



Research priorities relevant to development or updating of nano-relevant regulations and guidelines

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Outline and Introduction

The purpose of this document is to identify complete and ongoing activities associated with development of appropriate regulations relevant to promoting the safety of nanomaterials (NM). In doing so the document identifies research areas or priorities that need to be addressed further in order to facilitate this process.

The use of a diverse array of NM in wide array of consumer, industrial and medical applications has led to an increased interest in improving our understanding of issues pertaining to their safe use. The European Commission has funded many projects during the Framework programme 7 which initially set out to identify whether there were significant safety issues relating to nanomaterials (e.g. NANOIMMUNE, NANOGENOTOX, ENPRA, MARINA, NANOSOLUTIONS, MARINA, NanoTEST). Based upon the evidence generated by these studies, EU projects have now moved on to develop strategies for risk assessment and decision making (e.g. MARINA, SUN, GUIDENANO, NANOSOLUTIONS) and to identify whether nanomaterials are adequately regulated by current European legislation (e.g. NANoREG and RiPoN). These activities are being further enhanced in Horizon 2020 with the funding of PROSAFE, NANoREG II and other new projects.

There are already several regulations in EU that specifically mention nanoparticles or nanomaterials. The first EU regulation mentioning nanotechnology was the Food Additive regulation (EC) No 1333/2008. Since then, other adapted regulations have been incorporated in areas relevant to food, cosmetics, packaging and biocides. More details are provided below.

However, there remain significant gaps in knowledge and procedures that need to be addressed in order to generate appropriate and proportionate regulation for nanomaterials that is informed by a sound evidence base. The following short report outlines the current regulatory landscape as it pertains to nanomaterials and then identifies the additional major areas that need to be considered, pointing out relevant activities that have or are being conducted in relation to each, but highlighting those for which more work is needed.

Some of the proposed work is academic research, while a proportion is related to standardisation/validation, and the remainder involves integration of evidence with social and political considerations in order to better regulate nanomaterials. The major areas or knowledge gaps to be addressed are presented as hexagons in a diagram that provides an indication of how they might be prioritised over time in order to achieve this final goal. However, the diagram is flexible and can be updated as knowledge is acquired and the questions are adjusted. For each major area to be addressed a short narrative has been generated to further explain the relevance of the major area and to reference relevant activities that contribute to this major area.

The Current Regulatory Landscape Relevant to Nanomaterials

The RRR covers a very dynamic and rapidly changing environment, and so its content, at least in part will quickly go out of date. Some changes in regulation have already taken place, and where possible these are reflected in the document, while others are ongoing. Definitions and nanomaterial specifications used in international or national fora have been extensively discussed in the JRC Reports "Towards a review of the EC Recommendation for a definition of the term 'nanomaterial' Part 1: Compilation of information concerning the experience with the definition" (2014) and "Considerations on a Definition of Nanomaterial for Regulatory Purposes" (2010). Those most relevant for horizontal or sector-specific regulations in the EU are briefly discussed below.

In October 2011 the European Commission (EC) published a **Recommendation on the definition of nanomaterial (2011/696/EU)**,ⁱ here subsequently referred to as the **EC Definition**, which should be broadly applicable across different materials, application areas and regulatory sectors. The purpose of this definition is to enable determination when a material should be considered a NM for regulatory purposes in the European Union. The definition covers natural, incidental and manufactured materials and is based solely on the size of the constituent primary particles of a material, without regard to specific functional or hazard properties or risks.

The European Commission recommends the following definition of the term 'nanomaterial':

'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.

In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

The Recommendation further specifies:

By derogation [...], fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

[...] 'particle', 'agglomerate' and 'aggregate' are defined as follows:

(a) 'particle' means a minute piece of matter with defined physical boundaries;

(b) 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;

(c) 'aggregate' means a particle comprising of strongly bound or fused particles.

Where technically feasible and requested in specific legislation, compliance with the definition [...] may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition [...] where the specific surface area by volume of the material is greater than 60 m²/cm³. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition [...] even if the material has a specific surface area lower than 60 m²/cm³.

The EC Recommendation is being reviewed (Expected Dec 2016) in the light of experience as well as scientific and technological developments. Yet, the Commission is not considering any major alterations or changes in scope but rather minor clarifications in the text and ways to facilitate its implementation. The main elements (definition only related to size and particle number size distribution) are planned to remain the same ⁱⁱ.

The European Union sector specific legislations already including a legally binding nanomaterial definition are:

- the **Cosmetic Products Regulation No 1223/2009**,ⁱⁱⁱ
- the **Regulation on the Provision of Food Information to Consumers No 1169/2011**,^{iv} - The term used is "engineered nanomaterial". However, this regulation has been amended by the **Novel Foods Regulation No 2015/2283**. It has been stated in the Novel Foods Regulation No 2015/2283 that the definition in the Regulation (EU) No 1169/2011 should be deleted and replaced by a reference to the definition set out in the Novel Foods Regulation No 2015/2283 for consistency and coherence purposes. Both aforementioned Regulations have the same definition.
- the **Regulation on Plastic Materials and Articles Intended to Come into Contact with Food (EU) No 10/2011**
- the **Biocidal Products Regulation No 528/2012** ^v.

Nanoparticle/nanomaterial specifications are also included in the **EU Regulation No 10/2011 on Plastic Food Contact Materials and Articles**^{vi} and are foreseen in the **European Union Medical Devices Regulation**.^{vii}

The nanomaterial definition included in the **Cosmetic Products Regulation No 1223/2009**, limits the term nanomaterial to insoluble or biopersistent and intentionally manufactured materials with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm, therefore excluding all soluble and/or naturally occurring materials with dimensions at the nanoscale. The definition used in the Cosmetic Products Regulation is also relevant for the notification of nanomaterials as cosmetic ingredients in the Commission's Cosmetic Products Notification Portal (CPNP). It should be noted that a definition of soluble and biopersistent do not exist directly within the regulation itself^{viii}, but definitions of soluble exist elsewhere (e.g. the Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme: First Revision ^{ix}).

Article 3 of the **Novel Foods Regulation No 2015/2283** provides a definition of engineered nanomaterial. "Engineered nanomaterial" means any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. Properties that are characteristic of the nanoscale include: (i) those related to the large specific surface area of the materials considered; and/or (ii) specific physico-chemical properties that are different from those of the non-nanoform of the same material. In other words, this regulation has not used the standard EC definition for a NM.

Adaptations of the currently legally binding definitions in the latter two Regulations to that provided in **EC Recommendation 2011/696/EU** are foreseen, with sector specific provisions.

Article 3 of the **Biocidal Products Regulation (No 528/2012)** provides a definition of nanomaterial which was adapted from the EC definition. A "Proposal for a Regulation of the European Parliament and of the Council on Medical Devices, and amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009" is also under the scrutiny of the European Parliament. A nanodefinition adapted from the EC definition is included in Art 2.1 (15) of the draft Regulation.

The **Commission Regulation (EU) No 10/2011** of 14 January 2011 on plastic materials and articles intended to come into contact with food, provides some specifications for engineered nanoparticles without explicit definition of the term.

In spring 2017 the European Directorate-General for Environment is expected to publish EU Ecolabel criteria (Regulation (EC) No 66/2010) for dishwashing detergents, hand dishwashing detergents, hard surface cleaning products, industrial and institutional dishwasher detergents and laundry detergents. This regulation will include a

statement that 'All ingoing substances present in the form of nanomaterials shall be clearly indicated in the list with the word 'nano' written in brackets', where nanomaterial is defined as in the Commission Recommendation 2011/696/EU. This EU Ecolabel Regulation is not mandatory but it lays down rules for the establishment and application of the voluntary EU Ecolabel scheme.

Guidance on REACH: The European Regulation on Chemicals **REACH** does not include any nano-specific requirements. However, since REACH covers chemicals in whatever shape or size, nanomaterials are implicitly covered by REACH^x. In its guidance documentation on registration of nanomaterials for the purposes of REACH, the European Chemicals Agency (ECHA) explicitly refers to the EC Recommendation 2011/696/EU. Hence, the EC Recommendation is currently used as working definition for the purposes of REACH. The European Commission also intends to cover nanomaterials more explicitly in REACH and recent activities are aimed at amending REACH Annexes by including a definition of NM that is identical or very similar to the EC's Recommendation, and to include further nanomaterial-specific provisions in the REACH Annexes.

REACH (Annex XI, 1.5) outlines the conditions under which read-across and grouping can be used, although not specifically for NMs. A consultation document by ECHA currently considers grouping for NMs^{xi}. It is widely accepted that grouping and read-across have the potential to make safety testing more cost-efficient for regulatory testing. In fact in May 2016 ECHA initiated four different Partner Expert Groups (PEG) running currently that address:

- What is a nanoform
- Read Across and Grouping
- Human Health
- Environment

The final guidances generated from this process will be ready in april/may 2017.

Research Prioritisation Diagram

The research priorities are set out using hexagons according to the style established by ITS-NANO. The hexagon diagrams show that for each issue (hexagon) there is more than one way to progress. The hexagon diagram design has been chosen, since a strictly consecutive approach is considered inappropriate. The hexagon shape reflects the potential for each component to interact with multiple components, thus the organisation could be shuffled according to the specific research question addressed or as knowledge accumulates.

