A common European approach to the regulatory testing of nanomaterials

NANoREG

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Inventory of safety assessment issues and new approaches to research and governance

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1 Description of task

This deliverable provides an inventory of the range of obstacles currently affecting the ability of nanosafety researchers to deliver the required answers to prioritized regulatory questions. It then critically reviews some of strategies available for moving forward in the face of these obstacles (including both scientific/technical and social/governance types of strategies).

More specifically, this deliverable provides:
a) An **inventory of social and technical issues currently inhibiting robust safety assessment**[1] **of manufactured nanomaterials (MNMs)**,
b) A **summary of key bottlenecks facing EHS research** and currently inhibiting its ability to deliver reliable answers to regulatory questions,
c) A **review of high throughput screening and organ-on-a chip as new approaches to safety testing** that may help address safety assessment issues and research bottlenecks, including a discussion of the potential, challenges and limitations associated with these technical strategies,
d) A **characterization of different types of uncertainty important in science for policy,** highlighting those that are particularly relevant for safety assessment through the value chain and for which technical advances in test methods cannot fully eliminate,
e) A **critical review of two new approaches to governance - Safe-by-Design (SbD) and Responsible Research and Innovation (RRI)** – and an exploration of their potential integration as a novel way to assess and manage complex risks in the face of uncertainty,
f) An **outline of how challenges, obstacles and risks can also be seen as business opportunities**.

2 Description of work & main achievements

Summary

Through an extensive literature review and dedicated dialogue workshops with nanosafety researchers, this work has produced an integrated review of social and technical challenges facing the safety assessment of MNMs through the value chain. In addition to providing this easy to read overview of existing challenges, it critically reviews some of the strategies for

[1] In this deliverable our use of the term safety assessment includes formal assessments of risk. The term safety assessment was deemed preferable to that of risk assessment (in some cases) both because of the way it opens for an inclusion of broader factors than may be accounted for in formal risk assessment models, but also because of the way it frames the assessment in a more positive light, focusing on safety rather than risk. This means that the term risk assessment is used when referring to the use of specific formal processes of risk analysis, while safety assessment is used as a more general term for a range of activities performed in the interest of ensuring product safety.

tackling these, including both new technical approaches to scientific safety testing and new socio-political approaches to management and governance. It concludes that while new approaches to safety testing offer some significant advantages for safety assessment, there remains a need to find ways to move forward responsibly in the face of various forms of uncertainties. Here the work critically reviews the new approaches to management and governance of Safe-by-Design and Responsible Research and Innovation and offers a novel integration of the two with the stage gate model of innovation. This is specifically so as to advance the European commitment to having nanotechnology development that is both safe and responsible. The take home lessons from this work are that both technical and social (including political and economic) factors are creating significant bottlenecks for the ability of nanosafety science to deliver reliable answers to the regulatory questions identified and prioritised within NANoREG. Furthermore, since it will not be possible to eliminate uncertainties completely, new approaches to governance that are foresighted, flexible, transparent and broadly collaborative will be needed for the safe and responsible development of nanotechnology and can be integrated into a synthesis model. The interdisciplinary approach in this work, which seeks to illuminate both social and technical factors affecting safety assessment of MNMs as well as social and technical strategies for addressing these, is of significant value within the NANoREG project. Both the integrative model for safe and responsible innovation for nanotechnology and MNMs, and the proposals for how risks can be turned into business opportunities, provide useful points of action that can be followed up in the future.

Context

The **NANoREG** project brings together three key stakeholders in the development and regulation of manufactured nanomaterials (MNMs) – scientists, regulators and industry. In doing so, it aims to identify important regulatory questions for MNMs, develop a common approach to regulatory testing and provide scientific input for risk assessment and regulation.

Within NANoREG, **WP6** seeks to explore routes for filling the increasing gap between innovation and risk analysis, by:

a) *Being prepared* by developing effective foresight of emerging applications of MNMs and coupling this to risk assessment in a way that can advance relevant research and reduce uncertainties before market;

b) Integrating safety research directly into innovation through a *Safe-by-Design* approach to allow for the consideration of safety aspects (for both humans and the environment) right throughout the product/material design phase, and

c) *Turning risks into business opportunities* through highlighting how knowledge gaps and regulatory requirements can offer new business opportunities.

As a component of WP6, this **deliverable D6.2** provides an inventory of issues affecting safety assessment of MNMs and a screening of several approaches to address research bottlenecks currently affecting the ability of nanosafety researchers to deliver reliable answers to the identified regulatory questions. An overview of the specific areas and activities of the NANoREG project that this deliverable engages with and builds upon is provided below.

2.1.1 *Regulatory Questions*

In the **deliverable D1.1**, the NANoREG project identified a collection of regulatory questions concerning the health and environmental safety of MNMs requiring scientific answers. It also collated policy issues relevant for the regulation of MNMs. Following this identification, the NANoREG project seeks to have its research and contributions oriented towards answering

and addressing these prioritised regulatory questions and policy issues. Full details of the list of questions and issues, as well as the process used to develop it, can be found in D1.1. However, a condensed version of the themes and issues is provided below in Table 1.

Table 1: Condensed Summary of NANoREG Regulatory Questions and Policy Issues (marked with those aspects of particular focus within this deliverable)

Regulatory Question / Policy Issue		1 Definition and identification	2 Measurement and characterisation	3 Characterisation of transformation	4 Metrology and dose metrics	5 Extrapolation and grouping	6 Fate, persistence and long term effects	7 Kinetics and fate determination	8 Extrapolation of findings	9 Mode of action	10 Hazard	11 Human exposure	12 Environmental exposure a	13 Life cycle analysis	14 Risk assessment	15 Risk management	16 Health surveillance
1	Implementation of a harmonised definition across all regulatory frameworks																
2	Timely evaluation of both existing and new nanomaterials										**X**				**X**	**X**	
3	Tonnage threshold for registration within REACH																
4	Registration of nanomaterials and products for market surveillance																
5	Labelling of nanomaterials and products for consumer transparency																
6	Testing protocols and dossier requirements																
7	Lack of information on workers protection																
8	Governance approaches to deal with uncertain and complex risks														**X**	**X**	

This deliverable D6.2 specifically highlights where research bottlenecks are and how in some cases they are related to the identified policy issues. In exploring some technical options for addressing these bottlenecks, it contributes to policy issue 2 and regulatory questions 10 and 14. In presenting some governance options for handling these bottlenecks and safety

assessment issues, it also specifically seeks to explore options for question 15 and policy issue 8.

2.1.2 *Regulatory Data Gaps and Research Needs*

In **deliverable D1.2**, the NANoREG project produced an internal report on the knowledge gaps and research needs concerning the health and environmental safety of MNMs. This gap analysis involved reviewing the body of available work on EHS of MNMs and assessing where further work was required to address the regulatory questions identified in D1.1. This gap analysis revealed three key broad categories of knowledge gaps: a) characteristics that influence the risk posed by nanomaterials for human health and the environment, b) standardized methods able to determine these characteristics, and c) nano-specific risk assessment strategies and approaches. Analysing the regulatory questions in light of the research gaps and needs, the report highlighted needs for both the short and long term. In the short term, it was stated that to be able to answer the prioritised regulatory questions, it was important to better understand the implications of operationalising and implementing the EC definition within regulatory frameworks, and to predict and understand the transformation of MNMs under different conditions and processes. For the long term, it was highlighted as relevant to continue working to a) develop standardised test methods for characterisation and toxicity testing, b) improve our understanding of characteristics influencing both the hazard and exposure potential of MNMs, c) further develop and verify nano-specific assessment approaches and d) implement all of this in regulatory frameworks. Until standardised test methods and better information becomes available on the characteristics of MNMs, as well as their fate, behaviour, transformation and toxicity under various real world conditions, the deliverable argues that the implementation of governance approaches will have to accept and work with a significant amount of uncertainty. In this deliverable 6.2, we specifically aim to follow up on this conclusion by providing a detailed typology of relevant forms of uncertainty in science for policy on MNMs and elaborate on some of the factors that making filling these knowledge gaps particularly challenging. We also examine two particular governance approaches (namely Safe-by-Design and Responsible Research and Innovation) for the potential they offer for moving forward with risk management and innovation in the face of significant uncertainties.

2.1.3 *NANoREG framework and toolbox for safety assessment*

In **deliverable D1.10** a framework for safety assessment as developed within the NANoREG project will be presented. While that work is primarily focused on presenting how safety assessment of MNMs can be developed and applied under current REACH requirements and legislation, it also intends to outline some of the potential tools and overarching approaches that may also be relevant, including the approaches of lifecycle assessment and Safe-by-Design. This deliverable D6.2 further contributes to this framework by exploring how Safe-by-Design approaches may be broadened to also incorporate elements of Responsible Research and Innovation, which is currently being emphasised in European science and innovation policy and is aligned with the declared aim that European development of MNMs be both safe and responsible (European Commission 2005).

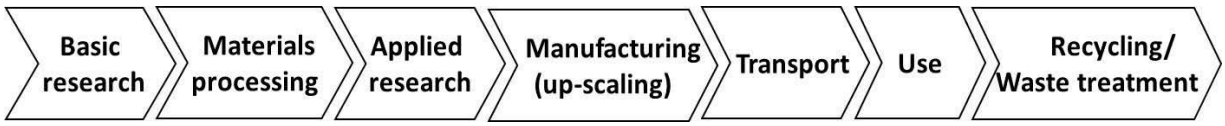
2.1.4 *Comparison of toxicity testing in drug development*

In **deliverable D6.3,** the challenges of nanosafety testing are related to the approach of toxicity testing that is used in drug development. This deliverable specifically works to explore the potential value of developing and applying a Safe-by-Design approach for MNMs. This is explored as a valuable approach for helping to narrow the gap between innovation and risk analysis, especially so as to enable safety testing to keep pace with innovation and

influence its orientation and development so that safety issues are considered and integrated at an early phase. The deliverable D6.3 specifically examines how this concept has been used within drug testing and explored its potential adoption and adaptation for innovation within MNMs. This deliverable D6.2 extends this work by relating the governance approach of Safe-by-Design to Responsible Research and Innovation and proposing ways in which they can be integrated for a safe and responsible development of MNMs.

2.1.5 *Value Chain Case Studies*

In the work deliverable D6.2 is doing to identify research bottlenecks and potential strategies for overcoming them, it has an interest in understanding and addressing MNMs from a value chain perspective. This is in line with the aims of the NANoREG project generally. This means that in considering what are the significant bottlenecks and obstacles affecting nano EHS research, and potential strategies for addressing these, it is important to consider them as potentially arising and affecting any stage of the value chain.



Within **WP1**, work is being done to develop value chain case studies to highlight how risk issues and research needs manifest throughout value chains. At the time of finalizing this deliverable, these specific case studies were not yet available to inform and structure the work in detail; however, the idea of the value chain was used for identifying safety assessment issues, research bottlenecks and potential strategies for addressing these.

2.1.6 *Content of this deliverable in the context of these other NANoREG activities*

The focus of this deliverable D6.2 is on providing an inventory of issues affecting safety assessment of MNMs and surveying potential strategies for overcoming them. This means that where D1.1. identified the important regulatory questions that need to be answered and D1.2 identified the knowledge gaps and research needs that exist, this document D6.2 places a spotlight on specifically identifying what is currently prohibiting the nanosafety research community from filling the identified gaps and delivering reliable answers to the prioritised regulatory questions. After doing this, the deliverable surveys the potential value of some new approaches to safety testing for filling the knowledge gaps in regulatory toxicology for MNMs, namely high throughput screening and organ-on-a-chip. The report then highlights how there are also types of uncertainties that exist beyond knowledge gaps and affect safety assessment through the value chain. Building on the work of D1.10 and D6.3, it then critically considers potential governance approaches for moving forward in the face of significant uncertainties, including a comparison and proposed integration of Safe-by-Design (SbD) with the related but significantly distinct approach known as Responsible Research and Innovation (RRI). It then concludes with an outline of how potential risks can be turned into business opportunities.

Research Method

The work presented in this report has been developed through both reviewing relevant literature across a range of different fields of both natural and social science and conducting dedicated workshops with nanosafety researchers. In the first instance, a literature review

(including deliverables from NANoREG and other European projects, academic publications, policy reports, and international initiatives on standardization and nanosafety) focused on challenges, obstacles and bottlenecks for research as well as risk assessment practices for MNMs (see Miller and Wickson 2015). Several resources either implicitly or explicitly discussed challenges in MNM characterisation, *in vivo* and *in vitro* nanotoxicity testing, and understanding environmental behaviour. Furthermore, literature on the governance approaches of SbD as well as RRI was also extensively reviewed. To supplement this literature review, the research then conducted several dedicated dialogue workshops with nanosafety researchers. The first such workshop was held in 2013 to map nanoscientists' conceptions of responsible research and innovation and involved participants from both industry and the nanosafety community (see Wickson and Carew 2014). In 2015, a further five dialogue workshops with different groups of nanosafety researchers were held to discuss the issues in more depth in a deliberative and dialogic manner. The issues primarily canvassed during these workshops were ideas concerning what constitutes good science and innovation, how this relates to the notion of responsible research and innovation, how responsibilities for the safety of MNMs are seen to be distributed and enacted, and what the obstacles for achieving responsible research and safe innovation are. Ideas from the nanosafety research community that were unearthed during these workshops have been directly integrated into this report.

Inventory of Safety Assessment Issues

Performing safety assessment of MNMs as they are developed and applied through value chains faces a number of obstacles. In this section we provide an inventory of broad and overarching issues that have emerged as challenging the ability to perform reliable and robust safety assessments of MNMs through the value chain.

