parameters have been identified by the research community for characterising individual particles including their chemical composition/structure, size and shape distributions, surface activities/structures, coatings and interactions (for dissolving particles as well as others), and dissolution/solubility. However, to further allow modelling, read-across of results and grouping of NMs, PC characterisation should be integrated with Exposure and Hazard characterisation.

The information discussed above should be based, as far as possible, on mechanistic understanding, i.e. how different particle characteristics relate to each other and to the measurement, sample preparation methods, and sampling strategies.

Ongoing research projects: a substantial part of the focus of NanoSolution, nanOxiMet, NANOHETER, NanoREG and NanoValid is on developing tiered approaches for characterisation.

Distant future (achievable in >15 years)

Identified gaps: Research is needed to improve understanding of NMs’ characteristics that are most strongly related to their toxicity, the conditions in which they should be assessed, and to identify strategies to group them based upon these characteristics (B.4).

Future research emphasis

Functional and rational grouping based on PC ID and Exposure parameters or PC ID and Exposure parameters

The developed techniques/measures should enable grouping of NMs (possibly in various ways, depending on life cycle stage) in relation to the conventional risk assessment terms of characterisation, fate (transport, persistence and changes in Physicochemical ID), and exposure in pure and complex media, and hazard (Figure 1.3). This should also allow predictions of risk and grouping in accordance with their full life cycle. Thus, when defining rational groups of NMs different approaches could be selected, based on the intended purpose.

One possible approach could be based on the reason why NMs are included in conventional materials and products. Generally, they are used to confer bulk materials with innovative characteristics, functions and activities. From another perspective, such functions and activities may be shared by NMs that differ in terms of conventional physicochemical descriptors, like size, shape and chemical composition. These innovative functions and activities may cause NMs to interact with
the environment and biological systems in novel ways. Thus, they can probably contribute to defining their risks, and defining such rational groups (on the basis of what they are and do) may provide a means to optimise NM toxicity tests.

Another possibility is to group materials according to the physicochemical characteristics that govern their fate when released in the environment (where they go). Indeed, when NMs are released into the environment they could also come into contact with organisms, and subsequently have various absorption routes and metabolic fates, i.e. bind to different biomolecules (nucleic acids, proteins, lipids etc.). Hence, investigations of effects of the different physicochemical characteristics on their environmental or biological fate can also help in the optimisation and prioritisation of toxicological tests, focusing them on compartments that the NMs are most likely to reach (where they go), and identifying concentration hotspots.

A final consideration relates to the necessity of considering NMs’ life cycles and changes that may occur during them. Determining such changes and developing suitable techniques for both characterising them in all stages and optimising toxicity testing strategies are fundamental priorities. This process can identify whether a new risk assessment of a given NM is needed (or not) at given life cycle stages, including storage, e.g. in cases where they dissolve and cease to be NMs.

Ongoing research projects: This area is a key focus of the NanoSolutions project.

Predictive PC IDs for biological/environmental effects (models requiring PC ID only)

Very few studies to date have attempted to develop QNAR tools for NMs (for more information, see the Hazard ID chapter). First and second generation QNARs are intended to provide most of the information required for risk assessment based on PC characteristics and in vitro data, thereby reducing or eliminating the need for

Figure 1.3. Example of functional grouping of NMs according to their inherent physicochemical characteristics (What they are), their activity (What they do) and the characteristics influencing their environmental and biological fate (Where they go).
in vivo testing. Development of a third generation of QNAR models enabling risk decisions with no need for hazard testing would be highly challenging, and has not been included in any strategies published to date (e.g. Toxicology in the 21st century).

Identified gaps: There is a need for more qualified experts trained in use of new and existing technologies/methods (B.6).

Future research emphasis
Education
The investigation of safety issues associated with NMs poses new challenges for toxicologists and environmental scientists, in terms of dealing with materials that differ from conventional chemicals. Therefore, training activities are required to equip scientists who are not experienced with nanofabrication/nanoscale relevant techniques to deal with such materials. This can be linked with the impact nanotechnologists may have in the introduction of new techniques to have a more efficient characterisation, then fostering the collaboration into new research projects for these specific training activities as well.

Ongoing research projects: Training is a key focus of the NanoIndEx and NANO-HETER projects.

Final recommendations
A Physicochemical Identity (Physicochemical ID) is defined as the dynamic pattern of physical and chemical characteristics (identified using appropriate analytical techniques) associated with one or several specified NMs during their life cycle. The physicochemical descriptors should describe key inherent features of the NMs (i.e. what they are), their influence on biological and environmental fates (i.e. where they go) and their inherent activity (i.e. what they do).

In the short-term (<5 years) it is recommended to provide different stakeholders with sets of standards/reference materials, validated instruments and standard protocols to be applied for their needs, with a tailored approach in order to maximise the cost-effectiveness of physicochemical characterisation. Quantitative standardised approaches, and relative validations, will improve the quality of PC characterisations.

In the mid-term (5-10 years), the availability of standard/reference materials, validated instruments and standard protocols will contribute to effective characterisation of NMs at different life cycle stages including following transformation by various processes and in complex matrices (bio-fluids, soils, etc.). They will also facilitate the development of novel physicochemical endpoints of NMs that could be helpful for unequivocal PC description of the NMs. Novel techniques (and instrumentation) with demonstrated advantages (high throughput and multimetric characteristics) will be another goal to facilitate determination of PC IDs. These instrumentalations will be helpful for analysing PC properties of NMs, possibly linking them to Exposure and Hazard IDs.

In the long-term (10-15 years), standardised protocols should be developed for PC monitoring and characterisation of NMs throughout their life cycles, in complex matrices, and in both in vitro and in vivo models. A further recommendation is to ensure that developed techniques are flexible and integrated (tailored or tiered) to satisfy the specific requirements of all stakeholders (e.g. regulatory authorities, industries and academics).
In the distant future (>15 years), it is recommended that the PC ID should be aimed at providing suitable high quality data for in vitro, in vivo and in silico approaches for exposure assessment and hazard identification. Although challenging, rigorous functional and rational grouping of NMs according to their inherent properties, and robust modelling, should reduce requirements for further assessments to minimal, highly focused Exposure and Hazard ID tests (or even ultimately solely in silico tests).
2 EXPOSURE IDENTITY

Ambition

The ambition is to define research priorities to develop focused parameters that can unequivocally identify the release of, and characterise exposure to NMs in different compartments and via different exposure routes (Exposure ID). This would provide stakeholders with a tailored framework of standard protocols that facilitate development of effective risk assessment and management practices, as well as maximising cost-effectiveness. The Exposure ID system should allow categorisation and grouping/ranking of NMs in relation to Physicochemical and Hazard IDs. Research priorities to meet this challenge are outlined below.

Definition

An Exposure Identity is defined as the pattern of concentrations of one or more NMs in different matrices (air, liquid or solid) and as a function of duration and variability over time during their life cycle. Exposure indicators should be selected according to criteria accounting for exposure routes (human and environmental), and the matrices in which exposure occurs. In risk assessment the Exposure ID critically links the physicochemical ID to the Hazard ID. The latter especially relates to how the characteristics of the organism or environment influences the exposure (e.g. the inhalation rate or depth for human inhalation studies).

Introduction

An Exposure ID could be defined as the pattern of exposure routes associated with NMs. This, combined with the Physicochemical Identity (PC ID) and Hazard Identity (Hazard ID), could be used to group NMs for risk assessment purposes. There is a need to establish how many parameters and dimensions (e.g. time, space, etc.) contribute to the effective concentration/dose of NMs. Key requirements for formulating a Hazard ID system is the development of appropriate testing guidelines or standardised protocols, and equipment allowing robust, efficient estimation of effects related to NM-related properties. To establish future research priorities, we need to fully define requirements for an Exposure ID, which can be divided into a number of broad categories (Figure 2.1):

Processes and factors determining exposure:
• Are available methods sufficiently efficient to identify, quantify and monitor NMs in the various stages of their life cycles?
• In particular, how do we discriminate engineered NMs from background particles? How do we address complex matrices? Can we quantify internal doses?

Monitoring:
• What are the best sampling strategies to identify human and environmental exposure hotspots?
• Can we more fully elucidate processes, emissions and exposure routes to improve monitoring by identifying optimal sampling times and places?

Exposure scenarios:
• Can we model NMs’ environmental fate, bioaccumulation and biodistribution? How can we relate measured concentrations and real exposure?
Future research emphasis and prioritisation

To obtain a rationale for grouping NMs based on their exposure identities, the following aspects should be emphasised, both for pristine and in situ NMs: (i) identification of research (in a broad context) that should be initiated, (ii) standardisation of tools and protocols to ensure that the results are useful in wider ITS and RA contexts, (iii) procedures to ensure that standardised protocols are appropriately and efficiently implemented, (iv) development of high-throughput systems to deliver fast evaluations, and finally (v) the development and improvement of models to extrapolate acquired information across NMs and media/biological endpoints.

As mentioned in the Introduction, the targets of future research must be founded on present knowledge and knowledge gaps. Hence, the future research emphasis has been framed in the context of the identified gaps in current knowledge (see Annexes II and III). The references e.g. “C.1” refer to corresponding points in the Knowledge Gaps and Research Priorities document (Annex II). Each gap is followed by approaches to address it.
Determine release

Robust characterisation of exposure is essential when assessing and managing risks associated with the use of NMs. For example, if there is no exposure even very hazardous NMs will pose no risk (or at most minimal risks), while repeated exposure to weakly hazardous materials can pose high risks, e.g. through non-specific effects. However, exposure assessment has not yet been clearly emphasised in ongoing research, and several issues need substantial consideration and clarification. It is important to stress that developments in the definition of Exposure IDs for NMs will have a direct impact on risk management procedures and safer-by-design approaches. Limiting exposure in processes, or choosing NMs with an intrinsically low likelihood of exposure, will be an important first step towards reducing and mitigating risks in occupational settings, consumer product applications, and environmental compartments where NMs are released during their life cycles or at their final destination. There are pressing needs to link exposure studies with physicochemical characterisation and to identify and prioritise groups of particles with high exposure likelihoods, which thus require most attention. However, to meet these needs further research is required to develop techniques that can accurately identify and quantify the release of NMs during the life cycle of products that contain them. Such techniques should be able to discriminate between engineered NMs and background particles, and to identify improved sampling strategies in order to produce relevant data for both research and regulation.

Standard protocols to quantify NMs in different matrices/techniques to discriminate from background

Identified gaps: Research should identify suitable methods for the detection, characterisation and extraction of NMs in complex matrices. Hence, there is a need for further optimisation and development of analytical methods (C.4).

Future research emphasis

In order to tackle these issues there is still a critical need to develop appropriate reference methods and protocols for exposure assessment, particularly for addressing complex mixtures of NMs and matrices. While several attempts have been made to establish such protocols for analysing particles dispersed in aerosols, there is a lack of techniques and protocols for analysing NMs in water, soil, sediment, biota and other solid matrices. More specifically, key requirements are to develop and validate quantitative on-line, hyphenated techniques for measuring NMs in aquatic matrices using appropriate separation devices coupled to sensitive detection systems, such as mass spectrometers (see also the Physicochemical ID chapter). Thus, for instance (as mentioned in the Physicochemical ID chapter), several groups are developing procedures for on-line coupling of Asymmetric Flow Field Flow Fractionation with Inductively coupled plasma mass spectrometry (ICP-MS), which can also be operated in a single-particle mode. Inter-laboratory comparisons should also be encouraged for the development of common protocols that can be used in human exposure assessment (particularly exposure to NMs in mixed matrices, e.g. in the food and cosmetics sectors) and the environmental detection of NMs.

To ensure the quality of results appropriate visualisation techniques should be developed, in parallel with the separation and detection techniques. Notably, electron microscopy could be used to investigate the agglomeration and aggregation of the materials, which should be clearly reported after the assays and contribute to the choice of dispersion protocols for toxicology and ecotoxicology studies. Such techniques are also crucial for evaluating fibrous NMs, for instance carbon nanotubes and nanocellulose, which are difficult to separate with size-based devices due to their high aspect ratios. Moreover, the use of techniques such as radiolabelling, neutron activation and isotope labelling, which allow quantification of
elements at very low concentrations, can be highly valuable in the development of reference methods for purposes such as monitoring changes in NMs’ states in complex media, providing NM-independent controls of the results.

Standard and reference materials

A key requirement for the development of techniques such as those mentioned above, and robust experimental verification of the results, is the availability of appropriate controls based on standard materials, since results of methods that separate particles according to their size or hydrodynamic diameter might be affected by both sample preparation procedures and agglomeration/aggregation phenomena in relevant experimental conditions. Addressing such limitations is even more critical for definitions of NMs published by the European Commission, in which the separation and particle number concentration of the individual components of a complex mixture are identifying NMs from a regulatory perspective. Standard NMs are crucial for rigorous validation of monitoring methods and evaluation of their applicability to different classes of NMs. For instance, labelled standards are essential for the development of techniques to track NMs within an organism or exposure medium, e.g. radiolabelled, neutron activated, or stable isotope-labelled materials can be used to assess the biodistribution or environmental fate of NMs, especially those composed of highly abundant materials.

Ongoing research projects: Several projects (Nanovalid, Scaff, ERANET-SINN/NANOHETER and NanoReg) will identify suitable methods for detecting and characterising NMs, and extracting them from complex matrices.

Education

Identified gaps: Research should develop practical handling guidelines and standards to train workers for activities involving NMs in the workplace (D.2).

Future research emphasis

In recent months, several guidelines have been developed to provide workers and occupational health and safety managers a means to handle NMs safely in research, development and production environments. For instance, Italy’s National Workers Compensation Authority (INAIL) has recently published guidelines entitled White Book – Exposure to engineered NMs and occupational health and safety effects, while during a workshop held in Berlin in November 2012, the German Federal Institute for Occupational Safety and Health (BAuA) launched guidelines called “Safe handling of NMs at workplaces – Practical Guidance for the Safe Use of NMs”, developed in the framework of the FP7 Project NanoValid. Risk management tools for occupational health and safety concerning NMs have also been released in Holland (Stoffenmanager Nano) and Denmark (NanoRiskCat), among other countries. In addition, the International Organization for Standardization (ISO) Technical Committee (TC) 229 Nanotechnologies has adopted use of the control-banding approach as a work item and is currently editing a technical specification on this topic. However, while these tools provide guidelines and risk mitigation strategies for NMs in the workplace, they share some limitations because the data that drive the application of risk reduction practices in several cases are not nano-specific and several measures are often based on a precautionary principle. Therefore, such guidelines need to be sufficiently flexible to allow appropriate adaptation in the light of new information that emerges from research.

Ongoing research projects: Several projects (Nanovalid, Scaffold, NanoREG, NanolndEX) emphasise the importance of developing practical handling guidelines and training workers for activities involving NMs in the workplace.
Short-term priorities (achievable in 5 years)

Exposure through NM life cycle stages, including end of life

Identified gaps: Research should focus on developing technologies and methodologies that can accurately identify NMs, monitor, quantify and measure their concentrations (number and/or mass) and physicochemical properties at various stages of their life-cycle (manufacture, use and disposal) [C.1].

Future research emphasis

Both the biological and environmental fate of NMs are strongly affected by some of their physicochemical characteristics, as illustrated for instance in Figure 2.2. Such characteristics may thus determine whether an NM is likely to cause adverse exposure effects to humans or the environment (or both). In addition, such characteristics may change during the life cycles of the materials, thus exposures may vary during their life cycles (Figure 2.2). Thus, there is a need for appropriate LCA (Life Cycle Assessment) and LCIA (Life Cycle Impact Assessment) procedures. Life cycle assessment procedures are incorporated in the ISO 14000 environmental management standards. Although various available tools for LCA have been applied to NMs in some projects, several aspects need to be improved, notably more detailed empirical data on release factors during their use phase are required and clarification of release scenarios at their end-of-life, during incineration, recycling, or disposal in landfills. These aspects must be treated as fundamental priorities, partly because of the strong and rising societal demands for sustainable waste disposal and management. Standard protocols are available for simulating disposal conditions, including incineration, composting and recycling. However, their applicability to NMs still has to be assessed and validated, and this must be considered a key long-term priority, when the NMs entering the market in future years will also need to be disposed of.

Therefore, both cradle-to-gate and cradle-to-grave approaches have to be applied to quantify all relevant inputs (e.g. material or energy) and outputs (e.g. emissions). The possibility and scope for defining similar life cycle behaviours across groups of NMs and products that include them in terms of physicochemical characteristics must also be assessed, for example: (i) groups that lead to the formation of dusts devoid of NMs during the incineration phase, (ii) groups of NMs that inhibit the potential recycling of products they are added to, and (iii) groups whose fabrication/inclusion of NMs lead to an increase or decrease of emissions in the manufacturing phase.

Ongoing research projects: Several projects addressing these issues (NanoValid, Scaffold, NanoSolution) are focused on developing technologies and methodologies that can accurately identify NMs, monitor, quantify and measure their concentrations (number and/or mass) and physicochemical properties at various stages of their life cycle (manufacture, use and disposal).

Exposure assessment priorities

Identified gaps: Research should focus on improving understanding of emissions, exposure routes and consequent risks (see C.3).

Future research emphasis

Ideally, elucidation of the Physicochemical ID of NMs during their life cycle, using knowledge of the processes involved (including their fabrication, use and end-of-life treatment) will enable robust assessment of their potential release, which is a prerequisite for exposure (Figure 2.2).
It would then be possible to formulate a prioritised list of exposure potential (and hence risk) by simply considering the processes NMs have been subjected to and their life cycle phase with no testing, which would be much quicker and cheaper for industrial users. These key exposure parameters (including duration of exposure, particle size distribution, elemental composition, particle surface area, mass and particle number) would also be valuable for assessing hazards associated with NMs at various life cycle stages, as illustrated in Figures 2.3 and 2.4.

**Ongoing research projects:** Several projects (Nanovalid, Scaffold, ERA-NETT/SINN) are focused on methods to improve understanding of emissions, exposure routes and consequent risks (see C.3).

**Monitoring strategies in occupational settings, to link workplace concentrations to real exposure**

Identified gaps: Research should focus on determining whether existing exposure assessment models are appropriate for NMs (C.2.), and how workplace air concentrations and personal exposure are related. Such techniques should consider near-field and far-field exposure modelling due to the rapid dilution and scavenging of small particles in air (D.1).

**Future research emphasis**

Current human exposure is probably principally related to occupational exposure, which essentially follows the inhalation route. Therefore, materials leading to a high likelihood of inhalation exposure should be considered of highest priority for
Figure 2.3. A flow diagram to aid prioritisation of hazard assessment according to human exposure routes.

Figure 2.4. A flow diagram to aid prioritisation of hazard assessment according to environmental exposure routes.
hazard testing (which is why hazard research has focused heavily on pulmonary models – Annex II). Within this framework, the importance of developing sampling strategies to determine relationships (models) between workplace concentrations and real personal exposure must also be stressed. For example, such models can be derived by integrating data obtained by personal exposure monitoring devices (including the timeframe of activities) and relate them to the NMs’ distribution within occupational sites, which can facilitate the formulation of appropriate risk management practices.

Ongoing research projects: Several projects (Nanovalid, NanoReg, Nanoindex) are assessing whether existing exposure assessment models are appropriate and relationships between workplace air concentrations and personal exposure.

Strategies for sampling NMs in sediments and soils

A key phenomenon that is exploited in nanotechnology, but raises several risks, is that properties of materials change as their dimensions diminish to the nanometre (hereafter NMs) range.

Innovative instrumentation

The development of innovative sensing techniques may be essential for HTP monitoring of environmental exposure to NMs. In detail, sensing platforms based on label-free detection provide cost-effective means to determine the presence of NM components in complex matrices. Such tools include, for instance, electrochemical sensing platforms and surface plasmon resonance instruments, systems that can be further enhanced by nanofabrication techniques. These techniques provide fast, reliable, flexible and quantitative measurements of the components of NMs, and thus could be valuable for screening prior to further investigation using more accurate, but more expensive and time-consuming techniques. The integrated involvement of scientists engaged in enhancing such systems in the development of innovative exposure monitoring techniques for EHS purposes should be encouraged in the future.

Innovative imaging techniques could be applied in the high content definition of internal doses in organisms, see the Distant future, Multimetric detection methods section for further details.

The mentioned techniques are clear examples (far from exhaustive) of ways in which nanosafety research may benefit from competences in nanobiotechnology and nanofabrication, integration of which in future basic research should be supported. They also provide a means for commercial exploitation and transfer of new knowledge to the anticipated growing market of nanosafety research that may be achieved through the upcoming regulation(s).

Ongoing research projects: Several research projects (Nanovalid, NanoReg, ERA-NET SINN/NANOETHER) are focused on aspects of environmental NM exposure, including identification and assessment of exposure routes, development of analytical methods to measure and verify concentrations of NMs, characterisation of released NMs and development of validated models for characterising the environmental fates of NMs. Several projects (Nanovalid, Nanosolution, ERA-NET SINN/NANOETHER) are also focused on improving understanding of hazards associated with nanoparticle dispersion in environmentally relevant media.
Mid-term priorities (achievable in 5-10 years)

Sampling strategies to assess exposure via ingestion

In 2011, the European Food Safety Agency (EFSA) published guidelines called Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain [2], following the increasing use of NMs in the food industry, which has also led to the publication of specific regulations for food contact materials [3], and the introduction of nano-specific requirements (labelling) in the new draft of the Food information to consumers regulation [4], due to come into force in 2014. Concerning NMs in food, identification of the correct exposure scenario according to the intended use is a crucial step, and must be accompanied by the identification and characterisation of NMs before they are added to food, in the food matrix, and during their passage through the gastrointestinal tract. For food contact materials containing NMs that could be released after exposure via ingestion, sampling protocols should be developed that allow the detection of NMs in the various food simulants used in regulatory risk assessments. In addition, it is even more important in such cases to obtain accurate, precise and sensitive quantifications, for both identification of threshold levels and understanding the applicability of the functional barrier concept.

Strategies for sampling NMs in organisms

Organisms comprise one of five compartments identified in MacKay fugacity models [5]. To ensure the applicability of such models to NMs, we need to identify how animals and plants adsorb, accumulate, transform and excrete NMs to build accurate environmental fate models. For these purposes, it is important to establish direct links between exposure and ecotoxicology information. In addition, sampling strategies to elucidate the transfer of NMs in the food chain require further development, since differences in rates of metabolism of NMs in different organisms may lead to variations in their accumulation, and hence toxicity.

Development of exposure scenarios

Identified gaps: Research should focus on determining whether existing exposure assessment models are appropriate for NMs (C.2).

Future research emphasis

A key horizontal issue in determining exposure scenarios is the need to integrate the expertise of industrial technologists, material scientists and experts in NM fabrication and synthesis processes to generate realistic data and scenarios. Indeed, crucial requirements for robust exposure assessment include the identification of where and when exposures are likely to occur (and thus should be assessed) throughout the life cycle of NMs. Furthermore, the integration of static monitoring tools with the use of personal, portable devices is of paramount importance for identifying whether (and if so how) air concentrations are related to personal exposure and then refining the monitoring process. This process could lead to a virtuous cycle, in which experts apply the new knowledge in safer-by-design approaches (using their experience in the development of lower-risk processes), involving pre-manufacturing risk assessments to determine whether or not exposure is a realistic concern. Further development is also required to address ontology-related issues, in particular appropriate description of the presence of aggregates, agglomerates, and bundles of both high aspect ratio and granular NMs.
Linking exposure to internal dose

Size and charge parameters are considered to be the main determinants of the extent of biological exposure. Measuring the fraction of intracellular NMs should be useful for elucidating the real dose (exposure) responsible for a certain toxic effect. However, current methods for measuring internal fractions of NMs often yield over estimates, due to the NMs “sticking” to the outer sides of cellular membranes. Thus, we need to implement effective methods to measure the NMs taken up by cells to relate their effects to doses, but this requires the resolution of several issues (which should be a short-term priority).

In realistic circumstances, NMs are typically coated by various biomolecules present in the surrounding environment when they interact with exposed cells. Hence, interfacial physicochemical properties of NMs and the specific chemical physical properties of the environment are key determinants of both NM adhesion to cells and subsequent internalisation. Accurate quantification of the effective amounts of NMs taken up by cells/organs/aquatic or soil organisms is essential for correlating their physicochemical characteristics, exposure and toxicity profiles. Moreover, knowledge of the internal doses will allow comparison of dose/response curves among different systems (in vitro and in vivo models).

To acquire the required information, it will be essential to:
• Develop robust techniques and protocols for characterising NM’s structural physicochemical characteristics (e.g. size, shape, surface charge, chemical composition) during all stages of their life cycles, and monitoring the dynamic changes;
• Determine their interfacial properties and how they evolve during their life cycles in relation (inter alia) to ionic strength, and contents of proteins, lipids and other molecules in biological fluids, aquatic and soil environments. The temporal evolution of some NM properties may strongly impact cellular internalisation. For example, the smallest NMs are generally taken up at the highest rates, and aggregation phenomena may dramatically reduce NM internalisation. However, increases in size may increase their specific surface reactivity. Changes in surface charge (due to the presence of a biomolecule layer) may also influence the adhesion of NM to cells and, hence, the degree of NM cellular internalisation (which is known to be mainly driven by endocytotic processes). Moreover, interactions between NMs and molecules may activate specific membrane cellular epitopes or damage proteins with consequent activation of adverse downstream cellular events;
• Improve analytical techniques. The most widely used techniques currently available for quantifying internal amounts of NMs in cells are fluorescence-microscopy, ICP-AES/MS and flow cytometry. However, these techniques may sometimes over estimate NM levels due to NMs adhering to the cells’ external membranes. Recently, the possibility of manipulating experimental conditions by (for instance) using low temperatures, agents that inhibit endocytosis or etchant molecules to overcome this problem has been explored, but not entirely successfully, due to the intrinsic toxicity of the treatments and/or creation of experimental artefacts. Another issue is the discrimination between NM and ions when measuring NMs’ bioaccumulation in different organs and tissues. To address this issue, the recommendation is to use hyphenated techniques that allow visualisation of NMs as well as analysis of their chemical contents (e.g. cryo-transmission electron microscopy and energy-dispersive X-ray spectroscopy).

Ongoing research projects: Several research projects (NanoReg, ERA-NET -SINN/ NANOHETER) are addressing whether existing exposure assessment models are appropriate for NMs.
Long-term priorities (achievable in 10-15 years)

Model bioaccumulation, biodistribution, and environmental fate

Identified gaps: Research should focus on improving understanding of NM emissions, exposure routes and consequent risks (see C.3).

Future research emphasis

With respect to the prioritisation of hazard testing, it is of paramount importance to identify ways to group NMs according to their exposure routes (including cross-matrix exposure) throughout their life cycles. This is essential because the optimal ways to systematically measure (and identify) exposure may vary during their life cycles.

In addition, there is a need to identify a set of physicochemical characteristics that may significantly interfere with determinations of effective internal doses (structural, morphological, and those related to interfacial interactions with the exposed media).

Sampling protocols for realistic exposures are also crucial requirements, notably the identification and modelling of exposure scenarios (for research and regulatory purposes), which will lead to the identification of where and when sampling is needed, and the required sampling duration. This will also facilitate prioritisation for hazard testing.

Sampling strategies for dermal exposure

Dermal exposure is considered a lower priority. This is not due to a lower likelihood of exposure per se, which is significant for instance in occupational settings, but because recent evidence shows that healthy human skin is an effective barrier for NMs (see Hazard ID). However, information about the penetration through hair follicles and damaged skin is scarce and further investigation of hand-to-mouth transfer is required.