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

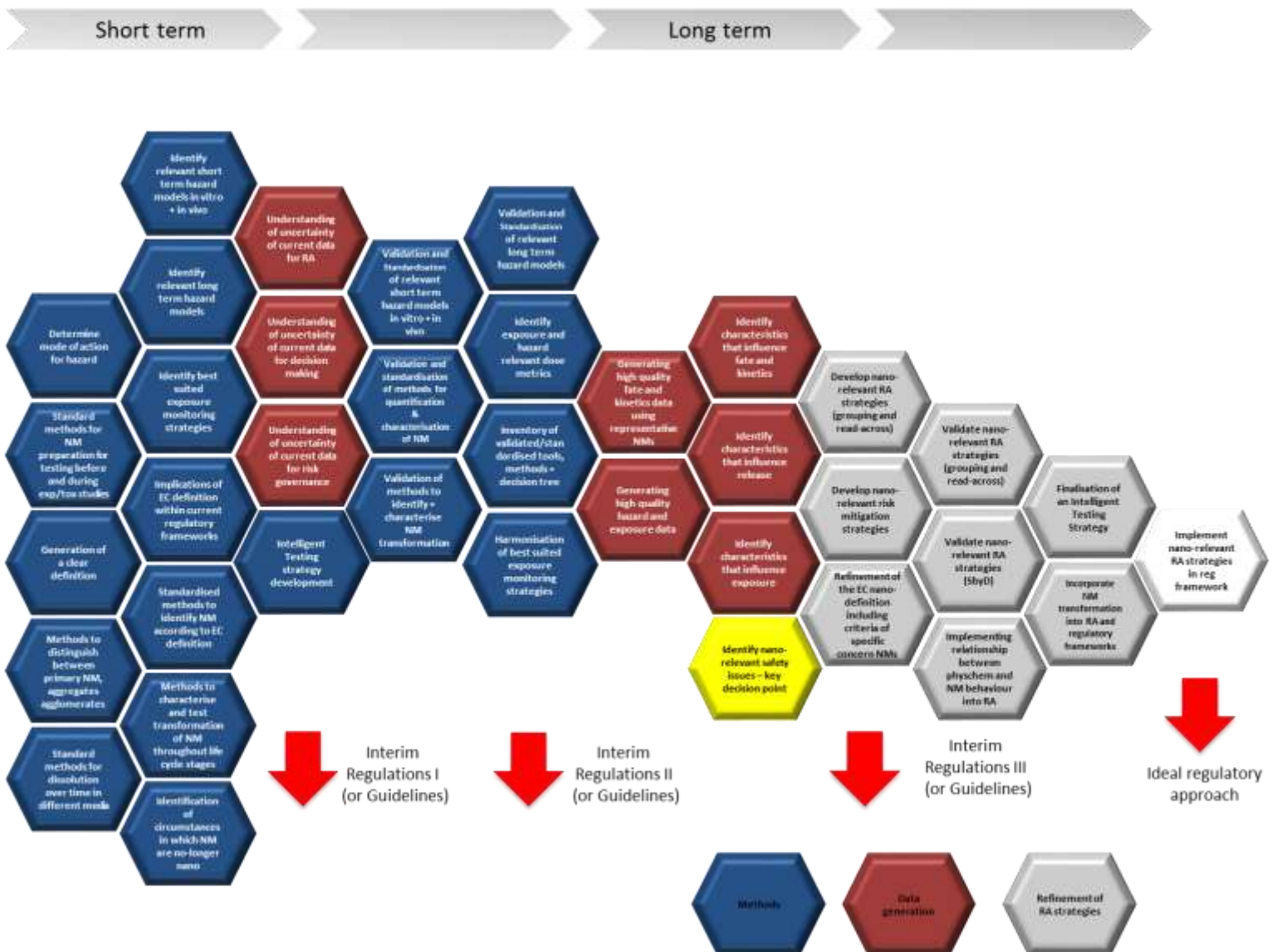


Colour coding of groups of research priorities are used in order to simplify understanding of the diagram.

For each major area, represented by a hexagon, the text provided aims to explain **why** the area identified is a priority, briefly summarise **what** is currently known relevant to this research priority, generate **recommended** actions that could be conducted to address the research priority and provide links to relevant **further sources of information**.


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 work required.


It is worth noting that not all of the major areas are academic research, but that this overall plan will also require input from standardisation and validation experts as well as risk assessors, policy makers and other interested stakeholders (e.g. industry and consumers). The diagram is flexible and can be updated as knowledge is acquired and the questions are adjusted.




Interim Regulation I (or guidelines)


The following research areas/priorities have been identified that are required to support regulation pertaining to nanosafety. Many of the research priorities in this section include significant information as many are areas that are relatively well advanced.


<p>1.1</p>		<p>Generation of a clear definition</p> <p>A clear definition of nanomaterial should be scientifically driven (i.e. evidence based), exhibit a well-defined scope and it should be possible to implement. The definition should be as uniform as possible across different legal frameworks and global locations, in order to prevent that a material is regarded as a nanomaterial in one framework and not in another.</p> <p>The European Commission (EC) published a recommendation in 2011 on the definition of a nanomaterial (2011/696/EU) ⁱ. Most probably the most distinguishing aspect of the EC nanomaterial definition is the use of particle size distributions based on the numbers of particles, and not on the mass or volume of the particles, as the main classification feature.</p> <p>In March 2014 the Joint Research Centre (JRC) Institute for Health and Consumer Protection published a review of the EC definition recommendation ^{xii}, followed by a second report in August 2014 by the JRC Institute for Reference Materials and Measurements ^{xiii}. Based on the feedback received regarding the current definition, compiled in the first report of the series, and its assessment, presented in this second report, the JRC has set of indications on how the definition could be modified to improve its clarity, effectiveness and issues relating to implementation in a third report in 2015 ^{xiv}. A further update with minor modifications is anticipated December 2016.</p> <p>In parallel, the Nanodefine FP7 project has prepared “Recommendations on a Revision of the EC Definition of Nanomaterial Based on Analytical Possibilities”, while NANOREG is concluding a forward-looking proposal for a revision of the substance and NM identification scheme for improved regulatory administration of materials in general. The observation is that the current substance identification scheme prevents the ability to clearly differentiate between different material modifications. It suggests that neither a scheme nor strategy, for higher generation materials exists. The NANOREG framework is supported by analytical methods for key criteria.</p> <p>Until recently, the USA there was no clear regulatory definition of "nano" and the term "definition" itself has been avoided. Building on earlier guidances, such as those from FDA in June 2014 ^{xv}, the EPA issued in March 2015 a draft for a non-public reporting scheme based on its authority by TSCA ^{xvi}. It contains a very specific description of which materials need to be reported:</p> <p>“Chemical substances that are solids at 25 °C and atmospheric pressure and that are manufactured or processed in a form where the primary particles, aggregates, or agglomerates are in the size range of 1-100 nanometers (nm) and exhibit unique and novel characteristics or properties because of their size, [specifically:] zeta potential, specific surface area, dispersion stability, surface reactivity”. But “would not apply to</p>
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		<p>chemical substances that only have trace amounts ..., such that the chemical substance does not exhibit the unique and novel characteristics or properties because of particle size.”</p> <p>Via the focus on size, substantiated by surface area, the de-facto-definition of the EPA reporting scheme draft is highly consistent with engineered NMs considered under the EC definition recommendation, but excludes materials not commonly considered as nanomaterials (traces of NM, natural NM).</p> <p>The actual reporting obligations in the EPA draft depend on similarity consideration by the term of “discrete forms” which is very closely related to the EU term of “nanoforms”, as it relies on parameters of Chemical identity and of Particle Characteristics (such as size, composition, surface area, surface coating, and more) and introduces parameter ranges to distinguish one nanoform from another.</p> <p>The OECD Environment Health and Safety Publications entitled "Safety of Manufactured Nanomaterials" includes 79 papers that consider risks, definitions and metrics related to NMs. Together with the standardisations edited by ISO TC 229, these OECD documents provide an approach to assessing health and safety of workers, consumers and the environment that uses structured frameworks or decision trees. This approach could be useful in the absence of existing relevant regulations.</p> <p>Vicki Stone Wilson Engelmann Serli Önlü Wendel Wohlleben Keld Alstrup Jensen Jacques-Aurélien Sergent</p>
<p>1.2</p>		<p>Methods to distinguish between primary NM, aggregates and agglomerates</p> <p>Currently, only electron microscopy (EM) can reliably be used to distinguish between primary particles from more complex structures (e.g. aggregates or agglomerates) in a sample. However, the quality of such analysis is strongly dependent on the adequacy of the sample preparation and some materials may be too sensitive for electron microscopy analysis. Currently, methods are becoming available to enable such analysis, but there is still a lack of consensus on the methodologies. Fully validated and standardised methods as well as reference materials are needed to fully enable calibration, validation and implementation of methods.</p> <p>While EM can help to distinguish between aggregates and agglomerates, this technique alone is not sufficient. The two states of aggregation and agglomeration are defined by the interaction forces between the particles. At very high resolution and with particles that show crystal lattices, EM can show if the lattice orientation is shared by two particles, what would point towards a stronger bonding (aggregate), while the missing of a similar lattice orientation points towards an agglomerate. However with amorphous particles this approach is not sufficient. Instead, for amorphous particles the shape of the particle curvature might be used.</p>

		<p>This research priority is relevant to pristine nanomaterials. Future work is in particular needed in regards to non-spherical and non-granular NMs, multiconstituent NMs, mixtures as well as those NMs dispersed in/applied to different media/matrices.</p> <p>NanoDefine is fully devoted to the implementation of the EC definition of a nanomaterial by a tool-box of methods. A “methods manual” and decision tree will be integrated in the “NanoDefiner” e-tool. Both nano- and non-nano- reference materials will emerge from the real-world substances from various industrial partners, including pigments and fillers, carbonaceous substances, polymers, metals with compact, platelet and fibre shapes. Consumer products include cosmetics, foods and plastics.</p> <p>The NANOREG FP7 project has developed sample item preparation and transmission electron microscopy (TEM) protocols to enable the identification of NM according to the minimum size-distribution of individual nano-objects and the aggregate size-distribution. The procedures have been demonstrated by inter-laboratory comparison using near-spherical and aggregated/agglomerated nanomaterials and fine pigments plus associated uncertainties have been determined. NANOREG has also produced improved analytical procedures for determination of relative density and to determine the non-porosity-derived specific surface areas as a modification to support the volume specific surface area (VSSA) criterion in the proposed EC-definition. However the generic use of VSSA is still questioned and further testing on diverse materials is required to identify limitations due to e.g., organic coatings/additives, agglomeration, and polydispersivity. The TEM results have been obtained using near-spherical reference materials and NM from the OECD sponsorship program. The procedures for VSSA assessment have been tested using both OECD NM and powder materials provided by the pigment industries.</p> <p>Jacques-Aurelien Sergent Keld Alstrup Jensen</p>
<p>1.3</p>		<p>Standard methods for dissolution over time in different media</p> <p>The transformation reactions of nanomaterials can be addressed by thermodynamic or kinetic concepts. For traditional chemicals the thermodynamic approach (solubility constants, solubility limits in a defined medium) is standard in the regulatory frameworks and information on the solubility limit in water at room temperature has to be given. However, there is general scientific consensus that for risk assessment of nanomaterials, knowing the solubility rate and biodurability is of greater importance than the solubility limit in water alone. Knowing the dissolution rate (release of ionic species) and behaviour of (nano)materials in relevant environmental and biological media is critical for assessment of both acute and long-term risks. Moreover, it may be difficult to separate dissolution data from reactivity (e.g., acid-base and redox activity) and information on phase transformations. Hence, the term should ultimately be considered in a wider perspective. Dissolution might be seen as a sub-category of transformation reactions (section 1.11).</p> <p>The solubility of a substance can be considered as an unambiguous characteristic of the substance. In contrast nanomaterials show a different solubility for each nanoform, depending on the intrinsic properties such as size, shape, coating. This means that nanomaterials would require a case-by-case assessment of solubility. Another point to</p>

