A common European approach to the regulatory testing of nanomaterials

NANoREG

Grant Agreement Number 310584

Deliverable D1.2

Results of GAP analysis

Regulatory data gaps and research needs

Due date of deliverable: 2013/06/30

Actual submission date: 2013/06/28

NANoREG Identifier:	Deliverable 1.2: Gap analysis
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Contributing partners:	ENEA, JRC, NRCWE, TEMAS, VN
Work package/task:	WP1 / Task1.2
Document status:	draft / final
Confidentiality:	confidential / restricted / public
Keywords:	Data gap, research needs, regulatory questions

Abstract:	In light of the NANoREG scientific questions related to urgent regulatory issues in the context of nanomaterials, we have performed a gap analysis in which we identified knowledge that is needed in the area of regulatory toxicology and risk assessment. The information for the analysis has been gathered from the NANoREG partners as well as numerous ongoing and previous initiatives and projects. The main objective is to ensure an efficient research strategy in NANoREG, as well as to guide the research community in terms of the information that is critically needed by regulatory authorities and policymakers. Our analysis will also facilitate the use and integration of existing data and results from other projects. The gap analysis revealed that most regulatory questions are related to the following three general knowledge gaps: a) characteristics that influence the risk of nanomaterials in the environment and humans, b) standardized methods to determine these characteristics, and c) nano-specific risk assessment strategies and approaches. The overview of the extent to which the knowledge needed for each regulatory question is already available or is expected to become available on short or long term, revealed that only the following short term research needs are not fully addressed by the ongoing and planned initiatives are: a) more insight into implications of the implementation of the EC definition within the regulatory frameworks, b) methods to test or predict the extent and rates of transformation of nanomaterials by incineration, chemical reactions and other processes. The long term research needs mainly concern: a) further standardization and validation of methods for identification, quantification, characterization and transformation of nanomaterials, b) further identification and verification of the key characteristics that influence the release, exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks, c) further development and verification of nano-specific risk assessment approaches or strategies, including extrapolation, interpolation, read across, grouping and approaches for safe design, d) implementation of these nano-specific risk assessment strategies and approaches within regulatory frameworks. There is also a lack in systematic sets of high quality data of well characterized nanomaterials on exposure, kinetics and toxicity to further develop, verify and validate nano-specific methods and approaches. Until then, the implementation of nano-specific risk assessment strategies and approaches within the regulatory frameworks strongly depends on the willingness to accept of a substantial amount of uncertainty in which the use of decision strategies and/or risk governance approaches seems essential.

DOCUMENT HISTORY

Version	*Date*	*Reason of change (responsible person)*
1	2013-05-31	first draft
2	2013-06-16	second draft
3	2013-06-24	final draft
4	2013-06-27	final report
5	2017-02-24	Project Office harmonized lay-out

This project has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no 310584

Table of Content

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Summary

In this document we present the outcomes of a gap analysis in the knowledge that is needed to provide answers to a set of key questions and issues in the area of regulatory toxicology and risk assessment of nanomaterials. Several knowledge gap analyses on risk assessment of nanomaterials have already been published, but none have specifically addressed the data gaps from a regulatory perspective. From a regulatory perspective, the risk assessment of nanomaterials should contain a comparable extent of uncertainty as that of conventional chemicals. It should therefore be realized that filling the nano-specific knowledge gaps will not reduce all uncertainties that are also relevant for conventional chemicals. The information for the analysis has been gathered from the NANoREG partners as well as numerous ongoing and previous initiatives and projects within the EU, OECD and other fora. The main objective is to ensure an efficient research strategy in NANoREG, as well as to guide the research community in terms of the information that is critically needed by regulatory authorities and policymakers. It also will facilitate the use and integration of existing data and results from other projects. Results of this gap analysis will be shared with the NANoREG advisory boards and, together with results of Task 1.1, will form the main basis for the work under Tasks 1.3 and 1.4. This document is the main deliverable D1.2, but the task will continue in close linkage with 1.3 and 1.4 in order to keep pace with both scientific and regulatory developments. In this document, more insight is given in how a list of policy issues will match (or not) with the regulatory question from the description of work (see task 1.1.).

The results of this gap analysis confirm that the major knowledge gaps as identified in the description of work still exist. Most regulatory questions are related to the following three general knowledge gaps: a) characteristics that influence the risk of nanomaterials in the environment and humans, b) standardized methods to determine these characteristics, and c) nano-specific risk assessment strategies and approaches. Based on an overview of the extent to which the knowledge needed for each regulatory question is already available or is expected to become available on short or long term, we identified only a few short term research needs that did not seem to be fully addressed by the ongoing and planned initiatives (including NANoREG). These short term research needs are: a) more insight into implications of the implementation of the EC definition within the regulatory frameworks, b) methods to test or predict the extent and rates of transformation of nanomaterials by incineration, chemical reactions and other processes. None of the long term research needs are expected to be fully addressed by the ongoing and planned initiatives. The long term research needs mainly concern: a) further standardization and validation of methods for identification, quantification, characterization and transformation of nanomaterials, b) further identification and verification of the key characteristics that influence the release, exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks, c) the identification of the most appropriate metrics for each type of nanomaterials within each specific route of exposure and toxicological endpoint, d) further development and validation of the approaches for extrapolation, interpolation, read across and grouping, e) further development of approaches for safe design, and f) implementation of all these methods and knowledge into nano-specific risk assessment strategies and approaches within regulatory frameworks. There is also a lack in systematic sets of high quality data of well characterized nanomaterials on exposure, kinetics and toxicity to further

develop, verify and validate nano-specific methods and approaches. Until then, the implementation of nano-specific risk assessment strategies and approaches within the regulatory frameworks strongly depends on the willingness to accept of a substantial amount of uncertainty in which the use of decision strategies and/or risk governance approaches seems essential.

1 Introduction

It is currently not clear to what extent current environmental and human risk assessment approaches can be applied to manufactured nanomaterials. Furthermore, there is a lack of adequate, reproducible data to validate existing risk assessment strategies and develop a science-informed understanding of how to quantify and predict the potential risks of these sophisticated materials. At the same time, there is a parallel and somewhat intertwined challenge: quantitative toxicology and risk assessment are unlikely to keep pace with the accelerating development of emerging nanomaterials, meaning there will be a growing knowledge gap between the materials being produced, and the knowledge needed to ensure their safe use. Bridging this gap will require new approaches to evaluating risk and making decisions in the face of potential risks where there is incomplete information on exposure, hazard, and response. Over the years, a variety of knowledge gap analyses in relation to the risk assessment of nanomaterials have been made (Aitken et al., 2011; Hankin et al., 2011; ITS-NANO, 2013; Micheletti, Riego Sintes, & Vegro, 2010; NRC, 2012; OECD, 2012b; Santropoulou, 2012; Savolainen et al., 2013), all of them relating to more or less the same list of topics:

1) Identification, quantification and characterization of nanomaterials
2) Dose metrics
3) Release, transformation and exposure of nanomaterials
4) Uptake, distribution, clearance and accumulation of nanomaterials.
5) Modes of action that lead to toxicity
6) Nano-specific risk assessment strategies and approaches, including extrapolation, read across and grouping approaches.

And for all these topics reference materials, standardized methods and basic understanding which information is necessary for what purpose, is needed in order to get these implemented into guidance. This document identifies the most important data gaps on the environmental and human risk assessment from a regulatory perspective and identifies the scientific research needs on short and long term to fill these data gaps. Numerous ongoing and previous initiatives and projects within the EU, OECD and other fora have been reviewed and evaluated with respect to these regulatory research needs. This is to ensure an efficient research strategy in NANoREG, as well as to guide the research community in terms of the information that is critically needed by regulatory authorities and policymakers. It will also facilitate the use and integration of existing data and results from other projects. This document only gives a general overview of which previous and ongoing projects are related to which regulatory research needs, cross referencing to other sources for a more comprehensive overview of the available (and expected) scientific knowledge from other projects and initiatives. This gap analysis is performed in close connection to Task 1.1. In Task 1.1 a virtual workshop with regulators (national coordinators) and other stakeholders is planned in which the collection of regulatory issues or questions will be elaborated. Additional or more refined regulatory issues or questions generated during this workshop or other initiatives may be incorporated into the project in a later stage. As such these have not been considered in the present gap analysis which had to be delivered timely in order to avoid further delay in the execution of other NANoREG project activities.

2 Scope and limitations

2.1 Initial set of key regulatory question

A set of key questions with respect to regulatory toxicology has been defined at the start of the project (and described in the description of work) to set the outline of NANoREG. These questions are already a translation of the regulatory issues and the NANoREG project description of work does not specify the relation between these. Table 1 gives an overview of these priority regulatory questions per domain. Because the results of the virtual workshops were not available in time for this deliverable, the results of this process are not taken into account in this gap analysis. Table 1 is a synthesis of the initial questions and demands from national regulation and legislation authorities, the RIP-oN reports and the lessons learned from FP6/7 projects, such as MARINA, NANODEVICE, ENPRA, HINAMOX, NanoSafe2, CellNanoTox, NANEX, NANOGENOTOX, NHECD, OBSERVATORY NANO, QualityNano and SIINN. Because the initial focus of the project was limited to regulatory toxicity testing, some regulatory questions on exposure and risk assessment are not included in this initial set of questions. However, the flexible architecture of NANoREG makes it possible to incorporate additional regulatory questions generated by the regulation and legislation authorities during the execution of the project.

Table 1: Initial set of key regulatory questions as formulated in the description of work at the start of the project to address the needs of regulatory authorities and the current policy issues.

Measurement and characterization	•How can nanomaterials be identified for the purpose of risk assessment as well as according to the EU definition of nanomaterials?
Identification	•Is a nanomaterial (particles, fibres) always a nanomaterial? Or are there circumstances that result in nanomaterials being transferred into something which does not fit into the EU definition of nanomaterials?
Metrology and dose metrics	•Which metrics (metrology) should be used for nanomaterials in regulatory toxicology?
Extrapolation	•What guidance can be provided on how to decide when information from different forms of nanomaterials (or from the bulk material) can be 're-used'?
Persistence and long term effects	•Will nanomaterials accumulate in man, the environment and environmental species and what are the driving forces?
Kinetics and fate	•To what extent is the dosimetry for nanomaterials (e.g. deposition, biodistribution) different from the bulk material and what are the options to extrapolate information on these aspects from bulk material or different sizes of the same chemical to the nano-range?
Mode of action	•What are critical characteristics of nanomaterials that need to be considered to develop safer nanomaterials?

Based on the kick off meeting of NANoREG (Amsterdam, 15 May 2013, WP7 meeting) and several recent policy related documents (Azoulay, Buonsante, Cameron, & Vengels, 2012; Bosman, 2013a, 2013b;

Christensen, 2012; Christensen & Larsen, 2013; EC, 2012; Fleischer, Jahnel, & Seitz, 2012; KEMI, 2013; UBA, 2013), it was recognized that the initial set of questions did not fully reflect the current policy issues. The initial set of regulatory questions has to be regarded as a first translation from policy questions and issues towards scientific questions. The table underneath shows which answers to the initial set of questions are expected to provide scientific knowledge on topics relevant to the current policy issues.

Table 2: Overview of which of the answers to the initial regulatory questions are expected to provide scientific knowledge relevant to the current policy issues.

Initial set of regulatory questions → **Policy issues* ↓**	How can nanomaterials be **identified** for the purpose of risk assessment as well as according to the EU definition of nanomaterials?	Is a nanomaterial (particles, fibers) always a nanomaterial? Or are there circumstances that result in nanomaterials being **transferred** into something which does not fit into the EU definition of nanomaterials?	Which **metrics** (metrology) should be used for nanomaterials in regulatory toxicology?	What guidance can be provided on how to decide when **information** from different forms of nanomaterials (or from the bulk material) can be '**re-used**'?	Will nanomaterials **accumulate** in man, the environment and environmental species and what are the driving forces?	To what extent is the dosimetry for nanomaterials (e.g. deposition, **biodistribution**) different from the bulk material? What are the options to **extrapolate** information on these aspects?	What are **critical characteristics** of nanomaterials that need to be considered to develop safer nanomaterials?
Implementation of a harmonized definition within all regulatory frameworks	✓	✓	✓				
Timely evaluation of both existing and new nanomaterial				✓			
Tonnage level/threshold for registration within REACH			✓				
Registration of nanomaterials and products for market surveillance	✓						
Labelling of nanomaterials and products for consumer transparency	✓						
Testing protocols and dossier requirements				✓	✓	✓	✓
Lack of information on workers protection		✓		✓	✓	✓	✓
Risk governance approaches to deal with uncertain and complex risks	✓						✓

* These policy issues are derived from several recent policy related documents (Azoulay, Buonsante, Cameron, & Vengels, 2012; Bosman, 2013a, 2013b; Christensen, 2012; Christensen & Larsen, 2013; EC, 2012; Fleischer, Jahnel, & Seitz, 2012; KEMI, 2013; UBA, 2013). Annex 2 gives more insight in which NANoREG work package the regulatory questions will be addressed.

