1. PUBLISHABLE SUMMARY

1.1. EXECUTIVE SUMMARY

ITS-NANO started in March 2012 with the aim to generate a research strategy that could lead in future to an Intelligent Testing Strategy for Nanomaterials in May 2013. The project had 9 partners from across Europe and was co-ordinated by Heriot-Watt, Edinburgh, UK.

Thousands of engineered nanomaterials exist and new ones are being developed in many different forms for use in a wide array of commercial products. Nanomaterials demonstrate many interesting proprieties, due to their nanoscale, and can show complex behaviour compared to both their corresponding chemical compounds and macroscale materials. Therefore it is important to assess their potential risks to human and environmental health associated with their production, use and disposal.

As for conventional chemicals variables when assessing risk include the basic materials, products containing materials, routes of release (air, water etc.), target species, routes of exposure, target tissues/cells and measures of toxicity (endpoints). Unlike conventional chemicals nanomaterials are extremely difficult to group, they could exhibit non-linear effects, and extrapolation of risk is very difficult and prone to errors. For these reasons assessment of risk on a case-by-case basis is too costly and slow to allow sustainable development of nanotechnology. Therefore a more Intelligent Testing Strategy (ITS), which is effective, efficient but accurate, is required.

The ITS-NANO document outlines a vision that is a way forward in which there is a knowledge-based sustainable development of engineered NMs. The research priorities required to achieve this vision are outlined, stretching from the short term to distant future, during which time it is predicted that there will be a decreased reliance on testing and a gradual increase in reliance on modelling/grouping/ranking approaches. The modelling approaches are not limited to hazard, but also include exposure and physicochemical characterisation.

A two-stage refinement process including a large number of stakeholders has devised the vision. First, ITS-NANO partners assessed current knowledge and research projects to identify gaps in knowledge. This information was combined with initial ideas pertaining to the structure of an ITS and both were discussed with stakeholders in an expert-workshop. This information along with feedback from an online questionnaire, was then used to generate the first draft of the research prioritisation needed to generate an ITS which was again discussed with stakeholders before generating a final version for dissemination.

The ITS-NANO document indicates that the future RA for NMs will be to follow a **risk evaluation paradigm** based upon that currently used for **chemicals**, but adapted where necessary to take into account **NM specific/relevant factors**. This paradigm relies primarily on **exposure and hazard**, but for NM the risk assessment will require increased emphasis on **physicochemical characterisation**, all of which are then composed into a RA.

The document outlines the research priorities in detail based on three essential sets of information: Physicochemical ID, Exposure ID, and Hazard ID. Grouping/Ranking that would lead to development of an ITS, as well as implementation of the research prioritisation into risk assessment

frameworks are also included. The research prioritisation for each chapter is depicted using hexagon diagrams, allowing quick identification of short, mid, long and distant future priorities and how each priority links to each other, as well as how to progress from the current position to an ITS in the future. The long-term and distant future priorities require consideration now in order to ensure that the short and mid-term research priorities generate the required information to allow the long-term priorities to be achieved.

The final document is available on line (http://www.its-nano.eu/wp-content/uploads/2013/06/ITS-NANO-PRIORITISATION-DOC.pdf) along with the executive summary (http://www.its-nano.eu/wp-content/uploads/2013/06/ITS-NANO-Executive-summary.pdf). The single research prioritization document is designed to be relevant to a wide variety of stakeholders and therefore abstracts explaining the relevance of the report to different stakeholder groups have also been generated. Links to all of these documents have been provided to stakeholders electronically.

The two stakeholder workshops proved to be an effective mechanism to directly inform relevant stakeholders about the project and its results. In addition, the final report has been presented at the NANoREG kick off meeting (May 2013) and at a MARINA project workshop (May 2013) in order to aid the input of ITS-NANO research prioritization into these two FP7 projects and therefore promote impact of the project. In addition, the report has been presented and debated at several international meetings. In June 2013 the report was officially launched via a Webinar as well as being presented to the NanoSafety Cluster partners.

1.2. A SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES

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 range. These changes may cause adverse (eco)-toxicological effects. As not required by regulation, 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 or physical dimensions of some materials. With the development of nanotechnology it has become increasingly clear that even 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 (i.e. typically as molecules).

The rapid introduction of engineered NMs to the market raises several challenges for current RA. 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 challenge is to devise novel appropriate, optimised approaches for screening of NMs and evaluating their risks.

