MODERN

MODelling the EnviRonmental and human health effects of Nanomaterials

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MODERN is cooperating with all ongoing modelling projects within the FP7-NMP Programme.

Contents

1   Summary

Nano-sized materials are a common element in many industrial processes mainly due to their unique properties that lead to the production of high technology products. The widespread use of nanotechnology requires the consideration of the environmental and human health risks that may result from the introduction of engineered nanoparticles (eNPs) into the environment. Although toxic effects for certain types of eNP have been recently reported, there is still a lack of knowledge about their possible long-term effects in biological systems.

The project focuses on the understanding of the processes governing the interactions of nanoparticles with biological systems and their associated mechanisms of toxicity, which are essential for eNP safety assessment. Information on the effects of well-characterized eNPs will be obtained from literature and other data repositories. Targeted in vivo and in vitro experiments will be also carried out to overcome the limitations of data availability and for model validation. Computational methods will be applied to model both nanostructure-property relationships and the complex and highly non-linear nano-bio interactions and to diminishing the need for animal testing.

The main goal of MODERN is to establish new modelling approaches suitable for relating nanotoxicity with the intrinsic molecular and physicochemical properties of eNPs at environmental exposure levels and to implement safe-by-design nanoparticle design strategies. This implies three specific objectives: (i) To apply computational models for the characterization of the structural and physicochemical properties leading to QNPRs and safe-by-design strategies for eNPs; (ii) to develop *in silico* models (QNAR) of biological activity of eNPs in the body and in the environment; and (iii) to establish a categorization and hazard ranking protocol for eNPs based on structural similarity principles and in the analysis of their toxicological profiles.

2   Introduction

As a growing applied science, nanotechnology has considerable global socioeconomic value, and the benefits afforded by nanoscale materials and processes are expected to have

significant impacts on almost all industries and areas of society. By 2015, the nanotechnology economy is estimated to be valued at 2.2 trillion €. Currently, in the conditions of the worldwide global economic recession, exponential growth of population, shortage of food, feed, fuel and raw materials and increasing environmental and societal problems, nanotechnologies have big expectations in almost every domain, from energy production to medicine. Moreover, nanotechnology has been referred to as the next industrial revolution. However, the safe application of nanotechnology at an industrial scale requires the careful consideration of the potential environmental and human health risks that may result from the introduction of engineered nanoparticles (eNPs) and nanomaterials in the environment. Nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm - 100 nm. ENPs may have a protective effect or pose new potential hazards towards living organisms, in part because their structure and properties are new and have not been previously introduced into the natural evolution processes. As hazard evaluation involves toxicity testing that is very laborious, limited (e.g., not all relevant issues can be tested), costly and still heavily relies on animal experiments, the introduction of in silico/QSAR (quantitative structure-activity relationships) approaches to nano(eco)toxicology is crucial, important and a challenging task. The need for cost-effective high throughput methods is even more obvious for eNPs than for 'regular' chemicals, since increasingly produced eNPs vary in composition, size and coating, leading to a huge number of combinations of chemical entities with different physicochemical properties (and thus different toxicity potential).

Understanding the implications of nanotechnology in our society and assuring its safe use requires a multidisciplinary research effort aimed to establish the basis for updating current regulatory framework regarding the safe use of these technologies. Indeed, there is an increasing risk of exposure to eNPs since the number of applications and consumer products on the market involving nanomaterials is increasing rapidly. For example, nanoscale iron for the remediation of contaminated groundwater, TiO_2 and ZnO nanoparticles for personal-care products (toothpaste, beauty products, sunscreens, and textiles) manufacturing, and nano-silver (n-Ag) as an antimicrobial additive in detergents, food packaging and clothing, such as socks and underwear. In addition, exposure at the workplace may adversely affect human health, especially in occupational exposure scenarios related to eNPs production, manipulation, and research. The study of eNP effects in diverse biological systems is currently of great interest. Although there is a growing availability of eNP data (primarily for n-Ag and TiO_2 and carbon-based eNPs), the potential harmful effects and mechanisms of action are still not fully understood. As of 27/01/2010, the ISI Web of Science had more than 100,000 publications retrievable by keyword "nanoparticles," and within this set of records only 87 were retrieved by refining the search by keyword "occupational", 73 by "hazard" and just 16 by "ecosystem". Although conventional toxicity data are rapidly emerging and High Throughput Screening (HTS) and High Content Screening (HCS) are contributing to the understanding of eNP - organism interactions, there is still a lack of fundamental understanding regarding these mechanisms. The acquisition of this knowledge is critical to characterize the hazard of eNPs, which is in turn indispensable for proper corresponding risk assessment and the development of appropriate environmental and health regulatory policies.