Sampling in air and water (environment)

The environmental release of NMs into both air and water bodies is also considered a lower priority, due to the extreme dilution NMs will have in such media. However, strategies for sampling them in these environmental matrices have to
be developed to investigate how NMs released in them contribute to the development of concentration hotspots in soils and sediments. Targeted monitoring strategies should also address major releases of NMs in industrial accidents.

**Ongoing research projects:** Several projects (Nanovalid, Scaffold, ERA-NET-T/SINN) are exploring methods to improve understanding of emissions, exposure routes and consequent risks (see C.3).

**Distant future priorities (achievable in >15 years)**

**Multimetric detection methods**

Innovative imaging techniques could be applied in the high content definition of internal doses in organisms. For instance, imaging mass spectrometry, which combines the acquisition of mass spectra with localisation of sources of the spectra within the samples, can be applied with high-resolution microscopy to juxtapose the spectra within the images. Hence, these techniques provide complementary information about the relative abundance of specific molecules and NMs in specific cellular compartments, at resolutions that can reach the low nanometre range. Plasmonic resonances of noble metal-NMs (which are within the visible and near infrared spectrum for spherical nanoparticles) may also be used in the localisation of such NMs in cells examined by confocal microscopy. This affords the possibility of tracking their dynamic uptake by cells and obtaining information that is strictly related to their chemical nature.
Final recommendations

Of paramount importance is methodological standardisation, which should include use of standard or reference materials for discriminating NMs from background particles in complex matrices, in all NM life cycle stages.

In the short-term (<5 years), for assessing human exposure particular attention should focus on inhalation in occupational settings and ingestion, with the aim of defining in parallel internal doses in cells. For assessing environmental exposure, attention should focus on identifying long-term accumulation and concentration hotspots, prioritising soils and sediments, which seem to be major accumulation sites. In both cases development and implementation of standardised protocols should be a continuous priority.

In the mid-term (5-10 years), concentrations of NMs in different matrices should be linked to actual exposures of cells and organisms. In addition, sampling strategies to characterise in detail mid-priority environmental compartments, for example biota, should be developed.

In the long-term (10-15 years), data should be available for lower priority exposure routes, such as dermal exposure, and environmental compartments, such as air and water. This information will enable grouping approaches based on matrices, exposure routes and/or compartments. In addition there is a requirement to model the exposure, bioaccumulation and fate of NMs in different life cycle stages.

In the distant future (>15 years), the development of standardised protocols for multimetric and innovative detection tools is recommended to enable faster acquisition of pertinent information and hence grouping and modelling.
3 HAZARD IDENTITY

Ambition

The ambition is to define research priorities to enable focused but effective assessment of human and environmental Hazard Identities (Hazard IDs). This would provide stakeholders with a tailored framework of standard protocols that facilitate development of effective risk assessment and management practices, as well as maximising cost-effectiveness. As modelling approaches develop, the testing required will become more focused. The Hazard ID system should allow for categorisation and grouping/ranking of NMs in relation to Physicochemical and Exposure IDs. Prioritised research needs to meet this challenge are outlined below.

Definition

A Hazard Identity is defined as the pattern of biological responses (determined using appropriate combinations of toxicological and ecotoxicological models, tests and endpoints) associated with one or several specified NMs. Human and environmental Hazard IDs are considered in an integrated chapter with the intention to promote collaboration and knowledge exchange between the two disciplines since it is likely that there will be many similarities between the modes of action of NMs underlying the observed toxic effects in different species and the techniques required to study hazard impacts.

Introduction

To establish future research priorities, we need to define what is required for a Hazard ID. The Hazard ID can be divided into a number of components (represented as hexagons in the figures) that can be applied to NMs during any stage of their life cycles and via any exposure route (Figure 3.1). The components making up the Hazard ID include:

• Use of a test model/system appropriate to the exposure route and relevant organism.
• Identification and use of the most relevant dose metric.
• Test concentrations/doses and exposure durations informed by realistic estimates of exposure (as provided by the Exposure ID).
• Determination of the mode of action related to the types of adverse effects that are likely to be induced.
• Assessment of whether any adverse effects may occur either locally at the entry portal, and/or distally.
• Assessment of NM bioavailability.
• Determination of the NMs’ toxicokinetics (absorption, distribution, metabolism, excretion, bioaccumulation).
• Assessment of whether adverse effects are irreversible or reversible.
• Determination of the timeline of induced effects, that is whether they are short-term (acute) or long-term (chronic) at relevant doses/concentrations.
• Assessment of dosimetry (relationship between doses and hazard impacts).
• Determination of target species/processes for the adverse effects (environmental specificity).
• Assessment of population-level effects.
• Assessment of the potential for NM metabolism/modification of their physicochemical characteristics and the subsequent impact on hazardous properties.
• Assessment of the potential for interactions of the NMs with other substances (antagonistic, potentiating or synergistic).
Hazard identification should be conducted in concert with Physicochemical identification (Chapter 1) to allow the development of Grouping and Ranking protocols (Chapter 4) as well as more advanced modelling approaches.

The above components form a comprehensive list of requirements for current generation of a Hazard ID (Figure 3.1), but ideally this list should be streamlined to reduce the burden of hazard testing. This would lead to a list of essential elements for the Hazard ID that could be used in parallel with a minimal set of requirements for a Physicochemical ID (Chapter 1) for more efficient risk assessment.

A key requirement for the Hazard ID system (Figure 3.1) is to relate the exposure and physicochemical identities to the mode(s) of action leading to adverse effects for different NMs. This is also true for chemicals, but the timeframe for chemicals and NMs could be different according to their fate, behaviour and toxicokinetics. This information allows the design of appropriate in vitro HTP assays for large-scale testing and thus generation of modelling approaches for streamlining strategies such as grouping, ranking and modelling approaches.

Factors such as NM life cycle stage and exposure route influence the optimal model for hazard investigation. Most models are constrained by limitations such as species or disease status, thus a thorough understanding of the potential impacts of these limitations or the development of improved models is required.

![Figure 3.1. The diagram identifies the components required to generate a Hazard ID (green hexagons), their integration with the information requirements relating to exposure ID (brown hexagons), physicochemical ID (blue hexagon), grey hexagons represent modelling components that will lead to the ITS. The hexagons with the same colour shade are related. The diagram is intended to start on the left (NM) and finish on the right, but there is no strict order of passage between the hexagons to achieve the final goal. This is one example of how the hexagons could fit together, but the hexagon shape reflects the potential for each component to interact with multiple components, thus the organisation could be shuffled according to the research question addressed and as knowledge accumulates.](www.its-nano.eu)
Formulation of a robust Exposure ID system combined with better understanding of NMs’ mode of action (see below) will also help future identification of the most appropriate dose metrics for NMs in vitro and in vivo. Suggested dose metrics include particle number and surface area, but with the ever-expanding understanding of the relationship of PC and Hazard IDs this is likely to change with time and perhaps become multi-factorial.

Mode of action is intimately related to the physicochemical ID of the NMs (Figure 3.1) and thus should include factors such as relating the hazard response to particle characters. This could include: the roles of soluble versus insoluble components of the NM; effects related to shape, size or crystal structure e.g. high aspect ratio NMs inducing inflammation and fibrosis; direct interactions with biological or environmentally relevant macromolecules and their effects on surface reactivity, fate, behaviour and hence bioavailability, and physical interactions or reactions with other chemicals. This list is not exhaustive, and more information can be found in the physicochemical ID chapter (Chapter 1).

The hazard responses considered within the mode of action should also include biological factors, such as:

- exposure concentration and duration and the relationship between dose/duration and the time course of biological responses (including short-term, long-term, acute, chronic, reversible and irreversible effects),
- route of exposure from all pertinent environmental compartments, e.g. water, soil, sediment, air, and food, as well as entry pathways, i.e. respiratory, oral or dermal,
- toxicokinetics,
- local or distal effects,
- mode of action including
  - cytotoxicity/lethality;
  - sublethal effects such as inflammation, oxidative stress, genotoxicity, growth and reproductive perturbations;
  - sensitivity across the organisms’ life cycle;
  - susceptibility (e.g. according to species, age, disease status or genetic variation).

A key requirement for formulation of a future Hazard ID is the availability of appropriate testing guidelines or standard protocols. For this, validation for target species or human responses is essential. Based on understanding of the mode of action, HTP technical approaches and equipment will allow robust, efficient estimation of the biological responses associated with NM-related properties. To rationalise testing and allow grouping, models should be developed that have thoroughly validated capacity for cross-NM, -species and -media extrapolation/interpolation.

Future research emphasis and prioritisation

The future research emphasis has been framed in the context of the identified gaps in current knowledge (see Annexes II and III). The references e.g. “G.5” refer to corresponding points in the Knowledge Gaps document (Annex II). Due to financial and practical constraints not all of the gaps can be addressed simultaneously, therefore the research ideas emerging from these gaps were prioritised following a large-scale stakeholder consultation (Venice, March 2013), and are presented below in priority order (Hazard-ID Figure 3.2). It is proposed that the topics deemed highest priority should be addressed (or begin to be addressed) in the short term, while lower priority areas should be addressed in the longer term (or if and when they become higher priority according to new research) based on when information is needed. This does not mean that the lower priority areas are less important, rather that they will be easier to address in the longer term when
information delivered by addressing the short-term priorities becomes available. Some work on the longer term goals needs to start now in order to frame the short-term research required.

Each set of gaps is followed by a proposed course to address them. The proposals to address the short-term priority gaps are relatively detailed, due to the greater availability of pertinent information. The proposed research to address longer term issues is less detailed but will become more detailed with time as knowledge is accrued.

**Short-term priorities (achievable in 5-10 years)**

**Dosimetry and dose metrics**

As indicated in Figure 3.1, dosimetry and dose metrics are reliant upon Exposure ID, as reflected in the following gaps.

Identified gaps: Research should address oral and dermal (including compromised and damaged skin) exposures (G5, G6), inter alia identifying the impact of these exposure routes to NMs’ physicochemical characteristics (F7).

**Future research emphasis**

For both human and environmental studies there is a need to determine dose metrics in hazard identification. The mass dose metric most commonly used to characterise hazardous effects of conventional chemicals may not be the optimal dose indicator for NMs. Due to the unique properties sometimes demonstrated by NMs they may have more potent effects than corresponding bulk materials. Other metrics that have been suggested to better describe doses include particle number and surface area. As information accumulates regarding the influence of their physicochemical characteristics on associated hazards other dose metrics could be developed.

Human hazard models: Much of the human hazard research to date has focused on exposure via the pulmonary route. It is now clear that pulmonary exposure to NMs is often more hazardous than inhalation of the corresponding bulk chemicals. Research on pulmonary exposure should therefore focus on elucidating the mode of action in relation to different adverse effects, including cancer, cardiovascular disease, asthma, COPD and developmental perturbations. There is also a need for studies on hazards associated with exposure via ingestion, especially long-term effects. Robust models need to be developed for such studies, which need to include local effects in the gastrointestinal tract, but also uptake (translocation) and distal effects. There have been relatively few studies on dermal exposure, and they have usually focused on uptake through undamaged skin. Therefore, more research is needed using skin disease models with compromised barrier function, such as tape-stripped ex vivo human skin.

NMs are likely to quickly become coated by biological molecules when they are in contact with a living organism. Components of the lung lining fluid, blood and gut rapidly form a corona around the NMs. Effects of the coatings acquired via different exposure routes on uptake and toxicity are still poorly understood.

Environmental hazard models: Current knowledge of NMs’ environmental effects (see Annex II) has been mostly acquired from short-term studies of their effects in aquatic environments. However, current environmental distribution models indicate that the main sinks for NMs are solid media, as NMs fall out from air or liquid media under gravity or simply agglomerate or aggregate with environmental sol-
ids. Hence, species should be tested in the most relevant media according to the environmental emission- and distribution-scenarios, i.e. sediments and soils according to present knowledge. The uptake routes (pulmonary, ingestion and dermal) are also relevant to environmental organisms, although of course non-animal species take up NMs through their surfaces (equivalent to dermal uptake) but not via ingestion or pulmonary routes. There is also a need to study uptake/elimination mechanisms (passive diffusion, cytosis etc.) and mechanisms governing tissue distribution. In addition, NMs will come into contact with exudates from cells and organisms, leading to NM-surface coating/interactions and alterations in their physicochemical characteristics, which are also likely to influence their availability and uptake rates/mechanisms. Thus, effects of the biological and environmental experimental media used in simulations of human or environmental exposures on NMs’ physicochemical characteristics (see Exposure ID) should also be studied. These dispersion media should be as environmentally or physiologically relevant as possible. Variations in these factors will also affect the uptake, translocation and biological responses induced by NMs.

Ongoing research projects: Several projects (e.g. NANOREG, SCAFFOLD, NanoValid) will focus on oral exposure, but dermal exposure remains poorly addressed.
The impact of exposure routes on NMs’ physicochemical characteristics is being extensively studied (e.g, NaNoREG, NanoSolutions, NanoValid). The NanoValid and MARINA projects, and to a lesser extent NANO REG, are acquiring some of the required information on the distributions and effects of NMs in different environmental compartments.

Mode of Action

Identified gaps: Determining NMs’ mode of action is essential (F.1). This should start from a better understanding of how key physicochemical characteristics (F.6, G.4), and not only the size of NMs (H.3), are associated with hazards. In addition, sub-lethal effects have to be characterised (H.4/5).

Future research emphasis

To aid identification of physicochemical characteristics that may be responsible for toxicity, where possible research should focus on NMs that have been manipulated in controlled ways, modifying one character at a time (e.g. size). However, technical modifications in one characteristic often alter other characteristics, making panels of NMs currently difficult to compose. The standard or reference materials proposed in the Physicochemical ID chapter will be useful in this respect.

Understanding the underlying mode of action (MoA) of observed toxic effects and relating in vivo responses to in vitro responses (see next section) is essential to achieve these goals because knowledge of MoA will allow identification of appropriate models and endpoints for HTP screening, as well as providing the data input required to generate grouping/ranking schemes and in silico models. Such responses may also serve as NM-related biomarkers for at least two purposes: (i) they can be used to identify relevant endpoints for HTP screening if they are an indicator of a detrimental response, (ii) alternatively, biomarkers of exposure can be used as an alternative to measuring exposure directly, providing that the biomarker is sufficiently specific.

Due to the relatively recent emergence of nano-ecotoxicology the range of endpoints, mechanisms, and species investigated in environmental models are relatively limited compared to human hazard models. Consequently there is limited understanding of NM uptake and mode of action. Hence, there is a need to study uptake mechanisms such as passive diffusion, phagocytosis etc., and mechanisms governing tissue distribution and elimination. Sub-lethal nano-relevant biomarkers that indicate population effects have been used in environmental studies, but these require further development, including comparisons across different organisms/models. For both human and environmental studies, analysis of omic responses (phenomic and epigenomic to metabolomic) will provide mechanistic knowledge with wide applicability. This implementation of omics should be pursued further.

In the short term, Hazard identification will need to include both in vitro methods that have been thoroughly validated for the intended uses and in vivo dose-response studies. How the mode of action relates to the Hazard ID is summarised in Figure 3.1.

Several components of the Physicochemical ID should be linked to the Hazard ID. Apart from environmental studies on effects of size, there have been very few published investigations of the potential contributions of NMs’ physicochemical properties to biological hazards. Knowledge of correlations between physicochemical properties and (eco)toxicological effects must be improved to support grouping of the NMs and enhance in silico approaches to risk assessment and decision-making.
Ongoing research projects: Determining NMs’ mode of action is crucial and a substantial focus of several projects (nanOxiMet, NanoSolutions, NanoValid). To our knowledge, only the NanoSolutions project includes specific efforts to improve understanding of associations between specific physicochemical characteristics and hazards. Sub-lethal environmental effects still seem to be poorly addressed.

Identifying relevant in vitro and in vivo models for future analyses

Identified gaps: Validated test systems, species and endpoints need to be developed (F.3, F.8, H.2), and the most appropriate dispersion protocols for risk assessment should be determined (F.8). Better interpretation of data is needed, both for in vitro-in vivo extrapolation and to differentiate between statistical and biological significance.

Future research emphasis

The in vivo and in vitro models chosen for hazard studies will depend upon the questions to be addressed, such as the exposure route, NM form, and endpoint of investigation. The following section outlines models that exist, but potentially need modification/extension, as well as gaps in model availability. The models, when combined, must be capable of covering all relevant biological life stages as well as NM life cycles, allowing hazard assessments to be carried out at all life cycle stages that represent realistic exposure scenarios.

In vivo human hazard studies: For risk assessment and regulatory purposes it is essential to have access to standard or validated in vivo methods and models. However, different methods may be required, depending on the focal type of NM. Such validated or standard methods need to cover diverse endpoints, including reproductive/developmental toxicity. In many cases in vivo methods have not been rigorously validated for reproducibility, reliability, or relevance to human health effects (for either NMs or traditional chemicals). Analysis of amassed historical animal data (for chemicals, particles and NM) should be conducted to re-assess currently accepted in vivo methods for their ability to predict human health effects.

For risk assessment of an inhaled NM, testing by inhalation in rodents, either by nose only or whole body exposure, is probably accepted as the most biologically representative type of study design currently in use (especially nose only). However, such testing is expensive, technically difficult and raises animal welfare concerns. Instillation in rodent models is a well-used alternative, and can provide useful information providing that controls are used to allow benchmarking of particle responses relative to each other. The dose response data generated from instillation models should be viewed with caution, as instillation differs markedly from inhalation in dose rate, deposition pattern, and format (wet suspension rather than dry cloud). This problem might be circumvented by better modelling and benchmarking of the data. However, because of the uncertainty about NM dose rates and formats, instillation may currently be better for hazard assessment and ranking of material than for quantitative RA. More research is required to allow such data to be used for RA and perhaps for the technique to be validated.

Compared to pulmonary exposure, much less is known about gastrointestinal or systemic responses to ingested NMs. Animal models are available for ingestion studies, including ingestion via food, water, or gavage. However, use of gavage should be considered with caution (in much the same way as instillation, as described above), and further work is required to establish appropriate protocols for NM delivery via these methods leading to their validation.
In vivo environmental studies: There is still a need to assess the suitability of current in vivo hazard test methods and to evaluate alternative (currently non-standard) biologically relevant endpoints. Examples of alternative endpoints include behavioural changes, respiratory stress and endpoints describing mechanistic effects, which can often be determined more quickly than endpoints used in current tests.

The most relevant species in regard to NM hazards should be identified for specific, realistic exposure scenarios and guidelines/standard protocols should be recommended. However, more information is needed to determine the relevance of species’ susceptibility when making species choices. Further, investigation is required as to whether different models or refinement of existing models are needed for improved ecotoxicity testing of NMs. This includes development of knowledge regarding the scope for extrapolating observed effects from one species (or group of species) to another, so information obtained from earthworm or fish studies, for example can be used with known confidence to classify, group or predict an NM’s biological activity in other organisms (see also Chapter 4). Finally, specific environmental model species should be considered as alternative biological models that are relevant to human health. Such models are already used, e.g., Drosophila (genetics), microbes (Ames test), zebrafish (cardiovascular disease). These models provide alternatives to rodent testing, although they still require animals in some cases.

In vitro human and environment hazard studies: As for the in vivo methods, it is essential to standardise or demonstrate the validity of in vitro human hazard models and methods for RA and regulatory purposes. In vitro hazard testing in physiological media requires the development of relevant dispersion protocols. The suitability of existing in vitro methods must be determined (and their validity demonstrated) to ensure in vitro tests provide robust predictions of human and environmental toxicity.

For hazard screening and uptake studies, in vitro models can be divided according to their complexity, which varies from simple single cell to multi-tissue types. A wide array of models are already available (mostly related to human health), but many of them are relatively simple. Further testing of existing models and the development of new models is required to ensure availability of suitable, reliable models that adequately cover all target systems in the body. Individual models might be more appropriate for some exposure scenarios than others. This requires further investigation, along with dispersion protocols appropriate to the route of exposure.

Simple single cell types - recent results from the European FP7 projects ENPRA and InLiveTox suggest that hepatocyte cell lines exposed to serum-dispersed NMs provide good predictions of hepatic responses (cytokine gene expression and oxidative stress) following intravenous (i.v.) exposure; they are relatively good at predicting hepatic responses following intratracheal exposure, but poor at predicting hepatic responses following gavage exposure. More work is required to verify these observations, as (if confirmed) these results would allow better identification and justification of relevant in vitro models for specific exposure and hazard scenarios.

Co-cultures of organ-specific cells and inflammatory cells such as macrophages might be more useful for mimicking organs in which inflammation plays a key role in defence responses to particles/pathogens (e.g., lung), but less important in organs where macrophages exhibit immune tolerance rather than inducing inflammation (e.g., liver and gastrointestinal tract). A number of co-culture and 3D models for the lung and gastrointestinal tract have been developed, but they have not yet been standardised or validated and thus require more investigation. Complex 3D models and fluidic/organ on a chip/multi-tissue/ex vivo models are discussed in the mid-term priorities section.
An alternative way to apply in vitro systems is to use them to screen models to identify susceptible organisms and exposure routes, which could then be examined in more detail using standardised in vivo test procedures. This is more likely to be a short-term use of in vitro tests, while using in vitro models as replacements for in vivo testing is a longer term goal.

Finally, interpolation/extrapolation and read-across can occur at many levels and is explored for NMs in Chapter 5. However, it would also be useful to consider the scope for using these approaches to apply hazard data across species, and between in vivo and in vitro models.

In vitro-in vivo comparison: A few studies have compared in vitro and in vivo responses, but only for relatively few NMs and a limited range of endpoints. Much more work is required to develop these models sufficiently, and generate sufficient data, to identify where and when these models can be usefully and validly applied (see In silico).

In order to validate in vitro systems there is a need for in vitro-in vivo extrapolation (IVIVE), i.e. toxicokinetic simulation using in vitro data, such as data on physico-chemical characteristics and clearance rates. Physiologically-based pharmacor toxicokinetic (PBPK) models mathematically describe absorption, distribution, metabolism and elimination (ADME). Substantial progress has been made towards creating PBPK models for preclinical assessment of drugs via different exposure routes. This is also described in long-term priorities (F.4.High-throughput/ High Content Screening).

Among humans, inter-person variability is observed in absorption, distribution, metabolism and elimination and PBPK models allow the introduction of anatomical and physiological characteristics to build a virtual population of patients. Consequently, the approach can be used to identify sub-populations with specific risk factors and predict interactions between materials/chemicals or genetic variation. There is now a need to develop these in silico approaches to accommodate nano-specific characteristics (e.g. NM size, surface charge/functionality etc.) alongside conventional molecular descriptors (e.g. MW, pKa, Log P etc.) to facilitate interpretation of in vitro data and bridge the findings to the human situation.

General comments: The lack of proven validity and standardisation of the methodology used in hazard testing of NMs is partly due to the lack of well characterised and harmonised controls/test items. Reference or standard NMs (e.g. OECD materials) should be developed that are suitable for benchmarking, and representative of the variation in physical and chemical composition of available NMs. Some NMs of this type are now available, but whether they are sufficient and the most suitable contexts for their use remain to be determined. The use of such reference or standard materials as controls will improve the ability to benchmark hazards of NMs and to make cross-study comparisons.

The lack of standardised methodology includes a lack of clarity regarding the best methods for dispersing NMs prior to hazard tests. However, the development of a universal method for dispersing NMs is unlikely due to the wide variations in their physicochemical characteristics, matrices and exposure scenarios/routes. Instead, in the future a matrix or decision tree of dispersion options will be required to guide researchers/hazard assessors towards the most appropriate protocol(s). Protocols may not necessarily be aimed at generating mono-dispersed suspensions. In many situations, they may be more relevant to mimic realistic situations with aggregated or agglomerated particles. Most published studies have applied a single exposure, so for all models future studies may need to incorporate repeated expo-
sures. Specific criteria for distinguishing which statistically significant responses are biologically relevant need to be considered, including their statistical power and toxicological significance.

The dispersion issues are also relevant for single organism tests and environmental mesocosm-models. Such tests are currently difficult to control and monitor, making it difficult to model the results. Therefore, there is an urgent need to improve understanding of dispersion characteristics and behaviour in environmentally relevant media. This will enable more detailed interpretation of controlled single organism studies (and in the longer term the results of the more complex mesocosm studies). There is also a need to develop dispersion procedures for studies of the fate and behaviour of NMs.

The need for dispersion methodologies also links to the need for better characterisation of doses, e.g. to take into consideration settling rates determined by particle size, density and other factors such as medium viscosity and time. Although these details are likely to change the absolute toxicity (e.g. LC50) values calculated from results of such assays, it is not yet clear whether they would sufficiently modify the conclusions drawn to warrant the additional resources required for more comprehensive tests. Again, this needs more investigation.

A better understanding of exposure doses in laboratory studies may also link to improved methods to measure and express environmental exposure, including repeated exposure. When combined, this knowledge will be useful for all hazard studies to identify “realistic” (or “realistic worst case”) dosing for relevant real-life scenarios to allow more appropriate and accurate extrapolation of risks.

Various models (e.g. in vivo, in vitro, QNAR etc.) can be developed and validated using a predefined panel of standard NMs with agreed and well-characterised positive and negative controls and outcomes. As stated previously, we cannot test the hazards of all NMs, nor can we use all possible methods to test each NM, instead, in the future there is a need to develop a specific and tailored toxicological testing strategy to use for different “kinds/groups” of NMs. Decision trees would again be useful to guide researchers to identify the most appropriate testing strategy for each NM group.

Ongoing research projects: This area of research is a key focus of several large projects, including NanoValid, NanoSolutions, and NANoREG.

Bioavailability, toxicokinetics and bioaccumulation

Identified gaps: The mode of action of a wider range of NMs should be studied in different environmental compartments, including sediments, soils, and the marine environment (H1, H7, H8). There is also still a major need to investigate their behaviour in complex matrices.

Future research emphasis

NMs' different stages of use: Very little is currently understood about how the toxicity of NMs is affected when they are parts of complex matrices, e.g. paint, toothpaste, plastics, soils, sediments etc. However, the limited literature available suggests that their toxicity changes dramatically during different stages of the use of engineered NMs.

Environmental hazard studies: Very little is currently known about the bioavailability of NMs to environmental species (see Exposure ID), mainly because actual exposure and internal dose measurements are difficult to acquire in the field and
knowledge of the general pathways of NMs’ interactions with organisms is poor. Hence, more research is required to link NMs’ actual exposure with the direct biological responses.

Very few bioaccumulation studies have been performed, partly because quantification of NMs’ bioaccumulation is not generally currently possible. In addition, current bioaccumulation standard protocols are not valid since they rely on an equilibrium being reached. Hence, appropriate methods for measuring bioaccumulation following NM exposures should be developed, and when technically possible used to determine relationships between NM’s bioaccumulation potential and characteristics/properties. Further, there is little indication of how actual uptake occurs, e.g. via the gut or outer surface, and the surface characters that influence uptake.

Human hazard studies: Understanding toxicokinetics is crucial for determining internal doses and the likely systemic effects of NM exposure in human tissues/cells. There is a reasonable amount of published research in this area, particularly in relation to toxicokinetics and systemic effects following pulmonary exposure (see section 4.1.1.2 of the Knowledge Gaps and Research Priorities document, Annex II). However, toxicokinetics following other exposure routes is generally lacking, and understanding this aspect of NM fate and behaviour is essential for determining biologically relevant doses in various cell/tissue types within the body. Determining the final destination of the NMs as well as the likely composition of the NMs and any resulting metabolites is a key step in identifying their potential toxicity.