		<p>consider is that nanomaterials come with a distribution of intrinsic properties such as size. This means that the dissolution rate might initially be determined by the smaller particles, while later being dominated by the larger ones. Hence neither dissolution rate nor solubility limit will be an unambiguous characteristic of a nanomaterial. Dissolution rates are dependent on agglomeration state and specific surface area (among others), but size distribution and size-dependent agglomeration states will hamper the normalization of rates to a mean size or mean specific surface area of the material. Read-across techniques between different nanoforms have to be established by experiment and adapted modelling.</p> <p>In any case, data comparability requires establishment of suitable standard methods, where there is agreement on the procedure, to establish the dissolution rate or solubility limit, and the definition of the soluble (dissolved) matter and in which media testing should be performed. An operational strategy would be to establish dissolution and reactivity data of test materials in a suite of accepted model fluids that can generate data for predictive reaction-fate modelling.</p> <p>The methodologies which are currently assessed to produce dissolution rates differ in their general approach. While batch-tests with periodic particle/solution separation and determination of the dissolved content work towards a dynamic equilibrium of solids and solutes, which would require an extrapolation to zero concentration or the fitting of a model function for the establishment of a dissolution rate, other approaches work against an infinite sink, avoiding these problems.</p> <p>Recent work published by Tantra et al. (2015) and conducted in parallel by NANoREG on solubility / dissolution, as well as NANoREG work conducted on biodurability, have concluded that the term 'solubility' is not suitable for materials/nanomaterials because dissolution is not considered to reach equilibrium. Instead, it is proposed to perform 'dissolution studies' and to specify the 'rates of dissolution' and/or the dissolution/solubility at a specific time-point assessed in a range of media relevant for risk assessment. This is discussed in NANoREG deliverables D2.08 and D2.09. http://www.nanoreg.eu/images/2016_10_17_NANoREG_Factsheet_D2.08.pdf http://www.nanoreg.eu/images/2017_01_06_NANoREG_Factsheet_D2.9.pdf and will be further elaborated on in forthcoming publications. A request for a guideline activity in OECD has also been launched based on these two NANoREG deliverables. In addition, one of the NANoREG SOPs developed by NRCWE is currently a working item in CEN for development of a technical specification of the test method. This is the Sensor Dish Reader Method for assessment of hydrochemical reactivity (pH and O₂ as a proxy for redox) and dissolution in <i>in vitro</i> studies (CEN-TC 352_WG1_PG2).</p> <p>ISO TC229 (ISO/DTR 19057) is also an activity relevant in regards to establishing a technical understanding of particle biodurability (ability to resist dissolution in human organ fluids such as the lung lining fluid).</p> <p>Future research needs in this area includes demonstration of how dissolution and reactivity data can be derived from first principles and modelling for grouping and read-across and how the obtained data can be used to improve the risk assessment of NMs. Further needed activities relate to establishment of improved methods for in situ analysis of dissolution and standardization of methodologies.</p>
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		<p>Links to 1.11 and 1.12. See also Priority 4.10.</p> <p>Keld Alstrup Jensen Vicki Stone Frank von der Kammer</p>
<p>1.4</p>		<p>Determine mode of action for hazard</p> <p>The hexagon to determine the modes of action is intimately related with the physicochemical characterisation of NMs in that it includes the relationship between hazard responses and factors such as surface area, surface reactivity (e.g. production of reactive species), solubility, shape, size or crystal structure. In addition this includes direct interaction with biological or environmentally relevant macromolecules and how this influences surface reactivity, fate, behaviour and hence bioavailability. Furthermore, this may also include physical interaction or chemical reaction with other chemicals. In addition, a hazardous effect may occur in cells or organs without direct contact with NMs due to secondary effects.</p> <p>NB: Provided that the chemical identity of nano-objects is stable at least during the assays, the biological identity should be better explored. For instance, the interaction with bacterial endotoxins can dramatically affect the inflammogenicity of some NMs in commonly used bioassays using immune-competent cells (e.g., macrophages). Although this may mirrors what actually happens in the real exposure scenarios, this could introduce a bias in laboratory assessment of NM hazard. This could be simply a component of a GPL.</p> <p>The assessment of mode of action will need to include the measurement of biologically relevant endpoints or biomarkers that relate to:</p> <ul style="list-style-type: none"> • cytotoxicity/lethality; • sub-lethal cellular and molecular effects such as adaptive responses, inflammation, oxidative stress, genotoxicity, cell cycle alterations, transformation, growth and proliferation; • sub-lethal whole organism effects such as weight gain/loss, adaptive responses, fibrosis, cardiovascular disease, neurological impacts, tumour development and reproduction impairment; • Individual, population, community level responses. <p>The hazard responses considered within the mode of action also include biological/environmental factors such as:</p> <ul style="list-style-type: none"> • exposure concentration and internal dose; • the relationship between dose/duration and the time course of biological response (including short term, long term, acute and chronic effects, as well as reversible and irreversible effects); • route of exposure from different environment media, e.g. water, soil, sediment, air, or food exposure as well as entry pathways, i.e. respiratory, oral or dermal entry; • toxicokinetics; • local, distal and systemic effects; • susceptibility/sensitivity according to species, age, life-cycle stage, disease status or genetic variation.


		<p>Toxicokinetics could be considered as a separate priority. It provides information relevant to read across, generation of human equivalent concentrations and it can help to guide the selection of <i>in vitro</i> tests.</p> <p>ITS-NANO Phil Sayre Ulla Vogel Vicki Stone Janeck J. Scott-Fordsmand Enrico Bergamaschi</p>
<p>1.5</p>		<p>Standard methods for NM preparation for testing before and during exposure/toxicology studies</p> <p>There is a need for more clarity regarding the best methods for NM dispersion for human and environmental hazard testing. This is true for <i>in vitro</i>, single organism tests and environmental mesocosm-models, although the level of importance and what it refers to clearly depends on the systems tested. Such tests are currently difficult to control and monitor, making it difficult to interpret the results. Therefore there is an urgent need to improve understanding of dispersion characteristics and behaviour in human and environmentally relevant media. This will enable better interpretation of <i>in vitro</i> and <i>in vivo</i> studies, but at the same time there is a need to link to the knowledge of what are relevant states of dispersion in the environment, in relation to the fate and behaviour of NM.</p> <p>The development of a single method for NM dispersion, for all types of studies is unlikely due to the variations in NM physicochemical characteristics, matrices and exposure scenario's/routes. In the future, a matrix or decision tree of dispersion options will be required to guide researchers/hazard assessors towards the most appropriate protocol(s). Protocols may not necessarily be aimed at generating mono-dispersed suspensions. In many situations, they may be more relevant to mimic realistic situations with aggregated or agglomerated particles.</p> <p>The following projects have, or are currently developing protocols and tools to improve distinction between primary NM, agglomerates and aggregates: ENPRA, NANoREG, SUN, SANOWORK, MARINA, NANOGENOTOX.</p> <p>The NANoREG guidance document for (eco-)toxicological testing predefines a number of dispersion SOPs and one harmonized sonication procedure for preparation of batch dispersion used for dosing into test systems as well as dispersion characterization SOPs. The revised dispersion SOPs are presented in deliverable D2.06 (http://www.nanoreg.eu/images/2016_05_31_NANoREG_Factsheet_D2.6.pdf). The entire guidance document and SOPs will become available from NANoREG in 2017 as well as via forthcoming scientific publications.</p> <p>For <i>in vitro</i> studies, NANoREG decided to use the NANOGENOTOX dispersion protocol (EtOH prewetting and 0.05% BSA water as dispersion medium). For <i>in vivo</i> studies, NANoREG decided to use the ENPRA dispersion protocol (optional EtOH prewetting and 2% serum water as dispersion medium). For ecotoxicology studies, it was decided to use a pure water-based dispersion medium or a NOM-water medium</p>

		<p>if reasonable dispersion could not be achieved in pure water. All dispersion protocols employ harmonized effective dispersion energy using an interlab validated probe-sonicator calibration protocol (see also http://www.cea.fr/cea-tech/pns/nanosafe/en/Documents/Session%205.1/PS5.1-16.pdf).</p> <p>These protocols were documented for 19 different NMs by comparative interlaboratory testing. Only a few NMs were observed to be a challenge due to material properties. The procedures are proposed for regulatory testing. Further work requires conclusions on whether the methodologies has significant (unacceptable) influence on the test results. Improved technologies to rapidly quality dispersions is also a need that can improve implementation in toxicological test laboratories.</p> <p>Keld Alstrup Jensen ITS-NANO Phil Sayre Ulla Vogel Janeck J. Scott-Fordsmand</p>
<p>1.6</p>		<p>Identify best suited exposure monitoring strategies</p> <p>The periodical assessment of the concentration of chemicals in environmental (but also biological) matrices (exposure monitoring) can be done for a variety of purposes, such as to provide risk assessment with quantitative exposure data, to link health endpoints with the magnitude of exposure in epidemiology, or simply to assess the compliance with and effectiveness of control measures (e.g., local exhaust ventilation systems, enclosures, etc.). The nature of the monitoring strategy will heavily depend on the reason for the assessment. For example, testing the effectiveness of control measures may be achieved by periodical assessment of airborne concentrations. However, testing compliance with standards (e.g. Occupational Exposure Limits (OELs) or quantitative assessment of risk, ideally should rely on the use of personal monitoring approaches which approximate the uptake by inhalation.</p> <p>Exposure monitoring for NMs is complicated as</p> <ul style="list-style-type: none"> • There is a lack of standard approaches and metrics (airborne mass, surface area, or other) that can be used to estimate exposure for risk assessment/management; • The majority of direct reading instruments for measuring nanomaterials are not specific to engineered nanomaterials and are affected by the environmental background; • Currently, only a few portable instruments that can be used for personal monitoring are available; most instruments are stationary; • In addition the accuracy of the measurements are sometimes poor because the sizing and counting principles are sensitive to the nature (type and state of agglomeration/aggregation) of the dust; • Offline analyses often rely on TEM/SEM, and are often used for qualitative characterisation, rather than quantification. • Comprehensive monitoring surveys require sophisticated equipment and can only be carried out by experts and are therefore expensive. • There is a lack of harmonization of the measurement data (i.e. data are collected using different instruments, given in different metrics, representing