None of the policy issues can be solved by scientific knowledge on the safety of nanomaterial alone, as policy making often is a matter of a mix of scientific evidence and politics. However, science is relevant for evidence-based policy and can provide impact on the scientific evidence on specific topics within a policy issue. One may for example use scientific knowledge on the limitations of the available methods for identification and quantification of nanomaterials in weighing the advantages and disadvantages of a

registration system for nanomaterials and products. Table 2 shows that the answers to the initial set of questions will provide scientific knowledge to all of the policy issues. Although some policy issues are expected to receive input from most regulatory questions, this does not mean that all essential scientific knowledge is provided, because this input is generally limited to one or two specific topics. No overall evaluation is made of the extent to which the essential scientific knowledge for each of the policy issues will be provided by answering the initial set of regulatory questions. However, it seems remarkably that none of the regulatory questions specifically address nano-specific information requirements. As noted above, exposure and risk assessment (including risk governance) are also not specifically addressed by any regulatory question, because the initial focus of the project was limited to regulatory toxicity testing. Scientific knowledge on these topics is especially useful for the last five policy issues. The link between the policy or regulatory issues and the essential scientific knowledge and data needs to become more clear and will need continuous attention during throughout the whole project duration of NANoREG.

2.2 Focus and exclusions of this gap analysis

Several gap analyses have already been published, but none have specifically addressed the data gaps from a regulatory perspective. Because of the limited time frame for this gap analysis, it uses previous gap analysis to describe the gaps and research needs with respect to a limited set of regulatory questions.

Focus: Inside the scope of this gap analysis are:

- Description of knowledge gaps and research needs with respect to the initial set of regulatory questions on environmental, health and safety assessment of nanomaterials
- General overview of previous and ongoing projects

Exclusions: Outside the scope of this gap analysis are:

- Description of knowledge gaps and research needs with respect to specific information requirements, life cycle assessment, exposure assessment or risk governance approaches
- Detailed description of previous and ongoing projects
- Improving the link between regulatory needs and scientific knowledge

2.3 Focus and exclusions of the NANoREG project

During the NANoREG project, additional regulatory questions will be added to the initial set and more detailed descriptions of results from previous projects and initiatives will form the start of the scientific research to be performed within WP2 through 6.

Focus: Inside the scope of NANoREG are:

- Addressing initial and additional regulatory questions (T1.1, 1.3 and 1.4)
- More detailed analysis of existing knowledge and tools (WP2-6)
- Improving the link between regulatory needs and scientific knowledge (T1.1, 1.3 and 1.4)
- Development of nano-specific methods for characterization, toxicity testing and exposure assessment.
- Development of nano-specific risk assessment and risk management approaches, including extrapolation, read across and grouping approaches
- Generate new data on to develop these methods and approaches (mainly on physicochemical characteristics and hazards)

Exclusions: Outside the scope of NANoREG are:

- Full acceptance, validation and implementation of newly developed methods and approaches into the various regulatory frameworks
- Generation large amounts of new data on the characterization, toxicity and exposure of a large amount of nanomaterials

3 Data gaps and research needs

Nanomaterials may behave differently compared to the molecular, ionic or larger bulk form of their chemical components because of their small size. Because of their complex interactions with their biological environment and their changing physical chemical characteristics throughout the life cycle, most data gaps are related to the lack of understanding of the behavior and effects of nanomaterials in the environment and living organisms. This leads to a large number of uncertainties on how to actually perform risk assessment of nanomaterials, especially with regard to the extrapolation of fate/effect data across media, biological species, and across the physical chemical properties of nanomaterials. These uncertainties need to be reduced by improving the applicability of common instruments and their ability to assess the risk of the nanomaterial under investigation. This will facilitate the development of further testing strategies as well as decision strategies under (persistent) uncertainty, and risk governance approaches.

In the following paragraphs, a description of the most relevant data gaps and research needs to reduce the uncertainties within the risk assessment of nanomaterials is given for each of the regulatory questions. Taking the objectives of ongoing and planned initiatives into account, the remaining short term (within the next couple of years) and long term research needs are identified. A more detailed description of the knowledge obtained from previous projects and initiatives can be found in the previous gap analysis (ITS-NANO, 2013; NRC, 2012; Santropoulou, 2012), the NanoSafety Cluster compendia for most FP7 projects (Riediker, 2013; Riediker & Katalagarianakis, 2012) and project websites of the individual project (Annex 1). These sources can also be referred to for more details on the expected contribution of the ongoing or planned initiatives. A more detailed description of the expected contribution of NANoREG to these research needs can be found in Annex 2.

3.1.1 Identification: Which materials and how to measure?

3.1.1.1 How can nanomaterials be identified for the purpose of risk assessment as well as according to the EU definition of nanomaterials?

To identify nanomaterials, both a clear definition and standardized detection and measurement methods are needed.

Although the need for a clear **definition** is also questioned (Maynard, 2011) and suggestions have been made to use flexible criteria instead (Feitshans, 2013), several institutions and countries have published definitions or descriptions of the term nanomaterial (BSI, 2011; ISO, 2008; JRC, 2011; NICNAS, 2010; SCCS, 2012; SCENIHR, 2010). For regulatory purposes the EC recommendation on the definition (EC, 2011) is the most logical choice, because this definition was primarily intended to provide unambiguous criteria to identify nanomaterials. This definition would then form the basis for specific regulatory provisions in various pieces of legislation.

To identify nanomaterials according to the EC definition the (primary) particle size distribution and/or volume specific surface area (VSSA) are regarded as the main characteristics to be determined within different media. The implementation of this definition in different regulatory frameworks is currently ground for intense debate. The main reason for this is that the definition can be used to identify nanomaterials, but not to identify the risks nanomaterials. It appeared to be complex to gain a good view on the impact of this definition on policy goals and along going regulatory consequences (Bleeker et al., 2013). Even for the purpose of consumer transparency only the identification of nanomaterials (irrespective the risks) might not be sufficient, because consumers might still consider the labeling of products with “nano” related to health risks. Another important purpose of including a definition for nanomaterials in different regulatory frameworks is to ask for some (additional) information on the material to assess the nano-specific risks. This is for instance illustrated by the inclusion of the wording “insoluble or biopersistent” in the current definition in the regulation for cosmetics (EU, 2009). The term insoluble or biopersistent is included because additional ‘nano-specific’ information seems unnecessary to assess the risk if a nanomaterial is not persistent, i.e. it easily loses its particle character. However, unless the same wording is implemented in all legislations, this may result in a situation where materials that are defined as nanomaterials in one specific regulatory framework, would not be defined as such in another framework. This situation can be prevented by clearly separating the purpose of identifying nanomaterials from the purpose of identifying the nano-specific risks. This can be achieved by implementing the same definition for “nanomaterial” in all regulatory frameworks and determine the specific requirements (to **identifying the nano-specific risks**) for nanomaterials as a second step which may be different for each regulatory framework. Several projects and initiatives have looked into nano-specific information requirements, within REACH (Christensen, 2012; Christensen & Larsen, 2013; ECHA, 2012; Hankin, et al., 2011) These specific requirements can, for example, follow a tiered approach, such as described in the EFSA guidance document (EFSA, 2011). Such a tiered approach could easily make the inclusion of e.g. “solubility” in the definition of a nanomaterial unnecessary by requiring no additional information for nanomaterials which easily dissolve in water, while requesting additional (toxicity) testing with the nanomaterial itself for persistent nanomaterials. To identify the specific requirements necessary for the risk assessment of nanomaterials, one need to determine when nano-specific risks should be considered and when these are unlikely to occur. However, based on the current scientific knowledge this is only possible to a limited extent. More knowledge on the **key characteristics/properties that influence the release, exposure, behavior** (fate and kinetics), **effects** (hazards) **and subsequent** environmental and human **risks** of nanomaterials is needed.

Next to a clear definition, **standardized methods** for identification are needed. To identify nanomaterials according to the EC definition, the main characteristics to be tested are the (primary) particle size distribution or VSSA (Bleeker, et al., 2013; Christensen, 2012). Although, there are several methods available to measure these characteristics, for most materials a combination of different methods is needed to determine if they fulfill the EC criteria. Still, several technical challenges remain. It is, for example, difficult to determine if aggregated materials fall under the EC definition, because it is usually not possible to measure the size distribution of their constituent primary particles. In such cases, it should be decided if it is acceptable to estimate the primary size distribution, based on the mean primary particle size and an assumed distribution of different sizes. Another option is the surface area may give more insight, because the surface area of agglomerates is similar to the sum of the surface area of the single particles. However, measuring the external surface area is at the moment only straight forward for powders (Bleeker, et al., 2013; Christensen, 2012). For risk assessment purposes, other characteristics that determine their release, exposure, behavior and effects in the environment and humans are also important. There are several methods to measure most of these characteristics for nanomaterials in the form they are produced (e.g. powders or liquid dispersions). However, these methods are not always suitable to characterize nanomaterials in different matrices (such as products, environmental compartments, test media and biological tissues) and after transformation, agglomeration and/or aggregation of the nanomaterials within these matrices (NRC, 2012; OECD, 2012a; Stone et al., 2013). In addition some of these methods, such as electron microscopy, are not readily accessible for everybody, because they require expensive equipment, highly trained personnel and a large amount of time (Linsinger et al., 2012). **Accessible, standardized methods to characterize nanomaterials in different media** are needed to identify, quantify and characterize the nanomaterials in all stages of their life cycle and within exposure and toxicity testing.

Short term remaining research needs to fill the data gaps with respect to **defining** nanomaterials are to gain more insight into implications of the implementation of the EC definition within the regulatory frameworks. With respect to **the identification of nano-specific risks**, insight into the influence of certain characteristics/properties of nanomaterials on the release, exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks of nanomaterials is required. For example the influence of particle size on hazard needs to be further established. Several projects have investigated the effect of size on the toxicity of nanomaterials *in vitro*, however, relatively few exposure, environmental fate and *in vivo* kinetic and toxicity studies have been performed with nanomaterials that only differ in size. The same holds for some other important properties that influence the risk of nanomaterials, such as surface area, crystalline structure and shape. In addition, a systemic analysis of all available data has not been performed, partly because the results of many experimental studies, including those performed in the OECD sponsorship program, are not publically available. Many current FP7 projects are investigating other properties that might influence the risk of nanomaterials, such as surface modification, dissolution and surface charge. Several of them, including NANOTRANSKINETICS, MODNANOTOX, NANOPUZZLES, ITS-NANO, MARINA and NANoREG, try to identify which specific *combination* of characteristics is most important in predicting the release, exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks and may therefore be used to develop nano-specific approaches for extrapolation, read across or grouping (see 3.1.4).

Long term remaining research needs are further identification of the key characteristics that influence the release, exposure, behavior (fate and kinetics), effects (hazards) and subsequent environmental and human risks and the implementation of some of this knowledge in the regulatory frameworks.

Short term research needs to fill the data gaps with respect to **standardized methods** are the identification or development of reasonably priced, accessible, standardized and validated methods and procedures to

quickly identify and quantify nanomaterials in different media according to the EC definition. In addition, standardized and validated methods to determine the most important characteristics of nanomaterials in different media are also needed to characterize the key properties of nanomaterials within the most critical stages of their life cycle and within the toxicity testing. Several projects have been investigating methods to identify, quantify and characterize nanomaterials (including NANOSUPPORT, OECD project on manufactured nanomaterials and test guidelines, NANOGENOTOX, ENPRA and NANODEVICE) and several others, such as SMART-NANO, NANOVALID, NANOSTAIR, MARINA and NANoREG will further develop and standardize those methods and procedures for which no standardized methods is available.

Long term remaining research needs are to further standardize and validate these methods for identification, quantification and characterization of nanomaterials in different media, including the development, standardization and validation of methods to measure additional key characteristics, for which no methods have been developed yet.

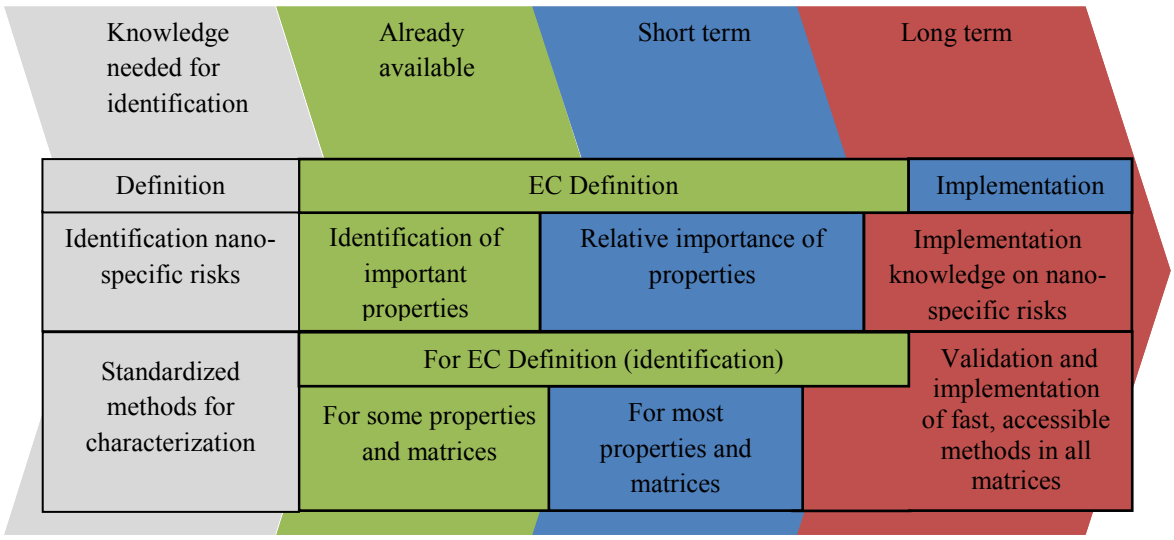

Figure 1: Overview of the extent to which the knowledge needed for identification (grey) is already available (green) or is expected to become available on short (blue) or long term (red).