Ongoing research projects: The necessity to address different environmental compartments is a priority area of the NanoValid project, and to a lesser extent NA-NoREG. Complex matrices are substantial parts of the focus of both NA-NoREG and NANOHETER.

Mid-term priorities (achievable in 5-10 years)

Identifying relevant long term in vitro and in vivo models

Identified gaps: There is a need for research into the mid to long-term effects as well as systemic effects of NMs (G.2).

Future research emphasis

A range of standardised and validated in vivo methods and models may be required, depending on the type of NM. Such methods need to cover long-term or chronic effects, reversible effects and repeated dose exposures.

In vivo studies on human hazards: For all exposure routes for human hazard studies, existing data generally concern “early stage” effects (e.g. cytotoxicity, inflammation) and studies on mid to long-term effects, such as fibrosis, carcinogenicity and reproduction, are less frequent. Systemic effects of inhaled NMs on the cardiovascular system have been frequently studied, but comparatively few studies have been carried out on other target organs/systems.

In vivo studies on environmental hazards: There is also a focus on the rather shorter term effects, with little focus on full organism life cycle testing and effects of long-term continuous or repeated exposure regimes, including for instance epigenetic responses and biomagnification.

Ongoing research projects: This area of research is a mid-priority focus of the NanoValid project.
Reliable in vitro and in vivo biomarkers

Identified gaps: the identification of a sub-group of relevant biomarkers is needed. Especially in ecotoxicology where there is a lack of validated biomarkers (F.9).

Future research emphasis

In vitro studies on human hazards: Identification of a minimum set of relevant in vitro biomarkers will help to focus and reduce the burden of hazard testing and allow identification of useful endpoints for use in HTP screens. Modelling approaches will be necessary to identify the most predictive hazard markers, especially the most predictive in vitro markers.

Ecotoxicity biomarkers: Whilst there are currently few nano-specific biomarkers for ecotoxicity testing, established human toxicity endpoints are commonly used, such as DNA strand breaks, oxidative stress assays, etc., that have been adapted and optimised for use in marine invertebrates and fish. However, there is an immediate need to develop tests that improve our understanding of biokinetic effects of body fluids with widely differing composition and metabolic states.

Ongoing research projects: The identification of validated biomarkers, including ecotoxicological markers, is a substantial focus of NanoValid.

More relevant/multi-tissue in vitro models

Identified gaps: Validated test systems, both in vivo and in vitro, need to be developed (F.3), and in vitro-in vivo extrapolations have to be implemented (F.4). Strategies to reduce vertebrate testing (alternative methods) are required (F.10).

Future research emphasis

In vitro studies on human hazards: There is a need to develop and validate a wider array of in vitro models, including complex 3D models, fluidic, organ on a chip, multi-tissue and ex vivo models of physiologically relevant systems that allow communication between different cell types to be investigated. Where possible, HTP/high content screening methods should be developed from these simple and more complex in vitro model, based on understanding of the NMs’ mode of action, and may be used to trigger future research in specific areas. Complex 3D models – less information is available on such models due to their complexity, but available data suggest that they can be useful for investigating mechanisms as well as longer-term and repeat-dose studies, as necessary.

Fluidic/organ on a chip/multi-tissue/ex vivo – again less data are available for such models, but they are suggested to provide feasible alternatives to animal testing for investigating complex multi-system responses. Organ-on-a-chip models have been maintained for several months and have potential utility for midterm studies. These chips include single organ systems, such as gut- or liver-on-a-chip (or multi-organ) systems. While it will be difficult to extend any of the in vitro models to examine much longer-term exposures, they often have capacity to allow such manipulations.

In vitro environmental studies: Few suitable in vitro systems are currently available for use in environmental sciences, and virtually none for regulatory testing, although this is likely to change in the future. To accelerate the identification of NM action modes, there is a need to develop such systems partly to reduce the use of vertebrate testing (e.g. fish) and partly because they may be efficient systems for studying modes of action, relevant for both environmental and human health studies. Such efficiency includes the capacity for HTP addition/implementation for in vitro assays.
As for human health studies, results obtained from in vitro tests need to be compared to data obtained from established (and thoroughly validated) in vivo models or, if possible, known human data, and their limitations must be clearly identified. In environmental studies, use of a broad range of organisms and in vitro studies coupled with HTP measures (e.g. gene responses) may provide excellent tools to study NM uptake and mechanism of action.

For co-cultures of specific, complex 3D models and fluidic/organ on a chip/multi-tissue/ex vivo models the same considerations apply as for human health studies (see above), although they are far less frequently used in ecotoxicology.

Ongoing research project: This area of research represents a key focus of several large projects, including NanoValid, NanoSolutions, and NANOREG.

**Short-/long-term, reversible/irreversible effects**

Identified gaps: There is a need for research into the mid to long-term effects as well as systemic effects of NMs (G.2).

Future research emphasis

The in vivo methods and models discussed above will be essential for determining time courses of responses to NMs and thus identifying whether they are short-term responses with impact or reversible with no significant impact, effects that manifest in the short term but persist (irreversible), or long-term effects with delayed onset.

While animal models are useful, they will always have limitations (relevance, cost and ethical concerns). For human hazard epidemiological studies of occupationally exposed subjects, or even human volunteer studies (e.g. nanomedicines) are therefore required. Likewise, prospective studies designed to reveal possible long-term adverse effects of exposure to low levels of NMs should be established. Such studies will provide further evidence to help interpretation of in vivo studies.

Ongoing research projects: This area of research is a mid-priority focus of the NanoValid project.

**Long-term priorities (achievable in 10-15 years)**

Cohort/Population relevant effects

Identified gaps: Information on the suitability of additional endpoints as indicators for the success of organisms at the population level (H.6.)

Future research emphasis

In vivo environmental studies: The results of potentially suitable additional endpoints identified by short term priority studies must be compared to the results of more advanced ecotoxicological tests (e.g. reproduction tests) to obtain information on the significance of the additional tests at the population level.

Identification of a minimum set of relevant biomarkers identifying NM-related effects will help to focus and reduce the burden of hazard testing, and allow identification of useful models and endpoints for HTP screening. Such biomarkers need to be relevant for information at the population level.

More research is required to identify sub-lethal endpoints/biomarkers (e.g. cytotoxicity, endocrine disruption and genotoxicity markers) linked to population effects of exposure to NMs, especially markers that directly reflect an effect of NMs.
Such biomarkers would also be useful in epidemiological studies. The shorter incubation time and smaller work load are advantages of biomarkers compared with direct population level endpoints (e.g. reproduction rates), and may enable the identification of effects particularly related to NMs. This makes them suitable for routine risk assessment studies in regulatory testing (and for field studies).

Human studies: Hazard data from human studies (e.g. occupational exposures) are relatively rare. Nanomedicine clinical studies will be useful in this respect, thus more effort is required to apply information obtained from such trials to general NM hazard considerations.

**Biomagnification**

*Identified gaps: There is a key gap in knowledge of whether existing exposure assessment models are appropriate for NMs (C.2).*

**Future research emphasis**

There is a need to understand the potential for trophic transfer of NMs. This requires the development of reliable techniques for detecting NMs in various biological media (body fluids, tissues etc.) and models describing the toxicokinetic behaviour and residence time of NMs in key organisms. The suitability and necessity of the standardised guidelines for accumulation testing, and whether in vivo chronic studies can be used to determine bioaccumulation (bioconcentration and bioaccumulation) should also be investigated. Food web interactions can result in uptake of NMs by organisms which would not be directly exposed. Therefore, investigation of communities of organisms and food webs for information on the distribution of NMs among the organisms and biomagnification is essential.

*Ongoing research projects: This area of research is a key focus of SCAFFOLD, nanoIndEx and NanoValid.*

**In vitro and in vivo models of susceptibility**

*Identified gaps: There are few in vivo models of disease that might be appropriate for investigating susceptibility to NM hazards (G.3).*

**Future research emphasis**

*In vivo studies on human hazard: Chronic inflammation is a primary response to pulmonary exposure to NMs. Such inflammation may exacerbate disease symptoms in susceptible individuals, as observed for air pollution. Pertinent health outcomes could include asthma, cancer, cardiovascular disease, lung diseases, and adverse fertility and reproduction effects. Further development of such models, both in vivo and in vitro, is therefore required.*

Similarly, responses such as inflammation following ingestion of NMs may also induce susceptibility to, or exacerbate, a wide variety of diseases. Little else is known about factors that are likely to influence susceptibility of humans to NM-induced hazards, therefore more work is required to identify significant factors and associated phenomena. A range of standard, validated in vivo methods and models (including susceptible or sensitive models) may be required, depending on the type of NM concerned.

*In vivo studies on environmental hazard: As outlined in the short-term priorities, the most relevant species in regard to NM hazards should be identified for specific scenarios (realistic exposures) and guidelines/standard protocols should be recommended. However, understanding of species’ susceptibility (sensitivity) is*
needed to inform choices and prioritisation of species to study, as well as to ensure a true reflection of the effects on a range of pertinent species.

Ongoing research projects: The development and identification of models of susceptibility is a focal area of NanoSolutions and NanoValid.

In vitro high throughput screening backed up by in vivo studies

Identified gaps: Strategies to reduce vertebrate testing (alternative methods) are required, including the use of HTP and computational models (F.4).

Future research emphasis

Initial HTP screening systems are unlikely to be sufficiently robust to provide all the hazard information required for RA. Therefore, research is required to establish the limitations of the systems designed and implemented and how they should best be supported by minimal animal testing.

Ongoing research projects: Aims of NanoSolutions and NanoValid include the development of such strategies.

Distant future priorities (achievable in >15 years)

In vitro HTP screening

See previous section. Once the limitations of HTP systems are established, efforts should be made to reduce them for human and environmental health assessments and thus obtain more robust in vitro HTP screening systems with reduced (or no) need for in vivo testing for comparison. For environmental studies in vivo studies with non-vertebrates will still be required.

NM grouping, ranking and modelling based on focused physicochemical, exposure and hazard IDs

Identified gaps: There is a need to develop appropriate “grouping” of NM based on their hazards and mechanisms of toxicity (F.2), and in silico modelling tools that incorporate the relationship between physicochemical properties and toxicological effects of NMs (F.5).

Future research emphasis

There have been very few attempts to develop advanced modelling tools for NMs, and toxicity and physicochemical based models are almost non-existent. There is an urgent need to determine and verify quantitative structure-activity relationships using Physicochemical and Hazard IDs for diverse NMs, so more work at all levels (characterisation, exposure, hazard and risk analysis) is required to generate the required data and assess the human relevance of such systems.

The first generation of model systems is unlikely to be sufficiently robust to allow risk decisions to be generated, so significant hazard data will be required to provide additional support. Therefore, research is required to establish the limitations of the designed systems and how they should best be supported by minimal in vivo and in vitro testing.

The second generation of model systems should be sufficiently robust to allow risk decisions to be generated with back-up provided by more focused hazard testing, preferably in vitro but also with animal testing where deemed absolutely neces-
sary. Therefore, the strategies developed to allow support by *in vivo* and *in vitro* data should be updated to reduce or remove the need for *in vivo* testing.

The feasibility of developing a third generation of QNAR systems that are sufficiently robust to allow risk decisions without any need for hazard testing is very controversial and has not yet been included in other strategies (e.g. Toxicology in the 21st Century). During establishment of the current research prioritisation this potential scenario was considered during document drafting and workshops in order to push the ITS as far as possible, as it represents the most efficient and ethical future for hazard testing of NM. However, discussion with partners and stakeholders suggested that a scenario with focused testing across hazards, exposure and physicochemical characterisation was more realistic.

*Ongoing research projects*: A key focus of NanOxiMet and NANoREG is the development of grouping approaches. Four modelling projects have recently started; please refer to the NanoSafety Cluster Modelling Working Group.

**Final Recommendations**

In the short term (<5 years) it will be necessary to develop more appropriate *dose metrics* that allow *dosimetry* studies to determine the *mode of action* of different NMs, which will require the identification of robust *in vitro* and *in vivo* *short term* or *acute models* of hazard for validation. These models can also be exploited to assess the *bioavailability* of NMs and their *toxicokinetics*.

In the mid-term (5-10 years) the range of *validated in vitro* and *in vivo models* and *standard protocols* should be expanded to include *long-term* or *chronic models*. The availability of validated models together with an improved understanding of NMs’ mode of action will allow identification of reliable *in vitro* and *in vivo biomarkers*. The *in vivo* models in particular will be used to determine *time courses* of responses to NM exposure, allowing distinction between *short- and long-term effects*. Long-term effects with *rapid and delayed onset*, as well as *reversible and irreversible* effects. There will also be a need to generate more relevant/multi-tissue *in vitro* models.

In the long term (10-15 years) knowledge of *population-level effects* and *biomagnification* will be required for environmental studies. For hazard studies in general we will require good *in vitro* and *in vivo models of susceptibility* to allow more focus on *vulnerable individuals or populations*. To *accelerate* the hazard testing of NM *in vitro HTP screening* will be essential, but initially this is likely to require back-up with *in vivo* and *in vitro* testing. In addition to the HTP testing, *modelling approaches* such as use of advanced models supported by both *in vivo* and *in vitro* hazard testing will be required.

In the distant future (>15 years) it is anticipated that *in vitro HTP screening* will become sufficiently robust to allow *reduced but focused hazard testing*, preferably without the need for *in vivo* tests, for human hazard estimation. Where sufficient information exists, hazard assessment will be possible for some NMs with no requirement for testing.
4 GROUPING/RANKING

Ambition

The ambition is to define research prioritisation to enable the grouping or ranking of NMs in the short term to prioritise NMs for hazard and risk assessment, and in the longer term to provide a framework for better focused physicochemical, exposure and hazard testing. The criteria applied to allow grouping and ranking will be tailored to the needs of specific stakeholders, thereby generating a selection of grouping or ranking models. The grouping/ranking of NMs for the purposes of risk assessment is a multi-factorial process and ideally should encompass critical components such as hazard and exposure of humans and the environment as well as key physicochemical properties of the NMs themselves.

Definition

Grouping and/or ranking will lead to reliable, accurate and efficient methods for testing, evaluation, decision analysis and risk guidance. In terms of definition, grouping simply refers to the arrangement of nanomaterials into groups based on common attributes. For risk assessment purposes, these groups must be based on an attribute that is relevant to risk such as a common hazardous physicochemical property, or an exposure potential that infers a greater harm or risk of exposure. Similarly, ranking is defined in this context as assigning a position in a scale, again with specific referral to risk, meaning that particles may be classified based on factors such as their potential for exposure (e.g. high dustiness) and/or potential to cause harm due to high intrinsic toxicity.

Ranking refers to the positions, in this context, of NMs on a scale (e.g. high to low hazard) that do not necessarily imply any relationship between the NMs on that scale. However, grouping does infer a relationship in (for instance) a common physicochemical attribute such as shape, or mode of action of toxicity. Groups can be ranked, and ranking can occur within groups.

Introduction

The use of grouping/ranking within hazard identification and risk assessment of substances such as chemicals and pharmaceuticals is not new. Indeed within current REACH guidance documents, chapter R.6 deals with grouping of chemicals and provides a clear introduction to grouping as well as read-across of chemicals:

“the terms category approach and analogue approach are used to describe techniques for grouping chemicals, whilst the term read-across is reserved for a technique of filling data gaps in either approach. A chemical category is a group of chemicals whose physico-chemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristic). The term analogue approach is used when the grouping is based on a very limited number of chemicals, where trends in properties are not apparent.”

Whilst grouping is recognised for its use with chemicals, its application to NMs is yet to be fully defined, and tools or approaches are still to be developed to allow its effective use in risk assessment of NMs.
In an ECHA/European Council for the Chemical Industry-Long Range Initiative (Cefic-LRI) co-hosted workshop discussion on read-across for chemical safety assessment under REACH [6], the main issues considered to impact the uncertainty, and thus the acceptance of a read-across prediction, were: (i) the experimental data used in the read-across, (ii) the chemical similarity on which the analogue or category is based, and (iii) the weight-of-evidence supporting the categorisation scheme employed. The discussion highlighted that the most acceptable categories for read-across are those based on integrating knowledge of how chemicals interact with biological systems with knowledge of the biological response(s) once compensatory systems are overcome (i.e. mechanistic information). Confidence in the read-across prediction would be reached when: (i) there is mechanistic transparency, (ii) experimental data for structural analogues allows for interpolation rather than extrapolation, (iii) the number of analogues within the chemical category increase (i.e. read-across from many to one), (iv) it is supplemented by toxicokinetic and absorption, distribution, metabolism, and excretion (ADME) information and (v) it is supplemented by data from relevant in vitro and in chemico (i.e. reactivity of a substance with biologically-important molecules) endpoints, all contributing to an increased weight-of-evidence. The key points articulated in the report by Patlewicz et al. for chemicals, but of direct relevance to the objectives of the ITS-NANO project and implementation of its outcomes, include:

• [Substance] category and analogue strategies were considered not to differ intrinsically in terms of scientific justification, but could differ with respect to weight-of-evidence and grounds for confidence. Categories were considered to offer the additional benefit of increasing confidence by the possible application of trend analysis.
• Confidence in read-across prediction would be enhanced when experimental data for structural analogues allowed for interpolation (i.e. to estimate between known values) rather than extrapolation (i.e. estimate by extension of known information). Analogue approaches should be structured using many lines of evidence (QSAR, in vitro, etc.) to justify the robustness and validity of the read-across, i.e. a weight-of-evidence basis.
• Demonstrating consistency in outcomes within and across endpoints as part of a data matrix would help increase confidence.
• Read-across for filling data gaps will be endpoint-specific in the first instance.
• Scientific principles and rigour should be identical regardless of whether the read-across predicted a presence or absence of toxicity.
• Access to good quality data and the information needed to characterise the target substance and source analogues as well as their respective impurity profiles is required for the read-across approach.
• Scientific challenges exist with determining similarity and, in turn, knowledge of the presumed mode of action (MoA) driving the endpoint under consideration. Knowledge of the mechanism of action at the molecular level may provide greater confidence in a read-across estimate, but this is not always available. The AOP (Adverse Outcome Pathway) concept, as it develops, may help in the future (see below).
• Toxicokinetic and ADME data including equivalents for environmental studies will help substantiate read-across.
• Under REACH, the starting point for the definition of a category has to be based on chemical structure.
• Attributes of substances and differences in structure that are, and are not, permitted to determine similarity will be based on integrating a variety of characteristics such as (i) functional group(s), (ii) bio-modification, (iii) a constant pattern of changes in a property across the category, (iv) common chemical reactions, and (v) 2D molecular similarity.
The OECD’s considerations of a proposal for a template, and guidance on developing and accessing the completeness of Adverse Outcome Pathways include that an AOP should be based on a single, defined molecular initiating event and linked to (a) stated in vivo hazard outcome(s) [7]. Any template used for AOP development should include a summary of the experimental support for the AOP, as well as a statement of: (i) the level of qualitative understanding of the AOP; (ii) consistency of the experimental data; (iii) confidence in the AOP, and (iv) level of quantitative understanding of the AOP. Moreover, a qualitative understanding should include documented identification of: (i) the molecular initiating event and molecular site of action; (ii) key cellular responses; (iii) target tissue/organ(s) and key tissue or organ responses; (iv) key organism responses, both physiological and anatomical, and (v) (if required) key population responses. It was further noted that the assessment of the evidence in support of an AOP should include criteria based on the International Programme on Chemical Safety (IPCS) mode of action framework. In order to use AOPs to develop chemical categories, three information libraries must be collated, programmed, and integrated: (i) a library of effects used in hazard assessment, (ii) a library of molecular initiating events, and (iii) a library of AOPs.

To develop the AOP, different types of data can be utilised. These include: structural alerts that reflect the types of chemicals that can initiate a pathway, data obtained using in chemico methods that measure the relative reactivity or chemical-biological interactions, in vitro assays that confirm the subsequent cellular responses (e.g. gene expression) and, ultimately, in vivo tests that measure endpoints that are directly relevant to the adverse outcome that drives regulatory decision-making. This information can be used to identify key steps in the AOP and provide scientific evidence supporting the AOP.

In 2010 the ISO published a Technical Report on the classification and categorisation of NMs [8]. The published classification system, termed the nano-tree, in which basic and common elements form the main trunk of the tree, but it also differentiates NMs in terms of their internal/external structures, their chemical nature, and their physical, mechanical, biological and other properties. The methodology is presented as an illustrative approach to improve communication and understanding between disciplines involved in nanotechnology and nanoscience, rather than providing an exhaustive consideration of the possible approaches to classifying NMs, and it is not intended to exclude other legitimate methods of classification that may be considered now or in the future. Nevertheless, it should be considered as an important node in the development of harmonised approach(es) to grouping/categorising NMs.

The principles and details on approaches to categorisation of chemicals and NMs introduced above, and the ongoing debate in the scientific community are consistent with the objectives of the ITS-NANO project. These are also considered to be compatible with the detailed “bottom-up” research strategy being developed to enhance the value of physicochemical and biological categorisation attributes for risk assessment purposes [Figure 4.1].

**Future research emphasis and prioritisation**

The grouping/ranking of NMs for risk assessment purposes is a multi-factorial process and ideally should encompass critical components such as hazard and exposure of humans and the environment as well as key physicochemical properties of the NMs themselves (e.g. Figure 1.3). Therefore, the future research emphasis for grouping/ranking is inherently reliant on and interlinked to those advances already stated for Physicochemical ID, Hazard-ID, and Exposure-ID. Consequently,
a degree of repetition is apparent in terms of topic, yet key knowledge gaps and areas requiring further research are given in relation to their importance for obtaining robust grouping/ranking approach(es) specifically and are highlighted below (cross-references in parentheses refer to the Gap Analysis Annex II), which serves to identify and integrate the priorities required for grouping/ranking as part of a future ITS.

To determine the future research efforts, we need to fully define what is required for grouping/ranking. A number of specific needs pertinent to grouping/ranking have arisen from consideration of the Physicochemical ID, Hazard-ID, and Exposure-ID in the context of the parameters required to group/rank nanomaterials and the

Figure 4.1. The diagram identifies the components required for the development of a grouping/ranking approach for NMs. Hexagon colours relate to PC ID (blue), Exposure (brown), Hazard (green), Cross-cutting issues, implementation into a RA framework (grey) and the final goal of the ITS (white). The diagram is intended to start on the left (NM) and finish on the right, but there is no strict order of passage between the hexagons to achieve the final goal. The order of priority is graded across the diagram, with hexagons to the left being of short-term-priority (<5 years) stretching to longer term and distant priorities on the right (≥15 years). It is important to note that contrary to similar representations in preceding chapters, the hexagons for grouping/ranking are not necessarily intrinsically linked, but overall contribute to progress towards grouping and/or ranking of NMs as well as modelling. This example is dominated by hazard, but in other scenarios the exposure or physicochemical hexagons may be more dominant.
Identified gaps: In silico modelling tools have not yet been developed and the relationships between physicochemical properties and toxicological effects of NMs have not yet been fully established (F.5). In detail, very few studies have attempted to develop QSAR tools for NMs, and toxicity-based nano-QSARs are almost non-existent. To begin to address this gap, there is an urgent need to determine and verify structure-activity relationships using an array of physicochemical properties and hazard endpoints for different NMs, therefore, more work at all levels (characterisation, exposure, hazard and risk analysis) is required to generate and validate such systems (F.5.1.2). In addition, in order to facilitate the IVIVE, there is a need to develop PBPK (physiologically-based pharmacokinetic) models that can successfully predict the ADME behaviour of NMs in both experimental animals and humans (F.5.3). In order to make use of read-across for NMs based on “analogous” materials, greater understanding of the fundamental drivers of toxicity based on physicochemical characteristics is needed. The use of extrapolation and read-across between materials of similar/related characteristics, species, human and environmental data, as well as in vivo and in vitro data should be exploited more (F.6).

Future research emphasis
The impact of exposure routes/pathways on PC characteristics (e.g. surface properties, protein corona etc.), and thus their impact on toxicity, needs investigation (F.7-8) There is indeed a need for better understanding of how NMs interact with their environment, as factors such as dispersion, aggregation and agglomeration can influence their characteristics in exposure and hazard studies (F.7.1), considering that experimental dispersions should aim to be realistic and not necessarily mono-dispersed (if that is not how the NMs will behave in situ) (F.8.1).

While some biomarkers (in vitro and in vivo) of hazard have been identified (e.g. indicators of inflammation, oxidative stress and cytotoxicity), the identification of a sub-group of the most reliable, specific and relevant biomarkers is lacking. Identification and validation of these biomarkers would be a priority for standard protocol development. Very little work has been carried out on biomarkers in ecotoxicology, making this a priority area for future research (F.9).

More guidance and examples are needed to allow differentiation between biological and statistical significance (i.e. responses that can be statistically distinguished from control responses, but merely homeostatic). Specific criteria to be considered are adequacy, reliability, relevance (partly represented in the Klimisch criteria) [9], statistical power and toxicological significance (F.11).

Several of these knowledge gaps are focus areas of ongoing research projects. For further identification of such activities, please refer to the respective chapters.

Physicochemical ID
Both grouping and ranking of NMs are almost certainly going to be based upon one or more physicochemical properties (e.g. ranking based on quantity of reactive transition metals). Therefore, determining a base set of particles’ physicochemical characteristics for characterisation is an important component, because missing information on key properties hinders comparison, grouping or ranking of materials.

Key physicochemical properties as they relate to risk (in terms of their influence on hazard and/or exposure) should be collated as and when they are identified to create
a base set of particles’ physicochemical characteristics that must be analysed to enable grouping/ranking. As knowledge is constantly evolving, this base set of physicochemical characteristics should also be allowed to evolve to reflect the current state of the art in terms of the addition of important new characteristics and removal of those no longer considered informative. This requirement is a cross-cutting issue relevant to Exposure ID and Hazard ID and links to several other identified gaps and associated needs for research. These cross-cutting issues are addressed in more detail below.

**Exposure ID**

Exposure is arguably the key component of risk, as the potential for exposure determines the potential for risk, while hazard is a key variable, but subject to exposure potential. Therefore, in terms of understanding risk, grouping or ranking of NMs or processes based around the potential for exposure of people and/or the environment is important.

**What physicochemical characteristics influence exposure and dose?**

The potential for exposure to NMs of either humans or the environment is a multi-factorial variable, that is influenced by the nature of the processes (e.g. production method) involved, the volume of material used, whether or not the NMs are combined within a matrix (master batch or product) etc., and the physicochemical characteristics of the materials themselves. Properties such as surface charge, density, shape, and aerodynamic diameter may influence a material’s propensity to become airborne and disperse within an environment resulting in exposure. As already stated in “Consideration of the life cycle”, these properties may differ markedly throughout the life cycle of a specific NM (depending for instance, on whether or not it is handled as a dry powder during primary production and thereafter incorporated in a matrix), and this must be considered in order to obtain an accurate evaluation of a material.

**Decision Tree – Exposure**

Similarly to hazard assessment, the grouping/ranking approach should be developed with the aim of integrating it in a decision tree approach to inform and prioritise testing/monitoring activities. Again, this may be in the form of performing a pre-screen of a material or process using grouping and/or ranking to identify where exposure may be likely. For example, this may be due to grouping materials based around physicochemical properties that increase the likelihood of them being dusty and becoming airborne or perhaps ranking activities or processes that are associated with high/low levels of exposure and therefore would call for exposure monitoring or controls. The inverse of this is using such approaches to determine where exposure is unlikely and, therefore, costly routine monitoring and control measures may not be required.

