Intelligent testing strategies for engineered nanomaterials

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ABSTRACT

The exciting properties exhibited by materials at the nano-scale have led to the development of vast numbers of different types of nanomaterials for a wide variety of applications. Such applications include medicines, cosmetics, sunscreens, clothing, food, food packaging, sporting equipment, paints and electronics. In fact, almost any application can include nanotechnology in some context. This has led to potential concerns about the safety of such nanomaterials due to the increasing likelihood of exposure to both humans and the environment. The unusual properties of nanomaterials make it difficult to predict their biological reactivity, making it essential to be able to assess the risk associated with their use.

National governments and the European Commission recognised the potential benefits of nanotechnology at a relatively early stage in their development, but at the same time they realised that for this potential to be maximised, the issues of risk needed to be better understood. However, we still lack sufficient knowledge to allow effective risk assessment of nanomaterials to be used by industry, regulators and for scientific research.

In order to capitalise upon the research knowledge available and in production, the European Commission has funded a project entitled 'Intelligent Testing Strategy for Engineered Nanomaterials - ITS-NANO'. ITS-NANO is a pan-European project aimed at identifying the most appropriate and effective research required to deliver an Intelligent Testing Strategy (ITS) for assessing exposure, hazard and the potential risks of engineered nanomaterials. Using the combination of an extensive gap analysis combined with expert opinion, ITS-NANO has already identified a range of research and knowledge gaps in data management, physicochemical properties of nanomaterials, exposure assessment, hazard assessment (toxicology and ecotoxicology) and risk assessment. A key focus across a number of these categories is the need for improved technologies, for harmonised protocols across a range of nanomaterials and scenarios, for the development of in silico tools, and for high throughput implementation of experimental procedures. The full gap analysis is available at the project website, www.its-nano.eu/the-project/project-output.

The ITS is now being designed to describe a research trajectory aimed at addressing the knowledge gaps and taking steps to greatly reduce the amount of uncertainty affecting this area of research. The strategy will be discussed with stakeholders from Europe and United States, integrating industry, regulators and scientists during a meeting in March, to be launched in May 2013. The ITS will be a fluid document which can be adapted as new information emerges and the current knowledge gaps are filled, and will provide a direction for new research to meet the increasing demands for risk assessment of nanomaterials..

Keywords: intelligent testing strategies, nanomaterials, nanosafety, risk assessment, grouping

1 INTRODUCTION

In order to provide a guideline for protecting life at all levels, from microbial, plant and animal to human, the ITS-nano project aims to develop an Intelligent Testing Strategy for engineered nanomaterials.

Nanosciences and Nanotechnologies are two of the fastest growing research areas of the last decade. Nanotechnologies promise benefits for a wide range of application – from information and communication technologies to health care, energy storage and consumer products. Numerous nano-enabled products are already available on the market in many countries worldwide and the total global market for nano-products is expected to reach $2.6 trillion (US) by the year 2015. As the market for nanotechnology increases, so will the likelihood of human and environmental exposure to the products of nanotechnology. Accordingly, the potential risks in these areas need to be assessed, although this is only feasible if there is relevant information on the exposure and hazard of the nanomaterials and nanomaterial-containing products.

Much work is being done in relation to exposure, hazard and risk assessment of nanomaterials (NM), but due to the vast number of materials that need to be assessed, and the lack of appropriate techniques or standard procedures, understanding of risk is not progressing at a rate that can enable sustainability and support adequate development of nanoscience and nanotechnologies. In addition, there are overlaps and gaps in the research landscape which need to be addressed in order to ensure that the information provided is appropriate, complete and of the best quality. Consequently there is an urgent need for new approaches which consider and exploit the unique aspects of engineered NM, and specifically an Intelligent Testing Strategy is required to guide future developments.

The terms integrated and intelligent testing strategy both appear in the literature, but with a lack of clarity over their meanings and differences. In the context of ITS-Nano

intelligent testing strategies means the development of a strategy that will provide an efficient but highly effective means of assessing the risks of nanomaterials, while integrated refers to the use of data from multiple sources and combining them into a single analysis to determine risk. Intelligent testing strategies are based on optimal use of all data generation sources and measures available [4] and take an integrated approach comprising multiple elements in order to speed up the risk assessment process while reducing costs and animal tests [5]. The ITS-nano aims to be "intelligent" both at the strategic level by identifying and setting a priority research agenda to reduce the research gaps according to the need of stakeholders (industry, regulators) and at the tactical level, to be economical and ethical (i.e. to adhere to the 3Rs principle). The project will propose a framework for grouping of engineered NM based on their chemical, physical and biological properties and on their subsequent exposure routes and biological impacts in order to intelligently design next-generation nanosafety evaluation and risk assessment strategies. At all stages of ITS development relevant stakeholders will be integrated into a dialog relating this strategy to their specific needs to ensure ITS-nano delivers a consent-driven strategy.

2 KNOWLEDGE GAPS

In order to facilitate the development of an intelligent testing strategy, it is necessary to start from an overview of the existing gaps in knowledge/research pertaining to hazard and risk assessment of engineered nanomaterials. This information was gathered from publically available information such as peer reviewed literature, published reports from governments and regulators and partner knowledge relating to current state-of-the-art. In combination with expert opinion, this information provided the evidence base upon which the intelligent testing strategy will be built. For a thorough description of the knowledge gaps, please refer to the ITS-NANO project website, www.its-nano.eu/the-project/project-output. Among all the identified gaps, however, several issues can be identified as key aspects, and are briefly discussed within this section.