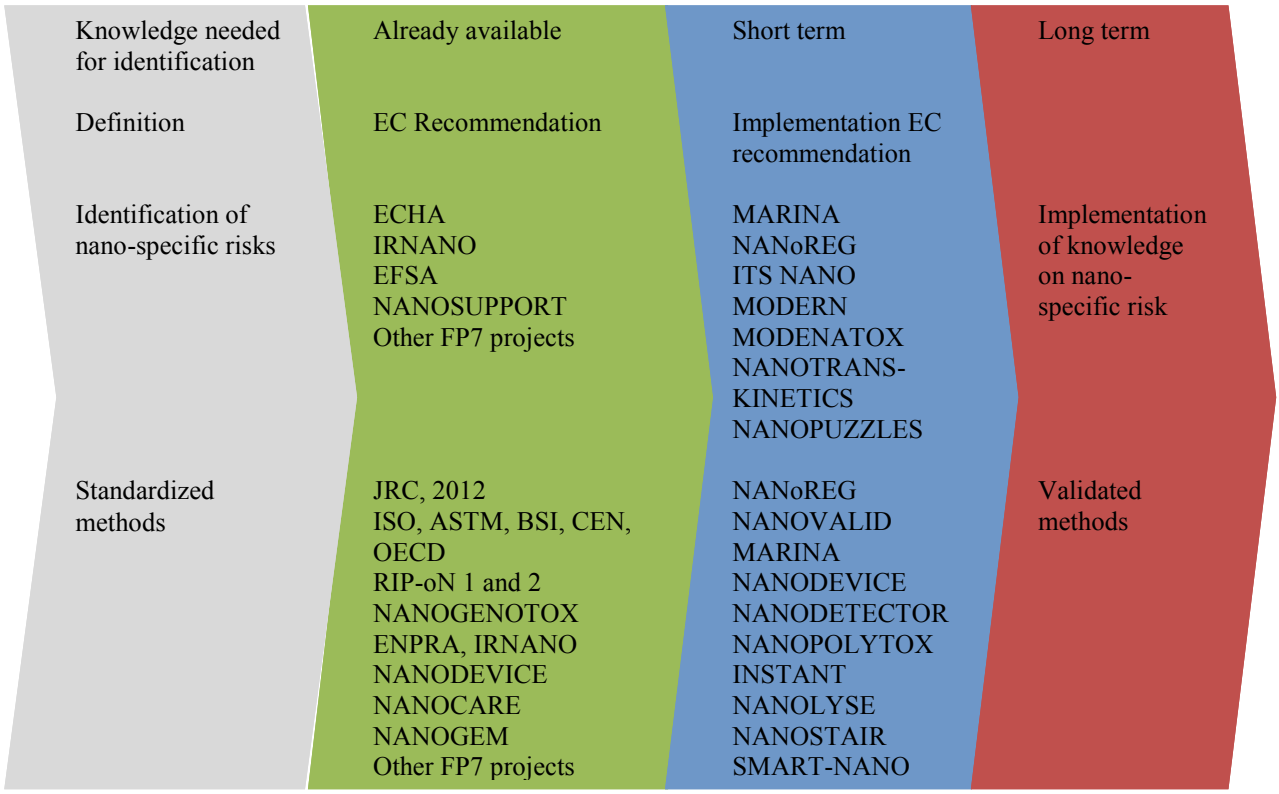

Figure 2: Overview of the initiatives and projects which have delivered (green) or are expected to deliver the knowledge needed for identification on short (blue) or long term (red).

3.1.2 Transformation: When is it no longer a nanomaterial?

3.1.2.1 Is a nanomaterial (particles, fibers) always a nanomaterial? Or are there circumstances that result in nanomaterials being transferred into something which does not fit into the EU definition of nanomaterials?

The physical chemical characteristics of nanomaterials can change throughout their life cycle, depending on the conditions and environment surrounding them (see also question 3.1.5). This may indeed lead to materials that do not meet the criteria set in the recommended EU definition for nanomaterials. If nanomaterials are dissolved into the molecular or ionic form of their chemical components, then they are of course also no longer nanomaterials and the behavior and related toxicity follows that of the molecular form of the chemical components (EFSA, 2011; Wijnhoven et al., 2009). However, dissolution is usually a gradual event which leads to situations in which over time the amount of nanomaterials gradually diminishes, while the amount of dissolved nanomaterials grows. This makes the risk assessment of gradually dissolving nanomaterials difficult (see option C in the figure underneath).

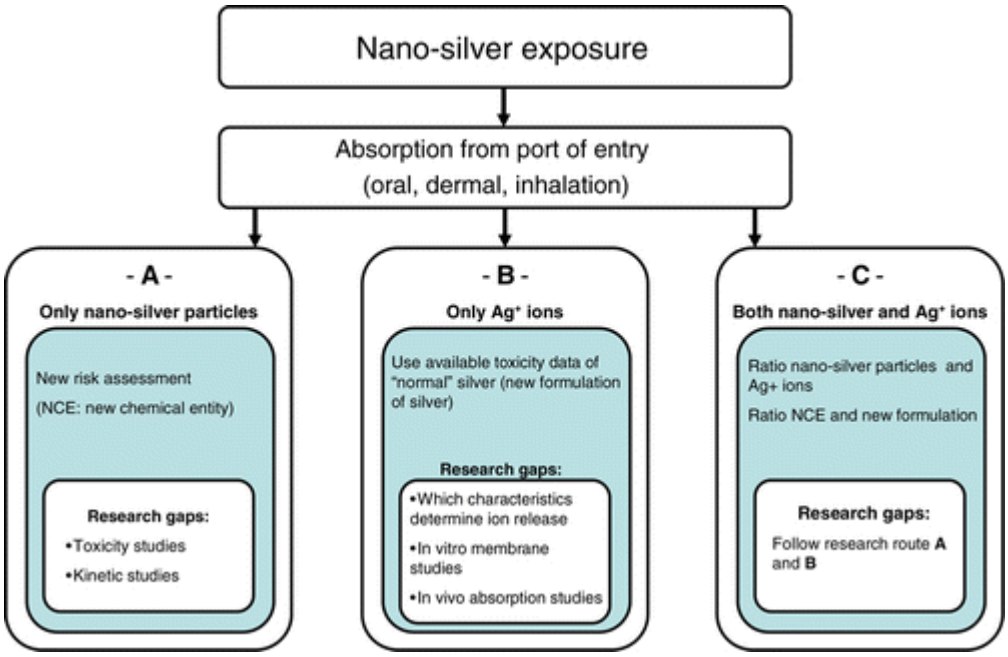

Figure 3: Overview of options for further toxicological research (Source: Wijnhoven et al., 2009).

There is limited **knowledge on the circumstances, extent and rate of dissolution**. Incineration, chemical reactions and other processes can also change or even destroy the (internal) structure of nanomaterials. Life Cycle Assessment (LCA) or other life cycle concepts can be used to identify these processes. However, not much is known about **the conditions that change or do not change the structure of nanomaterials** throughout the different stages of their life cycle. Aggregation and agglomeration changes the external size of the particles, which means that the material would still fall under the EU definition, because the primary particles size does not change. It should be noted that nanomaterials containing large (>100nm) aggregates and agglomerates may not always be identified as nanomaterial, because not all analytical methods are able to distinguish between primary particles, aggregates and agglomerates. Therefore, **methods that distinguish between primary particles and aggregated and agglomerates are essential in the identification of nanomaterials** (see also question 2.1.1).

Another related question is under which conditions nanomaterials can be formed. Although this might also be a relevant regulatory question, it was not included into the initial set of questions and will therefore not be discussed in this data gap analysis.

One of the short term research needs to fill data gaps with respect to **transformation of nanomaterials** into the molecular or ionic form of their chemical components is to further develop and **standardize methods** to test dissolution (rate) of nanomaterials in different biological and environmental matrices. Several FP7 projects, such as HINAMOX, NANORETOX and ENPRA, have already investigated methods to test dissolution. NANoREG, NANOVALID, NANOFATE, NANOPOLYTOX and a project within the OECD WPMN will further develop and standardize these methods. These methods can help to quickly scan the risk potential of certain nanomaterials and underscore the need for in vivo testing. In addition, these methods can be used to identify the most important circumstances under which nanomaterials will dissolve in different biological and environmental matrices (including air, water, soil, lung lining fluids, the skin, the gastrointestinal tract, macrophages, lysosomes, etc.). NANOSUSTAIN has investigated incineration of nanomaterials. Remaining short term research needs are to develop methods and further identify important

circumstances for incineration, chemical reactions and other processes which can lead to destruction or transformation of (the internal structure of) nanomaterials.

Long term remaining research needs are to further **standardize and validate methods** to **test or predict the extent and rates of the transformation of nanomaterials** into the molecular or ionic form of their chemical components by dissolution, incineration or other processes. Other remaining long term research needs are the implementation of some of these circumstances in risk assessment approaches and regulatory frameworks.

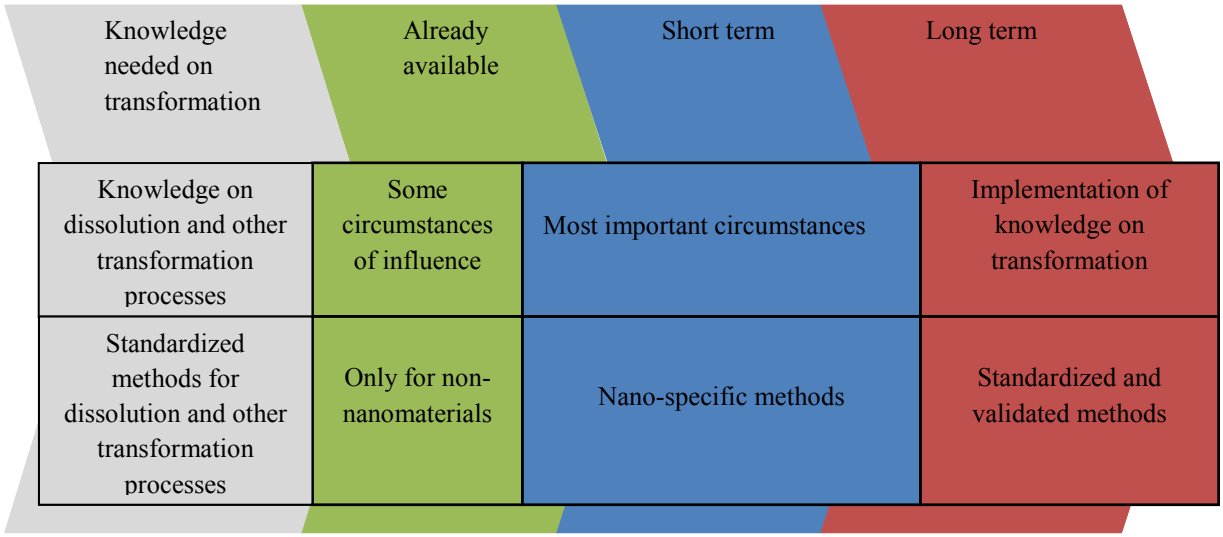

Figure 4: Overview of the extent to which the knowledge needed on transformation (grey) is already available (green) or is expected to become available on short (blue) or long term (red).

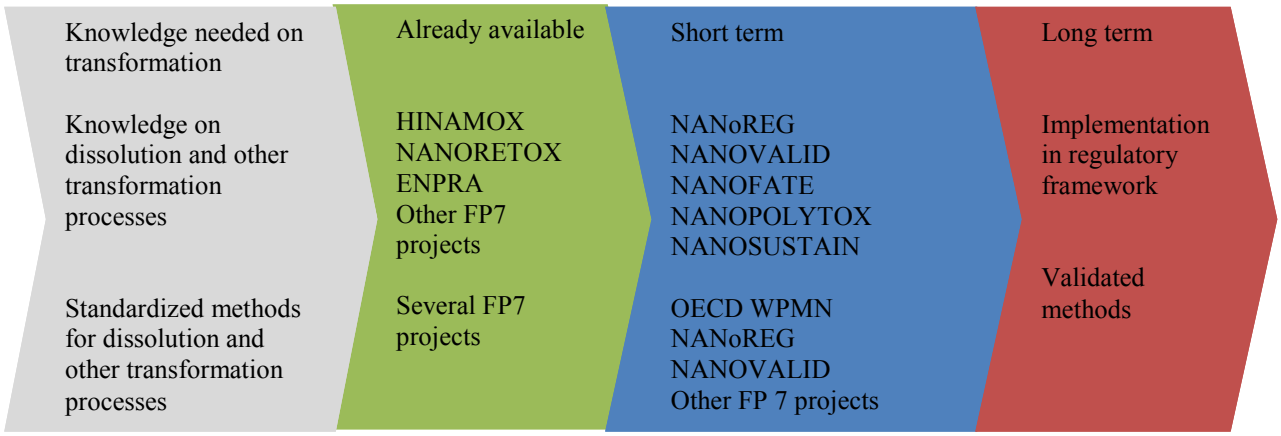

Figure 5: Overview of the initiatives and projects which have delivered (green) or are expected to deliver the knowledge needed on transformation on short (blue) or long term (red).