For MNMs, our literature review and dedicated workshops with nanosafety researchers (as described in the methods section) have identified several barriers as currently prohibiting the practice of risk analysis and safety assessment from a value chain perspective. This includes:

- The use of MNMs in a widely **diverse range of sectors** and applications, **without nano-specific regulatory requirements being consistently applied** across them
- The **lack of shared definitions** across the various regulatory spheres, as well as across different international institutes and nations
- The **nascent nature of validated and standardised test methods** for both nano(eco)toxicology research and measuring human and environmental exposure, which means reliability of some existing data may be questionable
- The **diversity of MNMs** and their use across a range of sectors (and therefore their potential to interact with various biological compartments, involve diverse exposure route etc.) challenging the development of standardised test methods
- The **limited basis of currently available scientific knowledge** on MNM toxicology, transformation, fate, exposure and behaviour in complex media and under real world conditions
- The use of digitalized high throughput research methods leading to **increased generation of data without the time to analyse the meaning** of this data in detail
- The **lack of reliable information on the commercial use** of MNMs and a practice of manufacturers sharing such information along the value chain

- The often **globally dispersed and distributed nature of various elements and actors along a value chain** and the challenges this creates for communication, information transfer, risk assessment, and regulation
- The **time lag** between the development of an innovative product and the development of reliable scientific knowledge on its possible safety implications
- The significant **pressure** placed on nanosafety researcher to keep pace with innovation at all costs
- The **limited funding** available for nanosafety research in relation to that available for advancing innovation, and the constraints placed on what this funding is available for (e.g. often an emphasis on research on hazards rather than issues of exposure or bioaccumulation)
- The **limited number of researchers** with high level competence and training for conducting nano(eco)toxicology research and MNM characterization
- The **lack of instrumentation and methods** capable of in situ characterization and dedicated MNM testing
- The **lack of training** for scientists in how to approach regulators, policy makers and publics and be able to communicate their work to audiences outside their discipline and area of expertise
- The **traditional scientific reward scheme** that gives little recognition or significance to transdisciplinary communication and collaboration with other stakeholders
- The **paucity of meeting spaces** able to bring together innovators, regulators, safety assessors and publics to develop shared goals, understandings, language and cooperative projects

Key EHS Research Bottlenecks

Moving from some of the general factors affecting the safety assessment of MNMs, there are also more specific constraints concerning the development of nanotoxicology research and the performance of MNM robust risk assessments resulting from insufficient knowledge. In particular this includes:

1 - **Knowledge of the Basic Properties of MNMs**
The novelty of many MNMs means that the basic nature of some of their core properties and behaviours is not well understood and without this foundational knowledge, developing scientific knowledge that can reliably inform safety assessment through the value chain is made more difficult. Examples where information about the basic properties of MNMs is still in its infancy and posing a research bottleneck includes:
- Understanding MNM surfaces and surface reactivity
- Understanding and predicting nucleation
- Measuring in real-time and in-situ the structure and reactivity of nanomaterials
- Quantitative characterization of surface composition and surface chemistry
- Documenting the effect and stability of any surface coatings or functionalisations
- Understanding nanoparticle-nanoparticle interactions, aggregation, and agglomeration potentials

2 - **Understanding of MNM Interactions with Biological Systems**
The potential influence of nanomaterials to adversely affect humans and other organisms in the environment revolves around understanding both the delivered dose/level of potential

exposure and mechanisms by which nanomaterials induce toxicological response in organisms. Understanding these phenomena is complicated by the still emergent basis of knowledge concerning factors such as:

- Nanomaterial uptake into biological systems (including all potential mechanisms for this)
- The dynamic interaction of MNMs with biological molecules
- The molecular basis of nanomaterial-induced biological response
- Relevant biological markers of nanomaterial exposure
- Nanomaterial metrics relevant to biological response
- Nanomaterials as delivery agents of other potential toxicants
- Released quantities of different forms of MNMs
- Analytical methods for the quantification of nanomaterials in complex environmental matrices and at low concentrations
- Heterogeneous distribution of MNMs, such as dilution, geoconcentration and bioaccumulation
- The persistence of MNMs in different environmental compartments and systems,
- Defining key transformation processes of nanomaterials in the environment and their effects on nanomaterial behaviour.

3 - **Required Infrastructure and Instrumentation**

A significant challenge facing measurement techniques for MNMs is to characterize, quantify, and track ensembles in situ, in vitro, and in vivo. To be effective, this requires the development of new infrastructure and instrumentation, including:

- Infrastructure for ensuring cross-laboratory validation using reproducible, well characterized MNMs
- Analytical tools for characterizing MNMs in environmentally relevant matrices
- Achieving computational accuracy at environmentally relevant scale lengths
- Addressing the configuration space of MNM interfaces
- Advancing computational methods able to handle large data sets and models within reasonable timeframes (e.g. the quantum computing)

New Approaches to EHS testing

2.1.7 *High Throughput Screening*

2.1.7.1 Concept

With the ever-growing numbers of MNMs in development and reaching the market, there is a huge demand from both the scientific community and legislative institutions to come up with rapid, cost-effective, and reliable ways to conduct MNM safety testing - preferably using in vitro approaches so as to comply with the commitment to reduce the use of animals in research. Large amounts of data are also needed for developing grouping and read across approaches for the risk assessment of MNMs. Generating this level of data in the rapid manner required is not possible using in vivo models or standard toxicity tests, and thus, high throughput approaches have emerged as having crucial importance for the safety testing and regulation of MNMs.

High throughput screening (HTS) is defined as the use of automated tools to facilitate rapid execution of a large number and variety of biological assays that may include several substances in each assay (Nel et al., 2013). HTS was originally introduced in the pharmaceutical and chemical industries as a quick way to evaluate the effects of many novel compounds. With the rapid growth of MNM production, HTS methods are needed to allow toxicity testing of large numbers of materials in a timely manner and with savings in labour costs. The adoption of HTS techniques for MNM toxicity testing therefore not only allows the

examination of large numbers of different materials at different concentrations and on different types of cells, but also makes substantial savings in testing time and variable costs for the research, as well as reducing the effect of inter-experimental variation.

An additional advantage of HTS *in vitro* is the way it facilitates the hazard ranking of MNMs, through the provision of significant amounts of data that can be collated in a database documenting all reported effects on biological and environmental systems. This then allows for analysis across different MNMs that can lead to a prioritization for which novel MNMs may require *in vivo* testing. Both high content analysis (HCA) and HTS approaches also offer possibilities to set up well-documented libraries of the key biological indicators of MNM-cell interactions such as cellular and molecular functions (e.g., cell populations, cellular morphology, membrane permeability, activation of transcription factor, mitochondrial membrane potential changes, oxidative stress monitoring, lysosomal mass/pH, and post-translational modification, as well as markers of genotoxicity) (Prina-Mello A. et al., 2013, Harris et al., 2015, Huk et al. 2014, 2015). This ability to generate relevant primary data may then need to be further aggregated and collated into forms that are specifically useful for regulatory or industrial decision-making.

Various methods have been applied with high throughput adaptations to study toxicity of MNMs, employing diverse physical, chemical and biological principles and endpoints. Some examples of these are briefly described in the section below on the current state of the art in this area.

2.1.7.2 State of the Art: Examples

Label-free cellular screening of NP uptake has been frequently applied for studying MNMs translocation and uptake. Label-free dosimetry and imaging techniques allow the study of authentic MNMs (avoiding fluorescent dyes) – a crucial requirement in the regulatory context. Methods with this potential are: Atom emission spectroscopy (AES), mass spectrometry with inductively coupled plasma (ICP-MS), Ion beam microscopy (IBM) techniques, such as micro-proton-induced X-ray emission (μPIXE) and micro-Rutherford backscattering (μRBS), Electron microprobe analysis (EMPA), Magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computer tomography (SPECT), Transmission electron microscopy (TEM), ultrahigh resolution microscopy (CytoViva^{TM}), time-of-flight secondary ion mass spectrometry (ToF-SIMS) and confocal Raman microspectroscopy (CRM), X-ray energy-dispersive spectrometry (EDS) and electron energy-loss spectrometry (EELS) in TEM, Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM) tomography, etc. These methods can be coupled with 3D reconstruction to provide in vitro models.

High content analysis Combining automated image acquisition and powerful algorithms designed to quantify and extract a maximum of information from a population of cells, high content analysis (HCA) generates great quantities of data for a large number of cellular characteristics, including changes in fluorescence intensity and distribution of intracellular targets, as well as detailed information on cellular and nuclear morphology. A number of commercial benchtop HCA instruments are currently available, each offering specific advantages for imaging and analysis. These systems are equipped with powerful image analysis software based on the automatic identification of cells and - depending on the instrument - considerable flexibility for analysis. The vast array of possible analyses of specific cellular endpoints has made HCA a key approach in various domains including toxicology, genotoxicology, oncology, neurobiology, and research on metabolic disorders (Thomas, 2010).

High throughput flow cytometry From simple cell-based fluorescent, colorimetric, luminescent, and radiologic plate reader assays, to high-content fluorescent imaging systems, the ability to screen MNMs in the living cells is essential in toxicology-screening

programs. High throughput flow cytometry is an ideal tool for cell-based applications involving screening of cells in suspension, where multiple readouts are desired (Peluso et al., 2007). Potential applications include cell viability and cell death, (apoptosis/necrosis), intracellular incorporation of MNMs or reactive oxygen species (ROS) detection. However, limits of use should also be considered, as MNMs may interfere with specific optical parameters influencing the obtained results. In addition, MNMs attached to the cellular membranes may not allow the access of fluorescent dyes to membrane structures, such as membrane receptors.

Impedance-based monitoring of adherent cells in real-time performs real-time cell monitoring using electrical properties (electric cell-substrate impedance sensing, or ECIS) and is a label-free, non-invasive, biophysical assay detecting dynamic cell responses. It provides a valuable tool for the investigation of MNM toxicity and early stage efficacy/toxicity testing of MNMs. It measures not only cytotoxicity but also other aspects of cell physiology and cell behaviour, including proliferation, growth, morphology and adhesion. A few systems are commercially available, such as ECIS and xCELLigence (USA). The impedance-based approach is useful and sensitive for MNM toxicity screening at different concentrations, on a range of cell lines simultaneously (Ke et al., 2011), without artefacts affecting the measured signal (Otero-Gonzalez et al., 2012, Paget et al., 2014, Paget et al., 2015a, Paget et al., 2015b). It can also be used to assess changes in cellular motility and adhesion in physiological conditions (Scrace et al., 2013).

In contrast, impedance-based flow cytometry (IFC) measures the impedance characteristics directly in cell suspension for each single cell. A microfluidic chip-based IFC developed by Amphasys AG (Switzerland) can analyse single cells without any specific sample preparation prior to measurement (Ke et al., 2011, Scrace et al., 2013, Cheung et al., 2010a, Cheung et al., 2010b). The method can cover impedance measurements at a broader frequency range, and thus yield information regarding the size and number of cells and, in addition, their membrane capacitance and cytoplasmic conductivity.

High throughput comet assay The comet assay measures DNA damage in cellular DNA. While the standard comet assay detects strand breaks, incorporating digestion with a lesion-specific endonuclease such as formamidopyrimidine DNA glycosylase (FPG) allows for measurement of specific DNA lesions. FPG modification measure oxidised bases (preferably 8-oxo-Guanine) and has therefore been employed also for MNMs to measure the effects of oxidative stress on DNA (Harris et al., 2015, Cowie et al., 2015, Huk et al., 2014, 2015). The throughput is increased with the 12, 48 or 96 gel arrays. Furthermore, automated scoring is being developed and several systems such as IMSTAR or Metafer are available. The 'CometChip' integrates a HTS comet assay with automated scoring in a novel way; cells are deposited at predefined positions stamped in a micro-array on an agarose-coated plate, so that it is possible to locate comets precisely for image capture and analysis (Wood et al., 2010)(Watson et al., 2014).

High throughput in vitro micronucleus assay The *in vitro* micronucleus (IVMN) assay measures clastogenicity and aneuploidy and is part of standard battery for genotoxicity and hazard assessment of chemicals as well as MNMs. HTS micronucleus includes the use of flow cytometry (Nusse and Kramer, 1984). Further modifications use 96-well in conjunction with a robotic auto-sampling device. This adaptation requires less test material than conventional test methods, and has a greater compatibility with HTS instrumentation (Bryce et al., 2007, Bryce et al., 2010, Bryce et al., 2013, Avlasevich et al., 2011).

The γH2AX assay C-termini phosphorylated histone protein, γH2AX was proposed as a potential biomarker of DNA double strand breaks (DSB) caused by genotoxicants (Bonner et al., 2008, Mah et al., 2010, Valdiglesias et al., 2013, Audebert et al., 2011) especially because DSB are considered to be the most critical kind of DNA damage, initiating genomic instability and, potentially, leading to cancer (O'Driscoll and Jeggo, 2006; McKinnon and Caldecott, 2007). Two types of methods are often used for γH2AX detection; a) counting foci or immunofluorescence microscopy, and b) measuring overall γH2AX protein levels (by

immunoblotting or flow cytometry). The first method is several orders of magnitude more sensitive and allows for the distinction between pan-nuclear staining and focus formation - it is this approach that is being employed in efforts to develop high throughput techniques (Bonner et al. , 2008). The computational approaches supported by image analysis software have been developed (Cai et al., 2009, Hou et al., 2009, Roch-Lefevre et al., 2010). High content imaging systems are also capable of γH2AX quantitation (Harris et al., 2015).