		<p>different scenarios: release measurements, personal or emission measurements at source and/or at background locations on the room) which hampers the use of this data for cross-comparison and risk assessment.</p> <ul style="list-style-type: none"> • Methods in complex media are only available yet for very few and specific cases where they are applicable only to a certain pairing of particle type and matrix. • Especially in environmental systems the differentiation between engineered, incidental and natural origin of the particles remains a challenge, although general concepts and first methodological approaches have been developed. <p>Tiered exposure monitoring approaches, involving the identification of NMs and monitoring of workplaces, have been proposed (eg. the NEAT approach by NIOSH). However, the available strategies mainly address risk management and are currently not integrated in (tiered) risk assessment approaches. In addition, biological markers of exposure/internal dose may be used in exposure assessments for preventive purpose; such exposure biomarkers should not only be correlated with external exposure, but with possible health outcomes ^{xvii}.</p> <p>Research priorities in the near future will be to develop tier- based exposure monitoring strategies that provide exposure estimates (as opposed to concentrations) that can be used within quantitative risk assessment approaches. Development of such tier-based strategies will require addressing uncertainties linked to exposure measurements and assessments and monitoring such as those associated with the technical capabilities of instruments, off-line analyses and the level of representativeness of the results.</p> <p>Different instruments are currently on the market that provide information on total or size-resolved particle mass and particle number and surface area, using a variety of principles that have been shown to not always provide the same results. Systematic comparison and calibration of instruments will be necessary if results from these instruments are used for quantitative risk assessment. However, size distributions obtained from instruments relying on different physical properties and reporting equivalent diameters (mostly applying the concept of a solid homogeneous sphere) must differ from each other in their results as soon as the particles do not meet the particle model employed in these techniques. i.e. when non-spherical particles are assessed by methods determining particle volume (laser diffraction), hydrodynamic diameter (DLS) and projected diameter (EM). This is not a shortcoming of the methods or a sign for erroneous measurement. On the contrary, these deviations between methods can be utilized to gain additional information about the particles, e.g. their elongation, fractal dimension or aspect ratio. These considerations are also relevant to general characterization of NMs (sections 1.11)</p> <p>Furthermore, little is currently known about the variability in exposure over time and space for NMs and their interaction with background nano-sized particles. Most monitoring assessments have been focused on short-term or task-based emission measurements and rarely the personal exposure. Long-term exposure has not yet been addressed. This area could also benefit from the identification, validation and use of biomarkers reflecting the body burden of foreign chemical . After release NM can alter important particle characteristics (e.g. size, shape and charge) which may affect their hazard. Studies investigating the spatio-temporal variability of background particles as well as engineered nanoparticles will be essential for the development of</p>
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		<p>comprehensive exposure monitoring strategies and calibration of predictive exposure assessment models.</p> <p>Most monitoring assessments have been focused on short-term or task-base exposure. Long-term exposure has not yet been addressed.</p> <p>Methods for individual NM exposure assessment are for the moment under development, either for conception of theoretical exposure models, or for practical use in epidemiological studies. International collaboration is essential to develop a concerted method of quantitative personal exposure assessment useful for epidemiology, risk assessment, and risk management in small companies.</p> <p>Most measurement strategies focus on inhalation exposure. However, dermal exposure and uptake (especially in damaged skin) may also need to be addressed. Currently, little guidance is available on the monitoring strategies for dermal exposure for chemicals, let alone NMs. Relevant workplace studies and exposure assessments models are developed in the SUN FP7 project.</p> <p>Soils and sediments are considered the final sinks of nanomaterials in the environment. Monitoring of nanomaterial exposure in the aquatic environment, soils and groundwaters is in its infancy and rarely ever a distinction of natural and engineered particles is performed, due to lack of methodology. Development of concepts, methods and instrumentation are required to increase specificity, improve applicability of methods and reduce costs.</p> <p>In addition to the collection of data, it is important to develop strategies for analysing and interpreting the results of the measurements. Development of methods that use results of measurements as well as estimates from available exposure models (for example, Bayesian methodologies) may lead to a reduction in uncertainty and improved accuracy.</p> <p>Martie Van Tongeren Monita Sharma Enrico Bergamaschi Janeck J. Scott-Fordsmand Keld Alstrup Jensen Frank von der Kammer</p>
<p>1.7</p>		<p>Identify relevant short term hazard models <i>in vitro</i> and <i>in vivo</i></p> <p>The models chosen for hazard studies will depend upon the questions to be addressed, such as the exposure route, NM form, and endpoint of investigation. The models, when combined, must be capable of covering all exposure routes, relevant biological life stages as well as NM life cycles, allowing hazard assessments to be carried out at all life cycle stages that represent realistic exposure scenarios.</p> <p>In the short term research priorities include improving the understanding of the relationships between physicochemical, exposure and hazard characteristics (e.g. by determining the mode-of-action underlying the hazardous effects), primarily to promote the development of grouping and or ranking approaches for NMs and to enable design of <i>in vitro</i> and high through-put (HTP) screening tools that target biological key</p>



		<p>processes. Such approaches are required to improve the efficiency of NM screening and risk assessment.</p> <p>Identification of the mode-of-action of hazardous effects will allow design of <i>in vitro</i> models targeting key and relevant processes rather than an indirect indicator or something that is simply easy to measure. In the distant future <i>in vitro</i> HTP testing and <i>in silico</i> models will allow focused hazard assessment with an eventual reduction in the burden of testing.</p> <p>Short-Term Inhalation Screening (STIS) and analogously oral (STOS) methodologies are used and developed in SUN (RIVM, BASF), NanoMile and nanoGRAVUR (BMBF). Such protocols can be used as a screening tool that will help in the design of subchronic and chronic tests. Recent H2020 calls will also address these issues.</p> <p>By linking to 1.4, these short term hazard models can be based on evidence of a link between the nanomaterial exposure and the mode of action leading to toxicity.</p> <p>ITS-NANO Phil Sayre Ulla Vogel Vicki Stone Jacques-Aurélien Sergent</p>
<p>1.8</p>		<p>Identify relevant long term hazard models</p> <p>Validated <i>in vitro</i> and <i>in vivo</i> models need to be developed that address long-term or chronic models. These models will also require the development of reliable biomarkers to estimate exposure and/or establish indicators for chronic effects. <i>In vivo</i> models will allow determination of time courses of responses including distinction between short and long term effects, rapid and delayed onset, reversible and irreversible effects, and underlying mode-of-action.</p> <p>In the longer term the goal is to develop and validate alternative non-animal models to replace <i>in vivo</i> models. These models should be based on the identification of key biological processes that drive the adverse effects. In addition to validation of simple culture systems, there will be a need to generate more relevant multi-cell and multi-tissue (e.g. pulmonary, gut, endothelium and liver) <i>in vitro</i> models. Only NANoREG (BMU, BASF, BfR) currently perform long-term <i>in vivo</i> testing. On the other hand, several long term inhalation studies using nanoparticles and carbon nanotubes have been published in the scientific literature, thus providing a means for validation of non-animal models of inhalation exposure.</p> <p>There are in fact some <i>in vitro</i> approaches that provide insights into possible long-term effects. Examples include:</p> <ol style="list-style-type: none"> 1. Fibrogenicity and epithelial-mesenchymal transition (EMT) tests (which occurs in organs and in the initiation of metastasis for cancer progression); 2. Assessment of genotoxicity as an indicator of potential carcinogenicity;



		<p>3. Cell transformation assays (e.g., BalbC 3T3)</p> <p>4. Epigenetic changes (e.g., imbalance DNA methylation, histone modification)</p> <p>In the long-term, knowledge of the population-level effects, bioaccumulation and biomagnification of NMs will also be required. A common approach to mammalian toxicology and ecotoxicology studies is encouraged. Studies will generally require robust, appropriate <i>in vitro</i> and <i>in vivo</i> models of susceptibility to focus on vulnerable individuals or populations. Environmental changes can impact on the quality of life/health of mammals. The transfer of nanomaterials from environment to human beings should be considered.</p> <p>By linking to 1.4, these long term hazard models can be based on evidence of a link between the nanomaterial exposure and the mode of action allowing prediction of toxicity.</p> <p>ITS-NANO Phil Sayre Ulla Vogel Vicki Stone Enrico Bergamaschi</p>
<p>1.9</p>		<p>Implications of EC definition within current regulatory frameworks</p> <p>Different definitions for different regulations may lead to confusion. One product could be subject to more than one regulation and therefore parity of definitions and regulations (where appropriate) would help to reduce the burden of testing for producers as well as the generation of conflicting advice/regulation for the same product. However, one strict definition might not fit all regulations, and so perhaps a core definition with annexes relevant to different scenarios may be required.</p> <p>The European Union sector specific legislations already including a legally binding nanomaterial definition are the Cosmetic Products Regulation No 1223/2009, the Regulation on the Provision of Food Information to Consumers No 1169/2011, the Biocidal Products Regulation No 528/2012, and the Novel Foods Regulation No 2015/2283.</p> <p>A "Proposal for a Regulation of the European Parliament and of the Council on Medical Devices, and amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009" is under scrutiny of the European Parliament. A nanodefinition adapted from the EC definition is included in Art 2.1 (15) of the draft Regulation.</p>


List of current regulations that are relevant to NM, with and without a NM definition:				
Application	Authorisation ¹	Nano-Definition	Nano-Label	Guidance
Cosmetic Products	(EC) No 1223/2009	Yes	Yes	SCCS guidance
Medical devices	COM(2012) 542 final, 2012	Yes	Yes	No
Plant protection products	(EC) No 1107/2009	No	No	EFSA guidance (for oral intake via food) (EFSA Scientific Committee 2011)
Novel food/feed	(EC) 258/97	COM(2013) 894 final 2013/0435 (COD) reference to (EU) No 1169/2011	(EU) No 1169/2011	EFSA guidance
Food additives	(EC) 1333/2008	No	(EU) No 1169/2011	EFSA guidance
Enzymes	(EC) 1332/2008	No		
Flavourings	(EC) 1334/2008	No		
Food supplements	Dir 2002/46/EC	No	No	No
Feed ((EC) 767/2009)	Not required	No	No	EFSA guidance
Feed additives	(EC) 1831/2003			
Food contact materials	(EC) 1935/2004	No	No	EFSA guidance
Plastic food contact materials	(EC) 20/2011	No	No	EFSA guidance
Active and Intelligent Materials and Articles	(EC) 450/2009	No	No	EFSA guidance
Biocides	(EU) No 528/2013	(EU) No 528/2013	(EU) No 528/2013	Pending (information requirements)
Chemical substances	(EC) 1907/2006 (REACH) (authorisation required for certain hazardous substances)	No	No	ECHA guidance (ECHA 2012) 4 guidance documents undergoing the PEG process (April 2017)


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Sonja Hartl
Vicki Stone
Jacques-Aurélien Sergent


¹ Authorisation required means that substances/products have to undergo pre-market approval