3.1.3 Dose metrics: How to quantify?

3.1.3.1 Which metrics (metrology) should be used for nanomaterials in regulatory toxicology?

The risk of nanomaterials is influenced by many characteristics and properties, which means that information on the administered weight (mass) alone is usually not sufficient to describe the dose that determines a particular response in a biological system. In order to identify which dose metrics should be used for nanomaterials in regulatory toxicology, one should first identify in which parts of the regulatory frameworks quantitative information on the amount of nanomaterials are used. Within REACH, for example, the first quantitative information that is used is the production volume of the substance. This production volume determines if registration is needed and which data requirements are applicable. Within the Classification, Labeling and Packaging (CLP) regulation, dose levels at which toxicity effects are observed within different toxicity tests and/or the elimination rate from and relative concentrations in organisms, determine the classification and labeling of the substance. Within most regulatory frameworks, dose levels at which effects are observed in experimental tests are used to determine exposure limits (DNELs, PNECs, OELs, ADIs, etc.) which can be compared to the estimated exposure levels to estimate the risk.

Most quantitative information used in regulatory frameworks is used to distinguish substances with a relatively low potential risk from those with a relatively high potential risk. The dose metrics that is most appropriate to compare the risks of nanomaterials is probably not the same for each situation, but seems to depend on the type of nanomaterial, the route of exposure, the kinetics and/or the toxicological endpoint. For example, the dose response curves for two sizes of otherwise identical titanium dioxide are markedly different from each other when the deposited mass in the lungs (A and B) are used. However, when the mass is converted in the total surface area of the deposited particles (E and F), there is a remarkable overlap in the dose response curves (see Figure 2) (Oberdorster, Oberdorster, & Oberdorster, 2007).

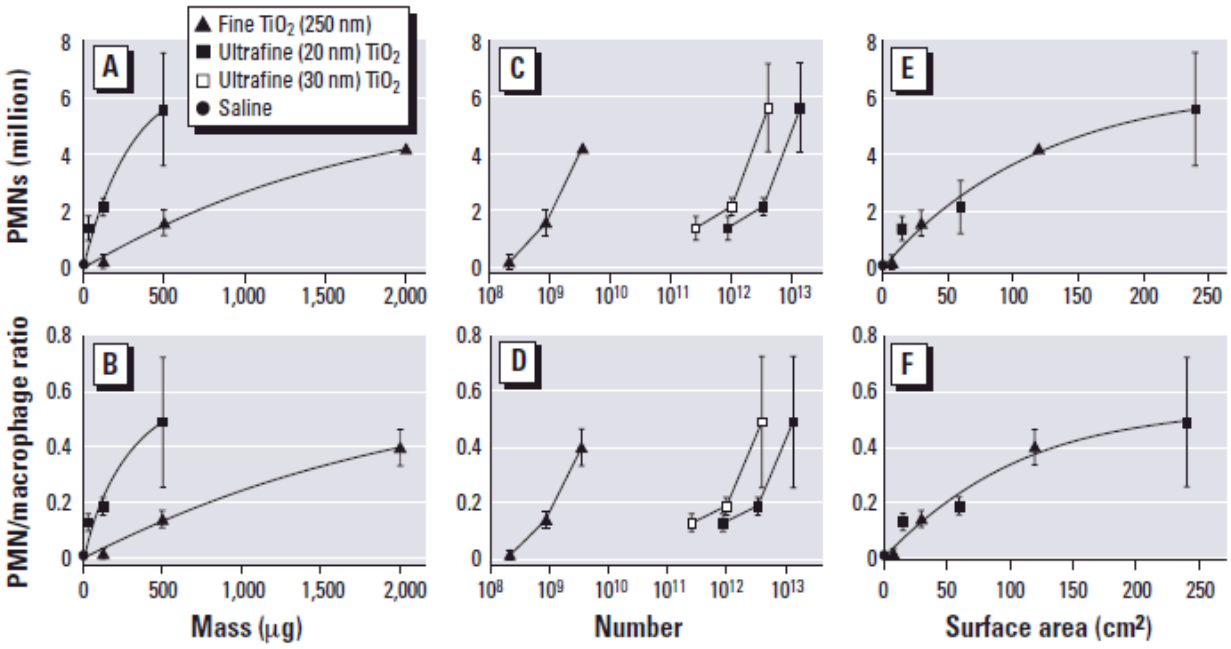

Figure 6: Inflammatory cell response in lung lavage 24 hr after intratracheal instillation of fine (~ 250 nm) and ultrafine (20-30 nm) TiO2 expressed by different dose metrics [particle mass (A, B), number (C, D), and surface area (E, F)] and different response metrics [number of PMNs (A, C, E) and PMN/macrophage ratio (B, D, F)] (Source: Oberdorster et al., 2007).

To determine which dose metrics should be used in which situation, more knowledge on the **key characteristics/properties that influence the exposure (release and fate in the environment), kinetics (internal dose at the target tissue) and subsequent toxicity** of nanomaterials is needed. Furthermore, knowledge on the implications of using different dose metrics in the different parts of the regulatory frameworks is needed. It is not practical to use all relevant characteristics in the dose description of nanomaterials in all parts of the regulatory frameworks. This would, for example, mean that different exposure limits would need to be derived with respect to each nanomaterial with (slightly) different characteristics, such as size or surface chemistry, etc. A more pragmatic way would be to use a reduced dose metric, in which, for example, the dose of nanomaterials consisting of the same chemical composition is characterized with fewer parameters (Park, de Jong, Oomen, & Delmaar, 2012). This approach can be justified if, for example, the role of some characteristics in the induced response is negligible compared to that of others. Alternatively, certain particle properties influencing the response may be uniquely related, such as particle size with surface area, reactivity and solubility, in such a way that only one parameter combining these properties needs to be included in the dose metric of all ENMs of the same chemical composition. Justification of the use of such a reduced dose metric should first be established by experimental study (Park, et al., 2012). However, the implications of using different dose metrics for different types of nanomaterials, routes of exposure and/or toxicological endpoints within the regulatory toxicology should also be considered. It may be difficult, for example, to compare the acute toxicity of nanomaterials and bulk materials if different dose metrics are used for the classification. The same holds true with respect to comparing different exposure routes or toxicological endpoints. Moreover, to be able to compare exposure and hazard the estimated exposure should be expressed in same dose metrics as the relevant exposure limit (e.g. OEL, ADI or PNEC).

Short term research needs to fill the data gaps with respect to the most appropriate **dose metric** are the development and use of **standardized protocols for sample preparation and the characterization** of nanomaterials **within exposure and toxicity studies** (including sampling strategy, data handling and the characterization of the nanomaterials that the cells or organisms are actually exposure to and the interaction of the materials with culture media, biological matrices, proteins, tissues and cells). These standardized protocols for sample preparation and characterization are needed to obtain a clear picture of the characteristics of the different nanoparticles (including their stability, homogeneity and aging) in realistic exposure situations and different stages of toxicity tests and their impact on the risk. Several initiatives and projects have been working on standardized protocols for sample preparation (OECD, 2012a) (NANOGENOTOX, ENPRA, etc.), exposure studies (NANEX) and characterization (see 2.1.1) and several others, such as NANOVALID and NANoREG will further develop and standardize these methods and procedures. Several FP7 projects have already identified the best dose metric within a specific situation (Oberdorster, et al., 2007; Park, et al., 2012). A more thorough analysis of the dose response data of existing studies might give more insight in the most appropriate metrics. Park et al. (2012) developed a practical mathematical based method to systematically study the question of what might be an appropriate dose metric. Several ongoing projects, such as NANoREG, are trying to gain further understanding for some other specific situations or types of nanomaterials. However, no clear picture has been developed to determine which metrics should be used under which circumstances for which type of nanomaterial yet.

Long term remaining research needs are further identification, verification and validation of **the key nanomaterial characteristics that influence the exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks** and the identification of **the most appropriate metrics for each type of nanomaterials within each specific route of exposure and toxicological endpoint**. In addition,

implementation of these most appropriate metrics and the approach to determine this metrics within the risk assessment approaches and regulatory frameworks is needed.

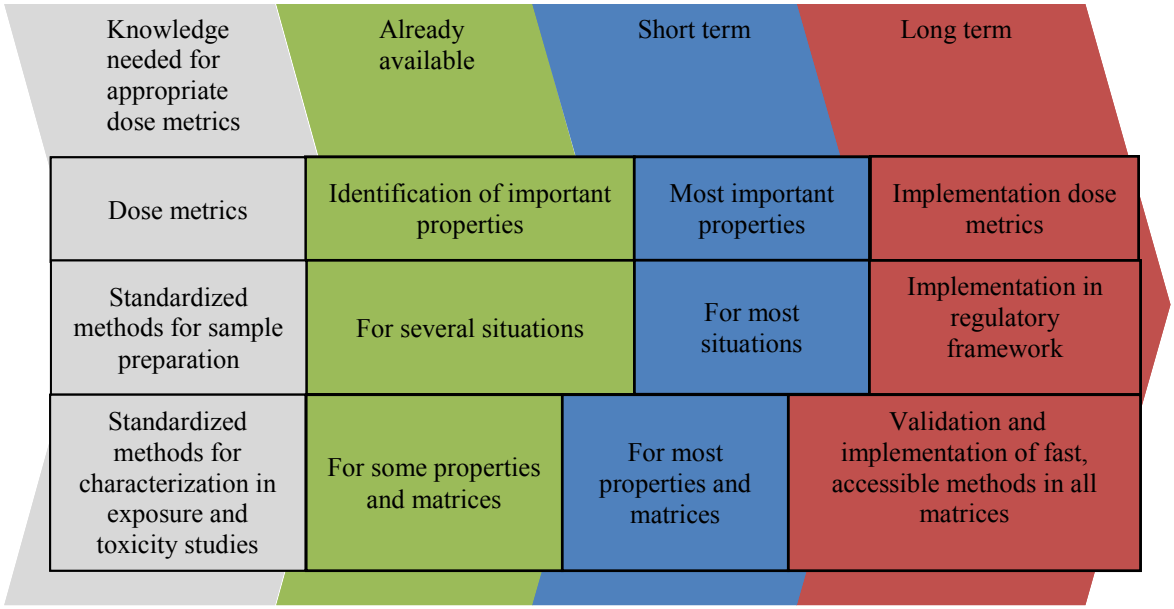

Figure 7: Overview of the extent to which the knowledge needed for appropriate dose metrics (grey) is already available (green) or is expected to become available on short (blue) or long term (red).

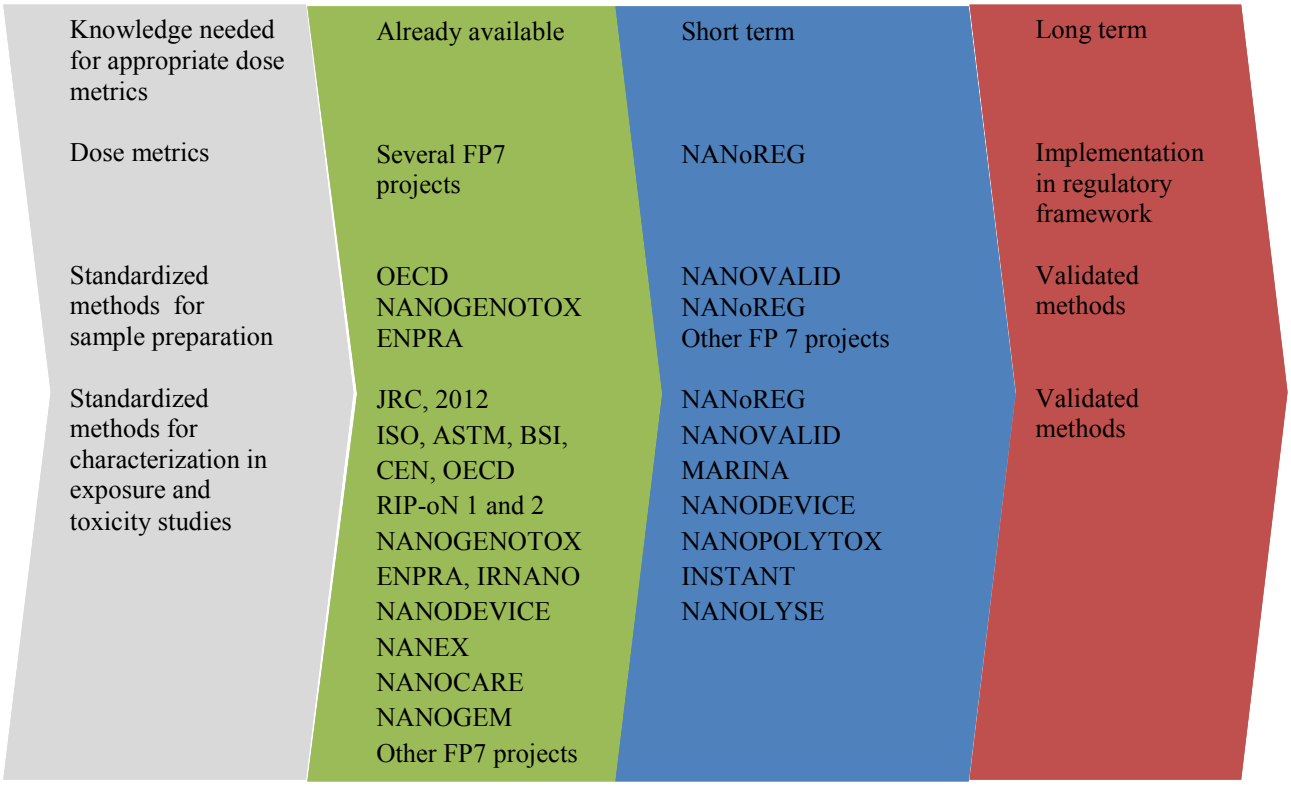

Figure 8: Overview of the initiatives and projects which have delivered (green) or are expected to deliver the knowledge needed for appropriate dose metrics on short (blue) or long term (red).