**Hazard ID**

In terms of testing the hazard potential of NMs, various components of the gaps identified within the Hazard ID section relating to human and environmental hazards are highly relevant to grouping and/or ranking. This is because when considering the potential risks involved with NMs, it is most informative to rank or group materials based on factors (e.g. properties) that influence these risks. Conversely, groupings based simply on arbitrary properties, such as name, may or may not provide information about relative risks due to the materials’ relative generic nature (i.e. they do not necessarily describe shape, size, crystallinity etc.). The gaps identified in the Knowledge Gaps and Research Priorities document (Annex II) and during the stakeholder consultations that are considered to be most relevant to grouping and/or ranking of NMs are described below in greater detail.
Mode of Action
The mode of action provides information about a material’s basis of toxicity, and for particle (eco)toxicology this most likely relates to one or more physicochemical characteristics of the particle in question. Therefore, to understand what physicochemical properties inform hazard (and subsequently risk) and to base grouping around these, one must first understand the mode of action. “Classical” particle toxicology (based on more conventional particles such as quartz, air pollutants etc.) provides a strong basis for understanding the mode of action of particle-mediated toxicity that serves nanoparticle toxicity well. However, NMs may have modes of action that are beyond current understanding, and it is these “unknowns” that are of most concern, as they may be missed. Therefore, understanding the mode of action enables us to: (i) see where toxicity is the same as conventional particle toxicity (probably most cases), bolstering confidence in current knowledge; and (ii) where toxicity differs, indicating the need for new approaches and new paradigms.

An additional key requirement is the ability to detect toxicity, both at a screening level and in limited, focused testing. If the mode of action is understood, especially in relation to a physicochemical property, then when materials are grouped and testing is tailored to a group (which ideally is the case) one can be sure of detecting a reliable indicator of toxicity and equally important, be confident in the absence of toxicity.

Linking Hazard ID to Physicochemical ID
Linking physicochemical properties to the mode of action in terms of toxicity allows materials to be grouped or ranked based on analysis of physicochemical properties without (eco)toxicological testing, which is likely to be both cheaper and more useful to a company (e.g. for quality control purposes). This can only be achieved if toxicity (ideally, but not necessarily, including mode of action) and a physicochemical property is linked. Where this is done, it is possible to rank materials based on the presence/absence or quantity of a property that confers toxicity (e.g. higher to lower surface area) or group them together based on the presence/absence of a property. When this is done, it may then be possible to infer a hazard status without testing and consequently apply control measures or select a different material for a process that has a lower hazard status. Alternatively, grouping/ranking can be used to identify those materials which are potentially high risk and therefore do require further or more detailed (and costly) testing and those which do not, rather than needing to take a blanket approach to testing. In this case, the presence of a “negative” physicochemical property (i.e. one that implies greater hazard) would mark a material as needing more thorough investigation, whilst its absence may mean a high level screening approach is considered sufficient. In addition, a link between mode of action and physicochemical properties allows testing to be more focused on detecting toxicity based on that mode of action. For example, a carcinogenic particle may not be detected as being hazardous based on an acute toxicity study unless it is known that the particle may be carcinogenic (or genotoxic etc.), due to a physicochemical characteristic acting as an indicator that is specifically tested for. This is important, as the consequences of failure to detect a carcinogenic particle may be very severe, but testing every particle (or combination of particle/functionalisation) for carcinogenicity is simply not possible.

Comparison to bulk (non-nano) particles/Read-across
An issue surrounding the testing of NMs is whether or not this is required if the “bulk” (i.e. non-nano) form has already been tested. This raises the issue of sameness and whether crossing into the “NM definition” causes a fundamental shift in toxicity of a material, especially as the definition of a NM has no basis in (eco)toxicology. Where toxicity is detected and this is linked to a physicochemical property, com-
Comparisons with the bulk form, if it exists, are useful for several reasons. The first is to determine if the basis for toxicity (i.e. the mode of action) is the same or not, and if so, is this equal or not between the two forms? If this is the case, then it may be possible to perform at least cursory grouping/ranking of materials based on the relative hazard status of the bulk material – i.e. read-across from the bulk material. For example, several studies have shown that nano-sized titanium dioxide particles are far more potent, on an equal mass basis, than (poorly soluble, low-toxicity) non-nano sized particles [10, 11]. The basis for this toxicity has been shown to be the particle surface [11], and because nano-sized particles have a far larger surface area per unit mass, the nano-form is more inflammmogenic; this has been shown by normalising with respect to surface area, which equalised the level of inflammation [12, 13]. Here, because the comparison has been made and it has been shown that the basis (or mechanism) of toxicity is fundamentally the same, despite the differences in mass-based potency, it may be possible to scale (based on surface area dose) between these particles and read-across to obtain information on the hazardous nature of nano-titanium dioxide based on the relative toxicity of the bulk form. This may also be potentially possible for other poorly soluble low toxicity particles in terms of bulk to nano read-across of the same materials, but also potentially bulk to nano read-across between different poorly soluble low toxicity particles. However, it should be stressed that this is only hypothetical and not relevant to other particles such as quartz or diesel soot that do not meet the definition of a poorly soluble low toxicity particle.

The second reason, also stated above in the “Determine Mode(s) of action” section is that some NMs may have fundamentally different modes of action from the bulk form, making a read-across or scaled comparison from bulk to nano forms inappropriate. Understanding these fundamental differences will enable us to group and rank materials based on bulk material toxicity if known more confidently, thereby waiving or focusing testing. For industry this means that where it is appropriate, there may be no need to repeat testing where it has already been performed and sufficient information is already available.

Decision Tree – Hazard Testing

The grouping/ranking approach should be developed with the aim of integrating it into a decision tree approach to inform and prioritise testing. This may be in the form of performing a pre-screen of a material using grouping/ranking to identify materials of concern and the most suitable testing approaches. For example, ranking materials based on their exposure potential may indicate which materials (or in which part of their life cycles) exposure is likely to be high, and therefore require a more in-depth understanding of potential hazards, and conversely cases where there will be very little or no exposure and thus lower concern and testing requirements. In addition, grouping materials based on physicochemical properties linked to a specific mode of toxicity (e.g. liver toxicity) may also allow testing to be focused or widened (as indicated by a decision tree matrix) to the most relevant target systems/endpoints (i.e. be more bespoke) to provide a more robust hazard assessment.

Risk

Whilst the grouping/ranking of NMs is most likely to be based on their hazard and/or exposure potential, the risk characterisation may also strongly influence the route to grouping/ranking of NMs and its utility (see Chapter 5). An important issue to address when dealing with grouping/ranking in relation to risk is uncertainty, as it is simply impossible to know the outcome of every eventuality, however unlikely, that may result from the use or mis-use of a substance or product. Therefore, it is important to understand where uncertainty may be acceptable (as in the case
of highly unlikely events or usage scenarios) and where it is unacceptable (as in the case of an acute response to exposure resulting from normal use). For further discussion on uncertainty see Chapter 5.

Weight of Evidence

One approach that is useful for ranking, grouping and prioritisation of chemicals in terms of attributes pertaining to physicochemical properties, hazard, exposure and risk is the Weight of Evidence (WoE). Linkov et al. (2009) [14] proposed a conceptual framework for categorising WoE methodologies as quantitative or qualitative. The most basic qualitative integration occurs through Listing Evidence where all evidence is made explicit and readers are allowed to make their personal judgments [15]. The Best Professional Judgment (BPJ) goes a step further by providing, in addition to the evidence, informed interpretation [16, 17]. Logic methods place WoE in structured frameworks to reach dichotomous conclusions [18, 19]. Causal Criterion methodology follows a similar approach using a structured framework, but seeks to identify cause-effect relationships [20]. Scoring and Indexing normalises evidence to numerical values for interpretation [21, 22]. Fully quantitative methods characterise problems numerically with statistical tools or Multi-criteria decision analysis (MCDA) [14, 23]. Qualitative methods are typically applied in situations where evidence is very limited and the assessment relies largely on expert judgment. Quantitative methods are useful when systems are complex and there are many types of data to consider.

WoE evaluation has been recommended for risk-ranking and prioritisation of NMs, but most WoE frameworks are qualitative in nature and do not satisfy the growing requirements for objectivity and transparency in regulatory decision-making [24, 25, 26]. For the first time Hristozov et al. [27] have implemented a quantitative WoE framework that utilises MCDA methodology for identifying hazard resulting from physicochemical and toxicological properties of NMs. The application of MCDA methods yields multiple benefits over qualitative approaches such as the ability to incorporate conflicting information, to facilitate trade-offs among competing alternatives, and to propagate uncertainty. The approach of Hristozov et al. [27] explicitly integrates expert evaluation of the quality of available data based on the above mentioned criteria: adequacy, reliability, statistical and toxicological significance. Although it is constrained by the lack of knowledge about the complete set of relevant physicochemical characteristics, and how they relate to the observed biological responses, when more knowledge and empirical data are acquired the approach can be extended to construct a logic model, which can link certain physicochemical properties of NMs to biological responses to provide insights about when read-across among slightly different NMs is possible and group them accordingly. Moreover, Hristozov et al. [28] proposed a complementary WoE approach for relative ranking and prioritisation of nano-exposure scenarios. This is the first exposure model explicitly implementing quantitative MCDA methods and using expert judgment for filling data gaps. In order to test how variations in input data influence the obtained results, both approaches use probabilistic Monte Carlo sensitivity/uncertainty analysis.

Since the grouping and risk analysis of NMs would be affected by severe uncertainty and data variability, it may be useful to address them in a probabilistic manner using stochastic approaches such as Monte Carlo or Latin Hypercube Simulations. Such approaches would yield distributions of hazard estimates instead of single values, which could be plotted against distributions of exposure estimates in order to identify central tendencies of expected risk and associated high-end probability of exposure. Quantitative WoE approaches such as MCDA allow this to be done.
Final Recommendations

The grouping/ranking of NMs in terms of risk requires the interaction of Physicochemical ID, Hazard-ID, and Exposure-ID as well as consideration of its integration and acceptance within current and future regulatory frameworks. Targeted experimental data is required to provide greater clarity and certainty about the basis of grouping, ranking and read-across between substances. This needs to be hypothesis-driven (rather than simple screening of arbitrary particle cohorts) in order to demonstrate the accuracy and consistency of outcomes and to provide a weight of evidence to counterbalance uncertainty. Understanding the mode of action of observed toxicity and/or exposure and how this relates to physicochemical characteristics of a particle is a central theme in the required research. This in turn is influenced by and influences other key research issues such as the base sets of physicochemical characteristics that need to be characterised to inform grouping/ranking etc. for risk assessment. Lastly, a recommendation for future research is to develop new approaches for grouping, ranking and the scientific basis for numerical extrapolation/interpolation of results between species/models (e.g. IVIVE), analogous materials or even the same material, but based on differing properties such as surface area or coatings. Whilst this does not necessarily involve new testing, it is arguably just as important to future development of acceptable grouping and ranking that will lead to a reduction and refinement in testing.

The ITS-NANO discussion and recommendations pertaining to grouping/ranking are consistent with those recognised as a cross-cutting theme in Nanosafety 2015-2025: A Strategic Research Agenda towards Safe and Sustainable Nanomaterial and Nanotechnology Innovations. Specifically these include: (i) develop systematic sets of NMs with properties varied in a stepwise manner that allows assessment of the significance of each property for toxicity, (ii) describe “reference” states and agreed media compositions etc. to enable identification of biomarkers of impact and enable a move towards predictive toxicity assessment, and (iii) elucidate the longer term fate of particles following interactions with living systems, with a view to developing a computational tool termed the ENM SAFETY CLASSIFIER in the Nanosafety 2015-25 Strategic Research Agenda, that will predict ENM (engineered nanomaterial) safety based on the utilisation of minimal but sufficient amounts of information to give a robust ENM safety classification.
5  PRACTICES FOR IMPLEMENTATION INTO THE RISK ASSESSMENT FRAMEWORK

Ambition
The ambition here is to define how the future outputs (data and tools) of the research prioritisation can be used and implemented into the risk assessment (RA) framework for NMs. The differences in the RA of NMs in comparison to “conventional chemicals” include the consideration of multiple forms and their variations over their life cycles, the relationship between physicochemical properties, and (eco)toxicity as well as exposure and the potential higher uncertainty due to bigger data gaps. The ambition includes identification of the relevance of the current RA frameworks for NM and definition of “best practices” for RA of NMs that need to be tailored according to NMs’ type and applications; future adaptations of the RA practices and framework (e.g. novels concepts, tools); increased confidence in and regulatory acceptance of the developed ITS-NANO strategy.

Definition
The RA framework in the context of this document is understood as “the entirety of EU law requiring a RA of substances for their safe use as such or in products/articles and the related guidance”. The RA framework is, as such, considered to be applicable to NMs, even if they are not always explicitly addressed. Some adaptations may be required to ensure the safety of current and future types and applications of NMs and the integration of new tools, to which this chapter aims to make recommendations.

Introduction
The development of practices for implementation of the ITS-NANO Strategy into the RA framework is relevant to a number of different stakeholders, including researchers, industry and regulators, who are likely to follow different approaches for RA. While research-based approaches for RA usually incorporate the use of newer, non-validated, non-standard tools under (sometimes unrealistic) worst case conditions, the regulatory RA makes use of established/validated tools referring to real life exposure situations, considering realistic worst case conditions. Research in the field of RA could be seen as contributing to the establishment of correlations and general conclusions on NM toxicity, exposure and risk, also addressing potential gaps in the regulatory RA and providing an “early warning system”. This may have a direct influence on the development of materials and products (safety by design), as well as on the development/adaptation of risk management tools. Regulatory RA is characterised by binding decisions with more wide-reaching consequences (e.g. inclusion or exclusion in a list, restrictions, protection of people and the environment) and hence a greater level of responsibility, requiring a solid decision-base. Although this chapter is in principal of great relevance for both research and regulatory RA, the main focus is on RA in a regulatory context.

A suitable framework for RA of NMs requires access to reliable and relevant data on their hazard and exposure. However, at present there is still insufficient data to establish such a detailed framework, especially in a regulatory context. Although the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) [29], and more recently the OECD [30], concluded that hazard identification and risk assessment methodologies for conventional substances can be considered applicable to NMs, specific aspects still require further research, development, vali-
dation and standardisation. For example, major issues to be addressed include the acquisition of high quality exposure data, decisions on the most appropriate (relevant and practical) dose metrics, and physicochemical characterisation – for both NMs as produced and NMs in realistic exposure conditions. A further challenge for the future is to develop an ITS to overcome the currently proposed “case-by-case” evaluation, which the ITS-NANO project goes some way to addressing. It is also relevant to include this testing strategy in a targeted RA strategy, which should be widely applicable to a variety of NMs. Tiered approaches as well as grouping/waiving options are essential to optimise testing, thus reducing costs and animal use while maximising the output. The applicability of other trends in RA methodologies to NMs, like the change from deterministic to probabilistic approaches and the use of Multi Criteria Decision Analysis (MCDA), should also be considered. The above mentioned aspects have also been considered in a new strategy for RA of NMs currently under development in the EU FP7 MARINA project. Finally, novel tools to address RA of NMs need to be validated and incorporated into practise within the respective framework.

**Current practices for RA of NMs**

European legislation provides a binding framework by explicitly or implicitly covering NMs and the assessment of their risks. REACH provides the main legislative framework on chemicals within the EU, and NMs are covered by its substance definition which addresses chemicals in whatever size, shape or physical state. NMs and their potential risks and/or safe use/application are also covered by sector-specific legislation (either implicitly or specifically), such as the General Products Safety Directive [31], Biocidal Products Regulation [32], Cosmetics Regulation [33], food-specific legislation [34], etc.

Besides legislation, guidance also contributes to the RA framework. Guidance is usually more specific and mainly serves to correctly interpret and apply the legal text for specific purposes, however it is not legally binding. It is more flexible and can, therefore, be adapted more rapidly to new requirements than the legal (main) text. For example, some REACH guidance documents [35] were updated in May 2012 to include specific provisions for NMs. Guidance for NM testing and/or RA is also available for cosmetics products [36] and food/feed [37, 38]. The regulatory framework and corresponding guidance determine the information requirements and methodology for carrying out a RA.

Given the conclusions of the second regulatory review on NMs that was recently published by the European Commission [39], substantial modifications of the RA framework are not envisaged within the next few years. However, minor amendments to REACH annexes, additional guidance by ECHA, market surveillance on consumers’ products, and a web-platform for sharing information are expected to better address NMs. Conversely, several Member States and NGOs consider the RA framework as insufficient to ensure a safe use of NMs and strongly advocate the need for a more targeted and effective “nano-specific” legislative tool (e.g. a “nano-patch” for REACH) [40, 41].

In general, the existing RA regulatory procedures are similar in terms of principles, but slightly differ in the methods used to characterise risks as well as in their assumptions and data requirements. A detailed description of the RA regulatory procedures as well as their specifications for NMs can be found in Section 2.3, 5.3, and Appendix III of the ITS-NANO Identification of Knowledge Gaps and Strategic Research Priorities report (Annex II). The most important issues of the current practices for RA of NMs identified are summarised below.
1. Definition of a “NM”

- A specific definition is included in an EC Recommendation (2011/696/EC) and several recent EU regulations.
- All current definitions are based on size (determination of surface area as an alternative option) and refer to a size range between 1-100 nm. The Biocidal Products Regulation (528/2012) directly implemented the recent EC recommendation on a definition [42], and therefore covers “natural, incidental or manufactured” materials; while the Regulations for Cosmetic Products (1223/2009) and for Food Information (1169/2011) only refer to “intentionally manufactured (or produced) materials”. Regulation 1223/2009 further restricts the definition to “insoluble or biopersistent material”. Currently, REACH does not include a definition of NM.
- Currently no single measurement method is available that is capable of determining whether a certain NM fulfills the EC’s recommended definition (number size distribution) or not. Due to this technical limitation, a combination of methods is recommended in a current JRC report [43]. An additional challenge is to discriminate between naturally contained, intentionally added and/or engineered NMs and to distinguish them from background particles. A further complication is the determination of NM within complex matrices.

2. Adequate physicochemical (PC) characterisation of NMs is generally recommended:

- ECHA guidance [35] requires characterisation of NMs prior to administration, during, and at the end of the test. Shape and surface area should be measured in addition to particle size distribution, and multiple techniques should be applied to cover the whole size range.
- A list of properties to be measured is provided by the OECD [44] and has been superseded by different NM-specific guidance (e.g. ECHA, EFSA).

3. Most frameworks build on tiered approaches and/or decision trees that allow targeted and reduced testing and encourage grouping, waiving, and read-across. In particular:

- Exposure-based adaptation (exposure-based waiving or exposure-driven testing) in general is established by Annex XI (adaptation of information requirements) of REACH and explicitly mentioned for NMs by the EFSA guidance [37]: e.g. reduced information can be provided when no exposure is verified by data indicating no migration from food contact materials or when complete degradation/dissolution is demonstrated with no absorption of NM as such. A central component of that RA decision tree is, therefore, the solubility and dissolution rate.
- A comparative approach, which makes full use of existing data on a relevant non-nano comparator, is also suggested by the EFSA guidance [37].
- Read-across between NMs and from bulk materials should be used with caution and needs to be scientifically justified [30].

4. Use of alternative, non-animal testing is generally encouraged by all relevant legislation and specifically considered as fundamental in the Cosmetics Regulation, where animal testing of ingredients and products is prohibited. However:

- Few alternative methods to animal testing have been validated so far and none of them have been validated for NMs [36].
- The use of non-testing methods to address data gaps for NMs in REACH must be scientifically justified and strictly applied on a case-by-case basis [37].
- The Scientific Committee on Consumer Safety (SCCS) recommends that in vitro assays should not be solely used for NMs at present [36].
5. A separate risk assessment for NM is generally recommended:
• Under REACH, NMs can be considered as separate substances from their bulk counterparts or as different forms of the same substance. This determines the submission of one dossier covering different forms or separate dossiers for each form and, consequently, the information requirements for the respective tonnage levels which have to be provided.
• A separate risk assessment of NMs is currently required by the Plastic Food Contact Materials Regulation, Cosmetic Products Regulation, and Biocidal Products Regulation and proposed in the Novel Food Regulation (under revision). In these regulations, nanoforms are in principle considered not to be covered by dossiers related to their bulk counterparts, unless specified.

6. Risk characterisation methods
• Different methods are used to characterise the risk, such as Risk Characterisation Ratio (RCR) (e.g. REACH), Margin of Safety (MoS) (e.g. Cosmetics Regulation) or Margin of Exposure (MoE) (e.g. Biocidal Products Regulation).
• Current risk assessment methods are usually deterministic and based on the use of assessment factors (AFs).

7. Assessment factors (AFs) for RA
• REACH guidance [36] provides sufficient flexibility to address areas of uncertainty, data gaps and if justified deviations from the default AFs. As an alternative approach for extrapolating from experimental animals to humans for inhalation exposure, the actual lung dose can be calculated.
• The use of an extra nano-specific AF is currently under debate. However, to date no evidence supports the need of such a factor. A more important issue is the use of a relevant starting point (e.g. NM-specific hazard data).
• Vulnerable subpopulations are usually accounted for by the intraspecies AF. This AF is higher for the general public (10) than for workers (REACH: 5), assuming that the most vulnerable people (children, pregnant, old and sick people) are not occupationally exposed. Deviations from the default intraspecies AFs in both directions are possible but should be justified.
• Route-to-route extrapolation might be especially difficult for NMs, as differences in effects are expected to be not only quantitative (different absorption rate), but also qualitative (different nature of effects).
• The use of aquatic data to predict terrestrial toxicity is not recommended [44].
• The use of acute data to predict chronic toxicity is currently not recommended for NMs (for either human health or the environment) [44], as different kinds of effects may occur.
• Any deviation from the standard approach must be scientifically justified on a case-by-case basis [35].

• Nano-specific regulatory occupational exposure levels (OELs) have not yet been established for NMs [44]. Recommendations for OELs/DNELs have been published for carbon nanotubes by NIOSH [45], Pauluhn [46] and Aschberger et al. [47] and for TiO2 by NIOSH [48] and Christensen et al. [49] and for both under the Japanese NEDO project.
• Use of an already established OEL as a DNEL for NMs is only possible when PC properties of the material in addition to the route and duration of exposure are comparable [35].

9. Additional metrics relevant for NMs are recommended.
Surface area concentration (for systemic exposure) and particle concentration (for inhalation) are suggested by ECHA guidance [7] beyond current practices for RA of NMs.
To date, various adaptations of the RA framework and related risk management issues to NMs have been proposed in the literature. Some important examples are illustrated in Table 1 in Annex V (5.3). In addition, a new targeted, tiered RA strategy for NMs, integrating both environmental and human health aspects, is under development within the EU FP7 MARINA project.

Important issues in RA are the amount, type and quality of the data used to characterise risk and the associated uncertainties. Currently a lot of research data is produced on NMs using non-validated methods that are mainly suitable to identify but not characterise the hazard. One aim should be to direct research (e.g. via dedicated funding) so that results could support and feed better into a regulatory RA (see also previous chapters).

All the investigated new approaches (Table 1, Annex V – 5.3) have been developed by large organisations or regulatory bodies and are based on conventional human health and ecological RA paradigms. Most of these new frameworks are considered as iterative, adaptive, and transparent, even if this is not demonstrated in any documented applications for some of them. Several of them also include a life cycle perspective on the examined NM. In many cases, a “base set” of nano-specific information (mostly physicochemical properties) is required in the first phase of the process. The fact that physicochemical properties of NMs and consequently their potential risk can change during their life cycles has to be accounted for in the RA.

The main limitation of these new frameworks is the lack of a quantitative uncertainty assessment. In most cases, uncertainties are handled in a qualitative manner through identification of sources and data gaps and by suggesting a reasonable worst case scenario\(^2\). In addition, none of the frameworks propose a strategy for managing and reducing the identified uncertainties.

Taking preventive action in the absence of scientific certainty is embodied by the Precautionary Principle stating that “where there are threats of serious or irreversible damage or harm, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent this potential damage or harm” [50]. This approach is used predominantly for chemicals where relatively more information is available, so it presents a challenge in the field of NM RA, mainly due to the paucity of information linking properties and effects [51]. Therefore, all available information should be considered when making informed decisions about hazard, risk and preventive actions. This could be facilitated by emerging Weight of Evidence (WoE) methods such as Multi-criteria Decision Analysis (MCDA) [52, 53, 54].

WoE-based safety analyses involve identification of measureable parameters derived from experimental or modelling results and their integration with expert judgement into conclusions concerned with degrees of exposure, hazard or risk. Depending on the scope of the assessment, various types of data can be used, including physicochemical, (eco)toxicological, in silico and exposure information as well as results from PBPK modelling and in vitro/vivo extrapolations. To date, WoE has been explicitly applied for nanosafety evaluation by a few authors. In addition, WoE methods have been implicitly used in a number of scoring and control banding approaches (see Table 2 in Annex V – 5.3).

\(^2\) Currently most hazard studies are designed to test the very worst case, by e.g. using pristine, monodispersed NMs at high concentrations. These results have to be translated to realistic exposure situations or the design of hazard studies has to be adapted.
It is important to stress that most of the above mentioned WoE approaches are qualitative in nature and do not satisfy the growing requirements for objectivity and transparency in regulatory decision-making of NMs. Instead, the proposed approaches serve as tools to help industry identify relevant sources of risk in the life cycles of synthetic NMs and pinpoint areas of knowledge deficits [55]. While these approaches may be valuable, e.g. providing regulators with early nano-risk estimates, a major limitation is that many of them have not been thoroughly tested and their robustness has not been confirmed [56].

Hristozov et al. [27, 28] proposed the first quantitative WoE approach that utilises MCDA for integrating individual studies on hazard and exposure. The WoE approach allows incorporation of heterogeneous information in combination with expert judgement to make informed decisions in the face of uncertainty. The WoE approach is also sufficiently flexible to incorporate a system for data quality evaluation and substitution of lacking information, which is essential when the evidence base is weak and dominated by less reliable, non-standardised data [55, 27], and is applicable to several NMs.

The call for quantitative, robust decision-making tools in the nano-safety area is unlikely to wane, and the importance placed on them will increase with time. The production of new information will naturally move modelling activities towards the quantitative region of the WoE spectrum [27, 57]. MCDA can potentially overcome many limitations of the existing qualitative WoE approaches, while simultaneously providing a flexible framework adapted to various needs. Therefore, practitioners should consider MCDA methodology as a valuable data integration technique [58], which can potentially contribute significantly to the early stage RA of NMs [27].

**Research strategy for the risk theme**

The key elements of the ITS-NANO research strategy in the field of RA of NMs are presented hereunder. Knowledge gaps and research recommendations were identified and defined in the ITS-NANO Identification of Knowledge Gaps and Strategic Research Priorities report (Annex II), based on a literature search and stakeholder consultation. This is used as a starting point and integrated with the considerations on possible adaptations of “current practices” for regulatory RA of NMs, as discussed above. On this basis, a set of key research elements for the risk theme of the ITS-NANO research prioritisation was identified. An overview of these key elements, as well as their inter-connection, is presented in Figure 5.1. These research elements support the identification of “best practices” for regulatory RA of NMs in the future and the analysis of their implementation into the “current framework”, which are illustrated in the next section.