2.1.7.3 Potential, challenges and limitations

Automation through the increased adoption and development of HTS approaches can further streamline testing procedures, while eliminating the possibility of operator bias. HTS can also encourage research groups to establish databases on relevant toxicological determinants of MNMs, which will facilitate grouping and read across approaches for both innovation and regulation. The availability of a large database of reliable information about MNM toxicity generated through *in vitro* HTS methods will also arguably facilitate the prioritization of those materials that require *in vivo* testing and thus reduce the number of animals used in testing. Another benefit worth noting is that the faster scanning possible using an automated platform can increase the statistical power of results, through maximising the number of cells scored, while still saving time compared with visual scoring (Rossnerova et al 2011).

The reduction in cost and time for testing is however perhaps where HTS methods hold the greatest potential. It has recently been estimated that the time taken to complete evaluation of existing MNMs would be more than 30 years and the costs for testing them on an individual basis would be tremendous (Choi, 2009). The aforementioned HTS approaches for hazard assessment of MNMs clearly allows for a reduction in the time required for toxicity testing while increasing data outcomes. However, the cost-effectiveness of these approaches also needs to be considered. Cost-effectiveness analyses involve complex economic indicators and have been used to measure the relative value of a new or modified technology in terms of the cost per benefit gained. This type of analysis takes into account short-term costs, e.g. the cost per new endpoint identified or per time saved, and long-term costs, e.g. the cost per hazardous MNM identified *versus* the gains in terms of human disease prevention and environment protection. Thus, while short-term costs comprise mainly the direct costs associated with laboratory expenditure, the long-term costs are related to the societal costs and are much more complex to measure.

Focusing merely on direct costs, HTS/HCA technologies are expected to reduce the costs of MNM development, as has happened in drug discovery (Szymański 2012), due to the greater number of NPs/NMs and experimental conditions simultaneously assayed, and the lower amounts of test samples and consumables required, provided that the adequate equipment or accessories are available in the laboratory. There should also be savings related to direct labour (e.g., decreased time required to complete each task and lower degree of expertise or training necessary). Finally, regarding long-term costs *versus* societal benefits, the promotion of more robust, diverse and adaptable HTS technologies for the safety assessment of MNM, providing information early in the process of MNM development, will further minimise the costs resulting from a delayed finding of potential harm to human health and/or the environment, thus maximising the benefits of innovation.

Despite the unequivocal direct gains from applying HTS to nanotoxicology, the costs incurred by the laboratory can be prohibitive if the acquisition of expensive laboratory equipment (e.g., fully automated equipment for image analysis), sophisticated tools for data analysis or on-line data storage capacity is needed for its implementation (Buchser et al., 2012). The investment can, however, be justified depending on the number of tests and samples to be analysed, among other factors related to laboratory management. The high initial investment costs for the equipment necessary for applying HTS to nanotoxicology may, however, prohibit certain laboratories from pursuing this approach to research and generate a “matthew effect” in

which the large laboratories get larger while the small laboratories unable to keep up with this development grow ever smaller.

There are also several technical challenges associated with HTS/HCA design that should be noted. This is due to the way toxicology screening needs to be coupled with the characterization of MNMs in exposure medium and thus appropriate HTS analytical methods need to be developed. The limitations in capacity to characterize MNMs immediately prior to the treatment of a cell line with MNMs can be partially overcome with testing the effect on several cell lines, in several exposure scenarios and with large number of concentrations. However, validation of *in vitro* HTS tests for *in vivo* situations, and the development of HTS approaches that can assess dose- and time-dependent toxicity that are predictive of *in vivo* adverse effects, are also still required.

Since MNMs display singular physicochemical properties that can bias the results of conventional toxicity assays (Stone et al., 2010, Kroll et al., 2012, Guadagnini et al., 2015) depending both on the assay and on the MNMs, positive and negative controls should be systematically included in experiments. This is important in order to confirm the sensitivity of the techniques used, to assess for potential MNMs interference with assays or detection systems, and to benchmark the toxic effects of tested MNMs. This means that one crucial aspect of HTS for all endpoints is the choice of suitable nanomaterial-based positive and negative controls. This issue has been one of the foci of many European Union funded projects such as NANOGENOTOX and FP7 QualityNANO or FP7 NanoTEST and recently, several suitable candidate control MNMs have been described (Cowie et al., 2015, Dusinska et al., 2015, Paget et al., 2014, Wang et al., 2013).

2.1.8 *Organ-on-a-chip*

2.1.8.1 Concept

Microfluidics is the science and technology of systems that process or manipulate fluids using channels with dimensions of tens to hundreds of micrometres (Whiteside, 2006). By smart combinations of microfluidics and cell cultures, dedicated Organ-on-a-Chip (OC) models have been developed that show a more accurate representation of human organs compared to current *in vitro* systems (e.g. Huh et al., 2012; Kim et al., 2013). An OC is a microfluidic device containing miniaturized bioreactors in which living cells are cultured in a precise spatial arrangement and under a continuous flow of culture fluid in order to simulate the physiology of an entire living organ. The device may allow high-resolution, real-time imaging of the cultured cells as if they were in a living organ. The term Lab-on-a-chip is sometimes used for such systems as well, but here lab-on-a-chip is used to demarcate and refer to a device containing a chemical analysis instrument (such as a gas chromatograph) on a microchip, without containing biological materials such as cells. Therefore, the term organ-on-a-chip (OC) is used for the devices containing cells. The goal of such a device is not to construct an entire organ but rather to fabricate the minimal functional units that can perform the functions of the organ at the level of tissue, if not an organ. All the devices contain cells that are cultured in microchannels or microchambers that are continuously perfused with a stream of culture medium. The OC architecture is usually composed of polymeric or glass microchannels. Microfabrication techniques such as replica moulding and micro-contact printing can create micro-scale structures and patterns that enable the control of the organ features and fluidic flow.

The simplest OC system is a single microfluidic chamber containing only one type of cell from a certain organ (e.g. hepatocytes, epithelial cells of the renal tubule, endothelial cells from the intestine, etc.). More complex systems include two or more microchannels connected by porous membranes whose opposite sides are plated with different cells so as to recreate the interface between different tissues (lung alveolus, blood-brain barrier). Indeed, the possibility of integrating porous materials to separate two microchannels enables the analysis of the barrier function of some tissues (such as the blood-brain barrier), of the trans-cellular transport and of the mechanisms of cellular absorption and secretion. This is

important because it allows the creation of an experimental model that can simulate the interactions of the vascular endothelium and the parenchymal tissue that define virtually all organs.

OCs are often seen as offering a more accurate representation of *in vivo* conditions than existing models. Implicitly this points to OCs as comprising more than one cell type or comprising disease tissues. It is, however, worthwhile to question whether risk assessment either for nanomaterials or other substances, requires such improvement. This will be further discussed under paragraph 2.1.8.3.

2.1.8.2 State of the Art: Examples

To provide an overview of the state of the art in this field, specifically in relation to different organs, information has been assembled into tables presented in *Annex 1.*

The overview presented in Annex 1 underscores that the development of OCs for toxicity testing is still in its infancy. Various models have been developed, but a clear analysis of the requirements from risk assessment are lacking. For this, one needs to be aware where information from OCs has added value or where they can usefully tackle bottlenecks facing current risk assessment processes. In general, three types of models can arguably be useful for toxicity testing through OCs: 1) tissues representing tests under healthy conditions, 2) tissues representing tests under pathological conditions or 3) tissues representing barriers such as the gut wall, blood-brain-barrier, placenta, etc.

The relatively long viability of OCs and the dynamic character of OCs may make these systems more suitable to mimic repeated dose experiments than conventional and more static *in vitro* systems. The feasibility of using OCs for repeated dose experiments, however, is still under investigation.

2.1.8.3 Potential, challenges and limitations

OCs arguably have several benefits compared to the current approach of *in vitro* testing. An overview of these perceived benefits of OCs over static models is compiled in **Table 2**.

Table 2: Overview of advantages of OC devices compared to traditional in vitro assays.

Shear stress	Shear stress is the stress on a surface (of e.g. a cell) caused by the physical force of the movement of liquid along this surface. It is established that cell behaviour is different in the presence of fluidic shear stress.
Defined concentrations gradients	Through the fluid flow of the culture medium, complex concentration grades are achievable. Repeated flow stream lamination can for example promote cell chemotaxis, i.e. the migration of cells (e.g. macrophages) in response to a stimulus.
Homogenous chemical distribution in medium	Only a small portion of the administered dose reaches the cell membrane. Colloidal behaviour, particle sedimentation and diffusion have to be taken into consideration when correcting for the initial dose at the start of the experiment. With microfluidics a homogenous suspension of chemicals in culture medium can be applied to the cell membrane under continuous perfusion, thereby creating a more physiological relevant situation.
Micropatterning possible	Micropatterning allows for improved control over homo- and heterotypic cell-cell interactions and easier 3D culturing. These micropatterns can be used to control the geometry of adhesion and therefore the orientation of the cell division axis and is needed for the maintenance of stem cells or the exact placement of cell on a sensor.
Sensor integration	With the integration of biosensors in the device itself, a more reliable and quantitative monitoring of cell behaviour can be obtained (e.g. electrical activity, cytotoxicity measurements, optical sensors, cell based bio-sensors, microscale patch clamp devices).
Mechanical strains	(Cyclic) mechanical strain can accentuate toxic and inflammatory effects and enhance the transport of particles over organ barriers
High throughput screening	Small size will eventually allow multiple tests in one plate and allow for robotic plating and reading. In addition, due to a highly controlled environment, it is possible to reduce the otherwise labour-intensive micromanipulations that can be required in experimental assays.
Expanded cell viability	High optimal control over environmental conditions for cell-based assays, including waste removal by medium flow, increases the cell-viability and culture time.

OC models seem to have an added value over current static *in vitro* models which makes this nanotechnology promising for advancing reliable non-animal tests for the safety assessment of MNMs.

The better simulation of organs or tissues, the better dosing, and the ability to culture tissues up to four weeks makes the OC models of particular interest for toxicokinetics assessment, acute toxicity testing, sub-acute and repeated dose testing and possibly reproductive toxicity testing. One needs however to keep in mind that these systems only give qualitative results and thereby provide only indications for the absence or presence of toxicity. These indications can support conclusions to be drawn from *in vivo* studies.

Besides the significant challenges of standardization and validation of these techniques, there are several other challenges to overcome. First of all, a set of the most pivotal tissues to be tested for screening of toxicity needs to be defined. For MNMs, organ models for toxicokinetics seem to have the most added value. Moreover, OC models representing barriers like the gut wall or lung are already in place, but as in the more conventional *in vitro* models, these need to be checked for their validity for MNMs.

OCs are often promoted by researchers for their contribution to the development of alternatives to animal testing in the context of risk assessment. This goal seems to be far off, but it is starting to gain increasing attention from large pharmaceutical companies. They are, for example, specifically interested in exploring the added value of OCs in testing earlier under pathological conditions or in

dose range finding studies. Through this process, the goal of contributing to alternatives to animal testing might become closer, although any alternative test methods would also need acceptance from regulators.

Under task 1.3 of NANoREG, regulatory questions are defined and in one of these questions the need for long term or repeated dose toxicity testing is emphasised. In parallel, NANoREG is tuned to comply with policy needs to reduce animal testing. OCs do have the potential to serve these two goals, however, there would need to be a thorough analysis of the stage in innovation where OCs could offer added value (e.g. pre-regulatory or for regulatory dossiers) as well as the requirements in terms of information needed as input, a precise description of output generated and how it would improve the next step in innovation, a precise description of results and their contribution to risk assessment in a regulatory dossier, criteria for acceptance of OCs by innovators, and criteria for acceptance of the results by regulators. Altogether, this information should describe the added value of OCs in terms of alternatives to animal testing and should describe the feasibility of having this new approach to testing implemented in pre-regulatory as well as regulatory settings.

Typology of Uncertainties & their Relevance for MNM Safety Assessment

While the use of new testing techniques and approaches such as HTS or OC can go a long way towards improving nano(eco)toxicology research and providing relevant inputs for safety assessment and regulatory decision-making on MNMs, it is also important to realize that such technical advances will not be able to eliminate all relevant uncertainties. This is both because there are qualitative forms of uncertainty that will always be significant when science is conducted and used for informing policy, but also because for understanding the impact of innovations such as MNMs applied in complex systems such as value chains, more research does not necessarily always reduce uncertainty, but can actually increase it and/or result in an increased awareness of the various forms of uncertainty characterising these systems. Several typologies characterising different types of uncertainty relevant in science for policy have emerged over the last 2 decades (e.g. Walker *et al.* 2003; Wynne 1992; Stirling 1999a&b; Faber *et al*. 1992; Felt & Wynne 2007; Funtowicz & Ravetz 1993, Wickson *et al.* 2010). While the typologies differ in how they draw boundaries of distinction and define what constitutes the various forms, some patterns can be extracted and developed into conceptually useful categories. The typology of different forms of uncertainty relevant for science for policy that is presented below in Table 3 is a synthesis that draws on the references listed above.