3.1.4 Extrapolation: Can information from bulk or other nanomaterials be used?

3.1.4.1 What guidance can be provided on how to decide when information from different forms of nanomaterials (or from the bulk material) can be 're-used'?

There are many ways to use information (on physical chemical characteristics, exposure and/or hazard) of different forms, types and sizes of nanomaterials (or the bulk material) for extrapolation, read across or grouping within the risk assessment of nanomaterials. It can be useful within both exposure and hazard assessment for different purposes, such as waiving, ranking or determining worst case situations. Several criteria for extrapolation, read across and grouping have already been identified, including chemical composition, shape, biological persistence/dissolvability, functionality, bio-interfaces, and biological effects. However, the precise combination and cut-off points that determine if extrapolation, read across and/or grouping can be used and for which purpose have not yet been determined. To do this, more insight in **the key characteristics/properties that influence the release, exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks** of nanomaterials is needed. Some ways of extrapolation, read across and grouping, for example approaches that assume common biological effects, are surrounded by a lot of uncertainty which makes them more difficult to use in a regulatory context. Other approaches, for example those assuming a similar extent of release from a particular matrix, might be less complicated to verify or validate, which would make them more attractive to use within a regulatory context.

Short term research needs to fill in the data gaps with respect to extrapolation are the **development of nano-specific approaches for extrapolation, interpolation, read across and grouping** based on the current knowledge on the most important characteristics/properties that influence the release, exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks of nanomaterials. In addition, some assumptions within these approaches need to be verified using existing or newly performed experimental data. Several current projects are investigating properties that influence the risk of nanomaterials, such as size, surface modification, dissolution and surface charge. Only a few of them, including NANOTRANSKINETICS, MODNANOTOX and NANoREG, try to identify which specific combination of characteristics is most important in predicting the release, exposure, behavior, effects and subsequent risks and may therefore be used to develop nano-specific risk assessment strategies and approaches, including extrapolation, interpolation, read across or grouping. Some of them, such as MARINA and NANoREG, also investigate how these approaches can be implemented within the risk assessment approaches and the regulatory frameworks.

Long term remaining research needs are further development and validation of the approaches for extrapolation, interpolation, read across and grouping and acceptance and final implementation within the risk assessment approaches and regulatory frameworks. For the implementation of these approaches it is important to know how much uncertainty is acceptable from a regulatory perspective. This kind of knowledge may be obtained using decision strategies and/or risk governance approaches which are able to cope with a substantial amount of uncertainty. Furthermore, quick tests to assign nanomaterials to the right group or to justify read-across, extrapolation, or interpolation should be developed.

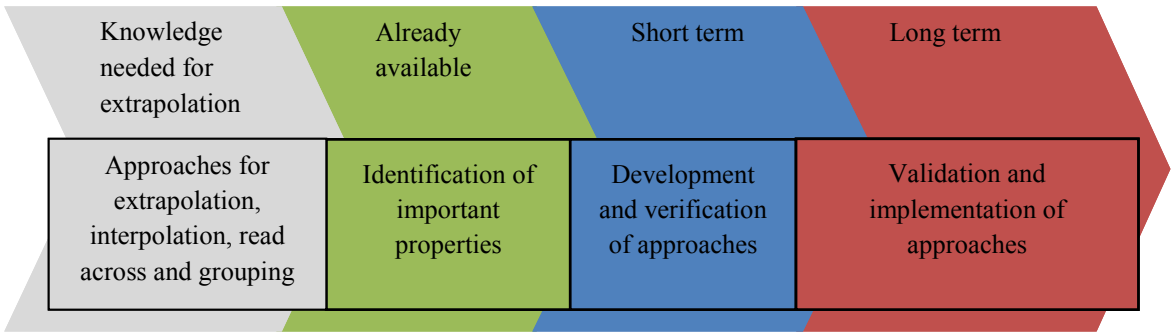

Figure 9: Overview of the extent to which the knowledge needed for extrapolation (grey) is already available (green) or is expected to become available on short (blue) or long term (red).

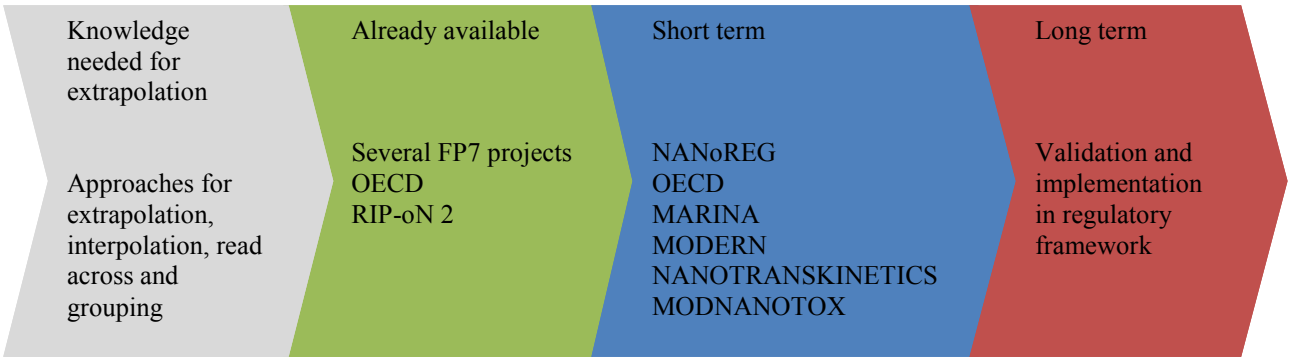

Figure 10: Overview of the initiatives and projects which have delivered (green) or are expected to deliver the knowledge needed for extrapolation on short (blue) or long term (red).

3.1.5 Fate and kinetics: Is the uptake, distribution and accumulation of nanomaterials different?

3.1.5.1 Will nanomaterials accumulate in man, the environment and environmental species and what are the driving forces?

The driving forces behind the behavior of nanomaterials in the environment and within environmental species and humans are not fully understood. Interaction of nanomaterials with their biological environment can change their physical chemical characteristics, including their ability to aggregate, agglomerate and/or dissolve and their surface composition (because they can surround themselves with a 'corona' of biomolecules). These changes are influenced by a variety of factors such as ionic strength, pH and the presence of natural organic and/or inorganic colloids. However, the precise distribution of the material between the dissolved and particular phases is largely unknown and quite variable between different types of nanomaterials (Pozzi-Mucelli, Duret, & Urbina, 2013).

Because of their small size nanomaterials do not seem to follow the same principles of passive diffusion and partitioning of the molecular or ionic form of their chemical components or the principles of not being able to cross cellular membranes of the larger bulk form of their chemical components (Malkiewicz et al., 2011). Therefore, the applicability of traditional approaches to estimate or model the biokinetics and biodistribution is limited. The nanomaterials can gain access to living systems across barriers in ways that small molecules and larger particles cannot, by receptor-mediated processes which can respond and adapt based on demand or other signals (Malkiewicz, et al., 2011). Although, very little is known about the parameters that

influence these processes, surface modifications and the corona are likely to play an important role in this. Once nanomaterials reach the circulation, they generally disappear rapidly from the blood by being taken up into tissues, mainly those containing phagocytic cells (Oomen et al., 2013). Nanomaterials are easily recognized as foreign to the body and usually taken up by macrophages and similar cell types of the lung, gastrointestinal tract or others tissues, following the endosomal pathway and generally end up almost entirely in the lysosomes. After uptake by these cells, nanomaterials do not easily exit cells and only undergo whole-body elimination to a fairly limited extent, indicating a significant potential for bioaccumulation of nanomaterials in organisms, particularly in the lysosomes of macrophages (Malkiewicz, et al., 2011; Oomen, et al., 2013). To be able to determine if and under which circumstances nanomaterials accumulate in man, the environment and environmental species, more knowledge on **the key characteristics that influence the fate, behavior and kinetics of nanomaterials** in the environment, environmental species and humans in needed.

Several related questions with respect to the life cycle assessment (LCA) of nanoproducts (including the release of nanomaterials from products), exposure, but also on absorption, distribution, excretion and the potential effects of accumulation of nanomaterials on the health of environmental species and humans might also be relevant from a regulatory perspective. However, these were not included into the initial set of questions and will therefore not be discussed in this data gap analysis.

Short term research needs to fill in the data gaps on fate, behavior and kinetics, including accumulation, are the identification of the characteristics that influence the kinetics and possible accumulation of nanomaterials. Several projects have investigated which properties influence the kinetics and possible accumulation of nanomaterials in the environment and organisms (e.g. NANOGENOTOX, NANOECOTOX). In addition, several current projects (e.g. MembraneNanoPart, NANOMILE, NANOTRANSKINETICS, MODNANOTOX and NANoREG) will generate more data on the fate, behavior and kinetics of nanomaterials. NANOTRANSKINETICS and MODNANOTOX will also try to identify the specific combination of characteristics that is most important in predicting the kinetics and possible accumulation of nanomaterials within organisms.

Long term remaining research needs are to identify and verify the most important characteristics/properties that influence the fate, behavior and kinetics of nanomaterials. In addition, research on the implications and implementation of this knowledge within the risk assessment approaches and regulatory frameworks is needed.

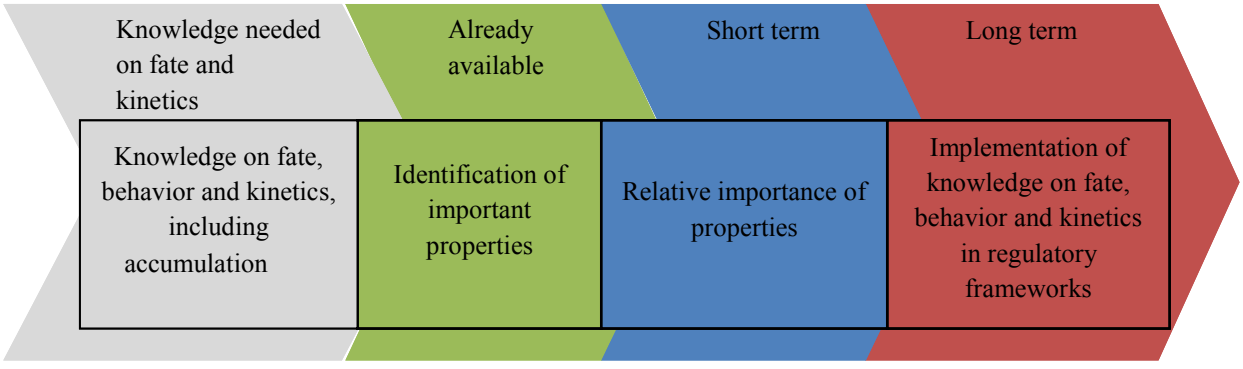

Figure 11: Overview of the extent to which the knowledge needed on fate and kinetics (grey) is already available (green) or is expected to become available on short (blue) or long term (red).