The initial objectives of the project were to develop:

• A framework for future research aiming at rational grouping, through well standardized methods, of engineered nanomaterials (ENM) according to their i) physical, ii) chemical, iii) biological characteristics.

• A framework for future research aiming at specific grouping of NM according to the specific health risk they present towards the immunological, respiratory, reproductive, circulatory, etc. systems.

• A strategy to increase the integration among stakeholders (food industry, nano-material manufacturers, pharma- and health-related industry) for a shared, agreed-upon risk assessment strategy and approach to conveying the appropriate, evidence-based information to the public.

During the project it was recognised that 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. An ultimate goal was therefore identified 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 the ITS-NANO final deliverable was therefore 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 was defined as a process that allows the risks of NMs to be assessed accurately (generates the right answers), effectively (generates useful answers) and efficiently (on time and on budget).

To achieve these objectives the following work was conducted:
To provide substantiated background knowledge for the strategy, the ITS-NANO project partners reviewed the current literature to identify knowledge gaps (Annex II of final research prioritisation document) and supplemented the acquired information with data acquired from responses to an online questionnaire distributed to participants in relevant on-going EU projects (Annex III of final research prioritisation document).

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.

1.3 A DESCRIPTION OF THE MAIN S&T RESULTS

ITS-NANO generated the following reports:

1.3.1 Gap analysis of the current published data
1.3.2 Gap analysis of current projects
1.3.3 Research prioritisation document leading to an intelligent testing strategy for nanomaterials

Reports 1 and 2 were used as background information on which to design the final research prioritisation document. The findings of each report are summarised below.

1.3.1 Identification of Knowledge Gaps and Strategic Priorities for Human and Environmental Hazard, Exposure and Risk Assessment of Engineered Nanomaterials

The full gap analysis is available on line at www.its-nano.eu, and a summary is provided as Annex II of the final research prioritisation document, also available at the same web link.

The current state-of-the-art was ascertained from review articles, a report from governments, regulatory bodies other relevant organisations and the expert knowledge of project partners. The hazard assessment section of this document was based on the number of publications identified using the NCBI PubMed (toxicology) or Web of Knowledge (ecotoxicology) search engines. The number of publications identified was used to construct 'heat maps' to indicate areas of high, medium and low research activity/output (figure 1). Finally, the knowledge and research gaps identified were moderated by a multidisciplinary panel of expert stakeholders at the first ITS-NANO Stakeholder Workshop (Edinburgh, September 2012).

The research and knowledge gaps listed below are shown in priority order with the most important issue in each area listed first.

Key:

few/no papers	n < 20
some papers	20 ≤ n ≤ 50
lots of papers	n > 50

n = number of publications (NCBI PubMed search)

				Biological impact			
Target	Bio-kinetics	Cyto-toxicity	Inflam-mation	Oxidati-ve stress	Fibrosis	Geno-tox	Carcino-genicity
Lung	381	250	543	207	44	45	246
Liver	76	28	39	19	3	6	23
Spleen/immune	52	19	79	21	4	2	16
CNS	54	20	47	32	2	3	18
GI Tract	29	21	29	12	2	5	19
kidney	30	9	17	6	1	1	4
CV	138	84	219	83	18	10	60
Repro/dev	5	1	6	1	1	2	1
pleura (retention)	23	23	47	20	4	3	11

Acute **chronic**

Figure 1. An example of a heat map taken from the gap analysis document, and used to assess the relative level of research activity across different human hazard research areas.

The following is an abbreviated list of the gaps in research knowledge and activity that were identified, however a more comprehensive and detailed list is available within the gap analysis document (www.its-nano.eu).

Data management

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

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

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

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

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

Physicochemical characterisation

B.1 There is a requirement to minimize the effort for characterisation in the future; therefore, a minimum set of parameters for characterisation should be defined.

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

B.3 Characterisation methodologies and technologies (instrumentation) should be developed and optimized to provide 'standard protocols' for detection, characterisation and extraction of NM in different media.

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.

B.5 The lack of fully characterised NM standards/ reference materials is a critical obstruction to research into the physicochemical characterisation of NM.

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).

Exposure Assessment

C.1 It is a pressing requirement to develop 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).

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

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.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 optimization and development of analytical methods.

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.

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

Environmental Exposure

E.1 Further research into environmental NM exposure is required.