As illustrated in Figure 5.1, in order to characterise the risk of NMs, all existing data and information need to be collected and properly stored in dedicated databases as well as evaluated in terms of reliability and relevance. A variety of testing and non-testing methods for hazard and exposure assessment of NMs is under development, e.g. *in vivo* and/or *in vitro* testing, QNAR/PBPK modelling and exposure modelling. Once validated, they will provide fundamental input to RA of NMs. Moreover, grouping and read-across can be used to exploit as much knowledge as possible and data from non-nano analogues or other nano-analogues, based on the assumption that physicochemical properties and consequent modes of action and effects are similar and can, therefore, be inferred. Ranking of NMs for their potential hazard, exposure or risk can be used to prioritise testing or risk management options.
The available information from different sources will be heterogeneous and needs to be properly interpreted and integrated to perform reliable risk evaluations. In this context, a standard harmonised approach for the estimation of risk is needed. This approach should go beyond current practices and address the peculiarities of NMs. Novel approaches for risk estimation have been already developed and proposed in the literature, but their robustness and applicability has to be demonstrated for a variety of NMs. Moreover, most of them deal with uncertainty in a qualitative manner, while a more quantitative approach is necessary. This gap may be filled using e.g. WoE-based approaches and ranking procedures. These novel tools may provide regulators with early stage risk estimates and, consequently, support short-term decision-making on NMs. To this end, it is essential to start the validation and standardisation process soon in order to increase the confidence and regulatory acceptance and consequently foster the integration into the RA framework.

The RA of the NMs process also needs to rely on an agreed acceptable risk level (i.e. the minimum margin of safety or the ratio between a dose descriptor and an exposure level), which is not a purely scientific issue, but is also influenced by policy. In addition, it relates to risk perception where several stakeholders (e.g. industry, NGOs, authorities, general public) are involved. Different risk management tools, such as applying “safety by design” in the development of new NMs and reducing the possibility of exposure to pristine NMs by production in closed systems or under liquid or accident avoidance can contribute considerably to the reduction of risk in consumer and occupational settings.
Towards best practices for RA of NMs

On the basis of the gaps and research recommendations identified in the gap analysis and subsequent stakeholder consultation (Annex II), and taking into account the key research elements for the risk theme illustrated in Figure 5.1, “best practices” for RA of NMs are defined and summarised in the following points below (from 1 to 13). In addition, recommendations to best address existing gaps to achieve the best practices are provided for each point. For each gap, reference to the corresponding code and section in the ITS-NANO Identification of Knowledge Gaps and Research Priorities document (Annex II) is reported in brackets. Figures 5.2, 5.3, and 5.4 illustrate in detail how the best practices are linked to the whole ITS-NANO research strategy. The cells that are linked to the best practices show the same numbers as in the bullet points presented below.

1. Definition of a NM
NMs’ specific requirements should refer to an internationally harmonised definition of NMs, for which a standardised and validated set of measurement methods [e.g. size, surface area] is available [47, 64]. Risk assessment and risk communication should however focus on the function (i.e. potential risk) of a NM.

2. Characterising exposure and addressing complexity of PC properties
The RA approach should take into account the complexity of PC properties of NMs resulting from their dynamic nature (e.g. over their life cycle from production to end of life), their diversity (e.g. different formulation processes and applications) and their inhomogeneity (e.g. different sizes ranges within one material) (J.1.1, J.1.2) and their consequences for hazard and exposure assessment.

• Exposure measurement techniques and models need to be developed and/or improved and validated to allow efficient and realistic exposure assessment and monitoring at different life cycle stages of the NM (see also exposure section) and to build up a valid database.
• As PC properties can change over time and are dynamic in test systems, they need to be determined prior to administration (pristine material) and monitored during the entire test duration. The tested NMs and their doses/concentrations should generally be realistic for real life exposure situations.
• PC characterisation and quantification is specifically challenging in complex media (tissue, environment) and after administration to living organisms [59], therefore further development and improvements of sample preparation, measurements methods and tracking systems are required.
• Validation of methods for PC characterisation of NMs is required. Reference NMs or representative “test materials” should be established [59].
• “Hotspots” of exposure need to be identified and defined to assess realistic worst case situations.
• Combined exposure and mixture effects of NMs and other chemicals and their interference should be considered.

3. Multiple metrics
RA should consider appropriate (multiple) metrics for NMs, which need to be defined and coherent/linked between different information requirements for the RA process. Current recommendations refer besides mass to specific surface area and particle number. Additional metrics may be considered in future.

• Risk characterisation should refer to the most relevant metric and/or be performed using different metrics.
4. Exposure-based adaptation
RA should apply exposure-based adaptation/waiving of hazard information requirements, if based on realistic exposure scenarios and validated exposure models.

Further guidance on RA of NMs allowing for reduced information when (external and/or internal) exposure to NMs can be excluded (verified by data) is required (J.3.3) – this should go beyond current recommendations, such as the decision tree of the guidance for NMs in food [38] based on solubility and dissolution rate.

- Exposure-based waiving requires development of realistic exposure scenarios, reliable exposure measurements and validated exposure models for NMs over their whole life cycles.
- More information on external and internal exposure assessment is necessary to inform (targeted) hazard assessment on the most relevant exposure route, target cells/organs, and realistic (worst case) doses and forms to be applied.

5. Integrated testing of NMs
Due to the huge variety of NMs, extensive testing of all individual NMs is not feasible. The RA process should, therefore, be based on a targeted, tiered approach including rational grouping of NMs and waiving of tests. Rational grouping of NMs should be based on their PC properties (especially in situ in complex milieu) and biological effects, including mode of actions (J.1.5) or fate. The aims of grouping are to enable read-across within groups and to best plan further testing, e.g. by testing one, two or more group members which could represent “representative” or “extreme” properties within the group (see also previous chapters, including Chapter 4 specifically on “Grouping and Ranking”).

- More research is needed to improve understanding of relationships between PC properties and toxicological effects of NMs and enable grouping; read-across and in silico modelling.
- Better knowledge on the toxicokinetics (from testing or modelling) of NMs is needed to improve target (in vitro) hazard tests and QNAR with respect to dose and form or to waive testing.

6. Use of data from multiple sources in a WoE approach
The use of information from other sources and its acceptability within RA is an important overarching theme. Therefore, the RA approach should exploit hazard and exposure data from multiple sources, including extrapolation from non-nano forms (J.2.4) or other nano-forms, qualitative information, a full spectrum of models (e.g. from “simple” in vitro or in silico screening data to “complex” ecosystem data) and consider them all together in a WoE approach. It could even include highly non-conventional sources of information, all of which may contribute to a weight of evidence to support or refute the potential risks of a substance.

- Further work is required to develop RA strategies for NMs, allowing better exploitation of data from multiple sources. (J.1.4)
- Epidemiologic data (positive and negative results) should be integrated in a WoE.
- More effort should be put on validation and optimisation of alternative methods for regulatory purposes to be more efficiently used in a WoE and with the final goal to be used as standalone methods.
- Research (funding) should focus on producing suitable data for hazard characterisation and regulatory risk assessment.
- Relevant nano-specific biomarkers should be determined (J.1.10).
- Effective tools for data mining need to be developed (see also below).
- Rigorous data analysis and interpretation is crucial (J.1.13).
7. Use of data from in silico tools
RA should exploit data from standardised and validated in vitro and in silico tools (e.g. QNAR, PBPK) for NMs, which should be developed for full application for regulatory purposes. It should also consider suitable data from non-validated tools in a WoE approach (see above).

- PBPK models need to be developed to facilitate in vitro-in vivo extrapolation and predict the ADME behaviour of NMs. They can also support the development of substance (NM)-specific assessment factors used in the RA (J.1.8).
- Nano-specific QSARs (QNARs) need to be developed based on suitable descriptors for NMs, to predict (environmental) changes of NM characteristics over time, their interactions with biological systems and mechanisms of toxicity. This will have to be based on a large body of data and experience mainly gained from HTP in vitro assays. A roadmap for developing nano-specific QSAR models for regulatory purposes has been presented by Winkler et al. [60].

8. Biomonitoring/biomarkers
RA should make best use of biomarker data from well-established biomonitoring programs:

- Relevant "nano-specific" biomarkers of early effects need to be identified/established and related to specific exposure values based on epidemiological studies.
- In order to incorporate mechanisms for timely and informed decisions, health and environmental surveillance programs need to be defined as safety net and early warning systems (J.4).
• Strategies need to be developed and agreed to measure, analyse and report biomonitoring data to enable future data pooling and data storage
• Health surveillance registries need to be developed.

9. Characterisation of uncertainty
RA should include a transparent characterisation of uncertainty [e.g. by addressing risks in a probabilistic manner using stochastic approaches] and mechanisms for updating this information applying an iterative approach. Characterisation of uncertainty should provide important criteria for decisions on an acceptable or tolerable risk, if required.

• For risk prediction of NMs, there is a need to quantify uncertainty and have mechanisms for updating this information every time new data becomes available (J.3.4).
• It is recommended that uncertainty be addressed in a probabilistic manner using stochastic approaches such as Monte Carlo and Latin Hypercube Simulations (J.1.6).
• Guidance, training, communication and stakeholder involvement should contribute to an increased level of confidence in the RA and regulatory acceptance of the methods applied.

Figure 5.3. This diagram summarises bullet points 5-12 in the chapter text of the ‘best practices’ for RA of NMs which relate to Data collection, interpretation and integration, RA method development and Risk management practices.
10. Use of non-conventional RA approaches

For (early) decision-making, non-conventional RA approaches should be considered in addition, or as complementary to, the traditional practises prescribed under existing regulations. It is recommended to use MCDA allowing trade-offs across different decision criteria even under conditions of high uncertainty (J.1.11). The updating of initial RA when more information or data become available can be optimised, e.g. by a recently proposed process based on the introduction of Bayesian probability networks [61].

- Non-conventional tools need to be further developed to overcome the current critical limitations and deliver robust risk estimations in the short term. The requirement is to enable appropriate decision-making at a regulatory level (and for producers) rather than absolute risk estimation (J.3.5).
- It is recommended that currently available and novel RA approaches are tested and validated using realistic exposure scenarios to fully evaluate their functionalities and limitations (J.2).
- Existing novel RA approaches should be thoroughly and robustly tested on a wide range of Reference or Representative NMs (J.2.1). To this end, a set of Reference NMs should be established at international level for validation purposes.
- The development of control banding tools is currently a dynamic area of research, and several other tools are also under development. Their current limitation to produce almost only qualitative results should be addressed (J.1.7).

11. Adaptive and responsive risk governance

- Future research is needed to develop adaptive and more responsive risk governance frameworks for timely and informed decisions. (J.3) More effective risk communication strategies need to be further developed.
- Stakeholder communication and dialogue need to be enforced (see below).

12. Promotion of stakeholder involvement

Stakeholders include inter alia:

- Industries that develop NMs and know their specific properties best,
- Companies (downstream users, often small and medium sized and often not experienced with RA) that use NM in their products or work with NM-containing products,
- Researchers who develop test methods and test the substances,
- Regulators who have to make responsible decisions on the presence or absence of risk based on available information,
- Workers and the general public who may be exposed to NMs, and NGOs that represent interests of other stakeholders or the environment.

Risk perception of different stakeholders can influence risk management decisions (e.g. restrictions) and steer future development of new technologies. Risk perception can be affected by media, trends, trust, confidence and uncertainty, which may in turn depend on the levels of transparency of the risk assessment process. The concern of different stakeholders needs to be assessed and managed.

- The importance of stakeholder involvement in developing RA frameworks is widely acknowledged; but the active incorporation of their perspectives in the RA and management of NMs should be further promoted (J.3.1).
- Stakeholder concerns and risk perceptions should be identified and structured in a logic model.
- Studies on risk perception, effectiveness of stakeholder dialogue and risk communication should be conducted.
• It is recommended to use MCDA in combination with VoI (Value of Information), which takes the research needs of all stakeholders simultaneously into account (J.1.11).

• Risk communication strategies need to be further developed.

• To build trust of stakeholders, evidence-based guidance in the areas of concern assessment and management, dialogue and risk communication has to be developed.

• Stakeholders need to be trained to be able to take an objective/unbiased but also critical position and to take responsibility within their respective fields. Transparent actions are necessary to build trust. Stakeholders’ confidence in regulatory decisions has to be increased.

• Guidance on risk evaluation including societal balancing of benefits and risk needs to be developed.

**Towards best practices for Data Management of NMs**

The RA framework is also linked to Data Collection and Management (DM). Identified “best practices” for DM include:

1. **Harmonised procedures for data collection, storage and analysis**
   • There is a need for harmonised collection and analysis of data, using metrics relevant and (mutually) meaningful for exposure, hazard and RA in a regulatory context (A.1).
   • Test protocols should include information on the data to be reported; on results, on methodologies, and interpretation of data (A.2.4) [35].
   • The development of harmonised templates for documentation (publications and reporting) is recommended (A.2). They should be user-friendly to be widely used (A.4.3).
   • A minimum list of requirements should be established, with room for additional information if necessary (A.2.2).
   • The information reporting requirements should be flexible and adapted over time (A.2.1).
   • Ontologies need to be further developed to provide a common framework for description of NM properties. This is of great importance for e.g. data collection, interpretation and mining.

2. **Centralised collection and sharing of data.**

Increased access to databases collecting information on NM properties can provide useful information to facilitate grouping and read-across, avoid repetition of testing, allow WoE and establish algorithms for QNAR.

• Strategies for handling large data sets are required, as well as better mechanisms for centralised data collection and sharing. There is a need to develop databases with appropriate access for relevant users (A.3).
• Build upon existing databases, e.g. OECD-JRC Nanohub, PEROSH (IGF and TNO-initiated) NECID (occ. exposure) or NanoSafer databases (process emission database).
• A harmonised EU “nanoproduct” register could provide useful information for exposure assessment (A.3.6).
• The inclusion of negative data and/or proprietary data etc. should be encouraged (A.3.1).
• The proprietary/business sensitivity of data must be respected (A.3.2, A.3.4). Rules need to be defined.
• Different levels of access should be provided for open and restricted access.
• A platform for researchers should be provided to use/exchange unpublished or raw test data. Information obtained from tests on NMs in different research projects (for description of current databases see Annex V 5.1).
• Data sharing should be promoted/encouraged, e.g. by an interactive reciprocity system.
• User-friendliness will increase the willingness to provide, share and exploit data.

3. Quality control and relevance of the data
Data quality is relevant for building up databases but is also generally crucial for all risk assessment steps. Data gathered based on standard testing guidelines often offer the most robust and transparent information due to stringent and diverse reporting requirements. However, such information is not always available and may not offer the required insight into mechanisms of toxicity etc. more often obtained with a more research-centred approach that can be crucial to developing the information base required to assess the risk of NMs. Additionally, when interpreting data from testing it is important to distinguish where results indicate a true functional impact rather than simply a deviation from the norm that is not associated with an adverse effect (e.g. may be dealt with as part of normal system homeostasis). For further discussion on this issue of Data Quality see Annex V.

• There is a need to ensure quality control (validity, reliability) and relevance of the data (for explanation see Annex V 5.2) (A.4).
• Data submitted must be quality checked (generate a list of minimum requirements e.g. “evidence based medicine” database), such as: quality of test design, relevance of the applied test concentration (A.4.1).
• Criteria to assess the quality of the data must be defined, as well the experts (individuals or expert groups/panels) who should carry out the assessments (A.4.2).
• There is a need for adaptation of existing guidance and/or development of additional guidance on methods for testing NMs’ physicochemical properties, fate, exposure and effects.
• Further development/validation of alternative methods to animal testing is needed.
• For more confident use of research-driven (non-standard) information, criteria need to be developed (and enforced). Further developments and improvements of tools to evaluate the quality of tests are required, for instance based on ToxRTool [62] and specific quality control recommendations for NM-studies [63]. This has to some extent already been put forward for toxicological research e.g. by Klimisch [9] and updated for its relevance to NMs [63].

Threshold assay values should be defined and linked to true biological thresholds.

4. Use of tools for data mining
• There is a need to develop tools for automated data and text mining in existing and future databases (A.5). Examples of current initiatives include e.g. TNO’s database, sectorial databases as well as the tool developed through the FP7 NHECD project.

5. Reference materials and appropriate/validated protocols.
• Advice and recommendations should be given, including which reference materials and appropriate/validated protocols to use (A.2.3).
• A library/database of standard, approved reference materials in defined media should be generated. This would contribute to reducing the need for expert interpretation, as results could be compared to standard data (A.2.5)
Final Recommendations

The RA framework in the context of this document is understood as the entirety of EU law requiring a RA of substances for their safe use as such or in products/articles and the related guidance. The RA framework is, as such, considered to be applicable to NMs, even if they are not always explicitly addressed. Some adaptations may be required to ensure the safety of current and future types and applications of NMs and the integration of new tools, to which this chapter aims to make recommendations.

Approaches are often required to make RA more efficient and to prioritise the activities conducted to this effect. The current RA framework prioritises hazard over exposure which is also logical for NMs.

Future research outputs from the application of the ITS-NANO research prioritisation will provide increased information on hazards and exposure associated with NMs from multiple sources, including in vitro tests, read-across/grouping/ranking, in silico models and validated exposure models. The use of alternative methods is already encouraged or even requested for regulatory risk assessment, provided that the methods are validated and/or a scientific justification is provided.

A short term (<5 years) priority is to improve implementation of the available methods in current risk assessment practises. Most priorities must be addressed by a continuous process, depending on the speed and success of the development, standardisation/validation and finally regulatory acceptance of the ITS methods. The collection and management of high quality data is a near-term but also continuous priority. In addition, harmonised definition of NMs, improved NM characterisation, reference materials and exposure-based adaptations are all key priorities that will aid implementation.

Training and additional guidance are required for correct interpretation and integration of the NM data and to further improve confidence in the methods and their regulatory acceptance.
The main foreseen requirements in the mid- to long-term (5-15 years) are for novel quantitative tools developed or applied for RA of NMs, such as MCDA-based or stochastic approaches, as these may require adaptations of the current regulatory framework, which is mainly based on deterministic approaches. In addition, mid-term priorities include development and implementation of quantitative weight of evidence approaches, while long-term priorities include use of in silico tools and probabilistic approaches. In the distant future, risk assessment based on modelled data (with minimum targeted testing) and probabilistic risk assessment approaches are prioritised.

Continuous priorities include stepwise integration of ITS (hazard/exposure), promotion of stakeholder involvement, risk communication, acquisition of weight of evidence, characterisation of uncertainty, adaptive and responsive risk governance, harmonised procedures for (centralised) data collection, storage, mining and analysis, data collection and management, quality control of data, safe product design and processes. Approaches are often required to make the RA more efficient and to prioritise the activities conducted to this effect. The current RA framework prioritises hazard over exposure which is also logical for NMs.
Proceeding with the research prioritisation

The ITS-NANO ambition is to outline research priorities over the next 20 years that allow structured research to lead to intelligent testing strategies for risk assessment and management. This also includes rapid screening, improved models, identification of high risk materials, and implementation of strategies to counter these risks. This research should cover NMs' physicochemical characters, exposure and potential hazards, and how these three areas can enable a modelling of risk. The research enables further development of novel materials with inherent characters that emphasise a sustainable development.

Within each of the areas of physicochemical, exposure and hazard ID, specific issues have been identified and prioritised. Although the individual areas can partially develop independently, highly integrated research is required to advance this field as rapidly as possible. Hence, the identified priorities from each area have been combined to allow their integration and better identify the optimal course for progress. In generating the cross-cutting priorities, additional ideas to support these priorities are suggested.

Cross-cutting priorities

The generation of libraries of standard protocols to allow a tailored or streamlined approach to testing. This library is likely to be quite extensive, it will therefore be necessary to support it with a decision tree or matrix to allow individual stakeholders to identify the protocols most relevant to them.

The generation of reference and standard materials. Different standard materials may be required for different applications (e.g. calibration of a microscope, versus toxicity testing), but it would be advisable to assess the array of potential reference and standard materials so that where possible a material can be used for multiple applications.

The research strategy infers that there will be reduced testing with time and increased reliance on grouping/ranking in the short term, and more advanced modelling approaches in the longer term. Research into grouping/ranking and other modelling approaches is therefore a key priority, and while they appear on the right hand side of all of the research prioritisation diagrams, this reflects the need to acquire the basic information required for their design and implementation. In order to ensure that the right information is generated to construct such models, it is necessary to consider the structure, content and design of these models in the near future.

The generation of HTP techniques is relevant for all aspects of NM testing (as reported in the respective chapters). They are often used with respect to toxicology endpoints, but are equally relevant to physicochemical ID and perhaps some exposure assessment scenarios. Where possible it will also be advantageous to make HTP approaches multi-metric, allowing multiple different endpoints to be assessed in single assays. It is even feasible that a single HTP assay could measure PC characteristics alongside hazard endpoints, or hazard alongside exposure.
Recommendations related to specific chapters

Recommendation – Physicochemical ID

A Physicochemical Identity (Physicochemical ID) is defined as the dynamic pattern of physical and chemical characteristics (identified using appropriate analytical techniques) associated with one or several specified NMs during their life cycle. The physicochemical descriptors should describe key inherent features of the NMs (i.e. what they are), their influence on biological and environmental fates (i.e. where they go) and their inherent activity (i.e. what they do).

**In the short-term (<5 years)** it is recommended to provide different stakeholders with sets of standards/reference materials, validated instruments and standard method protocols to be applied for their needs, with a tailored approach in order to maximise the cost-effectiveness of physicochemical characterisation. Quantitative standardised approaches, and relative validations, will improve the data quality of PC characterisations.

**In the mid-term (5-10 years),** the availability of standard/reference materials, validated instruments and standard protocols will contribute to effective characterisation of NMs at different life cycle stages including following transformation by various processes and in complex matrices (bio-fluids, soils, etc.). They will also facilitate the development of novel physicochemical endpoints of NMs that could be helpful for unequivocal PC description of the NMs. **Novel techniques (and instrumentation) with demonstrated advantages (high throughput and multimetric characteristics) will be another goal for determination of the PC ID framework.** These instrumentations will be helpful for the analysis of PC properties of NMs, possibly linking them to Exposure and Hazard IDs.

In the long-term (10-15 years), **standardised protocols should be developed for PC monitoring and characterisation of NMs throughout their life cycles, in complex matrices, and in both in vitro and in vivo models.** A further recommendation is to ensure that developed techniques are flexible and integrated (tailored or tiered) to satisfy the specific requirements of all stakeholders (e.g. regulatory authorities, industries and academics).

In the distant future (>15 years), it is recommended that the PC ID should be aimed at providing suitable high quality data for in vitro, in vivo and in silico approaches for exposure assessment and hazard identification. Although challenging, rigorous functional and rational grouping of NMs according to their inherent properties, and robust modelling, should reduce requirements for further assessments to minimal, highly focused Exposure and Hazard ID tests (or even ultimately solely in silico tests).

Recommendation – Exposure ID

Of paramount importance is the methodological standardisation, which should rely on standard or reference materials for discriminating NMs from background particles in complex matrices, during the whole NM life cycle.

**In the short-term (<5 years),** in detail, in human exposure particular attention should be given to inhalation in occupational settings and ingestion, with the aim of defining in parallel internal doses in cells. In environmental exposure, a major focus should be the identification of long-term accumulation and concentration hotspots, with prioritisation of soils and sediments, which seem to be major sites of
accumulation. In both cases development and implementation of standardised protocols should be a continuous priority.

In the mid-term (5-10 years), data relating the concentrations of NMs in different matrices should be linked to the actual exposure of cells and organisms. In addition, sampling strategies to characterise in detail mid-priority environmental compartments, for example biota, should be addressed.

In the long-term (10-15 years), data should be available for lower priority exposure routes, such as dermal exposure, and environmental compartments, such as air and water. This information will enable grouping approaches based on matrices, exposure routes and/or compartments. In addition there is a requirement to model the exposure, bioaccumulation and fate of NMs in different life cycle stages.

In the distant future (>15 years), the development of standardised protocols for multi-metric and innovative detection tools will be required to enable faster delivery of information for risk assessment and for grouping, ranking and modelling.

Recommendation – Hazard ID

In the short term (<5 years), for hazard identification it will be necessary to develop more appropriate dose metrics that allow dosimetry studies to be conducted, to determine the mode of action of different NMs, which will require the identification of robust in vitro and in vivo short term or acute models of hazard for validation. These models can also be exploited to assess the bioavailability of NM and their toxicokinetics.

In the mid-term (5-10 years) the range of validated in vitro and in vivo models and standard protocols should be expanded to include long-term or chronic models. The availability of validated models along with an improved understanding of the mode of action will allow identification of reliable in vitro and in vivo biomarkers. The in vivo models in particular will be used to determine time courses of responses to NM exposure, allowing distinction between short- and long-term effects, long-term effects with rapid or delayed onset, and reversible and irreversible effects. There will also be a need to generate more relevant/multi-tissue in vitro models.

In the long term (10-15 years) knowledge of population-level effects and biomagnification will be required for environmental studies. For hazard studies in general we will require good in vitro and in vivo models of susceptibility to allow more focus on vulnerable individuals or populations. To accelerate the hazard testing of NM in vitro HTP screening will be essential, but initially this is likely to require back-up with in vivo and in vitro testing. In addition to the HTP testing, modelling approaches such as use of advanced models supported by both in vivo and in vitro hazard testing will be required.

In the distant future (>15 years) it is anticipated that in vitro HTP screening will become sufficiently robust to allow reduced but focused hazard testing, preferably without the need for in vivo tests, for human hazard estimation. Where sufficient information exists, hazard assessment will be possible for some NMs with no requirement for testing.
Recommendation – Grouping/Ranking

The grouping/ranking of NMs in terms of risk requires the integration of Physicochemical ID, Hazard-ID, and Exposure-ID as well as consideration of its integration and acceptance within current and future regulatory frameworks. Targeted experimental data is required to provide greater clarity and certainty regarding the basis of grouping, ranking and read-across between substances. This needs to be hypothesis-driven (rather than simple screening of arbitrary particle cohorts) in order to demonstrate the accuracy and consistency of outcomes and to provide sufficient weight of evidence to counterbalance uncertainty. Understanding the mode of action of observed toxicity and/or exposure and how this relates to physicochemical characteristics of a particle is a central theme in the required research. This in turn is influenced by and influences other key research issues such as the base sets of physicochemical characteristics that need to be characterised to inform grouping/ranking etc. for risk assessment. Lastly, a recommendation for future research is for the development of new approaches for grouping, ranking and robust foundations for numerical extrapolation/interpolation of results between species/models (e.g. IVIVE), analogous materials or even the same material but with differing properties such as surface area or coatings.

Recommendation – Implementation into risk frameworks

Future research outputs from the application of the ITS-NANO research prioritisation will provide increased information on hazards and exposure associated with NMs from multiple sources, including in vitro tests, read-across/grouping/ranking, in silico models and validated exposure models. The use of alternative methods is already encouraged or even requested for regulatory risk assessment, provided that the methods are validated and/or a scientific justification is provided.

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Training and additional guidance are required for correct interpretation and integration of the NM data and to further improve confidence in the methods and their regulatory acceptance.

The main foreseen requirements in the mid- to long-term (5-15 years) are for novel quantitative tools developed or applied for RA of NMs, such as MCDA-based or stochastic approaches, as these may require adaptations of the current regulatory framework, which is mainly based on deterministic approaches. In addition, mid-term priorities include development and implementation of quantitative weight of evidence approaches, while long-term priorities include use of in silico tools and probabilistic approaches. In the distant future, risk assessment based on modelled data (with minimum targeted testing) and probabilistic risk assessment approaches are prioritised.

Continuous priorities include stepwise integration of ITS (hazard/exposure), promotion of stakeholder involvement, risk communication, acquisition of weight of evidence, characterisation of uncertainty, adaptive and responsive risk governance, harmonised procedures for (centralised) data collection, storage, mining and analysis, data collection and management, quality control of data, safe prod-
uct design and processes. Approaches are often required to make the RA more efficient and to prioritise the activities conducted to this effect. The current RA framework prioritises hazard over exposure which is also logical for NMs.