<p>1.10</p>		<p>Standardised methods to identify NM according to the EC definition</p> <p>The current EC definition draws on number-based size distributions of primary particles. In case of non-spherical particles (most real-life materials), the smallest dimension of each particle should be measured. The primary particles within aggregates and agglomerates should be measured and counted.</p> <p>At present, there are no standardised methods which meet such challenges. As mentioned above (section 1.2), methods have progressed well in NANoREG and NANODEFINE to solve the measurement challenges in the proposed EC-definition of a NM.</p> <p>Jacques-Aurelien Sergent Antoine Ghanem Keld Alstrup Jensen</p>
<p>1.11</p>		<p>Methods to characterize and test transformation of NM throughout life cycle stages</p> <p>Linked to the need to be able to detect and quantify NMs in complex environments throughout their life cycle, is the need to identify their potential modification or transformation. This is especially important if the modifications result in a change in hazard (or exposure) and therefore risk</p> <p>NM undergo numerous environmental transformation processes and therefore the effects measured for pristine NMs may be representative of exposures occurring at manufacturing or processing sites, whereas environmental exposures will consist solely of transformed NM and so data related to pristine NMs may not be predictive of real-world scenarios (except for major accidents during production and transport where major release of pristine NM may occur). It is not possible to assess the environmental risks by only studying the pristine material and alteration and transformation processes need to be considered when determining fate and effects. Understanding the transformations of pristine NMs is therefore essential to correctly extrapolate the fate and effects of particles during the use and disposal phases of a nano-enhanced-product's life cycle. By better understanding probable endpoints of aged materials, NM fate can be better incorporated into the general structure of risk assessment. Considering that life cycles of many NMs are determined by their application within products, it becomes clear that relevant exposure scenarios and NM aging/transformations are strongly dependent on the life cycle of the nano-enhanced products themselves. While the product manufacturing portion of the life cycle will likely be in a controlled, industrial setting, the use and disposal of nano-products in the consumer realm will be decidedly less predictable and include more variables.</p> <p>Research on release and transformation of materials from nano-products is growing and the next necessary step is now to investigate the behavior and the effects of the released and transformed materials in the environment and on humans. In order to make this possible, we need to have sufficient released materials available for further testing. In addition we also need NM-free reference materials because many processes not only release NM but are also producing nano-sized fragments from all kinds of materials that may interfere with further tests. In order to provide a material mimicking released materials, the SUN consortium (Project on "Sustainable Nanotechnologies", EU 7th Framework funding) has come up with an approach by producing what is called</p>

		<p>“Fragmented Products” (FP). It is possible to obtain a material fraction that is mimicking the materials that are formed under real-world conditions and it has been shown how one could potentially isolate nanomaterials in amounts needed for further testing. The FP obtained by the SUN approach are not the real materials, but they represent an approximation and allow the production of hundred gram quantities. A further size fractionation can be performed to isolate a fraction that is more suitable and representative for inhalation studies. The fragments can also undergo further weathering to produce “Weathered Fragmented Products” (WFP).</p> <p>This proposal for obtaining released and transformed materials should be critically evaluated and tested for various NM and products. It can form the basis onto which a realistic assessment of NM under the life-cycle thinking can be performed.</p> <p>Transformation links to 1.3 and 1.12 in that transformation can include dissolution or fragmentation from composites and lead to a situation where the NM no longer exists. The SUN project tracks these processes and evaluates the properties over the lifecycle.</p> <p>Bernd Nowack Wendel Wohleben SUN Frank von der Kammer</p>
<p>1.12</p>		<p>Identification of circumstances in which NM are no-longer nano</p> <p>During their lifecycle, nanoparticles may undergo dramatic changes in several characteristics, including size, shape and composition. For example, in case of total dissolution and pyrolysis, the particles simply cease to exist. In other circumstances (e.g. sintering), the particles might “fuse” together into structures whose size is larger than the 100 nm upper limit of the current EC definition. Additionally, the particles can get encapsulated in a rigid matrix (case of nanocomposites) and, may not be expected to be released. All these cases should be identified and based on a clear definition based on robust testing methods.</p> <p>Consideration of particles that may stay the same in size and composition, but lose their reactivity also needs to be considered.</p> <p>Links to 1.3 and 1.11 in that dissolution and transformation can lead to loss of the NM over time, but also transformation from one NM to another.</p> <p>LABEX Serenade (CEREGE, France) Jacques-Aurelien Sergent Antoine Ghanem</p>
<p>1.13</p>		<p>Understanding uncertainty of current data for risk assessment</p> <p>There is principally a need to understand uncertainty for all data relevant to risk assessment (RA). Historically, data uncertainties in RA have been tackled by safety factors or use of (realistic) worst case assessments at initial stages of RA. Improved data-quality and more precise information could then be used to increase the confidence in the results. However, the tendency to default to conservative estimates (often based on assumptions) could result in over- precautionary risk management that would hinder product development. In order to avoid this, the assessment of uncertainty</p>


		<p>is essential as it can greatly improve risk communication and make the RA more realistic.</p> <p>The predominant types of uncertainties in the RA of NM are the knowledge and data gaps connected to the physicochemical identity of NM (or NM-enabled products), their toxicity, human exposure and environmental release scenarios. Each RA can involve numerous such individual epistemic and parameter uncertainties that accumulate by “error-propagation”. Today these uncertainties are often poorly reported and possibly not well defined, or even not known. They can be analyzed with stochastic methods (e.g. Monte Carlo) that provide probability distributions of hazard and exposure parameters. However, the experience shows that in the lack of sufficient data these distributions may often be too broad to generate meaningful information for RA. In order to reduce the epistemic and parameter uncertainties and to develop capacities to better assess them, current research evolves along the lines of better data quality control.</p> <p>There is a scientific drive towards establishment and inclusion of predictive risk assessment models to support regulatory risk assessment. Moreover, the use of exposure and dose response models generates modelling uncertainty, which is rarely quantified in risk assessments. Occupational and consumer exposure models are often designed to provide conservative exposure estimates, while exposure modelling for ecological receptors is predominantly done on a case-by-case basis, which often introduces variability in the obtained results.</p> <p>Other types of uncertainties, which are not easily quantified, are uncertainties related to the reliability of the test methods or whether e.g., the toxicological end-point was investigated.</p> <p>The SUN EU FP7 project attempts to include the analysis of the above uncertainties in a data-based quantitative RA. However, it is evident that a transition from traditional precautionary RA towards statistical/probabilistic RA is not straightforward.</p> <p>Keld Alstrup Jensen Wilson Engelmann Enrico Bergamaschi Danail Hristozov</p>
<p>1.14</p>		<p>Understanding uncertainty of current data for risk decision making</p> <p>The fundamental decisions that companies have to make in regards to the risks from their products are whether to avoid, transfer, mitigate or accept them. These decisions should be informed by realistic RA results according to regulatory requirements. In order to facilitate the interpretation of these results, it is of high importance to clearly communicate the underlying uncertainties. This is because the misinterpretation of risks can lead to their negative perception, which may challenge their acceptance by regulators or costumers, and hinder their transfer to the insurance sector. The latter is a critical issue for R&D companies as the lack of access to insurance markets exposes them to dangerous liabilities. In addition to uncertainty, variability can also hinder objective risk-based decision making. In contrast to uncertainty, which can be reduced by generating additional data, variability relates to the inherent complexity of the NM-based product and testing systems and is far more difficult to address. Uncertainty can be characterized in both a qualitative and quantitative manner, while distinguishing the</p>




		<p>variability typically requires “two dimensional” analysis by means of probabilistic (e.g. Monte Carlo) approaches.</p> <p>In addition to risk, decision makers should consider also other sources of uncertainty in deciding the cost they are willing to pay for risk management. This is challenging as the still developing regulatory environment makes it difficult to assess the cost of launching a product and whether future changes in legislation will induce post-launch costs (e.g. in terms of safety documentation).</p> <p>A full mapping of the criteria contributing to cost/risk-benefit decisions requires company/branch-specific information. The integration of these criteria into an assessment that can transparently inform R&D investment, risk management or risk transfer decision making in a context of high uncertainty calls for employing tools from decision analysis (e.g. weight of evidence, MCDA, Vol). The combination of these approaches with probabilistic methods can help to transparently communicate the underlying uncertainties in order to facilitate more objective decision making.</p> <p>Keld Alstrup Jensen Wilson Engelmann Danail Hristozov</p>
<p>1.15</p>		<p>Understanding of uncertainty of current data for risk governance</p> <p>Risk governance is a unifying approach to decision making that involves the actors, conventions, rules and processes concerned with how relevant risk information is collected, analysed and communicated in order to enable more effective risk management that is convergent with other public and private policies. It seeks to reduce risk by filling gaps in risk policy in order to avoid or reduce social, environmental or economic costs. Risk governance therefore comprises a variety of processes including those at the institutions by which authority is exercised and decisions are taken and implemented.</p> <p>The main uncertainties in the risk governance of early generation NMs (e.g. nanoparticles, coatings) are associated with an ignorance of their interactions with biological organisms and gaps in the regulatory systems at the national and international levels. The main source of uncertainty for the more advanced generations of NM-enabled products (e.g. nanodevices, nano-bio applications, and nanosystems) could be the unknown evolution of the technology and its influence on both the level of the individual (e.g. human health effects) and the society (e.g. cognitive issues and human evolution), as well as unknowns around the framework through which organisations and policies can address such uncertainties. Resolving these uncertainties can be challenging due to: (i) relatively fragmented governmental institutions and legal authorities dealing with nanotechnologies at the national and global levels; (ii) lack of practices to improve the responsibility or increase the liability of the stakeholders involved in the process of nanotechnology innovation; (iii) weak coordination among the different actors and stakeholders (e.g. academia, industries, regulators, civil society) in addressing the risks posed by nanotechnologies.</p> <p>An important aspect in risk governance is that it also requires actions due to the perception of risk at the different stakeholders. Therefore, it is important to act differently and discriminate between the risk governance approaches required for</p>



		<p>example within a factory and the approaches that may be required in communication and risk transfer to the external stakeholders (e.g., insurance companies, downstream professional users, public consumers, and the regulatory and administrative bodies).</p> <p>The data uncertainties in risk governance are often large and may in some aspects require use of “big data”. The error propagation discussed above, expands further and the data now originates from hard science, financial science, social science and psychology.</p> <p>Key research needs to reduce the uncertainties and ambiguities for the risk governance of nanotechnologies are: (i) to develop methods and tools integrating data and judgment consistent with regulatory requirements and applicable for risk management in industry; (ii) to identify and assess the concerns of the major stakeholders involved in nanotechnology innovation; (iii) to investigate the ethical and social dimensions of the economic, environmental and social impacts of nanotechnologies; (iv) to develop decision making tools that incorporate the above aspects and integrate them with the subjective value systems of the decision makers; (v) develop capacity to apply these tools to address any controversial nanotechnology developments as part of a globally accepted risk governance framework.</p> <p>These research developments should be underpinned by a robust risk communication strategy that should be designed to emphasize the discussion about the benefits of nanotechnologies and their non-intended side effects and the means to identify and quantify those effects and estimate the trade-offs among them. This risk communication strategy should also address the debate on the ethical and social implications of nanotechnologies and it should transparently provide public information on the principles and procedures used to assess their potential safety implications as well as to inform the public on the policies for investment in nanotechnology R&D.</p> <p>The recently launched caLIBRAtE EU H2020 project will address some of these research needs in order to establish a next generation nano-risk governance framework for nanotechnology. A key objective in this project is to test and calibrate/performance test and demonstrate various models to reduce uncertainty and support the risk governance during the innovation, product launch and post launch monitoring. In addition, models for decision support and monitoring will be improved by improving the understanding of risk perception at the different stakeholders.</p> <p>Keld Alstrup Jensen Danail Hristozov</p>
<p>1.16</p>		<p>Intelligent Testing strategy development</p> <p>The rapid introduction of engineered NMs to the market raises several challenges to the traditional risk assessment approach. The problems include a lack of appropriate tools for effective NM risk decision-making and insufficient capacity (time and money) to fully assess or evaluate risks associated with all NMs. The challenges are, therefore, to develop both appropriate and optimal approaches to screen NMs and evaluate associated risks.</p>