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 models and biomarkers for environmental exposure, it is recommended that a holistic approach based on assessment of their sources, behaviour and sinks be adopted.

Hazard assessment

F.1 Determining mode of action is key.

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

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.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.

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.

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.

F.7 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.8 It should be determined which dispersion protocols are most appropriate for risk assessment purposes.

F.9 While some biomarkers (*in vitro* and *in vivo*) of hazard exist (e.g. indicators of inflammation, oxidative stress and cytotoxicity), the identification of a sub-group 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.10 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.11 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.12 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.*).

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.

G.3 There are too 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.

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.

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.

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

G.6 There are only 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.

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.*).

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.

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

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.

H.4 There are little published data relating to sub-lethal effects after environmental exposure to NM.

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.

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.

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.

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

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.

J.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.

J.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.

J.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.

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).

1.3.2 Gap validation from current on-going research projects

The full description of information obtained from on-going research projects is available on line at www.its-nano.eu, and a summary is provided as Annex III of the final research prioritisation document, also available at the same web link.

An online consultation was launched within the NanoSafety Cluster and SIINN ERANet to identify whether the knowledge gaps and research priorities identified within the gap analysis, 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. An online questionnaire was prepared www.its-nano.eu/questionnaire to allow 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). Figure 2 provides an example of the data analysis gained from the online questionnaire.

The following conclusions were drawn:

- 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 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 50% of the projects that replied, similar response rates were found for the identification of exposure routes in a life cycle perspective.
- 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 50% of the projects.
- Concerning human exposure, work is focused on occupational studies with little current activity related to general population exposures.
- 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.
- Concerning hazard assessment, 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.
- 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.

- 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 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.

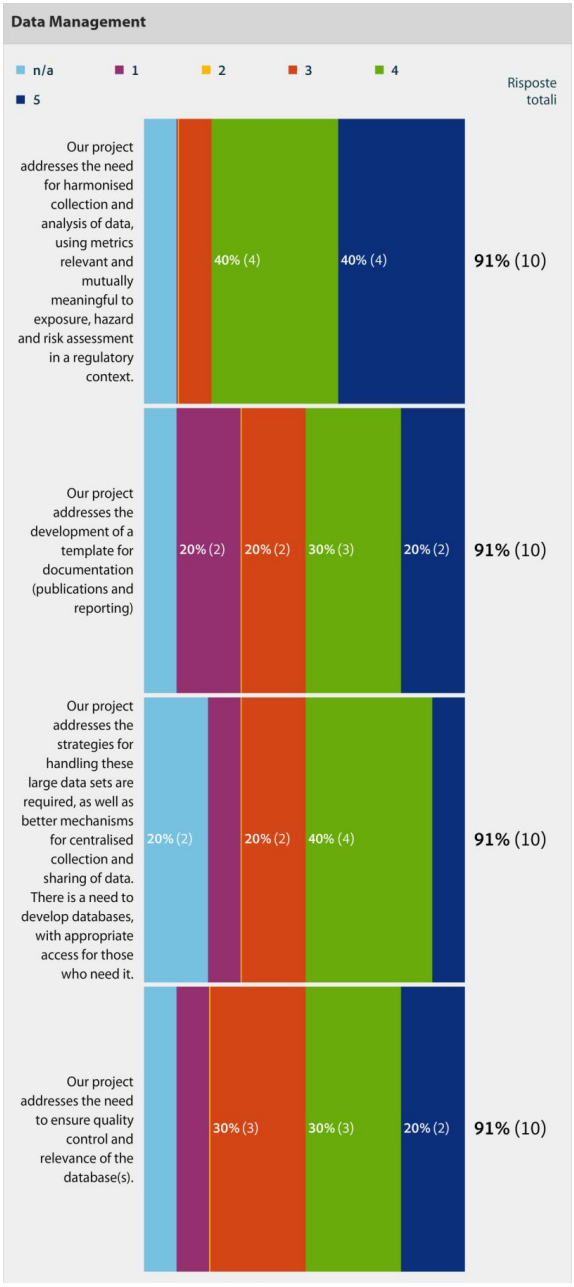

Figure 2. An example of the data analysis of the online questionnaire.