**General comments**

Although each component of the paradigm/strategy is addressed in individual chapters in this document, it is imperative that the future research strategy has a cross-cutting perspective, integrating several research areas. Thus, it is vital to continuously work towards goals such as the development and implementation of: (i) a common language (i.e. shared ontology, terminology and nomenclature); (ii) comprehensive, user-friendly information-sharing tools (e.g. databases); (iii) synergistically applicable advanced techniques (by providing, for instance, an efficient research framework and facilitating access to advanced analytical equipment); and (iv) in-depth RA methodologies. This work has already been initiated to a large extent through various EU nano-projects and the Nanosafety cluster.

It is necessary that the research links to other related areas already in progress e.g. infrastructure approaches (e.g. QualityNANO), database (NANOSAFETYCLUSTER), ontology (SEE EU PROJECTS), Specific risk-assessment and management approaches (e.g. MARINA and NANOVALID), and modelling approaches (See EU) etc.

Finally, the success of ITS-NANO will be the application of the research priorities in future research projects. Work in this respect has already commenced with Marina and NANOReg. It is likely that other projects may use the outputs of this project as well. This will greatly enhance harmonisation of approaches and outputs for these projects in the future, thereby providing added value to the research in this area. It is therefore anticipated that within the next three years significant gains will be made with respect to the priorities outlined above. Review of progress towards the ITS in the mid- to long-term will be required, and updates of the ITS-NANO research prioritisation document may be useful in this respect.
Annex I – member of ITS-Nano and Stakeholder panel

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Annex II – Identification of knowledge gaps and strategic priorities for human and environmental hazard, exposure and risk assessment of engineered nanomaterials

Executive Summary

Nanoscience and Nanotechnologies are among the fastest growing research areas of the last decade. As the market for nanotechnology increases, so will the likelihood of human and environmental exposure to the products of nanotechnology. Accordingly, the potential risks in these areas need to be assessed. However, it is broadly acknowledged that the current risk evaluation approach is not fully suitable for nanomaterials (NM), due to their unique characteristics. In addition, there are gaps in the research landscape which need to be addressed in order to ensure that the information provided can support an appropriate, complete and top quality risk evaluation. Consequently, there is an urgent need for new approaches which consider the unique aspects of engineered nanomaterials and specifically support the development of an Intelligent Testing Strategy (ITS).

In order to facilitate the development of an ITS, this report provides an overview of the current status and identifies existing gaps in knowledge/research pertaining to hazard and risk assessment of engineered nanomaterials. This information is gathered from publicly available information, such as peer reviewed literature, published reports from governments and regulators and partner knowledge relating to current state-of-the-art.

In drawing together the information to identify knowledge and research gaps in this area, a comprehensive list of potentially relevant documents was compiled. This consisted of review articles and reports from government, regulatory bodies and other relevant organisations. Each of these documents was then summarised by various experts working on the ITS-nano project, and the main knowledge gaps and potential areas for focussed future research were highlighted. In support of this, “heat maps” were generated to give an overview of current hazard-related research. These were based on the number of publications in each of several specific research areas. Lastly, this document was moderated, and the list of knowledge gaps prioritised by a multidisciplinary panel of expert stakeholders at the first ITS-nano Stakeholder Workshop (Edinburgh, September 2012).

The ITS-nano project complements various other projects working in this area, for example the Nanosafety Vision 2015-2020, and European Commission FP7 funded projects; MARINA, NanoValid and others, which aim to develop specific reference methods and approaches for the main steps in managing the potential risk of nanomaterials. ITS-nano will work in concert with these projects to ensure that a holistic and inclusive ITS is created.

To a large extent, current EU legislation allows NM risks to be dealt with under the existing framework without having nanospecific provisions (e.g. REACH, Consumer products). Some recently revised pieces of legislation do address NM directly (Cosmetics, Food, Biocides), however, further development in this area is still necessary. A provision of these pieces of legislation is to ensure a high level of safety of substances or products to humans and the environment. This can be achieved by a risk characterisation based on specific information requirements (e.g. REACH) or by fulfilling certain standards (e.g. for consumer products, medical devices). REACH Regulation EC 1907/2006 regulates chemical substances, including nanomate-
rials, and requires information for safety assessment depending on the tonnage level of the substance produced or imported in the EU. The information requirements usually refer to standard (guideline) tests which are defined for the different endpoints. However, these regulations also include provisions or rules to adapt the standard testing regimes to provide for substance tailored testing, alternative testing, grouping, read-across or waiving of testing. Vertebrate animal testing should only be performed as a last resort, however, no validated alternative methods (in general, not only for nanomaterials) are currently available for relevant endpoints, such as repeated dose toxicity and reproductive/developmental toxicity. Health and safety at work is another key regulatory area with work on-going to establish the suitability of the EU-OSH legal framework in its current form relative to nano-specific workplace risks. It is expected that the results of this study will help bridge the information gap in order to widen the evidence base as much as possible.

A further regulatory consideration is the emphasis being placed on adopting life cycle thinking-approaches or, more formally, a life cycle assessment (LCA) for emerging technologies, which includes consideration of health impacts.

Multiple uncertainties hindering regulatory Risk Assessment (RA) of nanomaterials have been identified, at least partially due to current insufficiencies in data. Likewise, there is also a lack of knowledge on how to group or rank these materials in order to identify and enable a risk assessment of the most potent material which, for example, could be done by grouping material according to their (i) physicochemical properties (especially in situ in complex milieu), and (ii) biological effects. Biological effect (including mode of action) is the key driver for understanding and grouping of NM. Taking into consideration the diversity of nanomaterials and the complexity of nanoscale systems, it is unlikely that a single physicochemical (PC) characteristic would be sufficient to describe the toxicity of NM. Moreover, different properties are likely to be related to different aspects of their interaction with living organisms and cells. NM must be adequately characterised at various stages in order to understand the changes to PC properties throughout the life cycle. This enables understanding of how NM behave in their environment and how interactions such as agglomeration and dispersion impact on real-life exposure scenarios (e.g. routes of exposure, internal dose received) and the various mechanisms of biological action.

While a number of techniques are available to characterise NM, it is not yet certain that the most appropriate PC properties have been identified. Many of the remaining limitations in this area result from a lack of technologies and standardised methodologies for measuring PC properties in complex matrices and discriminating between released and background NM at various stages of their life cycle. A critical obstruction to research in this area seems to be represented by a lack of NM standards or, specifically, a “gold standard” against which all measurements/tests would be compared. Since characterisation and testing at all stages of the NM life cycle may be sub-optimal or not possible (time and resource constraints), modelling approaches such as structure-activity relationships (SAR) should be encouraged. It is important that this information is assessed and verified and gaps identified in order to reduce the need for hazard testing by developing increasingly reliable models. While studies are on-going to generate quantitative SAR (QSAR) approaches for nanomaterials, no such methodology is currently available and validated on a broad array of materials. To support such development and reduce the high levels of uncertainty currently existing, work at all levels (characterisation, exposure, hazard and risk) is required to generate sufficient appropriate and reliable information. These in silico models should also enable read-across to conventional chemicals in bulk forms, as well as read across between various model species and between human and environmental studies. In order to make use of read-across for nanomaterials
based on informative materials, a greater understanding of the fundamental drivers of toxicity based on PC characteristics is needed.

The risks of human exposure to engineered NM have been comprehensively reviewed in several publications which arrive at similar conclusions about the priorities for future activities relevant to exposure assessment. These can be summarised as:

- identification of nanomaterials and description of exposure;
- measurement of exposures to nanomaterials and efficacy of risk management measures;
- training of workers and practical handling guidelines for activities involving nanomaterials in the workplace.

There are a number of issues affecting exposure estimation, including the need for better discrimination and characterisation of NM, the application of exposure models, the choice of metric, and instrument and measurement strategy. There is limited evidence of validation for occupational exposure, which indicates that model estimates should not be relied on alone without further confirmation of their validity in individual cases. There is also a need to further develop the evidence base about the potential for release of articles which contain NM or are coated with NM from a whole range of activities and processes. This would include measurements made in industrial scenarios as well as laboratory based simulation experiments and is relevant for both human and environmental exposure. There is also a need for harmonised collection and analysis of data using metrics identified from hazard studies, which are relevant and mutually meaningful to exposure, hazard and risk assessment in a regulatory context. This would enable a more extensive validation of methods and models to be carried out if required and, based on these validation exercises, standardised or new approaches could be adopted or developed.

Environmental exposure to nanomaterials occurs through various complex media including water, soil, sediment and air. In recent years, there has been an emergence of studies that attempt to calculate predicted environmental concentrations (PEC) for nanomaterials, however, as pointed out in the ENRHES final report, these studies have used quite simplistic exposure models. Some may lack reliability in their PEC-forecasts, and many are not currently validated (although further developments have taken place since the ENRHES report was published). An absence of validated models for estimation of environmental fate of NM for regulatory use has also been identified, including lack of knowledge in assessing and quantifying potential NM emissions to the environment, characterisation of released NM, and assessment and optimisation of exposure model efficacy. There is also a lack of nano-relevant analytical methods to measure actual exposure concentrations and verify behaviour and stability of nanomaterials (including aggregation or agglomeration etc.) in complex media. Modelling is a practical way of obtaining predicted first-level environmental concentrations taking into account the current lack of actual measured environmental concentrations, but requires the development of a database for model input parameters (e.g. transfer and partitioning coefficients, emission factors) which, at present, in some cases can only be based on crude assumptions. A few papers exist that physically detect and characterise nanomaterials in the environment. These papers point out the lack of suitable methods for detection and characterisation of nanomaterials when they are embedded in complex matrices, including water, soil, sediment and food and, hence, the need for further optimisation and development of analytical methods. A combination of several techniques is highlighted as a possible prerequisite for nanomaterials detection and characterisation of nanomaterials in a complex matrix.
The "hazard" of NM in terms of this report is expressed as nano toxicology and eco-
toxicology. There are several hundred publications describing research into the tox-
icity of NM in various media (human and environmental) dating back several years. 
These publications have been assessed to generate an overall picture of where the 
scientific field is now in terms of research into nanomaterials and their biological 
effects in humans and the environment. This overview is intended to provide only a 
general indication of the current level of knowledge, as the publications included in 
the heat maps have not been evaluated for "quality" or reliability.

Hazard to human health was classified according to the main exposure routes 
(pulmonary, dermal, ingestion) as well as broken down into local and systemic 
effects. There are far more publications relating to the hazards of pulmonary ex-
posure to NM than either dermal or gastrointestinal exposure routes, although little 
research has been published exploring disease or susceptibility models. The ma-
jority of studies investigating the relationship between PC properties and toxicity 
have largely focussed on the impact of NM size, surface area and solubility, with 
limited information on how other PC properties may result in toxicity. Most of the 
studies focus on "early stage" effects such as cytotoxicity and inflammation, with 
little investigation into the medium to long-term effects. There are a number of 
publications investigating the biokinetics of NM after initial exposure, yet few then 
proceed to investigate the systemic effects in other organs after distribution/trans-
location of NM. In comparison to the number of publications investigating the im-
 pact of NM on human health, there are very few studies looking directly at the im-
pact of NM in the environment. Following environmental exposure, NM size seems 
to be the only PC property that has been investigated in any depth in relation to 
hazard (and even that is limited). The most severe biological endpoints (e.g. lethali-
ity) appear to be more thoroughly researched in all environmental compartments 
apart from sediment. There are some biokinetics studies in freshwater and terres-
trial compartments, whilst other more mechanistic impacts are largely unknown.

In general, for both human and environmental nanotoxicology there appears to 
be a need for better and more reliable in vitro models (including validation of ex-
sting models and ensuring relevance to the in vivo state), high throughput in vitro 
models and computational models. Whilst the ultimate aim is to establish in silico 
modelling tools, there is still a long way to go in terms of acquiring sufficient haz-
ard data and relating that to PC characterisation. This data gap needs to be ad-
dressed and uncertainty more fully understood, in order to develop and validate 
these computational models.

Recent reviews of the published nano environmental health and safety data con-
cluded that the conventional RA paradigm is applicable to NM, if properly adapted 
to address their novelies and thoroughly and robustly tested on a wide range of 
NM. To address this, three key areas for future research have been recommended: 
(i) development of adaptive and more responsive risk governance frameworks; (ii) 
systematic evaluation of available alternative/complementary tools to RA and, 
if needed, further development of tools; (iii) definition of a health and environ-
mental surveillance program as safety net and early warning system. In general, 
most existing risk frameworks are "generic", i.e. in principle they address both hu-
man health and environmental concerns with a focus on occupational settings. 
Their structure is iterative, transparent and adaptive to some extent. Whilst some 
frameworks are quite specific in scope, most seem able to handle and adapt to 
multiple risk decision contexts related to NM, but none of them specifically incor-
porate mechanisms for timely and informed decision making. Real incorporation 
of stakeholder perspectives in the RA process for NM is still quite rare. The use of a 
tiered approach is recommended with the assessment of risks on a case-by-case 
basis. Most of the available non-conventional risk tools are not intended to facili-
tate regulatory decision making, but to serve as preliminary hazard/risk screening or research prioritisation tools. These tools are aimed at helping industry to identify relevant sources of risk in the lifecycle of synthetic NM and pinpoint areas of knowledge deficits. Most of the existing tools have not been thoroughly tested in terms of functionalities and limitations over a range of applications and/or NM. The RA provisions provided in EU chemicals and consumer protection legislation in principle apply to NM, but amendments have recently been discussed and published in some cases in order to specifically address NM and their novelties in a more appropriate way (e.g. new appendixes to REACH guidance documents).

Summary of major knowledge gaps and research priorities

This information will feed directly into the development of an ITS (Work Package 3 of the ITS-nano project) in order to provide the most appropriate direction for the strategy. The goals and priorities identified here have been checked for accuracy and prioritised by experts at a Stakeholder workshop in September 2012. The research and knowledge gaps listed below are shown in priority order with the most important issue in each area listed first.

Further details and recommendations for future research areas are given in the full Gap Analysis document (Identification of Knowledge Gaps and Strategic Priorities for Human and Environmental Hazard, Exposure and Risk Assessment of Engineered Nanomaterials), which is publicly available on the ITS-nano website (http://www.its-nano.eu/the-project/project-output).

A. Data management

A.1 There is a need for harmonised collection and analysis of data, using metrics relevant and mutually meaningful to exposure, hazard and risk assessment in a regulatory context. (section 3.1)

A.2 The development of a template for documentation (publications and reporting) is recommended.

A.2.1 The requirements should be flexible and adapted over time.

A.2.2 A minimum list of requirements should be listed, with room for additional information if desired.

A.2.3 Advice and recommendations should be given, including which reference materials and appropriate/validated protocols to use.

A.2.4 Protocols should include what is to be reported, methodologies, and interpretation of data. (This approach has already been started in the regulatory area (e.g. ECHA guidance, R7 REACH).

A.2.5 A library/database of standard, approved reference materials in defined media should be generated. This should reduce the need for expert interpretation, as results can be compared to standard data.

A.3 Strategies for handling these large data sets are required as well as better mechanisms for centralised collection and sharing of data. There is a need to develop databases with appropriate access for those who need it.

A.3.1 Negative data, commercial data etc. should be included.

A.3.2 The proprietary/business sensitivity of data must be considered (not all regulators publish submitted data (e.g. medical devices); REACH data are mainly public, but some parts remain confidential).

A.3.3 Toxicology and physicochemical data should be made available after registration of products.
A.3.4 Business aspects will remain confidential.

A.3.5 Perhaps a two-level database: research data, and data owned by industry?

A.3.6 Consider/build upon existing databases, e.g. OECD-JRC Nanohub, PEROSH (IGF and TNO-initiated) NECID database (occ. exposure.), or NanoSafer databases (process emission database); a nanoproduct register could provide useful information for exposure assessment.

A.4 There is a need to ensure quality control and relevance of the database(s).

A.4.1 Data submitted must be quality checked (generate a list of minimum requirements, e.g. “evidence based medicine” database), such as: quality of test design, relevance of the applied test concentration.

A.4.2 Criteria to assess the quality of the data must be identified, as well as who (individuals or expert groups/panels) will carry out the assessments.

A.4.3 The format for information submitted to databases must be specified. It should not be too onerous, otherwise it will not be widely used.

A.5 There is a need to develop tools for data mining in existing databases (e.g. TNO’s database, for instance, or sectorial databases).

B. Physicochemical characterisation

B.1 There is a requirement to minimise the effort for characterisation in the future, therefore, a minimum set of parameters for characterisation should be defined. (section 2.1.1)

B.1.1 “Nanoproperty” should be used rather than “structure” activity relationships (i.e. QNAR vs. QSAR) to further refine these NM physicochemical characterisation “wish lists” to make them more focused and financially viable. (QNAR is already used as an abbreviation for quantitative nanostructure activity relationship, but we would suggest changing this to nanoproperty).

B.2 There may be several lists of characterisation requirements (tiered approach?), depending on various scenarios or research questions across different NM.

B.2.1 Developing a decision tree is probably preferable to using matrices (matrices are more likely to be influenced by NM grouping).

B.2.2 Full characterisation (i.e. measuring every possible PC parameter) is not recommended in most cases, especially since there is no current definition of “full characterisation”. The PC properties characterised should, instead, be appropriate to the scenario under investigation.

B.2.3 The cost to industry must be considered, i.e. sufficient to characterise relevant properties and not lead to unnecessary costs. If the requirements are too great, it is unlikely that many industries, in particular SMEs, will be able to afford this effort, which may then reduce potential innovation.

B.2.4 Simple criteria are required, which must be achievable in commercial labs as well as for research.

B.3 Characterisation methodologies and technologies (instrumentation) should be developed and optimised to provide “standard protocols” for detection, characterisation and extraction of NM in different media (sections 3.2, 3.2.1).
B.3.1 New methods/protocols are required as well as identifying the preferred existing methods, their limitations, and the relevance of scale of the uncertainties compared to the regulatory needs.

B.3.2 Protocols need to be developed for all stages of the life-cycle (including various time points in vivo, and during storage).

B.3.3 Mechanisms affecting uncertainty need to be identified in order to reduce uncertainty.

B.3.4 Protocols should be developed for agglomerations, aggregations and dispersions.

B.3.5 A major issue is the identification and throughput of NM PC properties in complex matrices and nanoscale in situ analysis of coatings, charge, dissolution etc.

B.3.6 Formal validation of test protocols is not required during the early stages, as there is currently no “gold standard” against which protocols can be validated.

B.3.7 It should be possible to identify additional endpoints for physicochemical characterisation (catalytic activity, for instance), which are specifically relevant for nano.

B.3.8 Solubility kinetics is a key aspect, and a more suitable nano relevant alternative to log K<sub>ow</sub> should be identified. There is a need for a tool that utilises surface characteristics to determine how the NM is likely to behave and predict its transport, fate and impact.

B.4 Research is needed to better understand the characteristics of NM that best relate to their toxicity, the conditions in which these characteristics should be assessed, and to identify strategies to group NM based upon these characteristics (section 2.2).

B.4.1 The “reactivity” of a NM should be determined (how it is transformed, e.g. sulphated, redox, adsorption on other materials onto surface, agglomeration, dissolution, photosensitivity etc.), how this changes the PC properties of an NM, and how that influences the behaviour (and therefore effect) of the NM during its life cycle (i.e. corona formation, weathering or aging).

B.5 A critical obstruction to furthering knowledge and research into the physicochemical characterisation of NM is represented by a lack of fully characterised NM standards/reference materials (sections 2.1.1 and 3.2).

B.6 There is a need for more qualified, trained experts to use the new and existing technologies/methods (this extends to the need for expert analysis and interpretation of the resulting data).

B.6.1 Ensures that the techniques/methods are carried out properly to a standard level (quality control).

B.6.2 Alternatively, good guidelines, including how to interpret data, should be made available for key techniques.

NB. Using PC properties as a method for grouping NM must be fluid and reflective to accommodate increasing understanding of NM effects as research in this area progresses (section 2.1.3).
C. Exposure Assessment

C.1 It is a pressing requirement to develop technologies and methodologies that can accurately identify NM, monitor, quantitify and measure their concentrations [number and/or mass] and physicochemical properties at various stages of their life-cycle (manufacture, use and disposal) (sections 2.1.1, 2.1.3, 3.1, 3.2). For example:

C.1.1 Appropriate instrumentation and measurement strategies, metrics for exposure, and exposure models for both environmental and human exposure are required (section 3.1).

C.1.2 The recommended practice is that measurements should encompass assessment of at least mass, but where possible also number and/or surface area concentration (section 3.1).

C.1.3 In order to accurately estimate human and environmental exposure to NM, it is important to enable discrimination of engineered NM from background NM (natural or incidental) and spatial and temporal variability (sections 3.1, 3.2).

C.1.4 There is an absence of measurement methods and terminology to describe “bundles” or “clumps” of high aspect ratio NM or agglomerated “granular” NM, however, their presence should be noted in any exposure assessment (section 3.1).

C.1.5 Appropriate expert data analysis and interpretation are required alongside these new and modified tools.

C.1.6 Exposure should be considered with regard to real dosimetry and the in situ PC properties of NM (e.g. single particle or agglomerates). A better understanding of NM characteristics during real exposure scenarios will allow these to be better replicated in a research environment (section 2.2).

C.1.7 There is a need for a much improved sampling strategy to be implemented. In the context of REACH, the development of a strategy specifically for REACH compliance issues is necessary (section 3.1).

C.1.8 In relation to measuring NM exposure, research is needed to identify a set of PC properties that may influence the effective internal dose that cells or animals receive (section 2.1.2).

C.1.9 There is a research need to better understand the dustiness of nanopowders in general process-specific release rates (synthesis, powder dustiness, grinding, spraying etc.) and how this relates to airborne exposure of NM (section 3.1).

C.2 There is a key gap in determining whether existing exposure assessment models are appropriate for NM.

C.2.1 Substantial additional work is required in order to validate exposure models for use with NM. This includes generation of base data for validation e.g. through simulations and use of this data to test and adapt the models (section 3.1).

C.2.2 Models for prediction of hot spots and hot spot concentrations are essential. The critical exposures in the environment will be due to accumulation around the sources and in favourable bio-geo-chemical environments.

C.2.3 It is important to identify any bioaccumulation, bioconcentration, biomagnification effects that may occur (in human as well as environment, although this is particularly relevant to trophic transfer).
C.2.4 The limited evidence of validation for occupational and environmental exposure indicates that model estimates should not be relied on alone without further confirmation of their validity in individual cases. Compound-specific methods may need to be applied (as is done in quartz, asbestos, MMF, SVOC and VOC determinations) (section 3.1).

C.2.5 Exposure assessment should be considered along the whole value chain (from manufacture to product to disposal).

C.3 There is a need for better understanding of emissions and exposure routes (when and where exposure is likely to take place), as this is likely to influence the properties and behaviour of a NM and, therefore, the likely level of exposure (and consequently risk).

C.3.1 This also influences where and when monitoring should take place.

C.3.2 There is a need to know production amounts and production processes (research collaborations with industry required).

C.3.3 Different manufacturing processes/methods (e.g. by combustion, or underwater) will impact the likelihood and route of exposure as well as who is likely to be exposed.

C.3.4 At this point, (pre-manufacture) can carry out RA to determine whether exposure is a realistic concern or not.

C.3.5 There is a need to further develop the evidence base about the potential for release of NM from a whole range of types of activities and processes relevant to both release and exposure for humans and for the environment (sections 3.1, 3.2).

C.4 There are a lack of suitable methods for detection, characterisation and extraction of NM when they are embedded in complex matrices. Hence, there is a need for further optimisation and development of analytical methods (sections 3.2, 3.2.1).

C.4.1 High throughput techniques for the identification/quantification of NM in complex matrices are key.

C.4.2 Some techniques are available, but targeted procedures are not present and therefore there is no knowledge on detection limits for specific compounds in specific matrices. For certain exposure measurements, further understanding of instrument responses are required to get "true" values (e.g. airborne particle measurements).

NB. In a regulatory context, an emphasis is being placed on adopting life-cycle thinking approaches or, more formally, a life-cycle assessment (LCA) for emerging technologies (section 2.3.2.2).

D. Human Exposure

D.1 Strategies which encourage comparison between workplace air concentrations and personal exposure are recommended. They must consider near-field and far-field exposure modelling due to rapid dilution and scavenging of small particles in air (section 3.1).

D.2 It is important to develop practical handling guidelines and to train workers for activities involving NM in the workplace (section 3.1).

E. Environmental Exposure

E.1 Further research into environmental NM exposure is required, including:
E.1.1 Identification and assessments of exposure routes (e.g. atmospheric depositions, waste emissions from production and manufacturing, consumption and respiration processes, application of sludge from waste water treatment plants to landfills or (agricultural) soil) (section 3.2.3).

E.1.2 Development of analytical methods to measure/verify concentrations of NM in waste streams, soil and subsequently water. Characterisation of released NM in waste streams, soil and water (section 3.2).

E.1.3 Quantitative measurements of NM in aquatic matrices on-line techniques that can integrate Laser-Induced Breakdown Detection (LiBD) or Flow Field Flow Fractionation (FIFFF) with subsequent characterisation techniques (such as spectroscopic, microscopic, or biosensor measurement) are recommended for complete characterisation (section 3.2.1).

E.1.4 Development of validated models for estimation of environmental fate of NM (section 3.2).

E.2 There is a need to improve understanding of nanoparticle dispersion in environmentally relevant media with respect to both controlled single organism studies (and eventually mesocosm studies) and which component of the exposure mixture is responsible for the biologically relevant dose.

E.3 In developing appropriate indicator indices (models and biomarkers) for environmental exposure, it is recommended that a holistic approach based on assessment of their sources, behaviour and sinks is adopted (section 3.2.5).

NB. ECHA has recently published recommendations on how to characterise environmental dose-response relationships for NM under REACH Regulation (section 5.3.2).

F. Hazard assessment

F.1 Determining mode of action is key.

F.1.1 Both in vitro and in vivo studies should be undertaken to obtain dose-response data to characterise the biological ID at different levels (section 5.3.1).

F.1.2 Relevant/important to feed into risk assessments.

F.1.3 Important in the identification of relevant and useful biomarkers of exposure/hazard.

F.2 There is a need to develop appropriate “grouping” of NM based upon their hazard and mechanisms of toxicity.

F.2.1 Research is required to understand what the true “biological ID” is (i.e. how biological systems see and respond to the NM) in order to use this as a grouping tool.

F.3 Validated test systems, both in vivo and in vitro, need to be developed, and existing validated test systems need to be proved for appropriateness and if necessary adapted to ensure acceptance by regulators.

F.3.1 There is a lack of validation of NM hazard methodologies, in part at least because of the need for reference classifications and well characterised and harmonised controls/test items.

F.3.2 Different validated methods may be required, depending on the type of NM.
F.3.3 Validated methods for long-term or chronic effects are required.
F.3.4 Validated methods for repeated dose toxicity are required (section 2.3.2).
F.3.5 Validated methods for reproductive/developmental toxicity are required (section 2.3.2).
F.3.6 “Realistic” (or “Realistic worst case”) dosing for relevant real-life scenarios should be used in hazard studies, where this information is available.
F.3.7 There is a need for validated multi-tissue models (physiologically relevant systems) that allow communication between different cell types to be investigated.
F.3.8 Models of disease that impart susceptibility to NM induced toxicity are required.
F.3.9 The most relevant species should be identified for specific scenarios (realistic exposure) and guidelines/standard protocols recommended.
F.3.10 The suitability of existing in vitro methods must be determined (and validated) to ensure in vitro tests are predictive of in vivo toxicity.
F.3.11 Protocols must be identified that can be applied at all stages of the NM life-cycle and should be carried out at life-cycle stages that best represent realistic exposure scenarios.
F.3.12 There is potential to use low-level species as screening tools (need to consider the views/interests of industry and regulators).
F.3.13 High throughput/high content screening methods are key and may be used to trigger future research in different areas.