Multiple uncertainties hindering regulatory Risk Assessment (RA) of nanomaterials have been identified, at least partially due to current insufficiencies in data. Likewise, there is also a lack of knowledge on how to group or rank these materials in order to identify and enable a risk assessment of the most potent material which, for example, could be done by grouping material according to their (i) physicochemical properties (especially in situ in complex milieu), and (ii) biological effects. Biological effect (including mode of action) is the key driver for understanding and grouping of NM. Taking into consideration the diversity of nanomaterials and the complexity of nanoscale systems, it is unlikely that a single physicochemical (PC) characteristic would be sufficient to describe the toxicity of NM. Moreover different properties are likely to be related to different aspects of their interaction with living organisms and cells. NM must be adequately characterised at various stages in order to understand the changes to PC properties throughout the life cycle. This enables understanding of how NM behave in their environment and how interactions such as agglomeration and dispersion impact on real-life exposure scenarios (e.g. routes of exposure, internal dose received) and the various mechanisms of biological action. While a number of techniques are available to characterise NM, it is not yet certain that the most appropriate PC properties have been identified. Many of the remaining limitations in this area result from a lack of technologies and standardised methodologies for measuring PC properties in complex matrices and discriminating between released and background NM at various stages of their life cycle. A critical obstruction to research in this area seems to be represented by a lack of NM standards, or specifically, a 'gold standard' against which all measurements/tests would be compared. Since characterisation and testing at all stages of the NM life cycle may be sub-optimal or not possible (time and resource constraints), modelling approaches such as structure-activity relationships (SAR) should be encouraged. It is important that this information is assessed and verified, and gaps identified, in order to reduce the need for hazard testing by developing increasingly reliable models. While studies are ongoing to generate quantitative SAR (QSAR) approaches for nanomaterials, no such methodology is currently available and validated on a broad array of materials. To support such development, and reduce the high levels of uncertainty currently existing, work at all levels (characterisation, exposure, hazard and risk) is required to generate sufficient appropriate and reliable information. These in silico models should also enable read-across to conventional chemicals in bulk forms, as well as read across between various model species and between human and environmental studies. In order to make use of read-across for nanomaterials based on informative materials, a greater understanding of the fundamental drivers of toxicity based on PC characteristics is needed. The risks of human exposure to engineered NM have been comprehensively reviewed in several publications which arrive at similar conclusions about the priorities for future activities relevant to exposure assessment. These can be summarised as:

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

There are a number of issues affecting exposure estimation including the need for better discrimination and characterisation of NM, the application of exposure models, the choice of metric, and instrument and measurement strategy. There is limited evidence of validation for

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

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

3 INTELLIGENT TESTING STRATEGY

The ambition is to prepare the foundation for more structured future research concerning the interactions of NMs with living systems in order to intelligently design nanosafety evaluation and risk assessment strategies, to include rapid screening, computational models, identification of high risk materials, and implementation of strategies to counter these risks.

The strategy is to evolve general rules to modelling, grouping or ranking NMs. Such grouping or ranking can then be used to prioritize NMs for a full risk assessment. In the shorter term, the general rules will need to be subsidised by screening and modelling, either individually or in combination, to rank the NMs Ideally, the developed models will need to be stochastic and predictive, which can take into consideration the known uncertainty.

The future research strategy should focus on the issue species for nanomaterials (NMs) as compared to other materials in order to understand the properties that make them different from conventional chemicals. This research should cover identification of the physicochemical characters, the rules that govern exposure and hazards, and how these three areas can enable a grouping, ranking or modelling of the NMs in relation to risk. The research should also have a fundamental approach, which enables a further development of novel materials with inherent sustainable founded characters.

Within each of the above areas, specific issues have been prioritised. Although the individual areas do work – to some extent – independently, it is to perform integrated research in order to advance as fast as possible. Hence, it is necessary to combine the identified priorities (available in the Knowledge gaps document at the project website) in a combined supportive way in order to get the optimal course for progress

4 IMPLEMENTATION

The aim is to develop and provide best practices which can be implemented within the currently research and evolving regulatory framework for NMs. To achieve such 'best practices' for risk assessment, the specific knowledge gaps from the Knowledge Gaps report need to be addressed in a future research strategy.

Most of the points in the "best practice" relate to increased information on NM hazards and exposure by use of hazard data from multiple sources, including read-across and QNAR data as well as data on exposure from validated models. This is not expected to create any obstacles as such with respect to risk assessment of NMs within the current risk assessment framework. In fact, reduced vertebrate testing is already a key objective of the current framework. The use of alternative methods such as *in vitro* tests and *in silico* models is already foreseen, provided that the methods are validated and a scientific justification is given. Read-across from nano and non-nano analogues is also possible if

scientifically justified on a case-by-case basis. However, additional guidance will be required for correct interpretation and integration of the NM data and to further improve the regulatory acceptance.

5 CONCLUSIONS

The aim of this presentation is to provide a description of the work performed within the ITS-NANO Project (www.its-nano.eu) to develop an intelligent testing strategy for engineered nanomaterials.

The work started from the identification of the current knowledge gaps, followed by their prioritisation during a Stakeholders Workshop held in Edinburgh in September 2012. These input has been the basis to develop the Intelligent Testing Strategy, which latest draft version will be discussed during another workshop to be held in Venice, Italy, in March 2013, to achieve a succesful launch in May 2013.

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