3 Background

Recent advances in nanotechnology and the corresponding increase in nanomaterials use in everyday products have resulted in uncertainties regarding their environmental and human health impact. The environmental risk assessment of eNPs requires information on their potential emission sources, properties in the nanoscale, intermedia distribution, transformations and persistence, and on their adverse effects. Every step of this process is uncertain, starting from the emission estimates and rates of entry and mobility in the environment to the eNPs effects on specific endpoints. This is a huge task and there are extensive knowledge gaps. Thus, this task has to be simplified, to yield meaningful information in relatively short time and using reasonable amount of economic resources. Analogous challenges are currently met by chemical industries due to the implementation of REACH regulations where Integrated (or intelligent) Testing Strategies (ITS) play a key role.

Despite the impressive knowledge gaps, existing risk assessment/classification tools may still provide helpful insight. In addition, there is ample strategic information accumulated during several decades of human health and eco-toxicological studies on bulk chemicals (e.g., choice of test organisms and validation of test protocols) as well as new toxicogenomic methods suitable to obtain new mechanistic knowledge on eNP-induced stress response on relevant model organisms. This information should be considered when categorizing and ranking eNPs. Ranking of eNPs requires understanding the mechanisms that govern their interactions with biological entities. It has been recently demonstrated that it is possible to establish quantitative nanostructure–activity relationships (QNAR) to describe the effects induced by eNPs in living cells/organisms, as done for conventional chemicals.

The importance of developing QNARs for category formation, hazard ranking and ultimately risk assessment and safe nanoparticle design has been recognized in specific EU workshops (e.g., COST Exploratory Workshop on QNTR, Maastricht, 2011). The effects of eNPs in environmental conditions strongly depend on their bioavailability and toxicity mechanisms, which in turn are modulated by their physicochemical and structural properties. Therefore it is fundamental to gather and integrate the experimental data available in the literature and in public data repositories to develop a well-characterized nanoparticle knowledge base necessary to drive the computational effort required to develop predictive nanotoxicity models. In addition, it is essential to develop a new generation of molecular descriptors suitable to describe eNP property profiles from their structure. The above eNP property profiles together with the in vitro/in vivo screening of toxicity will facilitate the generation of eNP

signatures suitable for establishing categories based on similarity criteria.

Computational characterization of structural and physicochemical properties for safe-by-design nanoparticles

The identification of the hazard potential of nanoparticles to prevent harm to humans and the environment is of utmost importance for the widespread implementation and acceptance of nanotechnology. The production of safe nanomaterials requires understanding of the interactions between nanoparticle structure, properties and biological activity. Safe-by-design strategies for engineering nanoparticles can be implemented by introducing changes in the structure of eNPs, which in turn modify their intrinsic properties and effects. These strategies can be implemented by doping (introducing foreign element in the crystal lattice of the parent oxide - substitution or interstitial - to change the property of the material significantly with no detectable changes in the crystal structure) or by surface functionalization (i.e., surface adsorption of chemical species with a specific functional group responsible for the change in NP properties). The adoption of safe-by-design strategies either by introducing a dopant in the lattice or by functionalizing NP to generate a specific binding site for cellular proteins (protein corona) will help in the rational design of eNPs with reduced environmental and human health risks.

Therefore, specific nanoparticle descriptors have to be developed to describe intrinsic eNP properties such as surface chemistry, area, charge and reactivity, and structure-dependent electronic configuration using computational models. From the view point of molecular modelling, (1) nanoparticles are rather large and complex systems relative to single molecules and (2) the respective properties do not only depend on the molecular structure but also on physical properties of the nanoparticles. Currently, the number of existing models to address the physicochemical properties and biological activity of eNPs is very limited. However, until present no theoretical (molecular) descriptors have been developed that address the true properties of eNPs that depend on particle size. Moreover, available descriptors are only applicable to a limited range of eNPs (e.g., metals, metal oxides and carbon structures) without the capability to describe the properties of all possible eNPs. Therefore, a promising approach for developing QNARs is to construct integrated descriptors through chemoinformatics algorithms and to use them in subsequent regression analyses. This requires methods addressing the atomic and structural composition of nanoparticles in terms of their major building blocks, as well as considering particle size, shape and porosity. One possible approach would be to derive the information of the chemical composition from string notations (SMILES; InChI) as has been recently suggested. Chiral vector or atom-centred fragment type parameters, as proposed for carbon materials, can also be used as the basis for generating descriptors.