F.4 Strategies to reduce vertebrate testing (alternative methods) are required. These could involve improved and more reliable in vitro models (including validation of existing models), high throughput in vitro models and computational models (section 4.4).

F.5 In silico modelling tools are not yet developed and the relationship between physicochemical properties and toxicological effects of NM has not yet been fully established (section 2.3.2).
F.5.1 Very few studies have attempted to develop QSAR tools for NM and toxicity-based nano QSAR/QNARs are almost non-existent (section 5.2.1).
F.5.2 There is an urgent need to conduct and verify structure activity relationships using an array of PC properties and hazard endpoints for different NM, so more work at all levels (characterisation, exposure, hazard and risk data) is required to generate and validate such systems (section 2.1.2, 2.2).
F.5.3 In order to facilitate the IVIVE [in vitro-in vivo extrapolation] risk assessment tools of NM, there is a need to develop PBPK (physiologically-based pharmacokinetic) models, which can successfully predict the ADME (absorption, distribution, metabolism and excretion) behaviour of NM in both experimental animals and humans. (section 5.2.3).

F.6 In order to make use of read-across for NM based on “analogous” materials, a greater understanding of the fundamental drivers of toxicity based on physicochemical characteristics is needed (section 4.3).
F.6.1 The use of extrapolation and read-across between: materials of similar/related characteristics; species; human and environmental; as well as in vivo and in vitro should be more exploited.
F.6.2 The impact of exposure route/pathways on PC characteristics (e.g. surface properties, protein corona etc.) and, therefore, the impact on toxicity, needs investigation.

F.6.3 There is a need for better understanding of how NM interact with their environment, as factors such as dispersion, aggregations and agglomeration can influence NM characteristics in exposure and hazard studies (sections 2.2, 3.1).

F.7 It should be determined which dispersion protocols are most appropriate for risk assessment purposes (sections 2.2, 3.1).

F.7.1 Experimental dispersions should aim to be realistic and not necessarily monodispersed (if that is not how the NM will behave in situ).

F.7.2 Should monodispersed be used as “worst case”? 

F.8 While some biomarkers (in vitro and in vivo) of hazard exist (e.g. indicators of inflammation, oxidative stress and cytotoxicity), the identification of a subgroup of the most reliable and relevant biomarkers is lacking. Identification and validation of these biomarkers would be a priority for standard protocol development. Very little work has been carried out on biomarkers in ecotoxicology, making this an additional area for future research.

F.9 There is a need to investigate in vitro-in vivo extrapolations (IVIVE) to determine the relevance of in vitro methods and promote development of alternatives.

F.10 More guidance and examples are needed to allow differentiation between statistical significance and biological significance (i.e. what responses could be statistically relevant, and yet just be an example of a homeostatic response). Specific criteria to be considered are adequacy, reliability, relevance, (these three representing the Klimisch criteria) statistical power and toxicological significance.

F.11 There is limited knowledge on the behaviour (and, hence, hazard) of NM when part of a matrix (e.g. food, cosmetics, epoxy composite, paint, tissues/cells, sea water etc.) (section 4.1.1).

G. Human hazard

G.1 There is a need to implement regulatory changes where an effect of NM has been identified (e.g. nano effect following pulmonary exposure).

G.2 There is a need for further research into the medium to long-term impacts as well as systemic effects of NM (sections 4.1, 4.1.1, 4.1.1.3).

G.2.1 Research into the hazards associated with pulmonary exposure to NM generally focuses on the “early stage” effects (e.g. cytotoxicity, inflammation) with the medium to long-term effects (e.g. fibrosis, carcinogenicity) less frequently studied (section 4.1.1).

G.2.2 Systemic effects of inhaled NM on the cardiovascular system are frequently studied, but comparatively few studies have been carried out on other target organs/systems. There is a lack of dosimetry studies in this area. Nanomedicine and i.v. injection studies have purposefully been left out of this study (sections 4.1, 4.1.1.3).

G.3 There are few in vivo models of disease that might be relevant to investigate susceptibility to NM hazard.
G.4 There is a requirement to determine the pulmonary hazards (including mechanisms of action) associated with specific PC properties (other than size, surface area and solubility) which have not been extensively studied following exposure to NM (section 4.1.1).

G.5 Ingestion is a likely route of NM exposure, suggesting that research is required in this area to determine whether there is a nano (vs. bulk) related effect following exposure, and what those effects might be (section 4.1.2).

G.5.1 Very little research has been carried out with respect to hazard responses following ingestion of NM, with a dearth of studies investigating the medium to long-term effects (section 4.1.2).

G.5.2 Investigation is required to determine whether NM induced inflammation is a cause of disease.

G.6 There are few published studies of dermal exposure to NM, however, the current, generally accepted, understanding in this area (little or no size dependent effect) suggests the main research requirements for the future should be constrained to investigating NM toxicity in compromised/damaged skin models (section 4.1.3).

G.7 There is a need to develop criteria on how to make best use of human data for hazard/risk assessment (humans may already have been exposed to some NM [those without bulk form] for a long time), so this is relevant and useful information, although there may be drawbacks, e.g. statistical power, exposure assessment, poor knowledge to what people were exposed, cofounders etc.).

H. Environmental hazard

H.1 Given that sediments are considered a “sink” for insoluble NM released into the aquatic environment, there is a lack of research in this area which should be addressed (section 4.2.3).

H.1.1 Sediment studies are also useful for providing historical baseline exposure data.

H.1.2 As well as sediments, freshwater, marine, waste water and long term studies are also important.

H.2 More biologically relevant species could be studied to better understand ecotoxicity.

H.2.1 Species should be chosen based on the likely exposure route and uptake mechanism.

H.2.2 More understanding is needed on the relevance of species susceptibility (sensitivity).

H.2.3 The species tested may be different, depending on the aim of the study, e.g. a research approach versus a regulatory approach.

H.2.4 In aquatic environments (freshwater, estuarine, marine), the most frequently studied organisms are fish, algae and invertebrates. In sediments, the majority of research is carried out using invertebrates or, to a lesser extent, bacteria. Most terrestrial exposure studies currently focus on invertebrates (particularly worms) and bacteria. Further investigation is required as to whether different models, or refinement of existing models, are needed for improved ecotoxicity testing of NM (sections 4.2.1, 4.2.2, 4.2.3, 4.2.4).

H.2.5 The impacts of NM on ecosystem services should also be assessed.
H.3 NM size seems to be the only physicochemical property that has been investigated in any depth in relation to environmental hazard (and even that is limited). Apart from these studies on the effect of size, there are very few publications on any other PC properties investigating their potential correlation to biological hazard (sections 4.2.1, 4.2.2, 4.2.3, 4.2.4).

H.4 There are little published data relating to sub-lethal effects after environmental exposure to NM (sections 4.2.1, 4.2.2, 4.2.3, 4.2.4).

H.5 In general, there is a lack of studies describing mechanistic (local) effects following environmental exposure to NM. Biokinetics (e.g. translocation) and, to some extent, oxidative stress have been studied, however, cytotoxicity, endocrine disruption and genotoxicity are not well studied in any of the environmental compartments investigated (sections 4.2.1, 4.2.2, 4.2.3, 4.2.4).

H.6 There is a lack of information comparing standard ecotoxicological tests (e.g. reproduction tests) with additional potentially suitable endpoints to obtain information that is significant for describing the survival and success of organisms at the population level (section 4.2.1).

H.6.1 There is a need to develop/measure population level metrics in environmental hazard identification.

H.7 Although freshwater is by far the most studied habitat, there is still a very reduced range of materials and species tested, with very limited understanding of the mode of action (section 4.2.1).

H.8 There are very few publications on studies directly investigating terrestrial impacts of NM (section 4.2.4).

H.8.1 Most studies identified in the terrestrial keyword searches were related to air-borne NM with only a few going on to investigate effects after deposition/transport into natural waters. Very few then went on to investigate direct effects in soils or terrestrial organisms (section 4.2.4).

I. Risk assessment

The ultimate aim is to enable decision making, based upon predicted risk determined from the initial characterisation of PC properties and to reduce testing efforts (assisted by “grouping” based upon the (eco) toxicity data and exposure information as well as PC properties). To date, several frameworks and tools have been suggested in peer reviewed journals and international reports to support the implementation of risk assessment in the field of NM.

I.1 A key recommendation is that the traditional RA paradigm and related existing RA frameworks for NM are supported by the development of relevant non-conventional and complementary tools to overcome the current critical limitations and deliver robust risk estimations in the near term (section 5.2).

I.1.1 Standard RA approaches might not apply, as NM are dynamic and present a mixture of PC properties (e.g. a range of sizes). Due to the complexity of these systems, it might be more appropriate to use the analogy of viruses rather than chemicals.

I.1.2 Mode of action could be a key step in the development of better/more nano relevant RA. This will be related to the PC properties, which depend on a number of factors, including the formulation processes, likely exposure routes and exposure numbers (mass/conc.) etc.
I.1.3 Very few studies have attempted to develop QSAR/QNAR type tools for NM (section 5.2.1).

I.1.4 Further work is required to develop risk assessment strategies for NM, allowing better exploitation of data from multiple sources (section 5.2.1).

I.1.5 The current approach of risk assessment tends towards testing of individual NM, whereas we should aim for a research agenda resulting in rational grouping of NM according to their (i) physicochemical properties (especially in situ in complex milieu), and (ii) biological effects (section 2.2).

I.1.6 It is recommended that the RA of NM is addressed in a probabilistic manner using stochastic approaches such as the Monte Carlo and the Latin Hypercube Simulations (section 5.2.4).

I.1.7 The development of control banding tools is currently a dynamic area of research and several tools are still under development. However, with few exceptions, they currently all produce qualitative results (section 5.2.7).

I.1.8 The PBPK model can help to develop NM-specific assessment factors for interspecies differences; there is a need to develop PBPK modelled to facilitate in vitro-in vivo extrapolation and predict the ADME behaviour of NM (section 5.2.2).

I.1.9 In vitro data cannot always be used for RA of NM because of many current limitations, but it can provide useful information on relative hazard, kinetics and modes of action to be used in a weight of evidence approach (section 5.2.3).

I.1.10 There is potential to apply relevant nano-specific biomarkers.

I.1.11 It is recommended to develop a decision framework combining VoI (value of information) and MCDA (multi criteria decision analysis) to allow making trade-offs across different decision criteria, even under conditions of high uncertainty taking the research needs of all stakeholders simultaneously into account (section 5.2.8).

I.1.12 It should be determined whether a change in the legal framework would stimulate developments in this area.

I.1.13 Data analysis and interpretation are key.

I.2 It is recommended that currently available and new RA methodologies should be tested and validated using realistic exposure scenarios to fully evaluate their functionalities and limitations (section 5.2).

I.2.1 Existing risk assessment frameworks that are developed for NM should be thoroughly and robustly tested on a wide range of NM (section 5.1.1).

I.2.2 Most of the currently available tools are not intended to facilitate regulatory decision making, but to serve as a preliminary hazard/risk screening or research prioritisation tools (section 5.1).

I.2.3 There is a lack of standard tools and methods for RA of NM, and methods differ between regulators.

I.2.4 Comparative RA is recommended, which makes full use of existing data on a relevant non-nano comparator (section 2.4.2).

I.3 None of the existing RA frameworks specifically incorporate mechanisms for timely and informed decisions, but may be applicable if properly adapted to address NM. Future research is needed to tackle the development of adaptive and more responsive risk governance frameworks (sections 5, 5.1.1).
I.3.1 The importance of stakeholder involvement in developing RA frameworks is widely acknowledged, however, the real incorporation of their perspectives in the RA and management of NM is still quite rare (section 5.1.1).

I.3.2 None of the frameworks indicated in this report are specific for environmental RA; therefore, more specific RA frameworks addressing various environmental aspects of NM are needed (section 5.1.3).

I.3.3 Guidance on RA of NM allowing for reduced information to be provided when no exposure to NM occurs (verified by data) is to be recommended, e.g. EFSA “Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain” (section 2.3.2).

I.3.4 In order to predict risk from NM, there is a need to (i) characterise uncertainty and, (ii) have mechanisms for updating this information.

I.3.5 The requirement is not to get to an absolute, but to enable appropriate decision making at a regulatory level (and for producers).

I.3.6 There is a need to determine the acceptable risk (risk vs. benefit), but this will vary depending on stakeholder, sector and specific scenarios.

I.4 There is a requirement for the definition of a health and environmental surveillance program which may act as a safety net and early warning system (section 5).

This assessment of the current state-of-art, therefore, indicates that while much has been achieved, there remains a need to better characterise and quantify both exposure and hazard, for which identification of the most relevant physicochemical characteristics is paramount. The assessment also suggests that innovative ways to assess a broad spectrum of exposure and hazard information could provide a quicker, and perhaps more complete, indication of risk.

The full Gap Analysis document (Identification of Knowledge Gaps and Strategic Priorities for Human and Environmental Hazard, Exposure and Risk Assessment of Engineered Nanomaterials) is publically available on the ITS-nano website (http://www.its-nano.eu/the-project/project-output).
Annex III – Gap validation from current ongoing research projects

To maximise the integration of comments from the scientific communities and from a wide range of scientific communities, institutions and industries, and even non-scientific feedback, we will launch an open online consultation. This online consultation will gather comments and suggestions to be taken into account for the second draft of our framework that will be discussed at large during the international conference.

Description of work & main achievements

Preparation of the questionnaire

An online consultation was launched within the NanoSafety Cluster to identify whether the knowledge gaps and research priorities identified within WP2, and discussed during the stakeholders meeting in Edinburgh in October 2012, would remain as research priorities in the coming years, or if they were likely to be addressed by other projects. The gaps listed, both in its extensive format and in an executive summary version, were made available at the ITS-NANO project website, www.its-nano.eu/the-project/project-output, while an online questionnaire was prepared using a free online tool made available by Adobe at https://formscentral.acrobat.com/app.html, and linked at the project website, www.its-nano.eu/questionnaire. The graphic interface of the questionnaire, shown below in Figure 1 and attached as Annex 1 at the end of this report, allowed the participants to identify to what extent their projects were addressing the key gaps identified in a scale to 0 (not at all) to 5 (extensively).

The list of coordinators to be addressed was obtained from the Compendium and from the list of presenters at the SIINN workshop “Safe Implementation of Nanotechnologies: Common Challenges”, held in Grenoble, 29-31 May 2012. In addition, since the SIINN ERANet has just approved funding three new projects, the SIINN coordinators agreed to forward the questionnaire to their Principal Investigators.

**Figure 1.** Appearance of the online questionnaire, in particular for the data management section

Please tick in a grade from 1 (lower) to 5 (higher) how much your Project is addressing the knowledge gaps

<table>
<thead>
<tr>
<th>Data Management</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Our project addresses the need for harmonised collection and analysis of data, using metrics relevant and mutually meaningful to exposure, hazard and risk assessment in a regulatory context.</td>
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<td>Our project addresses the development of a template for documentation (publications and reporting)</td>
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<tr>
<td>Our project addresses the strategies for handling these large data sets are required, as well as better mechanisms for centralised collection and sharing of data. There is a need to develop databases, with appropriate access for those who need it.</td>
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<tr>
<td>Our project addresses the need to ensure traceability and relevance of the database(s)</td>
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</table>

Other comments about data management
The consultation opened on December 3rd, 2012, with a short deadline of December 15th, 2012. A reminder with an extended deadline was sent out on December 17th, 2012, but this did not increase the response rate, which was in total 11 out of 25.

**Analysis of the questionnaire results**

The online tool chosen for the preparation of the questionnaire allowed the results to be exported into both an Excel table and into a graphical format showing the distribution of the responses into the different levels. For ease of interpretation, only the graphical format is provided in this report.

**Data Management**

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**Figure 2.** Data Management is a key aspect of several projects, and the quality of the data in the databases is a very important issue. Harmonization of databases structure and the metrics reported in the databases is addressed extensively by the 80% of the projects which replied to the questionnaires, while the other questions show a 50% rate of high consideration within the projects.
Exposure assessment

Our project addresses the development of technologies and methodologies that can accurately identify NM, monitor, quantify and measure their concentrations (number and/or mass) and physicochemical properties at various stages of their life cycle (manufacture, use and disposal).

Our project aims to develop methods for detection, characterization and extraction of NM when they are embedded in complex matrices. Hence there is a need for further optimization and development of analytical methods.

Our project performs research to determine whether existing exposure assessment models are appropriate for NM.

In a regulatory context an emphasis is being placed on adopting life cycle thinking approaches, or more specifically, a life cycle assessment (LCA) for emerging technologies.

Our project addresses the need for better understanding of emissions and exposure routes (when and where exposure is likely to take place) as this is likely to influence the properties and behaviour of a NM and therefore the likely level of exposure (and consequently risk).

Figure 3: Exposure is a key component in Risk Assessment, however, from the analysis of the data in the questionnaire, only a few aspects of exposure are addressed extensively. In detail, techniques to quantify nanomaterials in complex matrices are addressed by the 50% of the projects that replied, similar response rates were found for the identification of exposure routes in a life cycle perspective.
Environmental exposure

Figure 4. Environmental exposure assessment shows several gaps in different key areas, including the identification of exposure routes, modelling of exposure and hyphenated techniques required for the development of high-throughput approaches.

Important areas of research, such as the development of techniques and general exposure assessment are studied by the 50% of the projects.
Figure 5. Concerning human exposure, work is focused on occupational studies with little current activity related to general population exposures.
Physicochemical characterisation

Figure 6. The cost effectiveness of PC characterisation is addressed by a relatively high percentage of the projects, including ITS standardisation. An intelligent approach on characterisation (addressed in the second question) is a key gap identified by ITS, but this was found to be a lower priority in the projects investigated. A general improvement of training is also a point to be taken into account.
Hazard assessment

Our project addresses the identification of mode of action.

Our project addresses the need to develop appropriate 'grouping' of NMs based upon their hazard and mechanisms of toxicity.

Our project aims to develop validated test systems both in vivo and in vitro, and to prove the appropriateness of existing validated test systems.

Our project addresses the need for more information on PC characteristics (e.g., surface properties, protein corona etc.), and therefore the impact on toxicity.

Our project addresses the need of in silico modelling tools are not yet developed and the relationship between physicochemical properties and toxicological effects of NMs has not yet been fully established.
Figure 7. Concerning hazard assessment (the low quality of the image is due to its size), while several projects address the mode of action and propose some grouping approaches, there is still a lack of effort in the use and development of in silico tools, in the validation of alternative methods and of in vivo/in vitro extrapolation methods, which remain key priorities to be addressed by the ITS.
Human hazard

Figure 8. While several projects are addressing human hazard, the relevance of the data for risk assessment and the regulation of nanomaterials are still a clear issue, which seems to not yet be addressed by the projects investigated. Among the different exposure routes, only ingestion seems to be appropriately considered, while there is still a lack of activities into long-term effects.
Figure 9. While ecotoxicology is an area in which there are many specific knowledge gaps, only a few of them seem to be extensively addressed, in particular with respect to the development of alternative models into more representative systems. As for human hazard, long-term studies seem to be a crucial gap.
Risk assessment

Our project addresses the need to develop non-conventional and complementary risk assessment tools, to overcome the current critical limitations of the traditional RA methodologies, and deliver robust risk estimations in the near term.

Our project addresses the need of testing and validating currently available and new RA methodologies, using realistic exposure scenarios to fully evaluate their functionalities and limitations.

None of the existing RA frameworks specifically incorporate mechanisms for timely and informed decisions, but may be applicable if properly adapted to address NAM. Our project prefers research needed to tackle the development of adaptive and more responsive risk governance frameworks.

Figure 10. Risk assessment tools are widely used and represent a clear focus of research in the investigated projects. While the conventional tools are thoroughly studied, a clear gap remains in the investigation of non-conventional and innovative tools.

Next steps

These results will be incorporated into the WP3 Strategy Documents, where with further analysis on the size of the projects providing the information, they will be the basis for the discussion on key research priorities.
## Annex IV – Glossary and definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Acceptable risk</strong></td>
<td>Risk management term. The acceptability of the risk depends on scientific data, social, economic and political factors, and on the perceived benefits arising from exposure to an agent.</td>
</tr>
<tr>
<td><strong>Acceptance values (TACs)</strong></td>
<td>Example of statistical criteria to define values of tolerance and/or of acceptance, which are relevant to develop quantitative reference models and quantitative validation (e.g. Tolerance of Acceptance Criteria, TAC).</td>
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<tr>
<td><strong>ADME</strong></td>
<td>Absorption, Distribution, Metabolism and Excretion of a substance following exposure. See toxicokinetics.</td>
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<tr>
<td><strong>Alternative methods</strong></td>
<td>Alternatives to animal testing, to minimise animal use.</td>
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<tr>
<td><strong>Assessment factor (AF)</strong></td>
<td>Numerical adjustment used to extrapolate from experimentally determined (dose-response) relationships to estimate the NMs exposure below which an adverse effect is not likely to occur (more general but often used as synonym for safety factor or uncertainty factor).</td>
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<tr>
<td><strong>Bioaccumulation</strong></td>
<td>The cell/tissue/organism/environmental compartment takes up more of a substance (NM) than is lost, causing a build up (accumulation) of the substance in the cell/tissue/organism environmental compartment. The net result of multiple physiological processes (ADME) of a substance due to all routes of exposure.</td>
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<tr>
<td><strong>Bioavailability</strong></td>
<td>The degree to which chemicals present in the environment may be absorbed or metabolised by human or ecological receptors or are available for interaction with biological systems. (ISO 11074).</td>
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<tr>
<td><strong>Bioconcentration</strong></td>
<td>The accumulation of a chemical in the tissues of an organism as a result of direct exposure to the surrounding medium (e.g. water); i.e. it does not include food web transfer. (MacDonald and Ingersoll, 2002; <a href="http://toxics.usgs.gov/definitions/bioconcentration.html">http://toxics.usgs.gov/definitions/bioconcentration.html</a>).</td>
</tr>
<tr>
<td><strong>(Bio)distribution</strong></td>
<td>Dispersal of a NM and its derivatives throughout an organism (or environmental matrix), including tissue binding and localisation.</td>
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<tr>
<td><strong>Biomagnification</strong></td>
<td>Result of the process of bioaccumulation and biotransfer by which tissue concentrations of chemicals in organisms at one trophic level exceed tissue concentrations in organisms at the next lower trophic level in a food chain. (Environmental Protection Agency, 2010).</td>
</tr>
<tr>
<td><strong>Biomarker</strong></td>
<td>The use of physiological, biochemical, and histological changes of exposure and/or effects of nanomaterials at the suborganismal and organismal level.</td>
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<tr>
<td>a) Of hazard – a measurable effect (biochemical, physiological, behavioural or other alteration in an organism) that is associated with NMs exposure and indicates toxicity, health impairment or disease.</td>
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<tr>
<td>b) Of exposure – a measurable biological effect in a compartment or organism that is indicative of NM exposure.</td>
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<tr>
<td>c) Of susceptibility – an indicator or an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific chemical substance.</td>
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<tr>
<td><strong>Biomonitoring (biological monitoring)</strong></td>
<td>Monitoring methods to better understand the complex relationships between external and internal exposure and, consequently, the potential adverse health and environmental effects. This may involve analysis of the amounts of potentially toxic NMs and their derivates present in body tissues and fluids as a means of assessing exposure to these NM and aiding timely action to prevent adverse effects.</td>
</tr>
<tr>
<td><strong>Biota</strong></td>
<td>The combined flora, fauna and microflora of a region or ecosystem.</td>
</tr>
<tr>
<td><strong>Cradle-to-gate</strong></td>
<td>Cradle-to-gate is an assessment of a partial product life cycle from resource extraction (“cradle”) to the factory gate.</td>
</tr>
<tr>
<td><strong>Cradle-to-grave</strong></td>
<td>Cradle-to-grave includes all Life Cycle stages from resource extraction (“cradle”), to production, use and disposal (“grave”).</td>
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</tbody>
</table>
Control banding  A technique used to guide the assessment and management of risk. It is a generic technique that determines a control measure (for example dilution ventilation, engineering controls, containment, etc.) based on a range or ‘band’ of hazards (such as skin/eye irritant, very toxic, carcinogenic, etc) and exposures (small, medium, large exposure). It is an approach that focuses resources on exposure controls and describes how strictly a risk needs to be managed. (http://www.cdc.gov/niosh/topics/ctrlbanding/).

Decision tree  A tree-like/flow chart of decisions and their possible consequences.

Deterministic analysis/model  Analysis in which all populations and environmental parameters are assumed to be constant and accurately specified. A deterministic model is fully specified and does not include a stochastic component.

Direct effect  A biological response induced by a NM.

Distal effect  A biological response occurring at a site other than the portal of exposure.

Dose metrics  A way of measuring/quantifying dose i.e. a unit of dose such as mg/kg. Dose is most commonly expressed in terms of mass, but may be provided in relation to other quantifiable characteristics such as surface area of particle number.

Dosimetry  The accurate measurement (the technique of measurement) of doses to determine the relationship between dose and effect.

ECHA  European Chemicals Agency. ECHA works with regulatory authorities to implementing the EU’s chemicals legislation for the benefit of human health and the environment as well as for innovation and competitiveness.

End of Life  When a product or material has reached the end of its useful life-time and will undergo a form of disposal (e.g. incineration, recycling, or disposal into landfills).

Environmental fate  The fate of a NM in the environment including the transport, persistence and changes in physicochemical ID. The pattern of distribution of a NM, its derivates (after release) in the environment as a result of transport, partitioning, transformation or degradation.

Exposure ID  The pattern of concentrations of one or more NMs in different matrices (air, liquid or solid) and as function of duration and variability over time during their life cycle. Exposure indicators are selected according to criteria accounting for the routes of exposure (both to humans and the environment), and the matrices in which exposure takes place. In risk assessment the exposure identity critically links the physicochemical ID to the Hazard ID.

Exposure Route/Pathway  The portal of entry into the organism (e.g. lungs, skin, GIT, blood) as well as entry pathways, i.e. respiratory, injection, oral or dermal entry.

Exposure scenario  The setting in which the exposure occurs (e.g. work place (occupational), consumer, environment) and how this influences the exposure route and dose.

OECD/IPCS: set of conditions or assumptions about sources, exposure pathways, amount or concentrations or agents(s) involved, and exposed organism, system or (sub)population (i.e. numbers, characteristics, habits) used to aid in the evaluation and quantification of exposure in a given situation.

REACH: set of conditions, including operational conditions and risk management measures, that describe how the NM is manufactured, used how exposure is controlled during its life-cycle.

Extrapolation (factor)  An estimation of a numerical value of an empirical (measured) function at a point outside the range of data used to calibrate the function or the use of data derived from observations to estimate values for unobserved entities or conditions (e.g. In vitro-in vivo, inter- intraspecies, bulk – nano, lab to real live exposure).

Extrapolation factor is a quantity used in effect and exposure assessment to adjust estimated exposure or concentrations/doses for uncertainties, to make corrections in the data, or to improve safety.