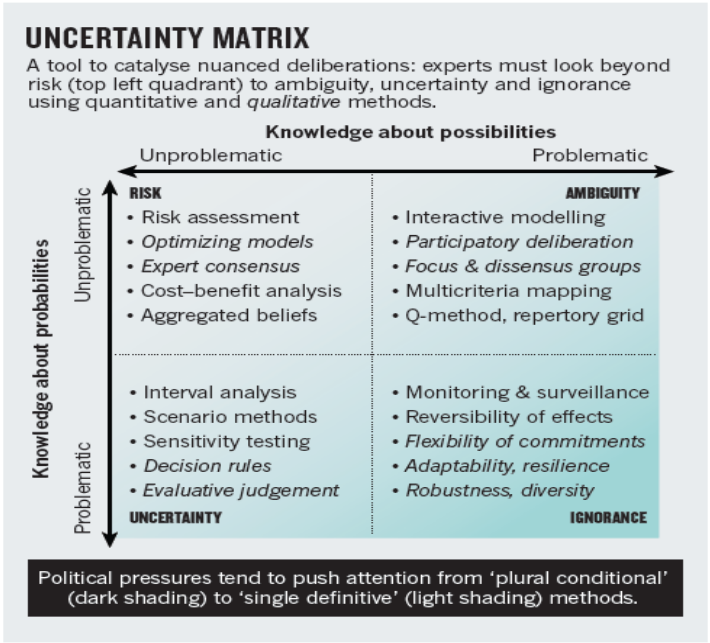
Table 3: Typology of Uncertainties Relevant for Safety Assessment of MNMs through the Value Chain

Type of Uncertainty	**Explanation**
QUANTITATIVE FORMS	
Risk (Probability calculated)	We can imagine a possible impact and calculate the probability of that impact occurring, even though whether it will occur or not remains unknown.
Uncertainty (As yet uncalculated)	We can imagine a possible impact but we don't know the probability that it will occur. It is possible to calculate that probability, but we haven't enough knowledge to do so yet.

QUALITATIVE FORMS	
Indeterminacy (Unable to calculate completely)	For complex, open, interacting systems, it is impossible to include all relevant factors and interactions in the calculations; therefore, knowledge is conditional and fallible.
Ambiguity (Various ways to frame a calculation)	We can variously frame both the impacts we are interested in and the way we approach, interpret and understand the knowledge and calculations generated.
Ignorance (Not aware of what to calculate)	We cannot imagine the possible impact. Not only have we not yet calculated the probability of the event, we are unaware of what we should make calculations for.

In the case of MNMs and concerns about their potential adverse effects on human and environmental health, one may argue that we are actually not in a position to assess risks at the current time. This is because there are serious knowledge gaps or uncertainties inhibiting our ability to carry out risk assessment in terms of imagining the range of impacts, calculating probabilities for these, and multiplying these probabilities by predicted magnitudes. This situation of uncertainty begins very basically with a lack of knowledge about how to accurately characterize various nanoparticles, as well as how to detect and measure them. As highlighted in earlier sections of this deliverable, there is also very limited information about likely exposure levels, dose-response relationships, modes of action, and fate in the environment - all of which are compounded by a lack of agreed and standardised test procedures and equipment. All of this is still arguably uncertainty that can be reduced through further research. However, not only is uncertainty (the current lack of knowledge) a problem for risk-based approaches to decision-making, other, more qualitative, types of uncertainty as described above also pose a serious challenge to policymakers and arguably become even more prominent and important as we seek to understand and assess potential safety issues throughout the value chain. Stirling (2010) has also provided a matrix of uncertainty that aligns with the typology presented above and describes a range of different methods and tools available for handling some of the different forms of uncertainty. The matrix is reproduced below in Figure 1.

Figure 1: Methods available for handling different types of uncertainty in science for policy (from Stirling 2010)



New Approaches to Governance

In addition to the specific tools and methods for handling different types of uncertainty provided by Stirling (2010), several more general and overarching approaches to the task of managing and governing new and emerging technologies have been developed in recent years as a way to work around knowledge and regulatory limitations. In this section of the deliverable, we survey two important new approaches to governance that are specifically being discussed for MNMs, namely Safe-by-Design (SbD) and Responsible Research and Innovation (RRI). Both of these approaches are designed to try and address some of the limitations that relying on regulation through risk assessment of final products faces for MNMs.

2.1.9 *Safety by Design*

2.1.9.1 Concept

Within WP 6 of NANoREG, the concept of Safe-by-Design has been developed to include the following key points:

a) different stakeholders contribute to a process in which health and environmental safety are considered at an early phase of the innovation process
b) there is a good interaction between nanosafety research and product development (innovation)
c) innovation processes make specific adaptations to design factors to take safety aspects into account

Within the agreed terminology for NANoREG more generally, the definition has been given as:

The 'Safe-by-Design' concept aims at reducing potential health and environmental risks at an early phase of the innovation process. The concept aims at creating an integrated research strategy, which enables the consideration of safety aspects for humans and the environment in the design process of a product/material to eliminate or minimise the risk of adverse effects during its life cycle including construction, use, maintenance and deconstruction.

Within the Safe-by-Design concept the functionality of a nanomaterial and its toxicity/safety are therefore considered in an integrated way. Such an approach maximises the use of resource and expedites the development of products containing nanomaterials and new nanomaterials that are safer by design (Annex I, Deliverable 1.10).

'Safe-by-Design' (SbD) therefore effectively refers to a process of anticipating potential impacts of a product/material on human and environmental health and addressing any identified safety concerns early in the innovation process through altering product design. This approach contrasts with traditional approaches to ensuring safety through an assessment of risks primarily performed after a product has already been developed for market. In the SbD approach, an identification and assessment of potential risks is considered right throughout the innovation process so as to guide the research and development in directions that not only account for the functionality of the product but also its safety. Considering how to integrate safety directly into the design of new products and materials can begin already in the early conceptual phase of a project and its selection of materials. It can then continue throughout the process of product development. It can also remain an important guiding principle during the experimental testing of any developed materials/products in the lead up to market and a consideration applied to a product's whole lifecycle.

This means that in addition to concerns around the research and development of a product during the innovation process, SbD can also extend across the whole value chain and influence choices concerning manufacturing processes, construction and production methods, transportation options

and distances, installation practices, use regimes, maintenance potentials and end of life disposal and/or degradation. In this way, safety concerns/and or risks can be considered and addressed not only during research and development of a product, but also taking into account how that product will be manufactured, transported, used and disposed across a full value chain perspective.

SbD is a concept that was first developed and applied in the engineering sector, particularly in the construction industry. Here the concept was traditionally focused on incorporating safety considerations into the design and development of engineered products as well as on construction sites. The concept was therefore initially adopted to prevent injuries on the work floor, reduce negative health effects of those constructing, using or maintaining a product and preventing human and environmental health issues from arising. Furthermore, its aspiration to predict and manage safety concerns both on the work floor and in end products was seen as having potential to reduce operational costs and facilitate a better alignment with legislative requirements. The concept of SbD was quickly taken up in other industries such as security, crime prevention and perhaps most notably, drug discovery, where the concept has been adapted and deployed further (see D6.3). The idea of enhancing the 'design' for drug development and products had already been given attention when the concept of 'Quality by Design' was adopted by the U.S. Food and Drug Administration in 2003. Quality by Design is a systematic approach that begins with pre-defined objectives, such as clinical relevance, efficacy and safety. It emphasises product and process understanding, and process control and builds on the idea that quality can be planned and built into a product (Yu, 2008). In recent years, however, the concept of SbD, with a stronger focus on eliminating risks and anticipating potential impacts of products or materials for human and environmental safety, has gained increased attention in fields such as 'green chemistry' and 'inherent safety', in which there is an enhanced attempt to design processes that have an intrinsically low level of hazard instead of simply trying to control hazards through protective systems. SbD is now considered to be a promising approach for reducing and eliminating risks of MNMs, products and technologies (e.g. Jacobs, Poel, & Osseweijer, 2010; Morose, 2010), although at the moment this is often being conceptualised and pursued primarily during the product design phase, rather than necessarily also through considering the whole value chain, including manufacturing phases, customer use or product disposal.

2.1.9.2 SbD & MNMs

To contribute to a safe and responsible development of nanotechnology, SbD approaches are being proposed as a way to mitigate the potential risks of MNMs. This turn towards SbD specifically for MNMs has emerged within both US and European contexts.

US

In the United States, the concept of 'Safety by Design' was first introduced into discussions around MNMs in 2004, as a way to create a bridge between the study of the implications and applications of nanomaterials and as 'an attempt to define safety as a fundamental property of materials' (Kelty, 2009; p. 91).

In 2001, the Center for Biological and Environmental Nanotechnology (CBEN) was founded to investigate potential applications of nanotechnology for environmental and biological issues. Shortly after its establishment, it became clear that there were several questions and areas of debate concerning potential safety issues. This has led to more research being performed on the *implications* of nanotechnology in addition to its *applications*. This work then went on to create the 'International Council on Nanotechnology' (ICON), an outgrowth of CBEN, which was funded primarily by corporations and supplemented by an NSF grant to bring stakeholders together to communicate across both the applications and implications of nanotechnology.

When CBEN and ICON decided to specifically research risks to human health and environment from nanotechnology/materials, they initially encountered a significant amount of resistance from scientists involved in developing applications. This resistance stemmed from the idea that studying safety concerns and implications for human and environmental health may threaten

nanotechnology development and could potentially lead to resistance from the public or a pullback by funders. The introduction of the notion of 'Safety-by-Design' into the debate by Vicky Colvin in 2004 immediately appealed to all sides as it was able to integrate the advance of engineering applications with research on the risks to human and environmental health. It was therefore embraced as a useful way to build a bridge between the different fields of research and offered the opportunity to investigate the different aspects simultaneously and harmoniously.

However, in addition to building bridges across communities, the introduction of 'Safety-by-Design' in the US context in the early 2000s also arguably opened up a new set of issues and questions. Studying the properties of new materials became an interesting field not only for physicists, chemists, materials scientists and nanotechnology developers, but also for (eco)toxicologists, biologists, ethicists and social scientists. This opened the field to a range of new people and perspectives. While `safety` became a core value for consideration in the experimental, theoretical and engineering work within MNM research and development, the term can have different meanings for people from different disciplines and perspectives. Safety is a relational value (e.g. to understand its meaning you first need to answer safety for whom or for what?) and since absolute safety can never be guaranteed, it raises further questions that need answering relating to how safe is safe enough, how do you know how safe is safe enough, who has the right to define this and how should it be defined. Safety-by-Design also reopened a discussion about the possibility for predictive toxicity by arguably changing the typical risk evaluation question of 'is it safe' to 'can we engineer it to be safe'? (Kelty, 2009).

The brief history of the emergence and use of the Safety-by-Design concept within the US setting over ten years illustrates that although it can be a useful bridging concept, able to bring together and unite the aims of communities developing applications of MNMs and those researching their potential impacts on human health and environmental safety, some significant challenges remain for its implementation in practice. These include the need to answer questions of safety for whom or what (noting that this becomes more complicated for the environmental context) as well as who gets to define acceptable levels of safety and how this is performed. These questions are particularly relevant as the concept is now being adopted for MNMs in the European context.

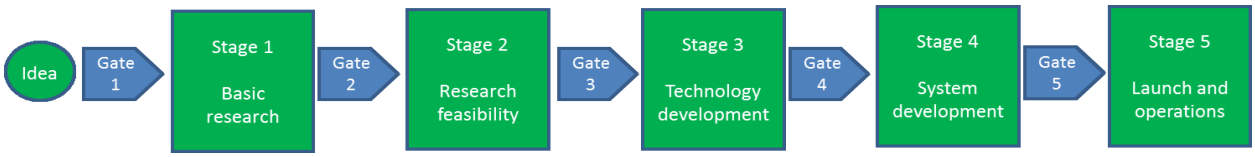
EU

Within NANoREG, the approach to 'Safe-by-Design' has been 'to develop new products where functionality and safety are tested in an integrated way through the development process phase' (D6.3, p. 14). The aims of the SbD concept are threefold. Firstly, it aims to have all stakeholders contribute to a process in which human health and environmental safety are considered in all stages of an innovation process not only during a regulatory evaluation of a finished product. Secondly, it aims for a good interaction between the nanosafety researchers and product developers and thirdly, it aims for innovation processes to be able to adapt and modify design factors to take safety aspects into account.

The NANoREG project has also specifically chosen to link the SbD concept to a stage gate model of innovation. A stage gate model is effectively a conceptual and operational map commonly used in innovation arenas for tracking and directing the development of new products to market. Linking the Safe-by-Design idea to the stage-gate innovation model provides some structure for the application of Safe-by-Design in the context of the development of MNMs.

The stage gate model being used within NANoREG presents the innovation process as consisting of various stages (see Figure 2 for an example). Each stage of this model has its own requirements that need to be met in order for the development to move on to the next stage. A gate then represents a decision point, or a control point, a point at which evaluation needs to occur before product development can continue and pass on to the next stage.