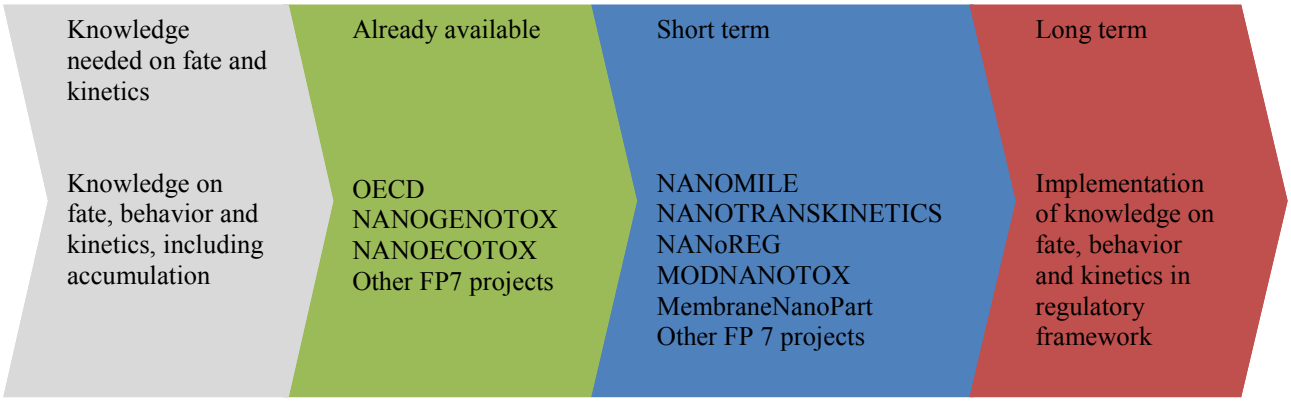

Figure 12: Overview of the initiatives and projects which have delivered (green) or are expected to deliver the knowledge needed on fate and kinetics on short (blue) or long term (red).

3.1.5.2 *To what extent is the dosimetry for nanomaterials (e.g. deposition pattern upon inhalation, biodistribution) different from the bulk material and what are the options to extrapolate information on these aspects from bulk material or different sizes of the same chemical to the nano-range?*

The deposition of nanomaterials is different from their larger counterparts, which makes it difficult to extrapolate information on the deposition pattern and distribution from bulk materials to the nanorange. More knowledge on the key characteristics that influence the behavior of nanomaterials in environmental species and humans is needed. The research needs to fill the data gaps on the extrapolation of kinetics have already been described in section 3.1.5.1 for kinetics (dosimetry) and 3.1.4 for extrapolation.

3.1.6 Safer nanomaterials: What makes them hazardous?

3.1.6.1 *What are critical characteristics of nanomaterials that need to be considered to develop safer nanomaterials?*

Several important characteristics that influence the release, exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks (or safety) of nanomaterials have already been identified (see also 3.1.4 on extrapolation). The size, shape, surface modification, chemical composition, dissolution, degradability, hydrophobicity and polarity all influence the release, exposure, fate, kinetics and toxicity of nanomaterials. Although, the precise combination of characteristics that is most important in predicting the risks (or safety) of nanomaterials is not known yet, approaches to use the knowledge on important characteristics in developing safer nanomaterials will be developed within NANoREG.

Short term research needs for the development of safer nanomaterials are to utilize the current knowledge on important characteristics/properties that influence the risk in the design of new nanomaterials and to use the current knowledge of the developers in the development of nano-specific risk assessment approaches.

Long term remaining research needs are further development and implementation of the approaches for safe design, which utilize the knowledge on the characteristics that influence the risks in the design of nanomaterials.

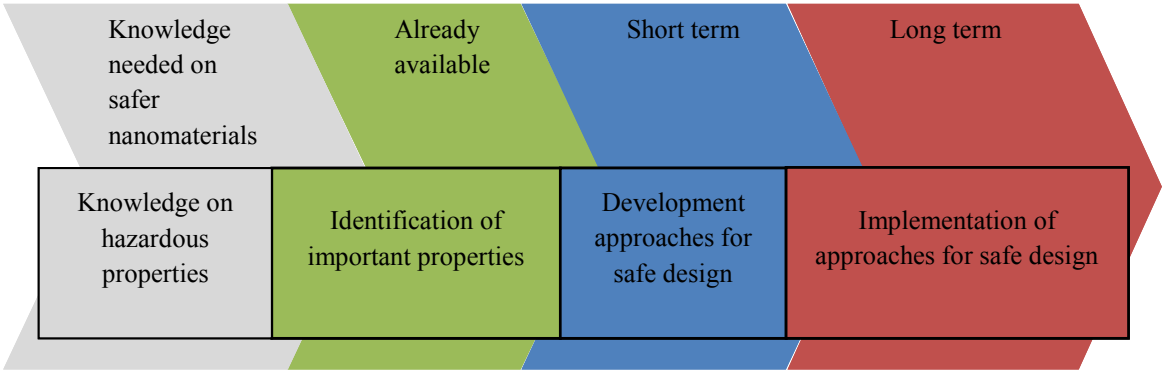

Figure 13: Overview of the extent to which the knowledge needed on safer nanomaterials (grey) is already available (green) or is expected to become available on short (blue) or long term (red).

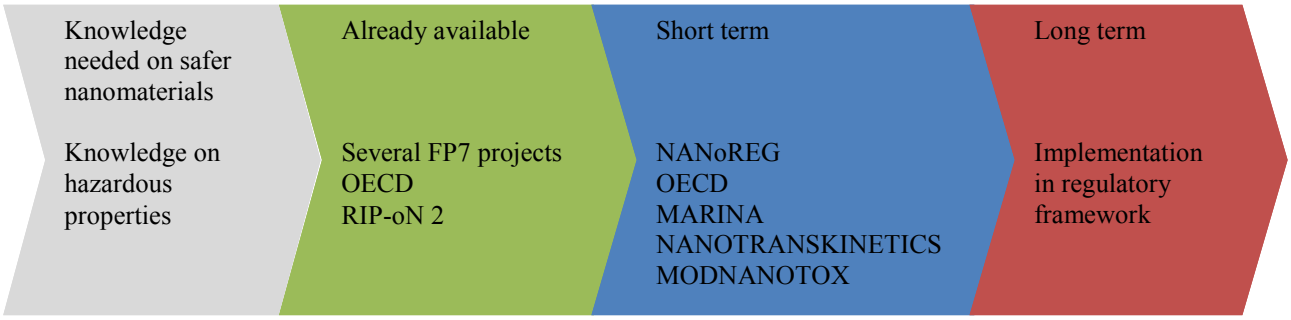

Figure 14: Overview of the initiatives and projects which have delivered (green) or are expected to deliver the knowledge needed on safer nanomaterials on short (blue) or long term (red).

4 Conclusions

The scientific knowledge needed to answer most regulatory questions is related to the following three general knowledge gaps:

- Identification of the key characteristics or properties that influence the release, exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks of nanomaterials
- Standardized methods for identification, quantification and characterization
- Nano-specific risk assessment strategies and approaches

Most importantly **the key characteristics or properties that influence the release, exposure, behavior (fate and kinetics), effects (hazards) and thus the subsequent risks** of nanomaterials need to be identified. Knowing which characteristics determine the release, exposure, behavior and effects of nanomaterials in the environment and humans, will provide information on when nano-specific risk assessment is necessary and which information is needed to allow such risk assessment. In addition, **standardized methods to determine the key characteristics** are needed. Finally, knowledge on these characteristics need to be implemented in **nano-specific risk assessment strategies and approaches**, including extrapolation, read across and grouping approaches. These strategies and approaches need to be

verified by experimental data obtained from standardized exposure measurements, kinetics and toxicity studies. In Figure 15 an overview is given of the general knowledge gaps that need to be filled to answer each regulatory question.

These three knowledge gaps will not be fully solved within the next couple of years (short term). Full understanding on how nanomaterials behave, change and cause effects within the environment and living organisms will probably never be achieved. However, some understanding is already available and more knowledge will be generated within the immediate future.

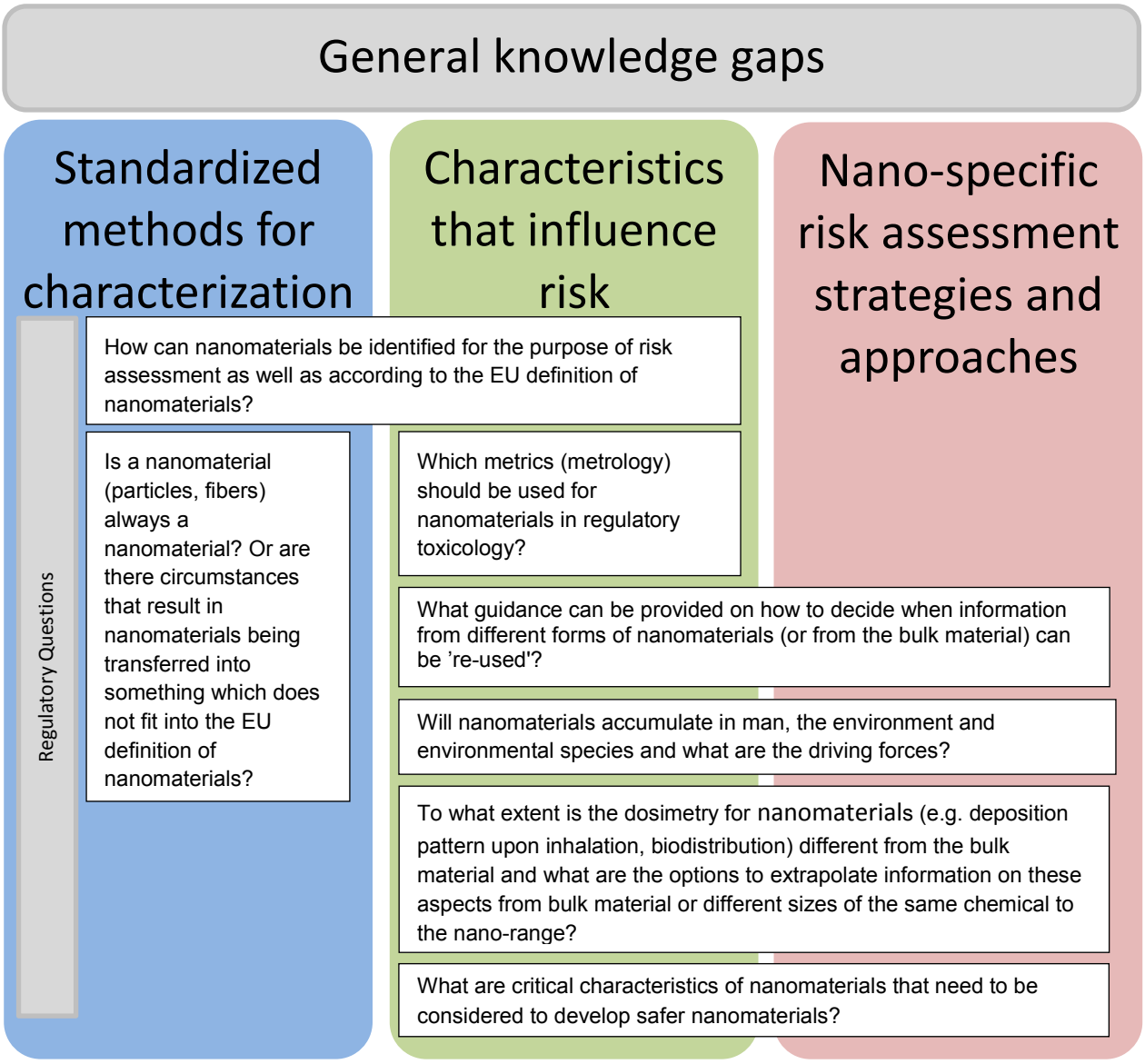

Figure 15: General knowledge needed to answer each of the regulatory questions as described in the description of work

An overview of the knowledge with respect to the regulatory research needs that is already available or expected to become available in the short or long term is given in Figure 4. This overview shows that

methods for characterization will probably be available for most characteristics within a couple of years, but validation of these methods is expected to take longer. With respect to the identification of characteristics that influence the risk, it is expected that most of them are already identified or will be in the next couple of years. However, more thorough knowledge on the specific combination of characteristics that is most important in predicting the risk will take longer, as will the implementation of this knowledge into nano-specific risk assessment strategies and approaches within the regulatory frameworks.

Most short term research needs are addressed by one or several of the ongoing or planned projects or initiatives. Only few short term research needs did not seem to be fully addressed. These remaining short term research needs are:

a) gaining more insight into implications of the implementation of the EC definition within the regulatory frameworks,
b) further identification of important circumstances for incineration, chemical reactions and other processes that can lead to destruction or transformation of nanomaterials, and
c) developing methods to test the extent and rates of transformation by these processes.