1.3.3 Final document – Research prioritisation to deliver an intelligent testing strategy for human and environmental safety of nanomaterials

The following text outlines the main conclusions of the research prioritisation document (taken from the executive summary):

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 into 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-relevant and 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 decreasing focus on *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 it would be available by specific and limited physicochemical, exposure and hazard testing**.**

A traditional risk assessment approach includes assessment of hazard, exposure and to a lesser extent physicochemical characterisation. The Intelligent Testing Strategy (ITS) presented here also uses exposure and hazard information, but elevates the importance of physicochemical (PC) characterisation of NMs.

All three aspects 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 at a meaningful speed. 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. 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 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 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 Exposure ID, 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 hotspots in soils and sediments. The development of standardised protocols must remain a continuous priority over time. In the mid-term, concentrations in different 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 hexagon priority diagrams from each chapter were combined to generate the diagram below (figure 3). This diagram 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.

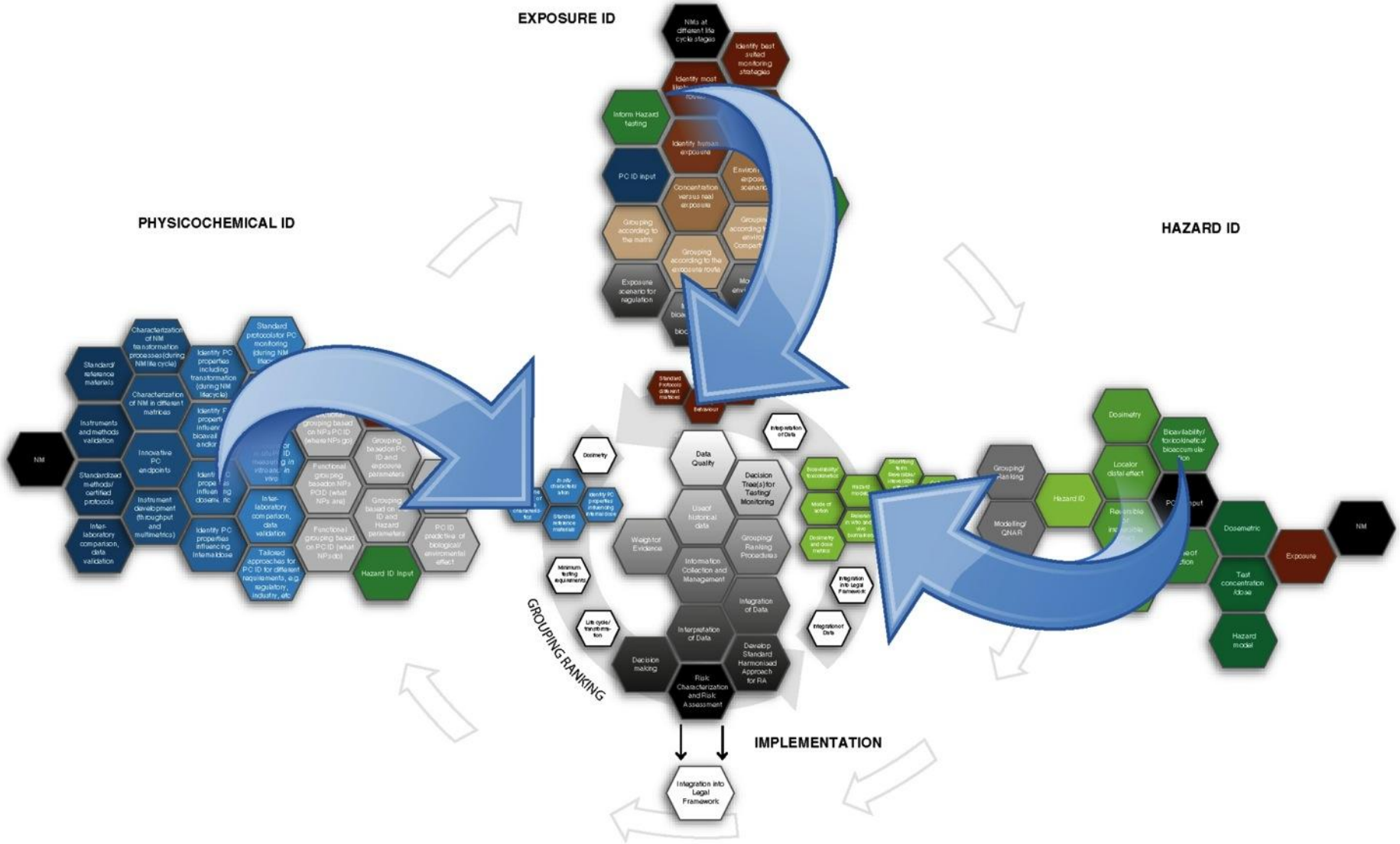

Figure 3: Summary of the ITS-NANO research prioritisation. This diagram illustrates the integration between the priority areas discussed in each of the above chapters. The outputs of the ITS-NANO feed towards the central, ITS which in turn feeds into the risk assessment frameworks.