Ex vivo  Experiments or measurements are carried out on tissue or cells (etc) collected from a model species (in vivo) which have been taken outside the organism from which they originate. This generally allows for more controlled experiments than in vivo, but still requires the use of animals.

Grouping  Arrangement of NM into a chemical category, whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity. (OECD).
Guideline
A principle put forward to set standards or determine a course of action. (Collins English Dictionary).

A guideline aims to streamline particular processes according to a set routine or sound practice.

Hazard assessment
A process designed to determine the possible adverse effects of a NM [or situation] to which an organism, system or [sub]population could be exposed. The process includes hazard identification and hazard characterisation.

Hazard characterisation
The qualitative and wherever possible, quantitative description of the inherent properties of a NM having the potential to cause adverse effects. This should where possible, include a dose-response assessment and its attendant uncertainties.

Hazard identification
Identification of type and nature of adverse effects that a NM has as inherent capacity to cause in an organism, system or [sub]population. It is the first step in the process of risk assessment.

Hazard ID
The pattern of biological responses (using different toxicological and ecotoxicological models, tests and endpoints) associated with one or several specified NMs.

Hazard model
An in vitro or in vivo or in silico, mammalian or environmental test system used as formal representation to assess the real life hazard of a NM.

High content
Screening of cells or molecules that provide multiple endpoints within one test. High content analysis (HCA), or visual screening is a method used in biological research and drug discovery to identify substances that alter the phenotype of a cell, including increases or decreases in the production of cellular products such as proteins and/or changes in the morphology (visual appearance) of the cell. High content screening includes any method used to analyze whole cells or components of cells with simultaneous readout of several parameters.

High throughput
Automation of testing allowing large scale repetition and numbers of samples to be assessed.

Hyphenated techniques
Historically this refers to a combination of two (or more) analytical techniques to separate and detect chemicals from a solution. The term hyphenation is widely applied in chromatography where many techniques have been combined (hyphenated) to obtain more information by a single analysis (e.g. gas chromatography-mass spectrometry). The application of hyphenated techniques (e.g. AFFFF-ICP-MS, TEM-EDX, Lab-on-chip, etc.) in nanotoxicology may be useful for the determination of more physicochemical properties of a NM by a single analysis (for example, separation and detection of a nano form from the soluble ions, distinction of size and chemical composition, etc.) while reducing artefacts (caused by the application of different methodologies and techniques on different batches of NPs).

See Multimetric.

**In silico**
Prediction of NM exposure, hazard or risk using computer modelling, e.g. QNAR, PBPK.

**In vitro**
A cell or molecule based system without the use of whole organisms.

"Relevant" in vitro models refer to those that have been shown to accurately and effectively represent in vivo responses.

"Multi-tissue" in vitro models include more than one cell type from different tissues.

**In vivo**
A test system that uses living organisms.

**in vitro-in vivo extrapolation (IVIVE)**
Evaluation of the ability of in vitro systems to predict the in vivo response.

**Indirect effects**
A biological response induced by cells or molecules activated following a NM exposure.

**Interfacial properties**
Extrinsic properties which result from interactions occurring at the interface (boundary) between NM (with specific intrinsic properties) and biological matrices. These properties describe hybrid NMs/biological entities.

**Internal dose**
The amount of NM that is internalised into the model cell or organism, or that can penetrating the absorption barriers (e.g. skin, lung tissue, intestinal tract) of an organism through either physical or biological processes.

**Intrinsic or structural properties**
Properties which take into account morphology and structure of NM after synthesis and before exposure and may comprise parameters such as purity, chemical composition, chemical stability of the coating, size, surface charge, solubility, crystallinity degree.

**Irreversible effect**
A biological response that persists over time and does not return to the pre-exposure phenotype.
Life cycle
a) Of NM – progression through all of the stages of a NM from resource extraction, to production, use and disposal.
b) Of organisms – A progression through a series of differing stages of development.

Life cycle stage
Any stage during the life cycle.
See life cycle.

Local effect
A biological response occurring at the portal of exposure.

Long term effect/Chronic effect
A biological response that persists for months or years and is likely to be irreversible. Chronic toxicity refers to the long-term adverse effects on an organism following constant dosing of a toxicant over a significant time period.
Ecotoxicology: an effect which lasts at least one complete life stage.

Matrix/matrices
A substance, situation, or environment in which something has its origin, takes form, or is enclosed. A binding substance, as cement in concrete.
Consists all possible interfaces of interactions (biofluids, soils, water, sediments, air, composites, etc.) for a NM.

Mode of action
The mechanism by which nanomaterials induce toxicity. A functional or anatomical change, at the cellular level, resulting from the exposure of a living organism to a substance.

Modelling
A process where a prediction of NM exposure, hazard or risk can be made using computational models.
See QNAR, PBPK.

Monitoring
Long-term, standardised measurement, evaluation, and reporting of specified properties of the environment, in order to define the current state of the environment, and to establish environmental trends.

Monitoring strategy
Understanding of NM emissions and exposure routes to determine when and where monitoring is performed.
See Sampling.

Monte Carlo Simulation
A technique used to obtain information about the propagation of uncertainty in mathematical simulation models. It is an iterative process involving the random selection of model parameter values from specified frequency distribution, simulation of the system, and output of predicted values. The distribution of the output values can be used to determine the probability of occurrence of any particular value, given the uncertainty in parameters.

Multimetric methods
Techniques using several measurable characteristics of a biological assemblage.
(Babylon online dictionary).
See Hyphenated techniques.

Nanomaterial
No strict definition is provided within this document, but is relevant to any materials between 1 and 100 nm.
See Nanoscale.

Nanoscale
Size range from approximately 1 nm to 100 nm.

Nanostructure
Having an internal or surface structure at the nanoscale.
(ISO TC 229).

PBPK
Physiologically based pharmacokinetic modelling, a mathematical modeling technique for predicting the absorption, distribution, metabolism and excretion (ADME) of NM in humans and other animal species.
Physicochemical descriptors: Physicochemical descriptors are a set of physicochemical characteristics selected using criteria including the description of key features of the NMs describing their inherent nature (i.e. what they are), their influence on biological and environmental fate (i.e. where they go) and their inherent activity (i.e. what they do). They can be used to obtain an unequivocal description and definition of a NM during the life cycle.

Physicochemical endpoints: Novel physicochemical endpoints of a NM to describe their physical and chemical behaviour in relation to matrices and life cycle (dissolutions, solubility, etc.) that presently are not considered within routine physicochemical characterisation.

Physicochemical ID: The dynamic pattern of physical and chemical characteristics (identified using a multi-method and techniques approach) associated with one or a group of specified NMs during their life cycle.

Population effect: Effect on a population of organisms.

Population level: In the context of this project, measurement parameter which give information on potential effects on a population of organisms (e.g. expected decrease of a population).

QSAR: Quantitative Structure Activity Relationship. A modelling approach to predict hazard based upon material structure. The relationship between the physical and/or chemical properties of substances and their ability to cause a particular effect, enter into certain reactions, etc. A quantitative relationship between a biological activity (e.g. toxicity) and one or more descriptors that are used to predict the activity.

QNAR: Quantitative Nanostructure Activity Relationship. A modelling approach to predict hazard based upon nanoscale physicochemical characteristics.

Ranking: A position in a scale, with specific referral to risk meaning that particles may be classified based on factors such as, their potential for exposure (e.g. high dustiness) and/or potential to cause harm due to high intrinsic toxicity.

REACH: Registration, Evaluation, Authorisation & restriction of Chemicals. REACH is a European Union regulation which came into force on 1st June 2007 and replaced a number of European Directives and Regulations with a single system.

Read-across: In the read-across approach, endpoint information for one chemical (the source chemical) is used to predict the same endpoint for another chemical (the target chemical), which is considered to be "similar" in some way (usually on the basis of structural similarity or on the basis of the same mode or mechanisms of action). In principle, read-across can be used to assess physicochemical properties, toxicity, environmental fate and ecotoxicity. For any of these endpoints, it may be performed in a qualitative or quantitative manner.

Reasonable worst case: Reasonable unfavourable but not unrealistic situation.

Reference material: A material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process. (ISO/Guide 35:2006(en), 3.1, https://www.iso.org/obp/ui/).

See Standard material.

Relevance: Establishing the meaningfulness and usefulness of the approach, method, process or assessment for a defined purpose.

Reliability: The reproducibility of outcome of the approach, method, process or assessment over time.

Reversible effect: A biological response that resolves over time returning to the pre-exposure phenotype.

Risk: The probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to a NM.

Risk analysis: A process for controlling situations where an organism, system or (sub=population could be exposed to a hazard. The risk analysis process consists of three components: risk assessment, risk management and risk communication, and is sometimes used an equivalent term to risk assessment.
Risk assessment
The use of physicochemical, exposure and hazard information to determine the risk of an adverse effect of NM on humans or the environment. The risk assessment process is intended to calculate or estimate the risk to a given target organism, system or (sub)population, including exposure to a NM, taking into account the inherent characterisation of the NM of concern as well as the characterisation of the specific target system. The risk assessment process includes four steps: hazard identification, hazard characterisation, exposure assessment, and risk characterisation. It is the first component in a risk analysis process.

Risk governance
Risk governance refers to the actions, processes, traditions and institutions by which authority is exercised and decisions are taken and implemented. Risk governance applies the principles of good governance to the identification, assessment, management and communication of risks. (http://www.irgc.org/risk-governance/what-is-risk-governance/).
Risk governance is required to be adaptive and responsive.

Risk management
Decision-making process involving considerations of political, social, economic, and technical factors with relevant risk assessment information relating to a hazard. It also includes the development, analysis and comparison of regulatory and non-regulatory options and the selection and implementation of the appropriate regulatory response to that hazard.

Risk perception
The subjective perception of the gravity or importance of the risk based on the individual’s knowledge of different risks and the moral and political judgement attached to them and their importance.

Risk characterisation
The qualitative and wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of a NM in a given organism, system or (sub)population, under defined exposure conditions.

Sampling
Collection of samples [e.g. from soil, water, sediment, air] from an environmental or occupational location.

Short term effect/Acute effect
A biological response that persists for hours, days or weeks, but within 1-2 month returns to the pre-exposure phenotype (i.e. reversible). Adverse effects occurring within a short time of exposure (relative to generation time).

Stakeholder
Stakeholder, an entity (person, group, organisation, member or system) that can be affected by the results of that in which they have a stake. Relevant stakeholders for ITS-NANO include: scientists, industry, downstream users, consumers, regulators, worker, NGO, environment.

Standard material
Material produced and delivered in accordance with a standard or specification. (ISO/TR 25901, https://www.iso.org/obp/ui/).
See Reference material.

Standard protocol
A standard protocol is a mandated, fixed procedure for completing a task. (www.ehow.com/facts_7382960_definition-standard-protocol.html).

SOP
Standard operating procedure.

Stochastic analysis/model
An analysis in which one or more parameters is represented by statistical distribution rather than a constant. A stochastic model is a mathematic model founded on the properties of probability so that a given input produces a range of possible outcomes which are due to random effects.

Structural or intrinsic properties
Properties which mostly take into account morphology and structure of NM after synthesis and before exposure and may comprise parameters such as purity, chemical composition, chemical stability of the coating, size, surface charge, solubility, crystallinity degree.

Susceptibility
Increased sensitivity to the adverse effects of nanomaterials due to factors such as species differences, age, disease status or genetics.
The condition of an organism or other ecological system lacking the ability to resist a particular disease, infection or intoxication. It is inversely proportional to the magnitude of the exposure required to cause the response.

TAC
Tolerance of Acceptance Criteria.
See Acceptance values.

Test concentration/dose
The quantity of NM added into a hazard model in order to assess toxicity.
See dose metric.
(OECD) Testing guidelines | Internationally agreed testing methods to identify and characterise the potential hazards and exposure (etc) of chemicals.

Tiered testing strategy | Sets out a structured approach to assessing the fate and effects of NMs, where test in higher tiers may be required depending upon the results of tests at earlier stages (i.e. lower tiers). Under a tiered structure, for example, data requirements for effects testing might progress from acute to chronic laboratory studies to field studies.

Toxicokinetics | The rate at which a substance enters the body, distributes, where it is distributed to, metabolised/transformed, accumulated or excreted.
See ADME.

Transformation | The change in NM physicochemical characteristics that occur by biotic or abiotic processes at different life cycle stages.

Uncertainty | Imperfect knowledge concerning the present or future state of an organism, system or (sub)population under consideration.

Validation | The process of determining the degree to which a model or simulation is an accurate, reproducible representation of the real world from the perspective of the defined purpose of the model.

Weight of evidence | The use and integration of different types of available data, information, and associated uncertainties as well expert judgment to assess NM risk and/or hazard. Available information is allocated a weighting considering the quality of data, experimental design, reproducibility of findings, nature and severity of response observed and the strength of the relationship between different pieces of information and the assessment endpoint.
Annex V – References and annexes to chapters

References to chapters


3. COMMISSION REGULATION (EU) No 10/2011. Plastic materials and articles intended to come into contact with food.


42. Linsinger TPJ et al. (2012). Requirements on measurements for the implementation of the European Commission definition of the term "NM": 2012, European Commission Joint Research Centre: Luxembourg.


Annex to chapter 5

5.1 Databases

1) NANOHub
JRC hosts the NANOhub database, a comprehensive, non-public IT platform designed for addressing and hosting information on nanomaterials. NANOhub is based on IUCLID (International Uniform Chemical Information Database) and on the OECD Harmonised Templates, and provides an accepted basis for regulatory use of data on chemicals in the EU. The sections in IUCLID have been expanded and additional entry fields have been added for nanomaterial-specific endpoints listed by the OECD WPMN in the Guidance Manual of the sponsorship programme.

The JRC NANOhub hosts, among other things, data and studies from the OECD Working Party on Manufactured Nanomaterials (WPMN) sponsorship programme on the safety testing of a representative set of nanomaterials, as well as test and measurement results on materials from the JRC Repository of Representative Nanomaterials. Results from research projects, such as for example ENPRA, Nanolyse, Nanogenotox or EcotoxNano (full list at: www.nanohub.eu), are also collected in NANOhub. NANOhub contains options to protect confidentiality, but also to share results between different consortia. Results from the OECD sponsorship programme are not yet available, but will become accessible in 2016.

2) EChem
The JRC also hosts EChem, an OECD initiative that makes information on chemical substances available across numerous different platforms and data providers. This could also include information on nanomaterials.

3) Nanomaterials product register
The European Parliament and some EU Member States have requested a register for products containing nanomaterials. France has already initiated a compulsory declaration scheme [64] for the identity, quantities and usage of nanoparticle substances or nanomaterials distributed or imported in France. Some other EU Member States will follow with different requirements: mandatory – voluntary, focus on product or specific nanomaterial. National initiatives could lead to divergence of rules and practices, and therefore a harmonised product register at EU level should be preferred to provide useful information on nanomaterials on the market and the potential exposure of consumers.

4) NHECD
A critical and commented database on the health, safety and environmental impact of nanoparticles has been created under the FP7 Support Action NHECD. The goal of this project is to build a free access robust and sustainable system that can meet the challenge of automatically keeping a rich and up-to-date scientific research repository, enabling a comprehensive analysis of published data on environment and health effects following exposure to nanoparticles. Methodology and automated ranking and commenting processes will be set up handling an unlimited number of detailed research results, so that a large scale knowledge base can be built and maintained on a long-term basis. This process will be accompanied and supported by automated data and text mining techniques capable of extracting the results from an unlimited number of published papers.
5.2 Data quality

Data quality is determined by the validity, reliability, relevance and adequacy of the data. **Validity** is applied especially in the absence of any formal, generally accepted guideline for a specific test or a non-testing method (e.g. QNAR). The method can be evaluated against official guidelines or against general validation principles, such as the OECD validation principles for Q(S)QRs. Although available OECD test guidelines have not (yet) been validated for NMs, OECD has concluded that, in most instances, nano-specific challenges can be addressed within existing test methods and risk assessment approaches. Hence, it would not be necessary to develop completely new approaches for nanomaterials. In some cases, it might be necessary to adapt methods of sample preparation and dosimetry for hazard (and fate?) testing.

There is a general requirement to reduce animal testing and to foster alternative approaches. Validated test methods are not available for all endpoints. The new guidance on the safety assessment of nanomaterials in cosmetics [36] gives an overview of available alternative test methods, their status and suitability to test nanomaterials in cosmetic products. For a risk assessment, especially for a weight of evidence approach, non-validated studies may be an important information source to reach a conclusion. Considering the long process it takes to validate new test methods, it is important to improve the regulatory acceptance of alternative methods, validated and non-validated. An important issue is always the quality of the data, which is further defined by the reliability and the relevance.

**Reliability** evaluates the inherent quality of a test report or publication relating to preferably standardised methodology (e.g. OECD test guideline) and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings (as defined by Klimisch [9]). Especially when studies have not been performed according to accepted guidelines and in compliance with good laboratory practise (GLP), it is important to evaluate the method used, characterisation of test substances, use of techniques and updates, comprehensiveness of reporting and the conclusions drawn.

The Klimisch categorisation system to assign reliability scores is generally accepted in regulatory reporting and risk assessment (e.g. Chemical safety assessment under REACH, in IUCLID). ToxRTool, as available from the JRC-ECVAM webpage [62], is a publicly available tool to rank the reliability of a study. It is especially useful for dealing with non-guideline studies and studies reported in open scientific literature. In addition, a method specifically developed to evaluate the quality of studies examining NMs has been proposed by Card and Magnusson [63]. In addition to the reliability score, it also includes a “nano-score”, which is determined by the completeness of the physico-chemical characterisation of nanomaterials assessed within the study and the adequacy of reporting of these parameters. For non-testing data, the following factors need to be considered: are the materials of interest within the applicability domain of the model or is similar to one or more chemicals in the training set; are there universal principles for their regulatory applicability? For “conventional” chemicals, there are OECD agreed principles for validating (Q)SAR models for their use in regulatory assessment of chemical safety.

**Relevance** covers the extent to which data and test results are appropriate for a particular hazard identification or risk characterisation. It refers to questions relating to the species relevant for target population, route of exposure relevant for population and exposure scenarios under consideration, whether the exposure regime is relevant for the population under consideration and whether the effect of concern is relevant with regard to the target population. Discussions are ongoing as to the
relevance of lung effects, including tumors observed in rats for human exposure situations, as the prolonged exposure does not give the animals the normal recovery period for lung clearance and as rats are particularly sensitive to developing lung tumours from lung overload to poorly soluble particles due to ineffective clearance.

### 5.3 Risk Assessment Frameworks

<table>
<thead>
<tr>
<th>Framework</th>
<th>Structure</th>
<th>Strength Points</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano Risk Framework, Environmental</td>
<td>The framework consists of six distinct steps:</td>
<td>- It allows both, quantitative and qualitative risk assessment, in accordance</td>
<td>- Although identified, uncertainties are handled in a qualitative way:</td>
</tr>
<tr>
<td>Defense and Du Pont [64]</td>
<td>1. Describe Material and Application</td>
<td>with the type and the quantity of available data.</td>
<td>the framework points out areas of uncertainty and data gaps and generally</td>
</tr>
<tr>
<td></td>
<td>2. Profile Lifecycles(s)</td>
<td>- It considers the potential exposure throughout the whole life cycle of the</td>
<td>suggests a “reasonable worst case” approach.</td>
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<tr>
<td></td>
<td>3. Evaluate Risks</td>
<td>examined nanomaterial.</td>
<td>- Some physicochemical properties (dissolution kinetics, dustiness, zeta</td>
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<td></td>
<td>4. Assess Risk Management</td>
<td>- The risk assessment is expected to be updated when new information becomes</td>
<td>potential, surface chemistry and lipophilicity) recognised as significant</td>
</tr>
<tr>
<td></td>
<td>5. Decide, Document and Act</td>
<td>available and reviews have to be planned when performing the first RA. Thus,</td>
<td>for a proper characterisation of a nanomaterial are not included in the base-</td>
</tr>
<tr>
<td></td>
<td>6. Review and Adapt</td>
<td>the framework is iterative and adaptive.</td>
<td>set of information required.</td>
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<tr>
<td>Nanotechnology Risk Governance, IRGC</td>
<td>The framework comprises five phases:</td>
<td>- A template for reporting the risk assessment procedure and communicating the</td>
<td>- The framework doesn’t specify which properties should be investigated, nor</td>
</tr>
<tr>
<td>(NRG) 65</td>
<td>1. Pre-assessment (problem framing, early warning and risk governance</td>
<td>risks is provided (Output Worksheet, Medley 2007).</td>
<td>which kinds of tests should be performed.</td>
</tr>
<tr>
<td></td>
<td>process organisation)</td>
<td>- Data sharing is encouraged.</td>
<td>- Uncertainties are identified, but no specific action is planned to deal</td>
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<td></td>
<td>2. Risk Appraisal (Risk and Concern assessments)</td>
<td>- Societal concerns and issues are taken into account.</td>
<td>with them.</td>
</tr>
<tr>
<td></td>
<td>3. Tolerability and Acceptability Judgement</td>
<td>- Risk communication is considered as an integral part of all stages of the</td>
<td>- Although adaptability and transparency are indicated as milestones of the</td>
</tr>
<tr>
<td></td>
<td>4. Risk Management</td>
<td>risk governance process and crucial for the effective linking the different</td>
<td>framework, these characteristics are not demonstrated in any documented</td>
</tr>
<tr>
<td></td>
<td>5. Communication</td>
<td>components.</td>
<td>application.</td>
</tr>
<tr>
<td>Comprehensive Environmental Assessment</td>
<td>The CEA framework is structured in 5 phases:</td>
<td>- It considers the risk assessment of a nanomaterial from a life cycle</td>
<td>- It doesn’t provide any specific procedure to deal with uncertainties.</td>
</tr>
<tr>
<td>(CEA) [48]</td>
<td>1. Life Cycle stages (feedstock, manufacture, distribution, storage, use</td>
<td>perspective.</td>
<td>- Although adaptability and transparency are indicated as milestones of the</td>
</tr>
<tr>
<td></td>
<td>and disposal)</td>
<td>- It takes into account the variability of the properties of nanomaterials by</td>
<td>framework, these characteristics are not demonstrated in any documented</td>
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<td></td>
<td>2. Environmental pathways, i.e. identification of the media where the</td>
<td>considering the formation of manufacturing by-products and environmental</td>
<td>application.</td>
</tr>
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<td></td>
<td>nanoparticles can spread</td>
<td>transformation products.</td>
<td>- It doesn’t provide any specific procedure to deal with uncertainties.</td>
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<td></td>
<td>3. Transport and Transformation</td>
<td>- Indications on some (but not all) properties relevant for the characterisation</td>
<td>- Although adaptability and transparency are indicated as milestones of the</td>
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<td></td>
<td>4. Exposure (Biota/Human) via inhalation, ingestion or dermal absorption</td>
<td>of nanomaterials are provided.</td>
<td>framework, these characteristics are not demonstrated in any documented</td>
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<td></td>
<td>5. Effects, i.e. evaluation of the health and ecological hazards</td>
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<td>application.</td>
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### Adaptive Screening-Level Life Cycle Risk Assessment Framework for Nanotechnology (Nano LCRA) [66]

The proposed framework consists of ten steps:

1. Description of the life cycle of the product
2. Identification of the materials and assess potential hazards in each life cycle stage
3. Qualitative exposure assessment for materials at each life cycle stage
4. Identification of stages of life cycle when exposure may occur
5. Evaluation of the potential human and non-human toxicity at key life cycle stages
6. Analysis of risk potential
7. Identification of key uncertainties and data gaps
8. Development of mitigation/risk management strategies
9. Gathering of additional information
10. Reiteration of the process

- Nano LCRA is a screening-level framework that can be applied when little information is available.
- "Mini" hazard and exposure assessments are conducted for each step of the life cycle, and further investigation (effects assessment) is performed only for the steps where exposure occurs, allowing saving time and resources.
- There is a whole step of the framework focused on the evaluation of the level of confidence in the assessment. However, although key sources of uncertainty are identified, the framework doesn’t suggest any specific approach for their management.

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### SCENIHR Framework [67]

The SCENIHR framework is a decision tree approach, composed of four stages:

1. Assessment of Need for Exposure Studies
2. Exposure Characterisation
3. Hazard Identification
4. Characterisation and Risk Assessment

- Within the framework, some significant physicochemical properties for the characterisation of nanomaterials are identified and suggested as required information when performing the RA procedure.
- The proposed staged approach is exposure driven and the need for in vivo tests has to be demonstrated on the basis the results of a set of alternative (in vitro and in silico) tests. Thanks to these characteristics, it is possible to decrease both the costs of experimental tests (dose-response assessment is not performed when no exposure occurs) and the number of animal testing.

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### Risk Assessment Framework for First Generation NMs under REACH, RIVM (FGNMs Risk Assessment) [51]

The framework proposed by RIVM represents a revision of the standard RA approach applied under REACH for the evaluation of bulk chemicals: all the steps of the processes which are relevant for the assessment of the NMs have been identified and modified in order to be applicable and reliable. A base set of information requirements for each of the phases of the process has been proposed.

- The standard risk assessment approach, of which the proposed framework is a revision, has already been in use for several years, analyzed and generally accepted by all the stakeholders involved in the RA process.
- A “base-set” of nanospecific information is required in the first phase of the process.
- Due to the nature of the standard RA approach, principles like transparency and precaution are guaranteed.
- Exposure is considered throughout the whole life cycle of the examined NM.

- The framework doesn’t provide any specific detail on the kind of tests to perform during the exposure and effect assessments.
- Life cycle perspective, transparency and adaption of the assessment are listed as features of the framework. However, there are no documented applications to demonstrate these characteristics.

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www.its-nano.eu
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<tr>
<th>Tool</th>
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<tbody>
<tr>
<td>US XL Insurance Database Methodology [68]</td>
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<tr>
<td>NanoRiskCat [69]</td>
</tr>
<tr>
<td>Risk-based classification system of nanomaterials [70]</td>
</tr>
<tr>
<td>Nano Hazard Assessment Approach [71]</td>
</tr>
<tr>
<td>Swiss precautionary matrix for synthetic nanomaterials [72]</td>
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<tr>
<td>French ANSES System [73]</td>
</tr>
<tr>
<td>Dutch StoffNManager Nano 1.0 [74]</td>
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<td>Danish NANOFAER [80]</td>
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<td>Hristozov et al. [2012a] [27]</td>
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<td>Hristozov et al. [2012b] [28]</td>
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<tr>
<th>Methods</th>
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<tr>
<td>Risk screening</td>
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<td>Risk screening</td>
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<td>Stochastic Multicriteria Acceptability Analysis (SMAA-TRI)</td>
</tr>
<tr>
<td>Indexing WoE, Expert judgment</td>
</tr>
<tr>
<td>Control banding</td>
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<td>Control banding</td>
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<td>Control banding (quantitative)</td>
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<td>Multi-criteria decision analysis</td>
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<tr>
<th>Outputs</th>
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<tr>
<td>Relative risk scores and classification</td>
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<tr>
<td>Hazard, consumer exposure classification</td>
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<td>Relative risk scores and classification</td>
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<tr>
<td>Relative hazard scores</td>
</tr>
<tr>
<td>Precautionary need scores and classification</td>
</tr>
<tr>
<td>Risk control bands</td>
</tr>
<tr>
<td>Hazard, exposure and risk bands</td>
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<td>Hazard, exposure and risk bands</td>
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<td>Relative hazard scores</td>
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<td>Relative exposure scores</td>
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<th>Potential users</th>
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<td>SME and industry</td>
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<td>Industry and academia</td>
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