None of the long term research needs are expected to be fully addressed by the ongoing and planned initiatives. The remaining long term research needs mainly concern:

a) further standardize and validate of methods for identification, quantification and characterization of nanomaterials in different media
b) further identification, verification and validation of the key nanomaterial characteristics that influence the exposure, behavior (fate and kinetics), effects (hazards) and subsequent risks
c) further standardize and validate methods to test or predict the extent and rates of the transformation of nanomaterials into the molecular or ionic form of their chemical components by dissolution, incineration or other processes,
d) the identification of the most appropriate metrics for each type of nanomaterials within each specific route of exposure and toxicological endpoint
e) further development and validation of the approaches for extrapolation, interpolation, read across and grouping
f) further development approaches for safe design, and
g) implementation of all these methods and knowledge into nano-specific risk assessment strategies and approaches within regulatory frameworks

Given the huge amount of different types and forms of nanomaterials, nano-specific risk assessment strategies and approaches, including extrapolation, interpolation, read across and grouping, are essential to efficiently evaluate all nanomaterials. For further development, verification and validation of these strategies and approaches, more data on exposure and toxicity is needed. Policy decisions can have a huge impact on the generation of such data. The implementation of the EC definition in the different regulatory frameworks may, for example, generate a lot of data. Registration of nanomaterials, products containing nanomaterials and/or occupational exposure to nanomaterials will give more insight into the potential occupational and consumer exposure and the release of nanomaterials into the environment. However, to be useful for the verification and validation of approaches, systematic sets of high quality data need to be obtained using standardized exposure measurements, kinetics and toxicity studies. For the development and verification of grouping approaches, for example, at least one complete data set of all relevant characteristics, exposure, kinetic and toxicity data is needed for the most representative nanomaterial of each group. In addition, enough data is needed to demonstrate a relative (lower) potency for toxicity for each individual nanomaterial within the group. Most of the ongoing and planned projects, including NANoREG, will not generate large amounts of these data, but some systematic sub-sets of data for a certain group of nanomaterials or a specific endpoint will be generated to develop and verify specific methods and approaches.

Most of the available knowledge has not yet been transformed into nano-specific risk assessment approaches which can be implemented into regulatory frameworks. Within the near future more knowledge

will be incorporated into nano-specific risk assessment approaches. However, these approaches will still be surrounded by a lot of uncertainty. Using the currently or soon to be available methods to identify, quantify and characterize nanomaterials in different stages of their life cycle, some of these uncertainties can be reduced. However, more thorough verification or validation of these approaches will probably take much longer. Therefore, the implementation of nano-specific risk assessment approaches within the regulatory frameworks strongly depends on the willingness to accept of a substantial amount of uncertainty in which the use of decision strategies and/or risk governance approaches seem essential.

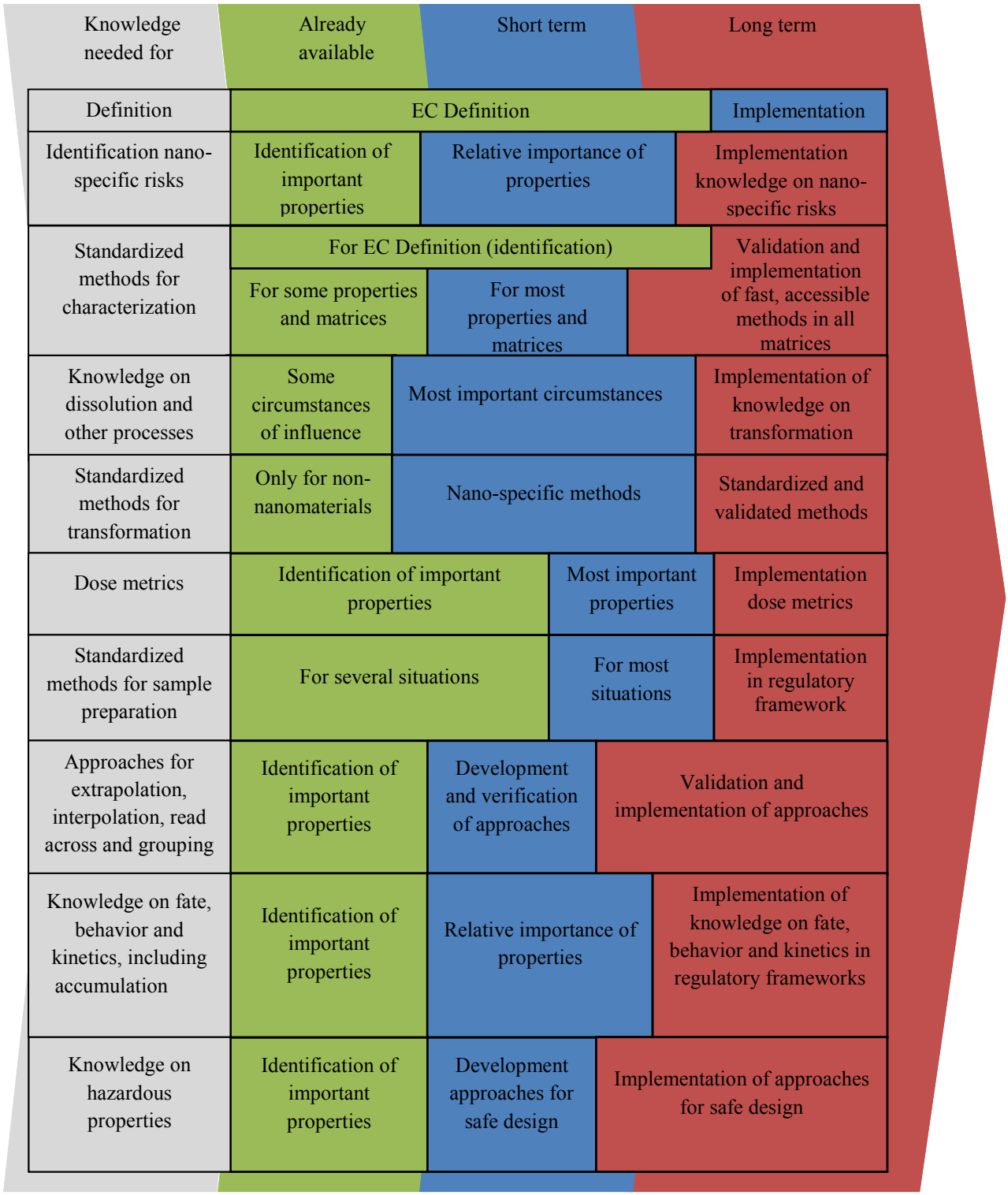

Figure 16: Overview of the already available knowledge (green) and the knowledge that is expected to become available on short (blue) and long (red) term with respect to the regulatory research questions.

Acknowledgements

We would like to thank the following people for participating in the internal review panel of this report:

Dick Sijm, National Institute for Public Health and the Environment (RIVM),

Eric Bleeker, National Institute for Public Health and the Environment (RIVM),

Grazia Barberio, Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA),

Håkan Wallin, National Research Centre for Working Environment (NRCWE),

Hugues Crutzen, Joint Research Centre (JRC),

Jan Roels, National Institute for Public Health and the Environment (RIVM),

Juan Riego Sintes, Joint Research Centre (JRC),

Karl Höhener, Temas,

Keld Alstrup Jensen, National Research Centre for Working Environment (NRCWE),

Monique Groenewold, National Institute for Public Health and the Environment (RIVM),

Stefano Castelli, Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA),

Stefano Pozzi Mucelli, Veneto Nanotech (VN),

Theo Vermeire, National Institute for Public Health and the Environment (RIVM), and

Tom van Teunenbroek, Ministry of Infrastructure and the Environment.

Annex 1: Abbreviations and Acronyms (including website of the research projects)

CellNanoTox	Cellular Interaction and Toxicology with Engineered Nanoparticles (http://www.fp6-cellnanotox.net/)
EC	European Commision
ENPRA	Risk Assessment of Engineered NanoParticles (http://www.enpra.eu/)
ENSSATOX	Engineered Nanoparticle Impact on Aquatic Environments: Structure, Activity and Toxicology (http://www.ennsatox.eu/?contentid=260).
HINAMOX	Health Impact of Engineered Metal and Metal Oxide Nanoparticles: Response, Bioimaging and Distribution at Cellular and Body Level (http://www.hinamox.eu/)
INSTANT	Innovative Sensor for the fast Analysis of Nanoparticles in Selected Target Products (http://www.instant-nps.eu/index.php?id=22)
ITS-NANO	Intelligent testing strategies for engineered nanomaterials (http://www.its-nano.eu/)
MARINA	Managing Risks of Nanomaterials (http://www.marina-fp7.eu/)
MembraneNanoPart	Modelling the mechanisms of nanoparticle-lipid interactions and nanoparticle effects on cell membrane structure and function (http://www.membranenanopart.eu/research.php)
MODERN	MODelling the EnviRonmental and human health effects of Nanomaterials (http://modern-fp7.biocenit.cat/)
ModNanoTox	Modelling nanoparticle toxicity: principles, methods, novel approaches (http://www.birmingham.ac.uk/generic/modnanotox/index.aspx)
Nanex	Development of Exposure Scenarios for Manufactured Nanomaterials (http://nanex-project.eu/)
Nanodetector	Ultrasensitive plasmonic detection of single nanoparticles (http://www.nanodetector.eu/)
NANODEVICE	Novel Concepts, Methods, and Technologies for the Production of Portable, Easy-to-Use Devices for the Measurement and Analysis of Airborne Engineered Nanoparticles in Workplace Air (http://www.nano-device.eu/)
NanoFATE	Nanoparticle Fate Assessment and Toxicity in the Environment. (http://www.nanofate.eu).
NANOGENOTOX	Towards method for detecting the potential genotoxicity of nanomaterials (http://www.nanogenotox.eu/)

NanoLyse Nanoparticles in food: Analytical methods for detection and characterisation (http://www.nanolyse.eu).

NanoMILE Engineered nanomaterial mechanisms of interactions with living systems and the environment: a universal framework for safe nanotechnology (http://www.nanomile.eu-vri.eu/)

NanoPolyTox Toxicological impact of nanomaterials derived from processing, weathering and recycling of polymer nanocomposites used in various industrial applications. (http://www.nanopolytox.eu)

NanoPUZZLES Modelling properties, interactions, toxicity and environmental behaviour of engineered nanoparticles. (no website available yet).

NanoSafe2 Safe production and use of nanomaterials (http://www.nanosafe.org/scripts/home/publigen/content/templates/show.asp?P=56&L=EN&ITEMID=4)

nanoSTAIR Establishing a process and a platform to support standardization for nanotechnologies implementing the STAIR approach. (http://www.nanostair.eu-vri.eu/)

NanoSustain Development of sustainable solutions for nanotechnology-based products based on hazard characterization and LCA. (http://www.nanosustain.eu)

NanoTransKinetics Modelling the basis and kinetics of nanoparticle cellular interaction and transport. (http://www.nanotranskinetics.eu)

NanoValid Development of reference methods for hazard identification, risk assessment and LCA of engineered nanomaterials. (www.nanovalid.eu).

NHECD Creation of a critical and commented database on the health, safety and environmental impact of nanoparticles (http://www.nhecd-fp7.eu/index.php?id=515)

Observatory Nano Observatory Nano (http://www.observatorynano.eu/project/)

QNano A pan-European infrastructure for quality in nanomaterials safety testing, 1st February 2013 re-launched as QualityNano.

QualityNano A pan-European infrastructure for quality in nanomaterials safety testing. (http://www.qnano-ri.eu).

SIINN Safe Implementation of Innovative Nanoscience and Nanotechnology. (http://www.siinn.eu).

SMART-NANO Sensitive MeAsuRemenT, detection, and identification of engineered NANOparticles in complex matrices (www.smartnano.org)

Annex 2: Description of the input on the regulatory questions form the different WPs within NANoREG

Table 3: Key regulatory questions and the WPs of NANoREG which are supposed to provide information to generate answers and feedback to regulatory bodies.

	Priority questions per domain	WP2: Synthesis, supplying and characterization	WP3: Exposure through life cycle analysis	WP4: Biokinetics and toxicity testing in vivo	WP5: Advancement of regulatory risk assessment and testing	WP6: Keeping pace with innovation
	Measurement and characterization:					
1	How can nanomaterials be identified for the purpose of risk assessment as well as according to the EU definition of nanomaterials?	✓	✓		✓	
	Identification:					
2	Is a nanomaterial (particles, fibers) always a nanomaterial? Or are there circumstances that result in nanomaterial s being transferred into something which does not fit into the EU definition of nanomaterials?	✓		✓	✓	✓
	Metrology and dose metrics:					
3	Which metrics (metrology) should be used for nanomaterial s in regulatory toxicology?	✓	✓	✓	✓	
	Extrapolation:					
4	What guidance can be provided on how to decide when information from different forms of nanomaterials (or from the bulk material) can be 're-used'?		✓		✓	
	Persistence and long term effects:					
5	Will nanomaterials accumulate in man, the environment and environmental species and what are the driving forces?	✓	✓	✓	✓	✓
	Kinetics and fate:					
6	To what extent is the dosimetry for nanomaterial s (e.g. deposition pattern upon inhalation, biodistribution) different from the bulk material and what are the options to extrapolate information on these aspects from bulk material or different sizes of the same chemical to the nano-range?		✓	✓		
	Mode of action:					
7	What are critical characteristics of nanomaterial s that need to be considered to develop safer nanomaterial s?	✓	✓	✓		✓

WP2: Synthesis, supplying and characterization

The following deliverables will give input to regulatory question number 1, 2, 3, 5, and 7.