	<p>This can 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.</p> <p>ITS-NANO outlined research priorities to allow: (i) intelligent design of nanosafety evaluation and risk assessment strategies, including rapid screening, and computational models, (ii) identification of high risk materials, and (iii) implementation of effective strategies to counter the risks.</p> <p>The Intelligent Testing Strategy (ITS) presented by ITS-NANO used exposure and hazard information, but also incorporates physicochemical (PC) characterisation of NMs. This paradigm therefore requires the systematic acquisition and implementation of key information related to exposures, hazards and physicochemical characteristics (IDs) of the materials. ITS-NANO provides research priorities spanning four timeframes:</p> <ul style="list-style-type: none"> • In the short-term (<5 years) needs include development of an understanding of the connections between NMs' physicochemical, exposure and hazard characteristics. 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 <i>in vitro</i> models) with more comprehensive and complex techniques (detailed methods and <i>in vivo</i> 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 increasing focus on <i>in vivo</i> and <i>in vitro</i> hazard testing is required. • In the distant future (>15 years) RA can be based on modelling and extrapolations, and only if additional information is required with focused physicochemical, exposure and hazard testing. <p>All three aspects are defined and the definitions are used to identify the research needed to deliver the tools and information required for robust RA. The ITS will require increased reliance on computational approaches and decreased reliance on testing with time, thus this prioritisation document identifies the research required to enable grouping/ranking of NMs to optimise the risk decision process in the short term, and modelling approaches in the longer term. Strategies for identifying PC, exposure and hazards of relevant materials could be developed independently to some extent, but highly integrated research is required in order to advance as rapidly as possible. Finally, a process for implementing research findings into current and future risk assessment frameworks has been established.</p> <p>ITS-NANO Executive summary nanoGRAVUR (BMBF, 2015 – 2018, coord. Kuhlbusch IUTA) Cefic LRI project (2014 – 2016, coord. Bouwmeester, RIKILT)</p>
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Interim Regulation II (or guidelines)



<p>2.1</p>		<p>Harmonisation of best suited exposure monitoring strategies</p> <p>In order to make progress in the field of exposure assessment of NMs and development of exposure models it is essential that results of studies are shared. Harmonization of monitoring strategies is essential if results between surveys are to be compared, combined and/or stored in an exposure database (eg Nano Exposure & Contextual Information Database NECID). Several workshops have been organised in recent years that have addressed harmonisation of measurement strategies for NMs, as well as analyses, interpretation and storage of measurement and contextual data ^{xviii, xix, xx}. OECD WPMN SG8 has published several reports on NM exposure assessment. In addition, the OECD in 2015 published a paper on a harmonised tiered strategy to measure NMs^{xxi}. ISO TR 27628 (2007) addresses “Nanotechnologies – Workplace atmosphere – Ultrafine, nanoparticles and nanostructured aerosols – inhalation exposure characterisation and assessment. The European Committee on Standardisation (CEN) is also developing a standard method to assess inhalation and dermal exposure to NMs. The decision criteria for moving up a tier are based on potential of exposure, rather than risk. Also, issues of exposure variability and uncertainty are not explicitly addressed.</p> <p>The best way for improving and harmonising the monitoring strategies will be to carry out monitoring surveys and use the data in the context of regulatory risk assessment of NMs. In addition, sharing of raw or processed data as well as experiences will also allow an evaluation and comparison of results from monitoring surveys, which will lead to overall, gradual improvements in the monitoring strategies.</p> <p>A key pre-requisite to enable a reliable monitoring strategy, is establishment of reliable nano-specific or proxy measurement methods. Currently, almost all exposure studies have been performed by measuring the particle number- and mass-concentrations and/or particle size-distributions by stationary measurements. Exposure to airborne nanomaterials needs to be measured in the personal breathing zone (PBZ) using nano-specific personal samplers or monitors. Such personal instruments have only become available over the recent years. The NanoIndex project (http://www.nanoindex.eu/) evaluated the performance and the field applicability of a number of personal samplers in laboratory and real exposure scenarios and provided a guidance document for measuring the individual exposure in the PBZ. Such studies showed that this accuracy and comparability is still applicable under real field conditions and that all tested instruments are robust and ready for field use.</p> <p>Although very promising, this approach need to be further validated and the choice of sampling tube material needs to be carefully considered. NanoIndex showed some polymer types to induce drastic artefacts when connected to monitoring devices. Unfortunately, none of the applied monitors could be used to measure exposure to fibrous aerosols, e.g. containing carbon nanotubes (CNTs).</p>
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		<p>Martie Van Tongeren Monita Sharma Enrico Bergamaschi Keld Alstrup Jensen</p>
2.2		<p>Validation and standardisation of relevant short term hazard models <i>in vitro</i> and <i>in vivo</i></p> <p>Key to understanding potential hazards of NMs is the availability of appropriate testing guidelines or standard protocols. For this, validation to target species or human responses is essential. Based on understanding of mode of action, high throughput (HTP) technical approaches and equipment will allow effective and efficient estimation of the biological response associated with NM-related properties. To rationalise testing and to allow for grouping, models should be developed allowing for cross -NM, -species and -media extrapolation/interpolation.</p> <p>The lack of proven validity and standardisation of the methodology used in hazard testing of NMs is, in part, caused by the need for well characterised and harmonised controls/test items/reference materials/positive nanomaterial controls. Reference or standard NMs (e.g. OECD materials) suitable for benchmarking should be available, which are representative of the variation in physical and chemical composition of available NMs. Some NMs of this type are now available, but whether these are sufficient and in what context they are best used remains to be determined. The use of such reference or standard materials as controls will improve the ability to benchmark hazard of NMs and to make comparisons across studies. In addition, positive nanomaterial controls in addition to non-nanomaterial controls would provide evidence that the employed testing strategy was appropriate and suitable for detection of nanomaterial-mediated hazardous effect.</p> <p>By linking to 1.4 and 1.7, these short term hazard models can be based on evidence of a link between the nanomaterial exposure and the mode of action leading to toxicity.</p> <p>ITS-NANO Ulla Vogel Vicki Stone</p>
2.3		<p>Validation and standardisation of relevant long term hazard models</p> <p>By linking to 1.4 and 1.8, these long term hazard models can be based on evidence of a link between the nanomaterial exposure and the mode of action leading to toxicity. The text for 2.2 is also relevant to 2.3.</p>
2.4		<p>Validation and standardisation of methods for quantification and characterisation of NM</p> <p>Validated and standardised techniques to characterise NMs are required in order to verify whether a product is or contains a specific type of NM, especially if that NM is considered to be hazardous. Validated and standardised methods are also required</p>


		<p>for characterisation and/or quantification of specific NMs in complex environments throughout the life cycle of a NM, ranging from detection within a commercial product (to allow regulatory compliance) through to release into the environment during all stages in the lifecycle. It is likely that a range of methods and tools will be needed to address these wide-ranging requirements. As mentioned above (section 1.2 and 1.10), methods have progressed well in NANoREG and NANODEFINE to solve the measurement challenges in the proposed EC-definition of a NM. These methods will require further work to allow validation and standardisation.</p> <p>Vicki Stone</p>
<p>2.5</p>		<p>Validation of methods to identify and characterize NM transformation</p> <p>Section 1.11 outlines why methods are required to identify and characterise NM transformation. Validation of these techniques will be required to facilitate their standardisation and use.</p> <p>The US-CAN-EU initiative NanoRelease (now hosted by ILSI Europe) has built on earlier inter-laboratory tests in MARINA and has performed two inter-laboratory tests on protocols that have been notified to ISO already: sanding and weathering. For such processes, validation is required for the steps of aging/transformation, sampling and analysis.</p> <p>ITS-NANO suggested that a library/inventory of such methods should be accompanied by a decision tree to allow stakeholders to identify the tools/standard methods most appropriate to them.</p> <p>The activities of NANOREG WP2 are relevant to this priority.</p> <p>ITS-NANO Wendel Wohlleben</p>
<p>2.6</p>		<p>Identify exposure and hazard relevant dose metrics</p> <p>For both human and environmental studies there is a need to determine appropriate dose and <u>dose metrics</u> in hazard and <u>exposure assessment</u>.</p> <p>1. Exposure. The mass dose metric most commonly used to characterise hazardous effects of conventional chemicals may not be the optimal dose indicator for NMs. Other metrics that have been suggested to better describe dose include particle number and deposited surface area in the lung. However, in the workplace atmosphere heterogeneous particle species of various shapes are present which would make such measurements difficult to interpret. Furthermore, currently available surface area measuring devices are tailored to assess spherical particles, which means that fibre-shaped particles may not be appropriately evaluated by such techniques. In addition, aggregation and agglomeration of NM in a workplace atmosphere may hamper the reliable counting of particles. This justifies an integrated approach in measurement, including multiple parameters (such as number, surface area and mass concentration) and multiple techniques. For example, the Nanoparticle Emission Assessment Technique (NEAT) developed by the National Institute for Occupational Safety and Health (NIOSH) employs a</p>




		<p>combination of direct-reading, handheld instruments (particle counters) to detect release of airborne NMs coupled with ficou-p-based air sampling and subsequent chemical and microscopic analyses for NM detection and elemental analysis ^{xxii}. As information accumulates regarding the influence of their physicochemical characteristics on associated hazards, such integrated dose metrics could be developed. With the ever-expanding understanding of the relationship of PC and Hazard this is likely to change with time.</p> <p>2. Hazard. NM hazard dose metrics also require consideration, and in fact the concept of Biologically Effective Dose (BED) has been described ^{xxiii}.</p> <p>In particle toxicology the dose is usually defined by the mass or concentration of particles per unit tissue, per number of cells or per surface area of cells in a cell culture. However, mass is not always the physical-chemical property that drives toxicity, but mass may be the most convenient practical measure of the quantity of particles present that provides a surrogate for the important dose since mass does not change over time, whereas particle number may change due to agglomeration.</p> <p>The BED in particle toxicology is defined as ‘the entity within any mass dose of particles that drives a critical pathophysiologically relevant form of toxicity in tissue, such as inflammation, genotoxicity or cellular proliferation’. Therefore the total mass dose includes the BED plus a fraction that is not biologically effective.</p> <p>The BED of some pathogenic particles has been identified (e.g. Quartz - unpassivated (active) surface area, asbestos - long, biopersistent fibres, low toxicity, insoluble nanoparticles – inflammation). However, despite knowledge of the BED for some particles, they are still measured by mass due to the difficulty in measuring the BED.</p> <p>In the future, development of instrumentation to directly measure the BED will allow improved metrics, which will improve dose-response assessments and thereby improve risk management.</p> <p>ITS-NANO Enrico Bergamaschi Ulla Vogel</p>
<p>2.7</p>		<p>Inventory of validated/standardised tools, methods and decision tree.</p> <p>The generation of libraries of standard protocols covering all aspects of NM RA (physicochemical characterisation, exposure and hazard testing) will allow a tailored or streamlined approach to testing. This library is likely to be quite extensive, it will therefore be necessary to support it with a decision tree or matrix to allow individual stakeholders to identify the protocols most relevant to them.</p> <p>In fact no such validated tools for NMs exist yet. This is the key goal of the H2020 project caLIBRAte and NANOREG.</p> <p>Keld Alstrup Jensen ITS-NANO Vicki Stone</p>