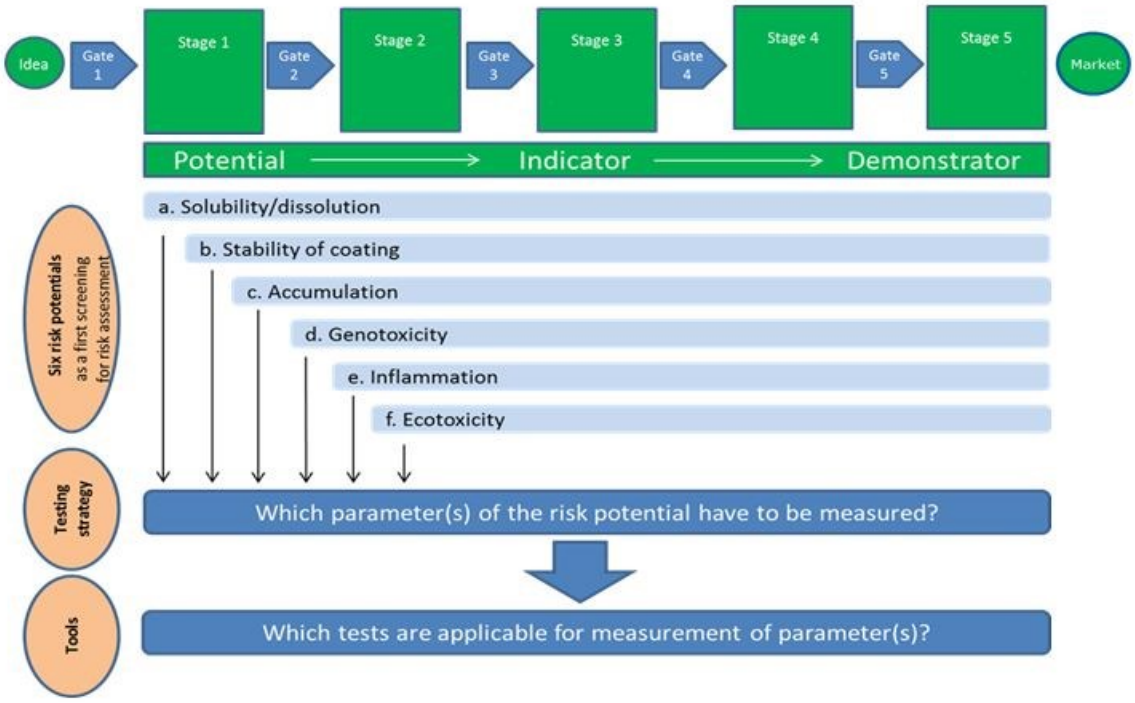
Figure 2: A stage-gate model of innovation (taken from D6.3)



Of course this figure is necessarily a simplification and many details of product development are not clearly depicted. For example, between the idea and basic research stages, several important (and often flexible) processes may take place, such as analysis of existing data, identification of knowledge gaps, consideration of regulatory requirements, specifications for R&D work, scoping, safety screening, preliminary risk analyses etc. It is therefore important to remember that, as for all models, this is necessarily a simplification of the process in practice but hopefully one that usefully illuminates fundamental elements of the stages through which innovation develops and the various points at which different types of changes may be implemented.

Since comprehensive data necessary to judge hazard and exposure is not available for all MNMs that are in development (and is unlikely to be available in the short term given the research bottlenecks outlined earlier in this document), the SbD concept being developed within NANoREG has also worked to develop the concept in relation to 'risk potentials'. This currently includes six key issues that can serve as indicators for potential hazards namely: solubility/dissolution rate, stability of coating, accumulation, genotoxicity, inflammation and ecotoxicity. These six risk potentials have been developed based on overlapping elements in several different approaches to risk assessment of nanomaterials such as those developed within MARINA, GUIDEnano, ITS NANO and NANoREG (Prosafe Report). The idea being that in the early stages of innovation, potentials for hazard can and should be screened. However, as product development moves closer to market readiness and application, it becomes important to move from understanding hazard potentials to investigating the presence of indicators and testing for demonstrations of harm. For this to be possible, it is important to identify the parameters of the risk potentials that have to be measured (currently under development within task 6.3) and the tools applicable for measurement of these parameters.

Figure 3: Investigating risk potentials in a stage-gate model.



Within NANoREG, NANoREG2 and ProSafe, the SbD concept will continue to be developed with the aim to help reduce uncertainties and risks associated with the development of novel nano-materials, products and technologies and increase and improve the interaction between innovators and safety research.

2.1.9.3 Potential, challenges and limitations

The SbD concept clearly aims to reduce uncertainties associated with MNMs while they are still in development and therefore before they reach any market application. This has the potential to ensure that the risks of products launched in the market are known and managed, that the predicted benefits outweigh any residual risks, and that industrial actors reach a situation of regulatory preparedness as their products develop. It could also enhance the public trust that innovators care about human health and environmental safety in addition to their profit margins and potentially increase acceptability for (consumer) products containing MNMs where this may prove problematic. Through its implementation, it also holds the potential to create a closer collaboration not only between product developers and safety scientists but also between scientists, innovators and regulators in which all work together to further common aims of technology development that is safe for human health and the environment.

However, there are some potential challenges for such an approach that should be recognised. Firstly, it is important to acknowledge that SbD was first utilised in an engineering industry in which there were *known* risks and uncertainties. For MNMs, however, we are still in the process of developing standards for safety testing and therefore we do not have a reliable body of knowledge on the risks that can simply be incorporated into design processes. This makes the applicability of this concept in practice very challenging for MNMs and the value and accuracy of any screening approaches, control banding, or grouping according to risk potentials still needs confirmation in practice.

Furthermore, a close collaboration between industry and scientists may actually create challenges around the independence and autonomy of nanosafety scientists. This may not only be a challenge for the scientists themselves but also for public trust in the research and any products it may have supported. This has certainly been the case for biotechnology in which safety research that has been funded, supported or done in collaboration with industry has been regarded as problematic and not necessarily trustworthy. Concerns can, for example, exist around what a close collaboration between innovators and scientists would entail for publishing results with negative findings, and tensions can be foreseen on potential conflicting interests in (not) publishing research findings, depending on how the results are viewed by innovation partners. This implies that if SbD facilitates a closer collaboration between nanosafety scientists and industrial innovators, the funding and publication model for this will need to be carefully considered. Such a close collaboration may also lead nanosafety science into a role of routine testing rather than creative research. In this case, it would be important to realise that not all trained scientists may be willing or able (according to their institutional policies) to engage in such contract style forms of work.

The concept of SbD also places all attention on the question of controlling safety and as a concept thereby fails to substantially engage with other potentially significant questions that can generate social debate and controversy over new technologies, such as: what is the underlying purpose and motivations behind the technology, how are the risks and benefits distributed, are the generated products sustainable, and what is the technology's contribution to solving key socio-ecological problems. At the same time, what is considered safe from the perspective of an innovator or a scientist might be different from what is considered safe from the perspective of a regulator or a citizen. This issue of defining and setting limits of acceptability is arguably particularly acute for questions of environmental safety when it is not clear whether harm to individual organisms, populations or ecosystems is of interest, or whether impacts on different types of organisms (e.g. vertebrates vs invertebrates) will be judged differently by different actors. Since what is meant by safety may differ from discipline to discipline, or stakeholder to stakeholder, a plurality of opinions

on questions such as safety for who or what and what is deemed a sufficient level of safety, are likely. Hence a transparent explication of and reflection on these issues (as well as the underlying values, norms and assumptions involved) is required.

It is also worth noting that to date SbD places significant emphasis on the properties of a new material for understanding its risk potential, rather than on the behaviour of the material in complex real world contexts in which the behaviour of humans and environmental actors could modulate the behaviour of the material. Focus on controlling the properties of the material as they manifest under the conditions of development may neglect to acknowledge the inherent indeterminacy involved when these materials are released into complex open-ended systems where control may no longer be possible. Attention therefore also needs to be given to how to handle the inherent indeterminacy and ambiguity in scientific knowledge and how to advance characteristics such as flexibility and reversibility in addition to safety in product design and development.

Another challenge SbD is facing is that the concept represents a general approach that would need to be adapted to specific contexts. The extensive diversity and complexity of MNMs and the wide spread applications of MNMs makes the need to tailor the generic approach of SbD to the specific contexts of innovation particularly acute. This is because it is not only important to consider *what* is addressed in the stages, but also how it is addressed, when, by whom and what levels of knowledge, reliability, safety and acceptance may be sufficient to pass the gate and continue to the next stage. Here, it may be important that the key actors using the SbD concept in a specific context discuss and adapt the questions and components of relevance so as to create appropriately tailored requirements to pass each gate.

A final limitation of the SbD concept is the lack of guidance provided on the distribution of responsibilities among different actors. It may be argued that it is legally clear that companies producing or importing products have responsibility for regulatory compliance and informing consumers of any potential risks, and that consumers have responsibility to use the products as intended and follow any described risk management procedures. However, within the proposed SbD approach in which safety researchers and industrial developers are being asked to collaborate in new ways, the distribution of responsibilities for ensuring product safety can become unclear. For example, do the industrial developers or the safety researchers have primary responsibility for ensuring safety in the SbD model? Furthermore, within a value chain perspective, many more actors are involved and responsibilities thereby diversify. For example, in NANoREG's D6.3 a general distribution of responsibility among different stakeholders is described in which it is suggested that risk-based researchers are responsible for their work being freely accessible and available for innovators, regulators and innovators are responsible to make sure there is enough funding to support the required safety research, international standards communities to develop appropriate standards, the media to report accurately and comprehensively, etc. However different actors may perceive their roles differently, placing the primary responsibility for ensuring safety elsewhere – e.g. scientists may highlight regulators as having the main responsibility for ensuring a consumer product is safe, while regulators may point to industrial developers and industry may point back to the scientists studying safety. This raises the question of how responsibility is seen as distributed among different actors involved in a SbD model.

2.1.10 *Responsible Research and Innovation*

2.1.10.1 Concept

The concept of "responsible innovation" (RI) or "responsible research and innovation" (RRI) is rapidly gaining currency in European policy discourse, as well as in international scholarship on the governance of new and emerging technologies. This rising emphasis on having the knowledge economy develop 'responsibly' is arguably the latest manifestation of a longer historical trend reimagining the relationship between science and society. This historical development of attempts to reimagine the science-society relationship and indeed, to enact it in new ways, is observable through the development of transdisciplinary forms of research, the use of practices such as technology assessment in its various forms, the increasing institutionalization of public engagement, the embedding of research on ethical, legal and social aspects (ELSA) into large technology development initiatives, and an increasing use of different forms of socio-technical

integration and midstream modulation (Stilgoe et al. 2013; Wickson & Carew 2014). As this historical telling suggests, RRI may be seen as an amalgamation or culmination of various activities and fields of practice and as a relatively recent term being used to bring these elements and practices together, a single definition has yet to firmly sediment. Surveying some of the different definitions being used within a European setting does, however, reveal overlapping and common features that can be said to characterise the concept. For an overview of the key definitions of RRI, see **Table 4**.

Table 4*: Key definitions of RRI within a European Policy Context*

European Commission 2012	**Von Schomberg 2013**	**Owen *et al.* 2013**	**Jacob *et al.* 2013**	**Rome Declaration 2014**
Responsible Research and Innovation means that societal actors work together during the whole research and innovation process in order to better align both the process and its outcomes, with the values, needs and expectations of European society. RRI is an ambitious challenge for the creation of Research and Innovation policy driven by the needs of society and engaging all societal actors via inclusive participatory approaches. RRI is also described as having 6 keys: engagement, gender equality, science education, open access, ethics and governance[2]	*Responsible Research and Innovation is a transparent, interactive process by which societal actors and innovators become mutually responsive to each other with a view to the (ethical) acceptability, sustainability and societal desirability of the innovation process and its marketable products (in order to allow a proper embedding of scientific and technological advances in our society)*	*Responsible Innovation is a collective commitment of care for the future through responsive stewardship of science and innovation in the present.* This is a process said to require that innovation be: *a) Anticipatory; b) Reflexive; c) Deliberative; d) Responsive*	*Responsible Research and Innovation refers to the comprehensive approach of proceeding in research and innovation in ways that allow all stakeholders that are involved in the processes of research and innovation at an early stage (A) to obtain relevant knowledge on the consequences of the outcomes of their actions and on the range of options open to them and (B) to effectively evaluate both outcomes and options in terms of societal needs and moral values and (C) to use these considerations (under A and B) as functional requirements for design and development of new research, products and services*	*RRI is an ongoing process of aligning research and innovation to the values, needs and expectations of society... RRI requires that all stakeholders including civil society are responsive to each other and take shared responsibility for the processes and outcomes of research and innovation. This means working together in: science education, the definition of research agendas; the conduct of research; the access to research results; and the application of new knowledge in society*

While all the proposed definitions of RRI given in **Table 4** can be seen to differ somewhat in the terminology, orientation, depth of description, and emphasis, certain characteristics can be seen as shared across them and therefore identified as central.

Accordingly, RRI can be said to involve:

1. A focus on addressing significant social and environmental challenges and needs;
2. A commitment to actively engaging a range of stakeholders early and throughout research and innovation processes for the purpose of achieving substantively better decision-making;
3. A dedicated attempt to anticipate potential problems, to assess available alternatives, and to reflect on underlying values, assumptions and beliefs;
4. A willingness to enhance equality, transparency and accessibility
5. A readiness amongst all participants to act and adapt according to 1-4.

[2] It is worth noting that in 2015 an EC expert group tasked with defining indicators for RRI (Strand et al. 2015) expanded this list to include sustainability and social inclusion so as to bring the keys closer in alignment with the Europe 2020 strategy.

From these characteristics it should be clear that RRI is a governance discourse that is primarily focused on changing the process of innovation and specifically directing it towards specific purposes rather than simply relying on downstream regulation of final applications as the way towards safe, acceptable and desirable products. This is not to suggest that regulation is not important, nor that products are necessarily less significant sites of engagement than process. It is just to highlight that when faced with the challenges of governing emerging technologies under conditions of complexity, uncertainty and competing values, rather than relying on risk assessment of final products, RRI calls for engaging in new approaches to orienting, organising, funding and conducting innovation processes as a way to facilitate the development of technological trajectories and future development in socially desirable directions.

2.1.10.2 RRI & MNMs

The development of nanoscale sciences and technologies actually represents one of the first areas in which there has been a dedicated effort to articulate and operationalize the concept of RRI. This is because within this field, a lack of nano specific regulatory frameworks (or agreed definitions and triggers across existing regulatory spheres), the existence of widespread scientific uncertainties, and a potential for social controversy, have all combined to generate a willingness to experiment with new approaches to governance. This application of RRI to nanoscale sciences and technologies is reflected in several EU policy documents (EC 2004; EC 2005; EC 2008) as well as international standards communities (OECD 2006; OECD 2007; ISO 2005, TC 229; ISO 2011; CEN 2004, TC 352; CEN 2011, TC 352).