In silico profiling of environmental and human health impact of nanoparticles

Despite the rapid increase in the number of reports on hazardous properties of eNPs, there is still no clear pattern as to what are the major eNPs issues regarding toxicity. A variety of eNPs have been tested for ecotoxicity, but it has hitherto not been possible to identify which eNP characteristics are more relevant to predict toxicity in general. Emphasis has been centred on the surface-area and degree of agglomeration. However, it is generally assumed that for easily soluble eNPs, such as zinc, toxicity may be related to both released ions and to particle properties. Thus, mechanistic data are controversial, mostly due to the limited amount of available information but also due to intrinsic methodical difficulties such as limited solubility and aggregation/agglomeration in the test media. Several *in silico* methods for bulk chemicals have been developed as an alternative for *in vivo* and *in vitro* toxicity data generation within the framework of REACH. For eNPs, there are still few self-consistent toxicity databases and *in silico* models despite the amount of data published in the literature. The lack of well-structured and complete data repositories regarding structure, properties and activity of eNPs hinders the development of reliable *in-silico* nanotoxicity models and the subsequent eNP hazard ranking.

Even though concentration and size effects for eNPs have been reported, there is still the need for more universal QNAR models capable of predicting *in vitro/in vivo* activity profiles of current and novel eNPs and to provide a sound basis for the design and manufacturing of better, cheaper and safer products. In addition, preliminary studies on the environmental distribution of nanomaterials delineate exposure scenarios involving much lower nanomaterial concentrations than the ones typically used for in vitro HTS nanotoxicity assays and modelling. Genomic and proteomic studies demonstrated that exposure to low concentrations of eNPs may disrupt basic biological functions at the cellular and sub-cellular levels, which not always translate to observable cytotoxicity effects. These perturbations, however, may act as early indicators (similar to biomarkers) of much severe nanoparticle impacts and long-term effects (Fig. 1).
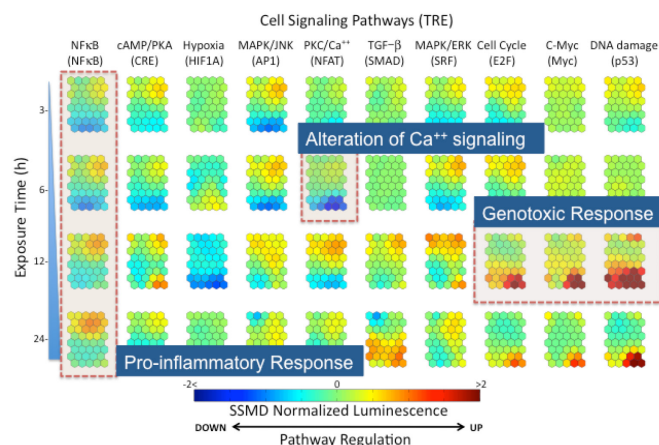

Figure 1. Knowledge extraction from HTS datasets

Nanoparticle categories and hazard identification and ranking

Despite the incipient efforts in nanotoxicity modelling, the large number of possible nanoparticle types (e.g., diverse combinations of core, surface modifications and functionalizations) hinders the development of universal QNPR and QNAR models. It is thus fundamental to develop similarity

metrics (based on nanostructure descriptors, physicochemical property profiles and biological activity) suitable to group nanoparticles into homogeneous categories where highly accurate and reliable models can be developed and validated. The establishment of eNP categories will also facilitate the ranking of their environmental and human health impact and will pave the way to the development of a risk assessment framework for nanomaterials.

4 Project Description and Organisation

The Project is structured in three research work packages (WP) designed to fulfil the specific objectives outlined above. Two additional non-research packages will account for the dissemination and exploitation of results and for the management and coordination of the project. The description of the S&T WPs and their knowledge domains (Fig. 2) is as follows:

WP1. Physicochemical, molecular and structural properties of eNPs (Lead: UFZ)

Main Research activities:

1. Development of new molecular descriptors and their associated calculation methodologies suitable to describe eNP molecular structure and properties.

2. Synthesis and characterization of eNPs targeted for QNPR model validation and to test safe-by-design hypothesis.

WP2. In silico profiling of environmental and human health impact of eNPs (Lead: URV)

Main Research activities:

1. Gathering literature data on *in vitro/in vivo* profiling of eNP effects on relevant environmental endpoints in aquatic and terrestrial ecosystems.

2. Development and analysis of integrated signatures describing structural, physicochemical and toxicity profiles of eNPs suitable for model development.