Interim Regulation III (or guidelines)

<p>3.1</p>		<p>Generating high quality hazard and exposure data</p> <p>Important issues in RA are the amount, type and quality of the data used to characterise risk and the associated uncertainties. Currently a lot of research data is produced on NMs using non-validated methods that are mainly suitable to identify but not characterise the hazard. One aim should be to direct research (e.g. via dedicated funding) so that results could support and feed better into a regulatory RA (see also previous chapters). This is not only true for exposure, but also for hazard and physicochemical characterisation.</p> <p>The relevance of this systematic high quality data needs to be careful checked to avoid the over-generation of data within the data being produced. Instead, focused data is needed to generated appropriate models, protocols and RA approaches in order to allow evidence based streamlining of testing in future (see below).</p> <p>Definition of high quality data</p> <p>High quality data are data that are reliable and relevant for the risk assessment required. Hence, in principle they are generated via validated methods and strict laboratory guidelines e.g. Good Laboratory Practice (GLP). Currently however, and likely in a foreseeable future these validated methods will not be widely used. Hence, a focus should be to define such validated methods and to ensure their wide distribution. Obviously simple methods will be more easily distributed and applied, but it is possible they will lack precision. Currently OECD and other groups (e.g. OECD Workshop Report 63, 2016) have suggested parameters to measure in order to obtain high quality information and schemes have been proposed on how to evaluate the quality of literature data e.g. various versions of the Klimisch approach ^{xxiv}. Regarding the latter however, it is important to realise that although a study may not seem high quality based on an evaluation using current standards it may indeed contain high standard information. It may be useful to have a decision tree approach, in which less comprehensive studies also provide information, although at a different level then very detailed and high quality studies. In addition, the use of reference materials and positive nanomaterial controls may facilitate benchmarking between different methods and laboratories.</p> <p>ITS-NANO Janeck J. Scott-Fordsmand Ulla Vogel</p>
<p>3.2</p>		<p>Generating high quality fate and kinetics data using representative NMs</p> <p>Consideration of fate and kinetics are relevant to both humans and the environment.</p>


		<p>For humans fate and kinetics relates to defining, characterising and quantifying the routes of uptake of NMs into the body via different exposure portals (e.g. lungs or gut), how they distribute around the body (e.g. via blood, lymph), the organs in which they accumulate (internal dose) and the routes of excretion/clearance. This includes a consideration of the rate of movement between compartments, the factors that influence this movement over time and the potential for bioaccumulation.</p> <p>Similarly for the environment, fate and kinetics relates to defining, characterising and quantifying the routes of release of NMs into different environmental compartments (e.g. air or fresh water), how they distribute within and between compartments, and consideration of whether they might break down over time or where they might accumulate. This includes a consideration of the rate of movement between compartments and the factors that influence this movement over time.</p> <p>Standardised protocols would be helpful for reliable characterisation of NMs (and the related PC properties) when present in complex bio-fluids or as product components throughout their life cycles. This will help to determine properties that influence bioaccumulation, but also fate, effective internal doses, and fate during their life cycles.</p> <p>These protocols require robust strategies for sampling and determining concentrations in appropriate indicator organisms and/or potentially sensitive environmental compartments need to be formulated and thoroughly validated.</p> <p>Another issue is the discrimination between NM and ions when measuring NMs' bioaccumulation in different organs and tissues. To address this issue, the recommendation is to use hyphenated techniques that allow visualisation of NMs as well as analysis of their chemical contents (e.g. cryo-transmission electron microscopy and energy-dispersive X-ray spectroscopy).</p> <p>See also sections 3.1 and 3.5</p> <p>Vicki Stone</p>
<p>3.3</p>		<p>Identify characteristics that influence release</p> <p>Release assessment regards the detachment of a fragment from a larger whole, such as a consumer product during use, and includes the release mechanism, form of the released entity, release scenario, probability of release, and lifecycle simulation, if relevant.</p> <p>Systematic series of release assessment identify key parameters that enable a ranking (and possibly even a grouping) of the probability and characteristics of release from nano-enabled products: aging intensity (photolysis, hydrolysis, leaching) and shear rates (abrasion, drilling, sanding), matrix properties (soft or brittle, resilient or labile), nanomaterial properties (particulate or fibrous, UV-absorbing, -reactive or -transparent).</p> <p>Data is available from around 70 publications, incl. several FP7 projects (nanohouse, NEPHH, MARINA, NanoPolyTox, SUN, NANoREG, to name a few) and recently progressed to real-world value-chains of in the SUN project.</p>

		<p>Key conclusions are 1) the importance decreases in the order: aging conditions – matrix properties – nanomaterial properties. 2) Reproducible protocols (aging - sampling – analysis) are possible, and are already required in specific legislation. 3) Many release phenomena are process-dominated with important background of nanoscale releases from conventional materials; accordingly, also the physical-chemical and toxicological properties of fragments released from nano-enabled products resemble primarily those of the matrix, modulated by properties of the nanomaterial.</p> <p>Wendel Wohlleben</p>
<p>3.4</p>		<p>Identify characteristics that influence exposure</p> <p>Identification of factors that influence exposure (exposure determinants) is essential for the development of exposure models, interpretation of exposure measurement results and identification of exposure control strategies.</p> <p>It should be realised that exposure is inherently variable, both over time and space. We know from experience that exposure can vary from one day to the next and between workers who are apparently doing the same activities. In addition, exposure can change from year to year, and there is evidence from several studies that occupational exposures in Western Europe and North America have been declining by approximately 5-10% each year. There is also evidence that exposure can be quite different between different sites with similar production facilities and between different countries. Some of this variability can be explained by investigating determinants of exposure, but generally there remains a relatively high level of apparently random variability.</p> <p>In order to study exposure determinants effectively, it is essential that</p> <ol style="list-style-type: none"> i. relatively large data sets are developed, ii. data are collected using the same or similar measurement strategies; and iii. detailed contextual information are available so that potential exposure determinants can be identified. <p>Statistical analyses of available exposure data can then be carried out to identify the determinants and quantify their effect on the level of exposure. The PEROSH group (http://www.perosh.eu/research-projects/perosh-projects/necid/) have developed the Nano Exposure & Contextual Information Database (NECID) for storing measurement results from NM exposure surveys. The use of this database structure should be widely encouraged within the NANOSAFETY Cluster and globally to facilitate sharing of the data.</p> <p>In addition, to investigating the determinants of exposure to NMs, it would also be useful to investigate the factors that determine the background exposure and its variability.</p> <p>Martie Van Tongeren, Monita Sharma, Enrico Bergamaschi</p>

<p>3.5</p>		<p>Identify characteristics that influence fate and kinetics</p> <p>To identify characteristics that influence fate kinetics and end of life scenarios for NM, requires a broader testing approach where a matrix of materials are tested covering the major NM characteristics e.g. surface area, size, surface charge etc. The materials in this matrix should obviously be studies using the most recent fate models including both colloidal models (for the particulate for) and free ion models (for possible dissolvable parts). Finally this should be coupled with NM character models, which enable an interpolation between various NM characters and hence enable predictive models based on NM characters, which is needed for new models and safer by design development. This approach will enable an interrelation between the current approach and the novel materials. The use of case studies here is simply a posterior exercise once the models have been derived. This work, will for the environmental part possible be performed over the next 3-5 years partly as an outcome of ongoing EU projects dealing with this, although such studies will need to employ tailored particle types (if available), that might not have any industrial relevance, but are able to fill the knowledge gaps still present.</p> <p>Janeck J. Scott-Fordsmand</p>
<p>3.6</p>		<p>Identify nano-relevant safety issues</p> <p>Identification of nano-relevant safety issues is a key decision point at which to determine whether refinement of regulations is actually required. These nano-relevant safety issues could relate to hazard (e.g. physical, such as explosion, or toxicological, as outlined in the “Classification, Labelling and Packaging” – CLP – regulation) or exposure and could be related to physicochemical properties (e.g., those leading to increased dustiness). This is not simply a trivial matter, but will require our efforts and resources for the years to come.</p> <p>ITS-NANO concludes that existing regulations may already be sufficient to cover nanomaterials through refinement for nano-relevant or nano-specific issues, but also to take into account the output styles of intelligent testing strategies.</p> <p>A key issue is how to ensure declaration of nanomaterials to make it possible for workers and consumers to realize that they handle nanomaterials or nanomaterial containing or releasing products.</p> <p>ITS-NANO Ulla Vogel</p>
<p>3.7</p>		<p>Refinement of the EC nano-definition including criteria of specific concern related to NMs</p> <p>Currently the EC nano-definition considers all NMs equally. As more information becomes available to identify the key physico-chemical characteristics, or use scenarios, the inferred significant risk, this nano-definition could be modified. This would have the advantage of allowing proportionate and targeted regulation of high risk NMs/uses, and avoiding over-regulation of low risk NMs/uses.</p>