The language of responsibility can be found threaded throughout European policy documents on nanoscale sciences and technologies. From its first communication “Towards a European strategy for nanotechnology” (EC 2004), the European Commission emphasized the importance that nanotechnology develop in a “responsible” manner, which was described as entailing adherence to ethical principles, addressing health, safety, environmental and societal concerns at an early stage, and including dialogue with stakeholders and members of the public (EC 2004). This sentiment was echoed and strengthened through the Action Plan that followed in which the overarching strategy for nanotechnology was characterized as ‘safe, integrated and responsible’ (EC 2005). The commitment was then further advanced through the development of a specific code of conduct for ‘responsible’ nanosciences and nanotechnologies research, which contained the following list of core principles: meaning, sustainability, precaution, inclusiveness, excellence, innovation and accountability (EC 2008). Interestingly, and in parallel, another code of conduct for responsible nanoscale science and technology was also developed in the same year as that recommended by the EC, spearheaded by the UK Royal Society, Insight Investment and the Nanotechnology Industries Association and generated in collaboration with a number of companies with a commercial interest in nanotechnology. This ‘Responsible NanoCode’ listed the following as its characterising principles: board accountability, stakeholder involvement, worker health and safety, public health, safety and environmental risks, wider social, health, ethical and environmental implications and impacts, engaging with business partners, transparency and disclosure (Responsible NanoCode 2008).

Around the same time that these European policy documents and industry initiatives demonstrating a specific interest in advancing responsible innovation in nanoscale sciences and technologies emerged, the international standards community also began to develop activities in the field. The OECD developed a working party on manufactured nanomaterials in 2006, which was focused on developing international cooperation and harmonization on human health and environmental safety testing. It then established a broader working party on nanotechnology in 2007 with the explicit intent “to advise upon emerging policy issues of science, technology and innovation related to the responsible development of nanotechnology” (OECD 2007). The International Organisation for Standardisation (ISO) created its technical committee on nanotechnologies (TC 229) in 2005, with a specific objective to “support the sustainable and responsible development of global dissemination of these emerging technologies”, alongside aims to facilitate global trade in nanotechnologies, ensure their health and environmental safety and promote good practices in

their production, use and disposal (ISO 2011). The European Committee for Standardisation (CEN) also established a technical committee on nanotechnologies (TC 352) in 2004, which has expressed a specific commitment to liaising with and coordinating its standards with the work taking place in both ISO and the OECD. In 2011, Working Group 2 of CEN's TC 352 on "Commercial and other stakeholder aspects" began a specific new work item on "Nano-responsible development: integration of risk and benefit assessment in the production, marketing, and use of nanotechnologies, nanomaterials and/or products incorporating nanomaterials".

As becomes clear, the emphasis on the need for a responsible development of MNMs has always been significant for European policy makers and is increasingly emphasised in standardization activities. Although the approaches adopted in the different documents, strategies and actions developed over time have slightly different framings and contexts in which they aim to contribute, there has been a consistent commitment to the importance of transparency and accessibility (acknowledging the way that this can be challenged by intellectual property rights and confidential business information), engagement and deliberation between different stakeholders, addressing environmental and health risks early in the innovation process, actively considering societal implications (such as socio-economic impacts and the distribution of risks, benefits and power) and having researchers in nanoscience and nanotechnology adhere to a code of conduct. As such, within European policy and international standard communities, responsible innovation has been specifically emphasized and embraced for nanomaterials, products and technologies.

2.1.10.3 Potential, challenges and limitations

The concept of RRI offers significant potential for reorienting innovation processes and practices so as to enhance the social acceptability and environmental sustainability of new products. It also offers significant potential for working as a supplement or extension for the framework of SbD that is increasingly being adopted within the NANoREG project. This is due to the way in which it a) brings in questions focused on the purposes of innovation, b) it extends collaborative engagement to include citizens, civil society and those with social science expertise, c) it incorporates broader questions and issues at stake such as those of ethics and sustainability, d) and it recognises the need to move forward in the face of pervasive uncertainty without a belief that risks can necessarily be thoroughly assessed and designed out.

However, despite its potential to improve innovation governance, the concept also faces a number of challenges and limitations. Much like SbD, the first of these refers to the question of how we define RRI and how we see it as distributed. Just as for safety, the meaning and interpretation of responsibility and/or product desirability can differ across individuals and stakeholder groups. There can also be a significant divergence between what people may recognise and say they are responsible for and how they enact their responsibilities in practice. There will also never be a single correct answer for these issues and they will always need to be deliberatively discussed and negotiated in practice. Moreover, with the focus of RRI on addressing social and environmental challenges and needs, questions may arise about how these needs are defined and prioritized, as well as what would constitute a desirable product or outcome. Furthermore, depending on how it is approached and defined, notions of RRI can be seen to challenge currently entrenched ideas of scientific and economic freedom, such as the autonomy of researchers and innovators to define what is interesting to work on and develop into products for the market. Indeed, RRI implies quite a different relationship between science and society than has dominated in recent years, namely one in which social actors and issues are given more influence and power over the shaping of science and innovation. This means that the concept can face significant resistance from actors benefiting from or comfortable with the current model.

The interpretive flexibility of RRI also has implications for the ability to be able to operationalise RRI and to find ways in which it may be usefully brought into innovation systems. Such operationalisation arguably requires the development of clear criteria for not only what RRI is but also indicators for how progress towards it may be achieved and measured. While work on this remains ongoing, three different approaches have been published recently (Strand et al. 2015; Stilgoe et al. 2013; Wickson and Carew 2014). Strand et al. (2015) emphasise that it is difficult to

specify precise indicators for something like RRI, which is dynamic and heterogeneous, and therefore suggest that the general framework of ideas they offer needs to be tailored to particular innovation networks by the actors involved. In the general framework they offer for this, however, emphasis is placed on the need for both *performance indicators* (for process and products) and *perception indicators* for all of the keys of RRI. Researchers in the UK have adopted a different approach, building on the four dimensions of RRI adopted by the engineering and physical sciences research council (namely anticipate, reflect, engage and act). The indicators used in this case included: a) risks identified, managed and deemed acceptable, b) compliant with relevant regulations, c) applications and impacts described and mechanisms put in place to review these, d) mechanisms identified to understand public and stakeholder views. In another approach, Wickson and Carew (2014) used a multi-stakeholder deliberative process to arrive at quality criteria and indicators for RRI. In this case, the authors used the defining characteristics that emerged to develop an evaluative rubric (describing routine, good, great and exceptional practices) that could be used by researchers, funders, innovators and other stakeholders to assess a project's performance against RRI criteria, also emphasising that this rubric should be tailored by the actors involved to their specific context. A more extensive description of all of these approaches to RRI indicators, including concrete examples is provided in Annex 2.

While challenges remain for the operationalization of both SbD and RRI, in the section that follows we seek to facilitate the uptake and implementation of these two new approaches to innovation governance by demonstrating how they may be integrated so as to advance innovation within the field of MNMs that has an increased chance of being safe, sustainable, socially acceptable and responsible in the face of scientific uncertainties and regulatory limitations.

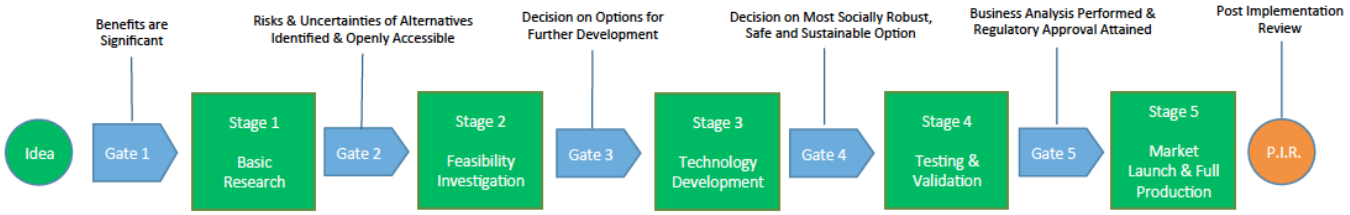
2.1.11 *RRI meets SbD*

While the emerging characteristics defining RRI have some overlap with the SbD approach (e.g. interaction between different types of stakeholders early in the innovation process), there are arguably some significant differences that create enhanced scope for the two governance approaches to come together in a complementary manner. Some of these differences include a) the focus of RRI not only on the products of innovation but also on the process of research and the purpose of innovation, particularly the need to align these with social needs, b) the extension of relevant stakeholders beyond regulators, innovators and EHS researchers to include civil society groups, non-governmental organisations and members of the public, c) an emphasis on reflecting on underlying values, assumptions and uncertainties throughout the innovation process, d) a specific commitment to transparency and accessibility, and e) an interest in anticipating impacts beyond risks to health and safety, including ethical issues, socio-economic impacts and questions regarding sustainability.

In the UK's ESPRC, the enactment of RRI has actually already been explored through a stage-gate model (see Stilgoe et al. 2013 and Owen & Goldberg 2010). This indicates the possibility that both RRI and SbD could be advanced and integrated into innovation practices through the expansion of this established model for managing innovation. In Figure 4, we build on the stage gate model that has been developed within NANoREG to date to advance SbD approaches in industrial innovation chains. Here we specifically indicate how the concerns related to RRI may be incorporated into this model.

While the expansion of the range of activities required at different stages indicated by our integrative model clearly makes significant additional demands on innovation actors (including an increased demand on human and financial resources) and would need to be amended and tailored to the needs of specific contexts, it arguably creates a more comprehensive form of assessment that significantly increases the chances of achieving safe, sustainable and socially robust innovation. For the value of this model to be demonstrated though, experiments with its implementation in practice would need to take place so as to provide rich feedback for its further refinement, development and spread.

Figure 4: An integration of RRI and SbD dimensions in a stage gate model (adapted from D6.3 as developed by RIVM and TEMAS)



Integration of Safe-by-Design in innovation processes involving MNMs	No Safe-by-Design activities	- Reduction of nano related uncertainties - List of potential nano related risks - Analysis of alternatives - Risk reduction	- Theoretical nano related risk analysis - Nano related risk mitigation - Grouping principles - Read across	Experimental nano related risk analysis	**Nano related risk assessment before launch**	**Update nano related risk assessment after launch** **Occupational health manangement during production**
		Occupational and product safety, Consumer safety, Environmental safety			**Nano related risk management**	
		Comparison with pre-defined safety levels				
		Organized Dossier shared by stakeholders (Robust nano safety data) (Pre-regulatory information)			**REACH Dossier**	
Integration of Responsible Research and Innovation in innovation processes involving MNMs	**Ask Questions about the Purpose of a Product** Will it address significant social or environmental challenges?	**Commit to Codes of Conduct for Responsible Research** **Evaluate Alternatives** With consideration given to questions of sustainability and ethics across the value chain in addtion to safety **Ensure Research Results & Assessments of Alternatives are Open & Accessible**	**Anticipate Potential Impacts** Draw on a wide range of perspectives to anticipate not only what are the potential risks, but also the potential ethical questions, socio-economic impacts, legal issues, consumer concerns etc and how these may be affected by the choice of different alternatives **Articulate the Distribution of Responsibilities** Consider who has responsibility for product safety, test quality and accountability if things go wrong	**Assess Sustainability through the Value Chain** e.g. through life cycle assessments **Engage with Stakeholders & the Public** Seek a broad range of views on the emerging technology and on the breadth and quality of the knowledge on potential impacts **Deliberate across diverse perspectives and positions**		**Transparent Communication** Ensure information on MNMs, potential impacts and uncertainties is clearly communicated and accessible throughout the value chain
	Reflect on Underlying Values, Assumptions & Beliefs					
	Who or what may benefit? How will any benefits be distributed?	Where are the limits of what is known and what is knowable? What gives you confidence in knowledge?	How should impacts and feasibility be defined and measured? By whom? How may research on impacts and feasibility be designed differently?	What defines quality in safety experiments and risk analysis? Who decides on acceptable levels of safety and how?	How may this product be used differently under market conditions? What may be required for testing and validation under value chain conditions?	How is communication framed and oriented? How are remaining uncertainties communicated?

Turning Risks into Business Opportunities

Although potential risks, safety issues and knowledge gaps may be conceived as negative, and in this deliverable we have so far largely focused on new approaches to testing and governance for addressing these issues, it is also valuable to consider the way in which they may offer a range of potential business opportunities. This could be through stimulating the development of new forms of required instrumentation, the performance of necessary tests for commercial actors, or the provision of needed reference materials. Some of the areas where we see the safety assessment issues identified in this deliverable as offering new business opportunities include:

1. Synthesis of well-defined nanomaterials

To develop reliable results in safety testing and innovation requires the synthesis, characterization, and modification of well-defined nanomaterials. This could include the development and provision of a set of standard materials for interlaboratory comparisons and benchmark reference points for theoretical and experimental studies. Development of reproducible, scalable methods for synthesizing nanomaterials with precisely controlled chemical and physical properties and the availability of sample sets that vary by only single properties (e.g., size, surface groups, shape) could significantly advance our understanding of how these properties affect response outcomes. The availability of such well-defined MNMs would represent a potential market for both researchers and innovators working towards Safe-by-Design. The production and provision of both MNMs for use as both safety testing reference materials and materials for innovation chains therefore represents a potential business opportunity.