3. Development, validation and mechanistic interpretation of QNPRs.

4. Development, validation and mechanistic interpretation of QNARs for eco-toxicity and human health.

WP3. Identification of eNP categories and basic hazard ranking (Lead: AU)

Main Research activities:

1. Development of a data mining framework suitable to identify similarity patterns on eNP signatures.

2. Development of a hazard ranking scheme suitable to rank eNPs and their categories according to their potential environmental and human health impact.
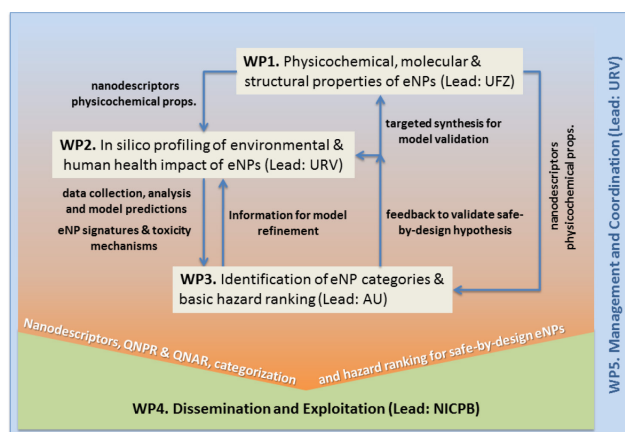

Figure 2. WP interdependencies and information flow

Project data sources and data quality control

The eNPs used in the project and their corresponding *in vivo/in vitro* toxicity data will be selected according to the last OECD, EU and US-EPA guidance documents. An initial subset of metal and metal oxide eNPs already available within the MODERN consortium will be considered in WP1 and shared across WPs. It will be used to develop QNPRs and safe-by-design approaches based on either solubility/redox potential mediated toxicity for metal oxide eNPs or on surface reactivity controlled by functionalization for metals. The MeOs in this subset with the necessary biological activity information will be used to develop nanotoxicity models and the hazard ranking scheme assuring that MODERN main goals can be attained without an excessively risky dependence on external data sources. Information on other nanomaterials, including those that could be outside the current EC definition, will also be collected from the literature and other sources (e.g., data available at the *Institut Catala de Nanotecnologia* (ICN) and the Centre for Nanobiosafety and Sustainability) (CNBSS); to complete the above first subset of eNPs. Additional nanomaterials may be added based on data availability and modelling requirements. The environmental effects of nano-scale chemicals (essentially metal and metal oxides) will be contrasted with the effects of the respective bulk chemicals whenever possible.

Consortium *in vitro* data will include diverse toxicity-related endpoints for protozoa, bacteria, yeast and algae. Data corresponding to *in vivo* assays will be collected for soil invertebrates, fish and mammalians. Regarding human health effects, human bronchial epithelial cells and murine macrophages will be used to evaluate toxic and inflammatory effects via a combination of single and multi-parameter assays. In addition to all the above data collected from diverse data sources available within the consortium, targeted *in vitro* experiments will be conducted to test the safe-by-design hypotheses.

5 MODERN activities

The global modelling-related challenges to be addressed within the current proposal are: (i) the development of nanoparticle categories based on their physicochemical, structural and toxicological properties, including their environmental and human health impacts, and (ii) the development of computational approaches for nanostructure characterization (nanodescriptors) and *in silico* models to assess nanoparticle effects.

The specific objectives to attain the main research goals are:

Build a well-characterized library of eNPs with a comprehensive description of their structural, molecular and physicochemical properties.

This will be accomplished by gathering available data with nanoinformatics tools and implementing physicochemical computational models to: (i) **determine** the relevant physicochemical properties of eNPs and characterize their molecular level structure by using specific descriptors suitable for nanoparticles (i.e., geometrical, topological, electronic, 3D, hydrophobic); and (ii) **perform** targeted synthesis and characterization of eNPs to validate QNPRs and to test hypothesis for safe-by-design strategies based on doping and/or surface functionalization.

Develop and validate *in silico* models of biological activity of eNPs in organisms and in the environment from *in vitro*/*in vivo* profiling data.

To attain this objective a project database system, interoperable with existing repositories, will be generated from literature information, collaboration with other NMP ongoing projects, and targeted new experimental findings for additional training and validation of *in silico* approaches to predict relevant physicochemical properties of eNPs from their structural information (QNPR) and their nano(eco)toxicity (QNAR). In addition, preliminary data management and analysis strategies that could be suitable to discover and study molecular signatures (e.g., genetic mapping) representative of sub-cellular process triggered at low concentrations will be screened from any available HTS data since these exposures that are more realistic in terms of emission rates could ultimately lead to observable long term adverse (e.g., toxicity) effects.

Define and implement a categorization and hazard ranking methodology for eNPs based on structural similarity principles and toxicological profiles.

This will be achieved by (i) using data mining algorithms to identify categories of nanoparticles with common signatures (i.e., structural, physicochemical and toxicological profiles); (ii) integrating QNPRs and QNARs into a hazard ranking/decision framework, which will be developed with weight factor approaches or multicriteria decision analysis (MDA).

6 Directory

Table 1 Directory of people involved in the MODERN project as beneficiaries*.

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