1.4 THE POTENTIAL IMPACT (INCLUDING THE SOCIO-ECONOMIC IMPACT AND THE WIDER SOCIETAL IMPLICATIONS OF THE PROJECT SO FAR) AND THE MAIN DISSEMINATION ACTIVITIES AND EXPLOITATION OF RESULTS

The project already has impact, via input into the FP7 projects MARINA and NANoREG. The final report has been presented at the NANoREG kick off meeting (May 2013) and at a MARINA project workshop (May 2013) in order to aid the input of ITS-NANO research prioritization into these two FP7 projects and therefore generating immediate impact of the project. For both MARINA and the future NANoREG, part of the DoW/call text involves development of an ITS. Rather than designing an ITS from scratch and identifying the research needed to generate this ITS, both projects will use the ITS-NANO final deliverable and build upon this work in order to work towards an ITS.

The two stakeholder workshops proved to be an effective mechanism of informing relevant stakeholders about the project and its outputs, leading to a number of the stakeholders facilitating the input of the ITS-NANO project findings into existing and future projects. The stakeholders involved in the two workshops are listed below:

Agnes Oomen, National Institute for Public Health and the Environment (RIVM); Andre Kleensang, Johns Hopkins University; Andrej Kobe, European Commission DG Environment; Andrew Owen, University of Liverpool; Barry Park, NanoKTN; Camelia Constantin, European Chemicals Agency; Cesare Rossini, Thermo Fisher Scientific; Christian Micheletti, Veneto Nanotech; Daniel Lyons, Ruđer Bošković Institute, David Carlander, Nanotechnology Industries Association; David Warheit, DuPont Haskell Global Centers, Derk Brouwer, Netherlands Organisation for Applied Scientific Research (TNO); Dik van de Meent, Radboud University Nijmegen; Federica Gallocchio, Istituto Zooprofilattico Sperimentale delle Venezie; Federico Benetti, Veneto Nanotech; Frieke Kuper, Netherlands Organisation for Applied Scientific Research (TNO); Gilly Stoddart, PETA UK;Igor Linkov, Carnegie Mellon University; James Wheeler, Health and Safety Executive; Jacques Bouillard, INERIS; Keld Alstrup Jensen, National Research Centre for the Working Environment (NRCWE); Ken Donaldson,Edinburgh University; Lang Tran, Institute of Occupational Medicine; Lara Stajner, Ruđer Bošković Institute; Laura Manodori, Veneto Nanotech; Lisa Bregoli, Veneto Nanotech; Marco Natali, National Research Council (CNR); Maria Letizia Polci, Ministry of Health; Mark Wiesner, Duke (CEINT); Michelle Kelly, Medicines and Healthcare Products Regulatory Agency; Neil Ebenezer, MHRA; Peter Bos, National Institute for Public Health and the Environment (RIVM); Péter Krüger, Bayer; Petra Buric, Ruđer Bošković Institute; Phil Sayre, Environmental Protection Agency; Sara Totaro, Veneto Nanotech; Silke Krol, Neurologic Institute "Carlo Besta"; Steve Morgan, Defra; Stuart Marshall,Unilever; Terry Wilkins, Leeds University; Thomas Kuhlbusch, IUTA; Tom van Teunenbroek, Ministry of Infrastructure and the Environment; Ulrike Bernauer, Federal Institute for Risk Assessment; Veronica Cappa,Istituto Zooprofilattico Sperimentale delle Venezie; Willie Peijnenburg, RIVM; Zeljko Jaksic, Ruđer Bošković Institute; Zuzana Klöslova, European Chemicals Agency.

In addition, the report has been presented and debated at the International Particle Toxicology Conference (June 2013) including 110 EU and US delegates In June 2013 the report was officially launched via a Webinar (approximately 70 attendees or groups) as well as being presented to the NanoSafety Cluster meeting in Dublin (June 2013). Future oral presentations are planned for Inhaled Particles (Sept 2013), for ECHA, SETAC, EU-US meeting including the Communities of Research, and an Ibook.