2. Development of new tools for studying MNMs in complex media

Identifying, observing, monitoring, mapping and understanding the behaviour and surface reactions of MNMs in complex media is currently a significant knowledge gap and filling it calls for the development of innovative new tools, instruments and methods for the research community. Tools that can characterise the structure and composition of MNMs in both complex test media but also environmental media (such as soil) and in vivo would represent a significant advance on what is currently available and would be a financial opportunity.

3. Development of experimental and theoretical methods for the study and control of nucleation processes

Improving the detection limits on instruments to investigate smaller particle/cluster sizes and lower concentrations to determine what species participate in new particle formation is important. In this regard, characterization of sub-10 nm particles is particularly important in both aqueous and air systems. Modelling nucleation clusters extends the use of advanced molecular computational techniques in a matter that could act as a bridge to investigating larger particles.

4. Development of new tools for MNM characterization

New tools are especially required for characterisation of MNMs under ambient conditions and in complex and heterogeneous media. Tools for characterising the surface composition of nanomaterials after environmental exposures would also be valuable.

5. Development of new computational tools for modelling and calculation

For MNMs, new computational tools that could bridge length and time scale gaps would be beneficial, as well as those that could model and be used to predict the dynamic and evolutionary properties of individual nanoscale materials in realistic, complex environments. Furthermore, validation of new multiscale computational methods for characterising MNM interactions with biological systems would be important.

6. Development of high-throughput screening methods and instruments

Developing methods and instrumentation that can enable well characterised MNMs to be screened for their potential toxicity of MNMs in a manner that is time efficient and cost effective would be highly beneficial.

7. Development of a database of MNMs

If such a database could be relatively standardized and available for distribution to multiple labs for comparative experimentation, it could represent a business opportunity.

8. Development of search engines for nanomaterial interactions.

The ability of computers to search a large number of interactions between natural biopolymers (e.g. carbohydrates, proteins, nucleic acids, polysaccharides and mixed biopolymers such as glycoproteins, lipoproteins, and glycolipids) and nanomaterials would be particularly useful in learning 'design rules' underlying nanomaterial toxicity. This would also allow researchers to focus on the most important biopolymer systems for more detailed experimental and computational study. This could not only lead to development of nanomaterials with high functionality and reduced adverse impacts on the environment, the development of such searching power could also be a commercial opportunity.

Evaluation and conclusions

Based on an extensive literature review and dialogue workshops with nanosafety researchers, this deliverable has created an inventory of the technical and social challenges that are currently inhibiting reliable safety assessment of manufactured nanomaterials, presented a characterisation of different types of uncertainty important in science for policy, and surveyed the potential of both new approaches to scientific research and to management and governance as ways to overcome the identified challenges and move forward in the face of uncertainties.

In the review of two new approaches to safety testing of MNMs – high throughput screening and organ-on-a-chip – the deliverable concludes that although these approaches offer significant benefits such as a potential reduction of costs, time and animal use in safety testing, technical challenges and limitations remain concerning issues such as the regulatory acceptability of such approaches, the added value of the knowledge generated, and the relationship to real world scenarios.

Having characterised various forms of uncertainty that are particularly relevant in safety assessment and that new technical approaches to safety testing cannot eliminate, the deliverable then provided a critical review of two new governance approaches seeking to manage complex risks in the face of uncertainty – Safe-by-Design and Responsible Research and Innovation. After highlighting the potential benefits and challenges associated with both approaches, this deliverable presented a way in which RRI and SbD could be combined within a stage-gate model of innovation for maximum benefit and a comprehensive assessment. This combination represents a unique contribution to the SbD stage gate model being developed within NANoREG. By including a broader range of actors and issues for consideration, it aims to enhance the potential for safe, sustainable and responsible innovation using MNMs, as called for by the European Commission. Even though challenges remain for the operationalisation and practical implementation of these new approaches to governance, the work in this deliverable offers a useful overview and critical review of the approaches and thereby a step forward towards the safe and responsible development of MNMs in the face of various forms of uncertainty.

Finally this deliverable contributes in a distinctive way to the NANoREG project by offering an overview of how the challenges facing the safety assessment of MNMs that have been identified within the document can also be turned into business opportunities. In this way it identifies challenges facing scientists, regulators and industry for developing safe MNMs and highlights not

only how these challenges may be addressed through new approaches to research and governance but also how they can be turned into new business opportunities.

The work in this deliverable has been particularly focused on offering useful inputs to regulatory questions concerning hazard identification (e.g. through surveying new approaches to research), risk assessment (e.g. by clarifying the range of existing obstacles) and risk management (e.g. by exploring and developing new approaches to governance). It has also been particularly occupied with addressing the identified policy issues of delivering timely evaluations and governing the development of MNMs in the face of complex risks and widespread uncertainty.

The new knowledge generated includes a clear overview of both the social and technical obstacles currently creating bottlenecks for safety assessment of MNMs, a critical review of both social and technical approaches to addressing these, an integrated approach for safe and responsible innovation using MNMs, and a list of ways in which the risks and uncertainties associated with MNMs development can be usefully turned into business opportunities.

3 Deviations from the work plan

The partners working on this deliverable applied for and received an extension of the deadline from the NANoREG management committee, allowing it to be delivered in December 2015 rather than August 2015 as originally planned.

This extension was important due to a delay some of the task partners experienced in securing their national co-financing and therefore the necessary staff to work on the deliverable, and a desire to wait for and build on the value chain case studies being developed within NANoREG. Furthermore, the extension was important to allow sufficient time for several dedicated workshops to be conducted with nanosafety scientists as part of the core research for this project. The deliverable has now been finalised within the extended deadline granted.

The title of the deliverable has been changed from the originally proposed “Inventory of safety issues in the innovation chain, including gap analysis” to “Inventory of safety assessment issues and new approaches to research and governance”. The change in the title was primarily made because it was felt that several gap analyses concerning safety issues for MNMs were already available, including some produced within the NANoREG project, and there was therefore a desire that this deliverable build on rather than repeat these existing analyses and work to offer novel information and insights. The decision was therefore made to place emphasis on articulating the technical research bottlenecks, as well as the social (including economic and political) issues, that are currently inhibiting the ability of the nanosafety research community to fill in the identified knowledge gaps. Furthermore, so as to offer constructive suggestions for ways to move forward, it was decided to dedicate significant space to exploring potential strategies for overcoming the identified obstacles, including not only the available technical strategies as originally planned (i.e. in terms of new test methods) but also including social strategies (i.e. in terms of new approaches to management and governance).

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Annex 1 State of the Art in the Organ-on-a-Chip Systems

State of the art in Organ-on-a-Chip systems

Organ	Properties	Characteristics (Mechanical/ Flow)	Model (Mono-culture/ Co-culture, cell type)	Advantages/ Main use	Limitations/ Issues	Suitable for toxicology/ drug/ developmental studies	References
Nerve	Axonal specification and pathfinding. Sensitivity of axons in response to for example gradients of proteins. Measuring electrophysiological responses.	Variability in fluid pressure and size of junctions between layers Microfluidic PDMS/APTES multiple chamber device	Mono-culture: primary hippocampal neurons (Xiao et al., 2013), primary neurons (Tsantoulas et al., 2013) Co-culture: mESC-MNS and myotubes (c2c12) (Park et al., 2013), Glia and (CNS) neuronal cells (Shi et al., 2013), hESC-derived Schwan Cells and hESC-derived axons (Ziegler et al., 2011)	Multiple gradients simultaneously in a single chip, easy to quantify Membrane protects the cell culture from damage from fluidic shear stress Increase in numbers and contact of synapses due to adding soluble factors Control over separated or connected different type of cells (Shi et al., 2013)		Motor Neuron recovery and desensitization studies	Xiao et al., 2013 Tsantoulas et al., 2013 (Alepee, Bahinski et al. 2014) Shi et al., 2013 Park et al., 2013 Ziegler et al., 2011
Fat	Lung-Liver-Fat (-Other tissue) model to mimic drug uptake system, measuring uptake of toxic material in fat tissue (µCCA device)	Fluid chambers (three/four), flow of culture medium or blood surrogate	Mono-culture in different chambers: L2 cells, C3A cell and 3T3- L1 adipocytes (Viravaidya et al., 2004), L2 and H4IIE cells (Sin et al., 2004)	Mimic complex multi-tissue interactions, parallel experimenting, fast	Difficult to culture three different kinds of cells in one device	Bio-accumulation, distribution and toxicity studies	Sin et al., 2004 Viravaidya et al., 2004
Skin	Response of skin barrier to chemical compounds	Four different chambers to test simultaneously different conditions on collagen patches, constant medium perfusion	Mono-culture: Human epidermal keratinocytes (HEK) cells	Increase in viability, growth and confluency of epidermal cells, more suitable for high throughput irritant and toxicity arrays		Dermal exposure to nanomaterials, irritation and toxicity evaluation, wound healing techniques	Oneill et al., 2008

Cornea	Mimic the stromal cell layer underneath the exposed epithelial cell sheet, and direct, layer-bylayer assembly of a purely cellular corneal construct	Collagen/PDMS microdevice, fluic acces to both sides of the bilayer	Co-culture: epithelium and stroma were isolated from rabbit eyes	More complete control over co-culture microarchitectures compared to the limited membrane thickness, pore size, porosity, and surface treatments		Studies on tissue development	Puleo et al., 2009
Heart	Record contractility by Muscular Thin Film (MTF) of multiple tissue Hypoxia tests	Fluidic control over drug wash-in/wash out experiments, electrical field stimulation, membrane potential changes Nitorgen flow to induce hypoxia (Khanal et al., 2011) Measuring electrophysiological properties of cells Pressure and Stretching differences	Mono-culture: primary culture cardiac myocytes (Agarwal et al., 2013, Khanal et al., 2011, Grosberg et al., 2011, Cheng et al., 2006), H9c2 cells (Giridharan et al., 2010)	Simple and reusable device (Agarwal et al., 2013)	Technically difficult to complete washout between dosages	Drug testing, diseased organ studies, high throughput studies, Ischemia/reperfusion studies Single animal can provide for multiple experiments (low shear)	Agarwal et al., 2013 Khanal et al., 2011 Grosberg et al., 2011 Cheng et al., 2006 Giridharan et al., 2010
Blood Vessel	Mechanical stimulation to mimic different fluidic properties of vascular structures Metabolic requirements by transport via convective pathway	Electrical circuits built by fluidic channels filled with ionic liquid. PDMS can be used to create a vascular geometry Elastomeric microfluidic cell regulated by a variable-speed pump	Mono-culture: HUVECs (Liu et al., 2013), Endothelial cells (Song et al., 2005) Epethilium and endothelium cells in different lines (Huh et al., 2010, Huh et al., 2012) Co-culture: umbilical vein endothelial cells, human embryonic stem cell-derived pericytes and rat tail collagen (Van der Meer et al., 2013), HMEC-1 cells (Shin et al.,	Simple, robust, easily scaled up for high troughput experiments		Drug testing, uptake of for example nanoparticles by the alveolar-capillary interface In vitro vasculature for tissue-engineered organs Differential shearing in multiple compartments performed on a single	Liu et al., 2013 Van der Meer et al., 2013 Shin et al., 2004 Song et al., 2005 Huh et al., 2010 Huh et al., 2012

			2004),			chip	
Muscle	Contractile performance and controle of muscle tissue	Muscular Thin Film (MTF) technology	Co-culture: Fibronectin and Human vascular smooth muscle cells (VSMCs) or cardiomyocytes (Grosberg et al., 2012)	Multiple tissue in one assay (two tissue chip)		Drug development and toxicity studies	Grosberg et al., 2012 Lam et al., 2009
Bone	Cyclic mechanical stimulation	Continuous-perfusion glass Laminar flow technologies	Mono-culture: Human Adipose tissue-derived stem cells (hASCs)m or human bone marrow-derived mesenchymal stem cells (hMSCs) (Park et al., 2012), Col1a1GFP MC-3T3 E1 cells (Jang et al., 2009), BMP-2 inducible expression cell line (C9 cell) (Zhang et al., 2011)	Increase in viability of cells Automated long-term monitoring of cells and sampling of the culture supernatant system for osteoblast differentiation assay using a small number of cells		Drug screening New bone formation studies Tissue engineering and regenerative medicine strategies	Park et al., 2012 Jang et al., 2009 Zhang et al., 2011 Zhang et al., 2014 Torisawa et al., 2014
Lung	Alveolar-capillary interface of the human lung Mimic the subatmospheric, pressure-driven stretching Three-chamber (lung-liver-other) microscale cell culture analog (mCCA) (Sin et al., 2004)	Two chamber device (air-liquid model) divided by PDMS membrane with small pores	Mono-culture: primary human small airway epithelial cells (SAECs) (Huh et al., 2007) L2 and H4IIE cell lines in separate chambers (Sin et al., 2004), mouse lung epithelium cells (MLE-12) (Fritsche et al., 2009) A549 cells (Tavana et al., 2011) Co-cultyure: human alveolar epithelial cells and human pulmonary microvascular endothelial cells (Huh et al., 2010 & 2012)	Complex physiological phenomena in a single device Innovative and low-cost screening platform Smaller size of the mCCA device, multiple experiments can be performed in parallel		High-throughput analysis and screening of cellular responses Alternatives to animal and clinical studies for drug screening and toxicology applications	Huh et al., 2007 Huh et al., 2010 Huh et al., 2012 Sin et al., 2004 Fritsche et al., 2009 Tavana et al., 2011
Liver	Hepatic clearance by flow	Overall flow housing maintained by a peristaltic pump	Mono culture: human hepatocytes in CCA (Chao et al., 2009), pri- mary rat hepatocytes	Beneficial effect on viability and cellular morphology	Primary cells retained specific hepatocyte properties closest	Investigate drug protein binding, drug–drug interactions	Chao et al., 2009 Cheng et al., 2012