		<p>To allow such refinement of the definition, many research priorities outlined above, that relate to characterization, the quantification of NMs in products, in the environment, resulting in exposure, resulting in hazard (and others) will need to be addressed.</p> <p>Vicki Stone</p>
3.10		<p>Develop nano- relevant RA strategies (grouping and read-across)</p> <p>Grouping refers to <i>the arrangement of NMs into groups based on common attributes</i>. For risk assessment purposes, these groups must be based on an attribute that is <i>relevant to risk</i> such as a common hazardous physicochemical property, or an exposure potential that infers a greater harm or risk of exposure. Whilst grouping is recognised for its use with chemicals, its application to NMs is yet to be fully defined, and tools or approaches are still to be developed to allow its effective use in risk assessment of NMs. Ranking is defined as <i>assigning a position in a scale</i>, again with specific referral to risk, meaning that particles may be classified based on factors such as their potential for exposure (e.g. high dustiness) and/or potential to cause harm due to high intrinsic toxicity.</p> <p>Ranking refers to the positions, in this context, of NMs on a scale (e.g. high to low hazard) that do not necessarily imply any physicochemical relationship between the NMs on that scale. However, grouping does infer a relationship in (for instance) a common physicochemical attribute such as shape, or a common mode of action of toxicity. Groups can be ranked, and ranking can occur within groups.</p> <p>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 intelligent testing strategy for nanomaterials. Informative grouping/ranking requires precise, accurate Physicochemical-, Hazard-, and Exposure 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.</p> <p>The grouping and ranking of NMs for risk assessment purposes is a multi-factorial process and ideally should encompass critical components such as hazard and exposure of humans and the environment as well as key physicochemical properties of the NMs themselves. Therefore, the future research emphasis for grouping/ranking is inherently reliant on and interlinked to those advances already stated for Physicochemical ID, Hazard-ID, and Exposure-ID.</p> <p>Read-across ^{xxv} entails the use of relevant information from analogous substances (the 'source' information such as another NM or substance) to predict properties for the 'target' substance(s) under consideration. In an ECHA/European Council for the Chemical Industry-Long Range Initiative (Cefic-LRI) co-hosted workshop discussion on read-across for chemical safety assessment under REACH, the main issues considered to impact the uncertainty, and thus the acceptance of a read-across prediction, were: (i) the experimental data used in the read-across, (ii) the chemical</p>

	<p>similarity on which the analogue or category is based, and (iii) the weight-of-evidence supporting the categorisation scheme employed. The discussion highlighted that the most acceptable categories for read-across are those based on integrating knowledge of how chemicals interact with biological systems with knowledge of the biological response(s) once compensatory systems are overcome (i.e. mechanistic information). Confidence in the read-across prediction would be reached when: (i) there is mechanistic transparency, (ii) experimental data for structural analogues allows for interpolation rather than extrapolation, (iii) the number of analogues within the chemical category increase (i.e. read-across from many to one), (iv) it is supplemented by toxicokinetic and absorption, distribution, metabolism, and excretion (ADME) information and (v) it is supplemented by data from relevant <i>in vitro</i> and <i>in chemico</i> (i.e. reactivity of a substance with biologically-important molecules) endpoints, all contributing to an increased weight-of-evidence.</p> <p>In March 2016, ECHA used the outcome of the MARINA project ^{xxvi}, where an RIVM proposal ^{xxvii} was harmonized to large extents with a ECETOC proposal ^{xxviii}, all from 2015, to publish their summary on grouping and read-across ^{xxix}.</p> <p>REACH (Annex XI, 1.5) now outlines the conditions under which read-across and grouping can be used, although not specifically for NMs although a consultation document by ECHA currently considers grouping for NMs ^{xxx}. It is widely accepted that grouping and read-across have the potential to make safety testing more cost-efficient.</p> <p>As indicated in the introduction, ECHA now has four different Partner Expert Groups (PEG) running currently, one of which is addressing Read Across and Grouping, expected to deliver in april/may 2017.</p> <p>Proposed schemes addressing human health or environmental questions differ in the hierarchy of properties to be prioritised. Occupational relevant schemes use „morphology“ as first-tier decision, motivated by the concern of inhalation of fibrous materials⁶. Schemes developed from an environmental perspective^{xxxi}, often prioritize hydrophobicity and charge. Both perspectives regard the reactivity and the shedding of metal ions as criteria. The differences are not contradictions, but would benefit from integration in a consistent, multi-perspective framework which then requires development into a tool.</p> <p>Confidence in read-across prediction will be enhanced when experimental data for structural analogues allows interpolation (i.e. to estimate between known values) rather than extrapolation (i.e. estimate by extension of known information). Analogue approaches need to be structured using many lines of evidence (QSAR, <i>in vitro</i>, etc.) to justify the robustness and validity of the read-across, i.e. a weight-of-evidence basis.</p> <p>Modelling work relevant to this has been conducted in projects such as MODENA and eNANOMAPPER.</p> <p>ITS-NANO nanoGRAVUR (BMBF, 2015 – 2018, coord. Kuhlbusch IUTA) Cefic LRI project (2014 – 2016, coord. Bouwmeester, RIKILT) Vicki Stone</p>
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<p>3.11</p>		<p>Develop nano- relevant risk mitigation strategies</p> <p>Risk mitigation strategies may be focused on reducing the toxicity of the particles or on reducing the exposure, or preferably both. Moreover, the exposure may occur by different pathways: Direct exposure (occupational, consumer and/or environment) or indirect (general population exposure through the environment), each one with its own mitigation measures. Thus, very diverse mitigation measures may be applied and they can be group in three different general strategies (e.g. as described in GUIDEnano):</p> <ul style="list-style-type: none"> • Safer-by-design: Design and synthesis of safer materials (less hazardous, more compatible with the matrix, less persistent in the environment...) without affecting their main functionality • Occupational Exposure control: Reducing potential workers exposure by using measures that reduce particle concentration in the workplace or by using personal protective equipment (see section 1.6) • Waste management: Reducing potential environmental exposure (and exposure through the environment) by application of novel and known waste treatment processes, including proposals for the implementation of recycling strategies <p>Safer-by-design (SbyD) approaches intend to re-design and refine NMs to mitigate their potential hazard, while maintaining the desired properties that make them attractive for various purposes. This involves:</p> <ul style="list-style-type: none"> • Identification of the feature(s) that make NMs potentially toxic • Evaluation of their desired properties and how they are correlated with the NM features identified • Re-design of the synthesis strategy in terms of the composition, morphology, structure and surface chemistry of NMs <p>Safety by Design strategies based on NM surface engineering, have the real possibility to control exposure and hazard potential, mitigating occupational risk. From this point of view SbyD is more a risk management approach than a RA approach, nevertheless it can exist and be fruitfully developed only if NMs characteristics that influence release, exposure, fate / kinetics, hazard, bioaccumulation have been identified with much information as possible on mechanism.</p> <p>Sanowork and SUN projects introduced some SbyD solutions and tested them through product case studies. Some of the proposed solutions actually sound promising because such approaches decreased some risk factors, whilst preserving and in some cases improving product performance. Nevertheless what appeared very complex and far to be validated is the process of selection and justification of design solutions in a SbyD framework.</p> <p>From this prospective, an intelligent and tiered testing strategy is highly recommended. It should include validated read-across tools and screening-level tools able to predict risk and provide a risk-benefit analysis of nano-enabled products in comparison to existing conventional products, under life cycle thinking.</p> <p>Briefly, it should be able to answer the following questions and take decision accordingly:</p>
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		<ol style="list-style-type: none">1) Does any modification introduced actually affect design properties (chemical composition, crystallinity, surface chemistry/charge, primary size, particle size distribution and its evolution in testing and life-cycle media)?2) If yes, does modification affect risk determinant properties (structural alerts) that can be considered relevant for estimating hazardous potential?3) If yes, do the changes in risk determinant properties reduce <i>in vitro</i> or <i>in vivo</i> toxicity according to the established biomarkers of mode of action? Evidence collected can be used to read-across risk for human health and establishing new exposure limits.4) If yes, different scenarios can occur.<ol style="list-style-type: none">i) Tested toxicology endpoints do not show a coherent response (e.g. some results show a reduction of toxicity potential, while others demonstrate an increase); in this case a further mechanistic investigation is necessary to better correlate changes in design and risk determinant properties and toxicology end-points)ii) Tested toxicology endpoints show a coherent response (e.g. all the results for the selected modification show an increase or decrease of toxicity potential); in this case a cost/effectiveness evaluation should be considered before discharging or validating the design solution proposed. <p>Development of SbyD approaches and frameworks are included in SUN, GuideNano and NANOREG.</p> <p>Anna Costa Socorro Vázquez-Campos</p>
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Ideal Regulatory Approach

4.1		<p>Validate nano- relevant RA strategies (grouping and read across)</p> <p>Once appropriate frameworks and tools for grouping and read across exist, these tools require validation in order to ensure their suitability to support risk decision making by industry and regulators.</p>
4.2		<p>Validate nano- relevant RA strategies (SbyD)</p> <p>Again appropriate tools for SbyD will require validation in order to ensure their suitability to support risk decision making by industry and regulators. SbyD strategies based on surface engineering to be effective should be validated within real industrial processing line and more generally along life cycle steps. Risk mitigation effects should be evaluated by comparing risk ranking before and after their introduction and product-process performances assessed in order to verify the sustainability of proposed modifications. A cost/benefit analysis would be necessary to improve stakeholders reception of such new preventive risk management measures.</p>
4.3		<p>Finalisation of an Intelligent Testing Strategy</p> <p>See 1.16. If the research prioritisation set out in ITS-NANO is followed then this provides a framework on which to establish a functional ITS for nanosafety testing within the next 10 years. As expertise and knowledge evolves it is likely that a functional ITS would also evolve.</p>
4.4		<p>Incorporate NM transformation into RA and regulatory frameworks</p> <p>To date there are no known considerations of how NM transformation would be addressed in any regulations. If research to characterise transformation becomes sufficiently advanced in future, this may be a consideration that is required. This is especially relevant if the transformation modifies the NM hazard or availability for exposure.</p>
4.5		<p>Implement relationships between physicochemical and NM behaviour into RA</p> <p>There needs to be consideration of how specific NM characteristics can be incorporated into the calculation of risk. One example relates to dissolution of NMs. Currently a number of regulations (e.g. cosmetics) exclude NM exhibiting solubility, but there is no distinction between rapidly and slowly dissolving materials. Therefore some materials may persist for long enough to induce risk but are not currently covered by the regulations. Inversely, materials commonly considered as insoluble are cleared faster than expected after inhalation, so that solubility underestimates abiotic dissolution in physiological fluids which again underestimates dissolution <i>in vivo</i>. Practical cut-offs need to be defined.</p> <p>See also research priority 1.3.</p>
4.6		<p>Implement nano-relevant RA strategies into regulatory frameworks</p> <p>The inclusion of NMs within regulations has been outlined in the introduction to this document. At the moment these regulations treat all NMs similarly and do not provide clear RA strategies for nano-relevant risks. Addressing the research priorities outlined in this document will facilitate evidence based nano-relevant RA, risk decision, risk management and governance of NMs.</p>

Final comments

The Research Regulatory Roadmap identifies and outlines the key activities completed, ongoing and required in the future to deliver effective and proportionate regulation of nanomaterials where required. The key activities do not just include research, but also standardisation and regulation development. Within the RRR, identification of **nano-relevant safety issues** is a key decision point at which to identifying whether refinement of regulations is actually required. These nano-relevant safety issues could relate to **hazard or exposure** and could be related to **physicochemical properties**. The detail provided for each priority is rather variable, reflecting the level of achievement and activity in each area, and so, priorities in the more distant future contain very little information currently.

The rapid evolution of this field means that the content may need updating with time, and in fact we already plan to combine the outputs of this report with other activities such as Closer To The Market Roadmap (CTTM) and to consider these reports in the generation to a NanoSafety Cluster Research Agenda.

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