Deliverable number	Deliverable title	Lead beneficiary number	Input to priority questions
D2.1	Establishment of primary MNM sample suite and web-based resource for sample order	2	General NANoREG
D2.2	Material data sheets on primary MNM sample suite up-linked with internet resource	14	General NANoREG
D2.3	Experimental evaluation of OECD methods for analysis of physicochemical MNM properties	20	Question 1
D2.4	Protocol for quantitative analysis of inorganic and organic MNM surface coatings	4	Question 1,3
D2.5	Protocol for characterization and categorization of MNM in powders and liquid dispersions	4	Question 1,2
D2.6	Validated protocol(s) for test item preparation for key in vitro studies Here we will contribute to establishment of methods the dissolution and solubility tests as well as direct test in media relevant for hazard testing	31	General NANoREG
D2.7	Validated protocol(s) for inhalation exposure and test item preparation for in vivo studies Here we will contribute to establishment of methods the dissolution and solubility tests as well as direct test in media relevant for hazard testing	21	General NANoREG
D2.8	Protocols for exposure-fate characterization in ecotoxicity and in vitro studies Here we will contribute to establishment of methods the dissolution and solubility tests as well as direct test in media relevant for hazard testing	22	Question 5,6
D2.9	Revised OECD methods for determination of physicochemical MNM properties for REACH end-points	29	Question 1,3
D2.10	Protocol(s) for size-distribution analysis of primary MNM objects in powders and liquids for compliance with the EU definition	26	Question 1
D2.11	Protocol(s) for VSSA analysis of primary MNM objects in air, powders, and liquids for compliance with the EU definition	NEW	Question 1
D2.12	Framework and procedures for characterization and reporting of MNM for regulatory needs	4	Question 1,3

WP3: Exposure through life cycle analysis

WP 3 will develop methods and approaches to measure and characterize nanomaterials within realistic exposure situations to humans (workers and consumers) and the environment throughout the different stages of the life cycle of nanomaterials (Question 1). In addition, WP3 will identify critical stages on the life cycle of nanomaterials for which exposure is expected to be high and evaluate the applicability of existing exposure models to nanomaterials, which will give input into the best dose metrics (Question 3), extrapolation (Question 4), the behavior of nanomaterials within the environment (Question 5), deposition patterns (Question 6) and critical characteristics that need to be considered to develop safer nanomaterials (Question 7).

WP4: Biokinetics and toxicity testing in vivo

WP4 will carry out toxicological and ecotoxicological studies focusing on standard regulatory testing and on in vivo studies. Tasks 4.4 and 4.5.4.1 will apply different methods to visualize MNM in tissues and cells (Question 2). All in vivo studies investigate acute and longer-term effects of representative granular and fibrous MNM. The results obtained with these studies will yield further information which dose metrics or which combination thereof will best explain the effects observed (Question 3). The majority of the in vivo studies will include investigation on the kinetic behavior (i.e. also a putative accumulation) including long-term exposure (Question 5). All these results will offer information whether the kinetic behavior of the tested nanomaterials is different to what is already known for the respective bulk materials (Question 6). One main aim of WP4 is to fill relevant gaps in the mode of action for biopersistent granular and fibrous nanomaterials. This will yield relevant information how nanomaterials need to be designed to be safer (Question 7).

WP5: Advancement of regulatory risk assessment and testing

WP5 will develop solubility testing procedures (Task 5.2), which is a very important characteristic in the risk assessment of nanomaterials (Question 1) and one of the major characteristic which determines the transformation of nanomaterials into materials that do not fit the EC definition of nanomaterials (Question 2).
Task 5.1 will develop criteria for categorization, read-across, extrapolation and interpolation within and between different types of nanomaterials and bulk materials (Question 4 and 6).
Within Task 5.3 this WP will develop an in vitro screening methodology for absorption/crossing barriers, one of the steps essential in the accumulation of nanomaterials in humans and environmental species (Question 5).
Task 5.4 (Inhalation toxicity modeling in vitro) and Task 5.5 (In vitro toxicity assays connected to regulatory questions) will unravel which nanomaterial characteristics are toxic primarily for the respiratory track and more generally by following standard in vitro procedures (Question 7).
Task 5.4 and Task 5.5 will also address Question 3 as results from different metrics may be compared.

WP6: Keeping pace with innovation

In WP6 is a strong focus on avoiding the development of hazardous (near) market ready nanomaterials. The chosen route is along two lines, i.e. 1) how lines of innovation and regulatory safety can be tuned better, and 2) gaining insight how functionality, physico-chemical characteristics of nanomaterials and toxicity can be derived in a structured and were relevant in a regulatory acceptable way. Task 6.1 deals with horizon scanning techniques and linking them to a first risk formulation. This risk formulation has direct links to almost all regulatory questions but it will certainly address the issue whether the solutions to these questions will also hold for new types of nanomaterials. In task 6.2 among others the role of dissolution and the required tests or tests in place will play an important role. In conjunction with foreseen activities in WP2 and WP5, this will give input to Questions 2 and more indirectly also to Question 5. Task 6.3 deals with the

classical safe-by-design approach in which in an iterative process functionality and toxicity are tuned. This task is foreseen to address Question 7 substantially.

References

Aitken, R. J., Bassan, A., Friedrichs, S., Hankin, S. M., Hansen, S. F., Holmqvist, J., et al. (2011). *Specific Advice on Exposure Assessment and Hazard/Risk Characterisation for Nanomaterials under REACH (RIP-oN 3): Final Project Report*.

Azoulay, D., Buonsante, V., Cameron, P., & Vengels, J. (2012). *High time to act on nanomaterials: A proposal for a 'nano-patch'for EU regulation*. Berlin, Germany: Bund für Umwelt und Naturschutz Deutschland (BUND), Centre for International Environmental Law (CIEL), ClientEarth.

Bleeker, E. A., de Jong, W. H., Geertsma, R. E., Groenewold, M., Heugens, E. H., Koers-Jacquemijns, M., et al. (2013). Considerations on the EU definition of a nanomaterial: science to support policy making. *Regul Toxicol Pharmacol, 65*(1), 119-125.

Bosman, M. T. M. (2013a). *Chairman's Report: Pragmatic steps towards a more effective EU nano policy.* The Hague, The Netherlands: Ministry of Infrastructure and the Environment.

Bosman, M. T. M. (2013b). *Conference 'Building Blocks for Completing EU Regulation of Nanomaterials' Major Agreements*. The Hague, The Netherlands: Ministry of Infrastructure and the Environment.

BSI. (2011). Nanoparticles: Vocubulary (Vol. PAS 71:2011). London, UK: British Standards Institution (BSI).

Christensen, F. M. (2012). *Scientific technical support on assessment of nanomaterials in REACH registration dossiers and adequacy of available information*. Ispra, Italy: NANO SUPPORT.

Christensen, F. M., & Larsen, P. B. (2013). *Information Requirements for nanomaterials*. Copenhagen, Denmark: IRNANO.

EC. (2011). Commission recommendation of 18 October 2011 on the definition of nanomaterial. *Official Journal L 275*, 38-40.

EC. (2012). *Communication from the commission to the European Parliament, the Council and the European Economic and Social Committee: Second Regulatory Review on Nanomaterials*. Brussels, Belgium: Commission of the European Communities (EC).

ECHA. (2012). *Guidance on information requirements and chemical safety assessment: Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a, b and c Endpoint specific guidance*. Helsinki, Finland: European Chemicals Agency (ECHA).

EFSA. (2011). Guidance on the risk assesment of the applicationof nanoscience and nanotechnologies in the food and feed chain. *EFSA Journal, 9*(5), 2140.

EU. (2009). Regulation (EC) No 1223/2009 of the European parliament and of the council of 30 November 2009 on cosmetic products (recast.). *Official Journal, L 342*, 59-209.

Feitshans, L. (2013). *Nanotechnology: balancing benefits and risks to public health and the environment*. Strassbourgh, France: Council of Europe.

Fleischer, T., Jahnel, J., & Seitz, S. B. (2012). *NanoSafety: Risk Governance of Manufactured Nanoparticles*. Brussels, Belgium: Institute for Technology Assessment and Systems Analysis (ITAS), Karlsruhe Institute of Technology (KIT) and Institute of Technology Assessment (ITA), Austrian Academy of Sciences.

Hankin, S. M., Peters, S. A. K., Poland, C. A., Hansen, S. F., Holmqvist, J., Ross, B. L., et al. (2011). *Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2): Final Project Report*.

ISO. (2008). Nanotechnologies: Terminology and definitions for nano-objects: Nanparticle, nanofibre and nanoplate. Geneva: International Organization for Standardization (ISO).

ITS-NANO. (2013). *Identification of Knowledge Gaps and Strategic Priorities for Human and Environmental Hazard, Exposure and Risk Assessment of Engineered Nanomaterials*. Edinburgh, Scotland: ITS-NANO.

JRC. (2011). *REACH Implementation Project Substance Identification of Nanomaterials (RIP-oN 1)*. Ispra, Italy: Joint Research Centre.

KEMI. (2013). *Draft proposal for a regulation for nanomaterials*. Sundbyberg, Sweden: Swedish Chemical Agency (KEMI).

Linsinger, T., Roebben, G., Gilliland, D., Galzolai, L., Rossi, F., Gibson, N., et al. (2012). *Requirements on measurements for the implementation of the European Commission definition of the term nanomaterial*. Ispra, Italy: JRC.

Malkiewicz, M., Pettitt, M., Dawson, K. A., Toikka, A., Hansson, S. O., Hukkinen, J., et al. (2011). *Nanomaterials in REACH*. Stockholm, Sweden: SKEP ERA-NET.

Maynard, A. D. (2011). Don't define nanomaterials. *Nature, 475*(7354), 31.

Micheletti, C., Riego Sintes, J., & Vegro, S. (2010). *Report on Early Harvest of Research Results on Nanosafety: Summary of the 1st Join CASG Nano and ENPRA Workshop held on 14-15 april 2010 at the Institute for Health and Consumer Protection, IHCP (Ispra, Italy)*.

NICNAS. (2010). Adjustment of NICNAS new chemicals for insustrial nanomaterials. *Chemical Gazette*.

NRC. (2012). *A Research Strategy for Environmental, Health, and Safety Aspects of Engineered Nanomaterials*. Washington, US: National Academy of Sciences.

Oberdorster, G., Oberdorster, E., & Oberdorster, J. (2007). Concepts of nanoparticle dose metric and response metric. [Comment]. *Environ Health Perspect, 115*(6), A290.

OECD. (2012a). *Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomarials*. Paris: Organisation for Economic Co-operation and Development (OECD).

OECD. (2012b). *Important issues on risk assessmentof manufactured nanomaterials* Paris: Organistation of Economic Co-operation and Development (OECD).

Oomen, A. G., Bos, P. M., Fernandes, T. F., Hund-Rinke, K., Boraschi, D., Byrne, H. J., et al. (2013). Concern-driven integrated approaches to nanomaterial testing and assessment - report of the NanoSafety Cluster Working Group 10. *Nanotoxicology, 2013 May 28. [Epub ahead of print]*.

Park, M. V. D. Z., de Jong, W. H., Oomen, A. G., & Delmaar, C. J. (2012). *Nanotoxicology – an in vitro approach: A practival way forward to determine appropriate dose metrics for engineered nanomaterials.* Maastricht University, Maastricht, The Netherlands.

Pozzi-Mucelli, S., Duret, D., & Urbina, M. (2013). *Draft: Roadmaps for the safe handling of nano-objects, safe processes, safe products and safe transportation of nano products addressing identified gaps: first map*. Padova, Italy: ERA-NET SIINN.

Riediker, M. (2013). *Compendium of Projects in the European NanoSafety Cluster: 2013 Edition*. Lausanne, Switzerland: Institute for Work and Health.

Riediker, M., & Katalagarianakis, G. (2012). *Compendium of Projects in the European NanoSafety Cluster: 2012 Edition*. Lausanne, Switserland: Institute of Work and Health.

Santropoulou, E. (2012). *Draft: Knowledge Gaps 2: Identification of knowledge gaps on the correlation between epidemiological health reports and possible safety and handling of nanomaterials*. Athens, Greece: ERA-NET SINN.

Savolainen, K., Backman, U., Brouwer, D., Fadeel, B., Fernandes, T., Kuhlbusch, T., et al. (2013). *Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations*. Helsinki: Finnish Institute of Occupational Health.

SCCS. (2012). *Guidance on the safety assessment of nanomaterials in cosmetics* Brussels, Belgium: Scienfific Committee on Consumer Safety (SCCS).

SCENIHR. (2010). *Opinion on the sceintific basis for the definition of the term "nanomaterials"* Brussels, Belgium: Scientifific Committee on Emerging and Newly Identified Health Risks (SCENIHR), European Commission.

Stone, V., Pozzi-Mucelli, S., Tran, L., Aschberger, K., Sabella, S., Vogel, U. B., et al. (2013). *Research prioritisation to deliver an intelligent testing strategy for the human and environmental safety of nanomaterials*. Edinburgh, Scotland: ITS NANO.

UBA, B. a. B. (2013). *Nanomaterials and REACH: Background Paper on the Poition of German Competent Authorities.* Dessau-Roßlau, Germany: UBA, BAuA, BfR.

Wijnhoven, S. W. P., Peijnenburg, W. J. G. M., Herberts, C. A., Hagens, W. I., Oomen, A. G., Heugens, E. H., et al. (2009). Nano-silver: a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology, 3*(2), 109-138.