			(Legendre et al., 2013), The human HCC cell line HepG2/C3A (Cheng et al., 2012) monoculture HEPG2/C3a and co-culture HEPG2/C3a-MDCK (Shintu et al., 2012)	Maintaining cellular metabolic competency for longer periods of time	to physiological levels, although it cannot reproduce the in vivo state perfectly	Drug discovery process to identify potential drugs and thereby reduce late stage failures or animal testing Metabolism testing for toxicology kinetic endpoints (shintu et al., 2012)	Shintu et al., 2012 Legendre et al., 2013 Other: Carraro et al., 2008 Lee et al., 2007 Powers et al., 2002 Khetani et al., 2007 Kane et al., 2006 Viravaidya et al., 2004 Sin et al., 2004 Allen et al., 2003 Sivaraman et al., 2005
Blood Brain Barier	BBB with a dynamic environment and a comparatively thin culture membrane	Fluid shear stress, and biochemically, by stimulation with for example TNF-α Analyze barrier tightness by measuring the transendothelial electrical resistance (TEER)	Mono-culture: hCMEC/D3 (Griep et al., 2013) , SV-HCEC (Harris et al., 2003), primary brain microvascular endothelial cells (BMEC) (Shayan et al., 2011 a&b) Co-culture: End3 endothelial cells and C8-D1A astrocytes (Booth et al., 2012). neurons (4%), astrocytes (95%), and microglia (1%) (Achyuta et al., 2013)	After 3–4 days in vitro (DIV), cells that are plated into the somal compartment have axons that extend across the barrier through the microgrooves, more physiological representation of the BBB Protocol (Park et al., 2006) can be finished in 1-2 days		CNS disease progression and drug delivery	Booth et al., 2012 Griep et al., 2013 Park et al., 2006 Harris et al., 2003 Shayan et al., 2011a Shayan et al., 2011b Achyuta et al., 2013
Intestine	Peristaltic motion, intraluminal fluid flow and	Top and bottom channel divided by a thin layer of	Co-culture: Caco-2 cells and LGG (Kim et al., 2012), Caco-	Long term culturing		Drug screening, intestinal health and	Mahler et al., 2009

	coexistence of microbial flora	PDMS porous membrane Vacuum chambers to mimic mechanical shear stress	2 cells and HT29-MTX (Mahler et al, 2009)	Villi forming of cultured cells Promote accelerated intestinal epithelial cell differentiation, increase intestinal barrier function µCCA is cheap to produce and use very little of the compound of interest, simulates multiple organs and human cells can be used.		disease studies	Kim et al., 2012 Kim et al., 2013
Kidney	Fluidic flow that mimics key functions of the human kidney proximal tubule. Regulating water and ion balance via molecular transport by hormonal stimulation. Creating biological tubule environments, laminar shear stress.	Multi-layer microfluidic device (MMD), two compartments (one flow chamber + one static chamber) divided by a porous membrane	Mono-culture: primary rat inner medullary collecting duct (IMCD) cells (Jang et al., 2010) Renal cells (Madin Darby Canine Kidney, MDCK) (Baudoin, et al., 2007) Madin–Darby canine kidney cells (Snouber et al., 2012) Primary kidney epithelial cells (Jang et al., 2013)	Models for toxicology using fewer animals and less expense, avoiding an intensive labor Mimic the in vivo responses than results obtained with cells maintained under conventional culture conditions		Disease model system, drug screening, preclinical safety studies	Jang et al., 2010 Baudoin et al., 2007 Snouber et al., 2012 Jang et al., 2013

Annex 2 Indicators for RRI

As described in section 2.1.10.3 on the potential, challenges and limitations of RRI, different indicators for RRI implementation in practice has been developed by different groups. In this annex, the tables containing more detailed information on the proposed indicators and approaches to measuring progress towards RRI are provided.

The expert group of the European Commission working on the development of indicators for RRI used the six keys as described by the commission as the criteria for monitoring the performance of RRI. The general framework that networks could adapt with their own selection of context specific relevant indicators is provided below, and examples of indicators for each key are available in the original publication.

Table 5: General framework for RRI performance indicators (originally published in Strand et al. 2012)

Criteria	Performance indicators		Perception indicators	Key actors
	Process indicators	Outcome indicators		
Public engagement				
Gender equality				
Science education				
Open access				
Ethics				
Governance				
Sustainability				
Social justice/inclusion				

The groups of UK researchers that have worked most closely with the implementation of RRI within the EPSRC describe RRI as involving four dimensions - anticipation, reflection, inclusion and responsiveness - and have pointed to techniques and approaches that can be applied to advance each of these dimensions, as well as identified factors affecting their implementation.

Table 6: RRI: Techniques and factors affecting implementation (originally published in Stilgoe et al. 2013)

Dimension	Indicative techniques and approaches	Factors affecting implementation
Anticipation	Foresight Technology assessment Horizon scanning Scenarios Vision assessment Socio-literary techniques	Engaging with existing imaginaries Participation rather than prediction Plausibility Investment in scenario-building Scientific autonomy and reluctance to anticipate
Reflexivity	Multidisciplinary collaboration and training Embedded social scientists and ethicists in laboratories Ethical technology assessment Codes of conduct Moratoriums	Rethinking moral division of labour Enlarging or redefining role responsibilities Reflexive capacity among scientists and within institutions Connections made between research practice and governance
Inclusion	Consensus conferences Citizens' juries and panels Focus groups Science shops Deliberative mapping Deliberative polling Lay membership of expert bodies User-centred design Open innovation	Questionable legitimacy of deliberative exercises Need for clarity about, purposes of and motivation for dialogue Deliberation on framing assumptions Ability to consider power imbalances Ability to interrogate the social and ethical stakes associated with new science and technology Quality of dialogue as a learning exercise
Responsiveness	Constitution of grand challenges and thematic research programmes Regulation Standards Open access and other mechanisms of transparency Niche management[a] Value-sensitive design Moratoriums Stage-gates[b] Alternative intellectual property regimes	Strategic policies and technology 'roadmaps' Science-policy culture Institutional structure Prevailing policy discourses Institutional cultures Institutional leadership Openness and transparency Intellectual property regimes Technological standards

[a] Schot and Geels (2008).
[b] See below and Macnaghten and Owen (2011) for an example of this.

The quality criteria and indicators for RRI developed by Wickson and Carew (2014) start with an outline of important elements for each of the defining characteristics for RRI that were developed through their multi-stakeholder deliberative process. These important elements are then developed into an evaluative rubric to assess RRI performance. It is important to note that the authors emphasise that it may be impossible for innovation actors and projects to perform exceptionally across all RRI criteria, however, reflecting on how they are positioned against each can usefully reveal both their strengths and areas for future improvement.

Table 7: RRI characteristics and important elements (originally published in Wickson & Carew 2014)

Defining Criteria of RRI	**Important elements for evaluation**
Socially Relevant and Solution Oriented	(a) Type of problem addressed (b) Type of solution sought
Sustainability Centred and Future Scanning	(a) Anticipating potential futures (b) Identifying potential risks and benefits (c) Considering social, economic and environmental sustainability
Diverse and Deliberative	(a) Level of cross-disciplinarity involved (b) Where stakeholders are involved (c) How stakeholders are involved
Reflexive and Responsive	(a) Recognition of preconditions in context and group (b) Exploration of underlying values, assumptions and choices (c) Openness to critical scrutiny (d) Ability to change after internal reflective practice and external feedback
Rigorous and Robust	(a) Aspects of the problem considered (b) Repeatability across actors and settings (c) Reliability of outcomes under real-world conditions
Creative and Elegant	(a) Novelty and daring (b) Sufficiency and beauty
Honest and Accountable	(a) Identification of uncertainties and limitations (b) Lines of delegation and ownership (c) Compliance with research ethics and governance requirements (d) Policies on open access and information sharing (e) Ownership over positive and negative outcomes

Table 8: Evaluative rubric for RRI

Criteria	Exemplary	Great	Good	Routine
Socially relevant and Solution oriented	Addressing a grand social challenge. Ongoing analysis of objectives and processes to favor the delivery of 'wicked solutions' (solving multiple challenges simultaneously)	Addressing a significant social need. Ongoing analysis of objectives and processes to maintain a focus on delivering a successful solution	Focused on a marginal or self-defined problem. Employing processes aimed at generating insights toward a solution, or a partial solution	Pursuing a purely personal interest. Possibility that process/product will only result in the creation of decontextualized knowledge or new problems
Sustainability centered and Future scanning	Inclusion of formal processes of future casting to at various points throughout the research and innovation process. Generating a range of positive and negative future scenarios and identifying and assessing associated risks and benefits of these for social, environmental and economic sustainability. Clear avenues for embedding responses to these possible futures and risk/benefit assessments into the project development	Inclusion of future casting activities at some point during the research and innovation process. Some attempt to integrate an assessment of the risks and benefits for social, environmental and economic sustainability. Identifiable points and possibilities for adaptation of the process to respond to the future scanning and risk/benefit assessment activities	Informal attempts to future cast at limited points in the project. A consideration of some associated risks and benefits in terms of one or more of the three dimensions of sustainability. Little indication of how the research and innovation process may adapt and respond to either identified possible futures or their risks to sustainability	A singular optimistic prognosis for future project outcomes with no clear effort to identify risks or survey possible future scenarios
Diverse and Deliberative	Openly and actively seeking ongoing critical input, feedback and feed-forward from a range of stakeholders. Encouraging and rewarding transformative mutual learning. Employing an evolving integrative method and consciously employing a TD process	Inviting, incorporating and integrating stakeholder views at various points along the research and innovation process. Actively seeking dialogic interaction with stakeholders and open to mutual learning. Encompassing a wide range of methods and adopting an interdisciplinary process	Limited stages of the research and innovation process open for stakeholder engagement. Tendency toward one-way forms of communication with stakeholders but open to some interaction. Involving some level of methodological diversity and multidisciplinary practice	Communicating with stakeholders only toward the end of the research and innovation process. Use of one-way communication approaches and defensive in the face of counter-views or stakeholder questions. Mono-methodological and mono-disciplinary
Reflexive and Responsive	Clear and explicit identification of institutional and contextual limitations and a structured effort to acknowledge and improve upon these conditions. Structured, purposeful periodic analytical review of underlying values, assumptions and choices. Actively seeking critical feedback from a wide group of sources and actors. Evidence of potential to adapt at a range of points in response to in-train reflective practice and external review/input/feedback	Explicit effort to identify institutional and contextual limitations and awareness of their significance for practice. Occasional use of structured process for reflecting on underlying values, assumptions and choices. Actively seeking critical feedback from select sources and actors. Clear indications of a capacity to adapt in response to reflective practice and external feedback	Some indication of awareness concerning limitations posed by institutional structure and contextual realities. Informal, one-off or ad hoc process for considering underlying values, assumptions and choices. Accepting critical feedback when offered. Stated willingness to accept change in response to internal reflective practice or external review and critique	No explicit consideration or recognition of the limitations posed by institutional structure and contextual realities. No process for facilitating reflective practice. No critique sought. No evidence for potential for change in response to criticism/unsolicited feedback
Rigorous and Robust	Comprehensive investigation of all aspects of the problem and the interconnections between them. Results repeatable by a variety of different actors operating across a range of relevant conditions. Outcomes work reliably under real-world conditions	Considering multiple dimensions of the problem and their interrelations. Results repeatable by the same actors operating under a range of relevant conditions. Outcomes with demonstrated functionality under real-world conditions	Interest in several dimensions of the problem although not necessarily their interrelations. Results repeatable by the same actors operating under similar conditions. Outcomes remain untested under real-world conditions	Narrow focus on one element or aspect of a problem. Results not able to be replicated. Outcomes unable to be reliably applied in real-world contexts
Creative and Elegant	The problem has been reframed in innovative directions, with new ideas being pursued through appropriate methods. Resources are carefully considered and allocated to efficiently achieve maximum utility and impact. Esthetical consideration is given to preconditions, process and products	New methods are being developed according to new ideas within an established problem framing. The use of resources is explicitly justified. Esthetical consideration is given to preconditions and products	New ideas are being pursued through established methods within an accepted problem framing. Considerable resources are inefficiently employed. Esthetical consideration is given to envisaged products	Problem framing, ideas and methods fall within established paradigms. Extensive resources (e.g. time, money, personnel, etc.) are dedicated to work with minimal significance or potential impact. No consideration is given to the esthetics of operating preconditions, research and innovation process or envisaged products

Honest and Accountable	Transparent identification of a range of uncertainties and limitations that may be relevant for various stakeholders. Openly communicated lines of delegation and ownership able to respond to process dynamics and contextual change. Documented compliance with highest-level governance requirements, research ethics and voluntary codes of conduct, all actively monitored throughout. Consistent use of open access to information policies. Preparedness to accept accountability for both potential positive and negative impacts	Identification of uncertainties and limitations deemed to be significant by those involved. Established lines of delegation and ownership. Compliance with governance requirements and research ethics with evidence of active monitoring throughout. Favoring open access to information policies. Willingness to accept accountability for potential positive and negative impacts	Some statement indicating uncertainties and limitations. Indications of potential lines of delegation and ownership. Complying with minimum standards of governance requirements and research ethics. Occasionally employing open access to information policies Willingness to accept accountability for positive impacts and some negative impacts	No transparency concerning limitations and uncertainties. Untraceable ownership of components. No specific acknowledgement of standards concerning governance requirements or research ethics. No demonstrated commitment to open access information policies. Accountability only accepted for positive outcomes