MODERN

MODeling the EnviRonmental and human health effects of Nanomaterials

Contract Agreement: 309314 Website: http://modern-fp7.biocenit.cat
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Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	Universitat Rovira i Virgili	URV	Spain
2	Helmholtz-Zentrum fuer Umweltforschung GMBH-UFZ	UFZ	Germany
3	Aarhus Universitet	AU	Denmark
4	Keemillise Ja Bioloogilise Fuusika Instituut	NICPB	Estonia
5	Universitaet Bremen	UNIHB	Germany
6	Tartu Ulikool	UT	Estonia
7	University of California Los Angeles, UC CEIN	UCLA	United States

Contents

1 Summary

Project Duration: 3 years

Project Funding: 999,816 €

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 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 a 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 modeling, (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-centered 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 centered 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 modeling. 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).

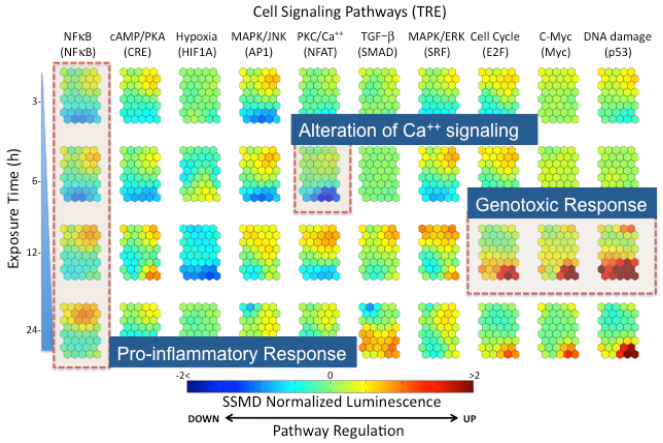


Figure 1. Knowledge extraction from HTS datasets.

Nanoparticle categories and hazard identification and ranking

Despite the incipient efforts in nanotoxicity modeling, the large number of possible nanoparticle types (e.g., diverse combinations of core, surface modifications and functionalization) 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.

3 Scientific and technological challenges

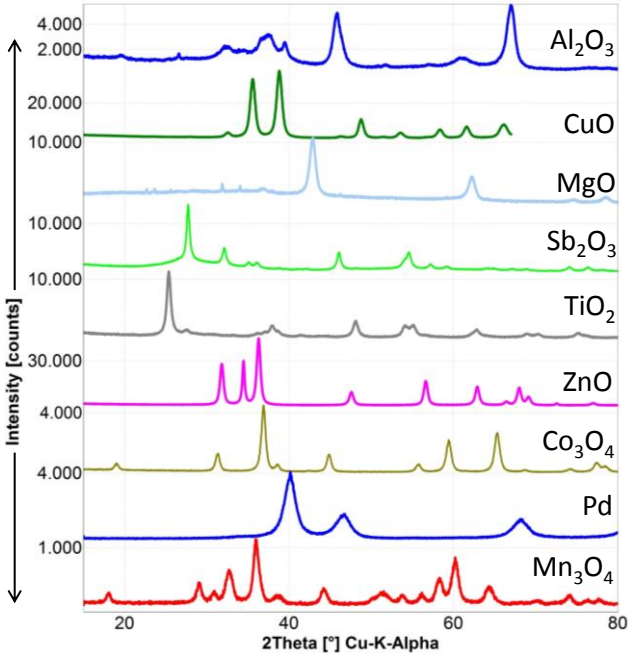
The global modeling-related challenges that are being addressed within MODERN are: (i) the development of nanoparticle categories based on their physicochemical, structural and toxicological properties, including their environmental and human health impacts, and (ii) the development of computational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

4 Objectives

The specific objectives to attain the main research goals are:

To 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 (Fig. 2).



Sample	Specific surface area (m^2/g)	BET size (d_{BET}) nm	Crystallite size (d_{XRD}) nm
CuO	72.4	13.1	12.2
ZnO	52.5	20.4	19.2
Al_2O_3	133.5	11.4	9.9
SiO_2	289.1	7.8	-
TiO_2	122.6	12.2	Anatase= 14.5 nm Rutile = 8.5 nm
Sb_2O_3	56.1	20.5	-
Mn_3O_4	80.9	15.2	11.6
Co_3O_4	102.3	9.6	8.6
Fe_3O_4	120.1	9.7	Fe_3O_4 = 8.2 nm, Fe_2O_3= 9.4 nm
WO_3	79.3	10.6	11.9
MgO	122.8	13.64	-
Pd	-	-	15.1

Figure 2. X-ray diffraction data and size characterization of the MODERN eNP library.

To 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 (Fig. 3).

$$P(T|\boldsymbol{x}) = 1/(1+\mathrm{e}^{-f(\boldsymbol{x})}) \qquad f(\boldsymbol{x}) = \sum_{i=1}^{6} \alpha_i \mathrm{e}^{-2\left[(x_{i,1}-x_1)^2+(x_{i,2}-x_2)^2\right]} + b$$

Support vectors:{ZnO, Ni_2O_3, Mn_2O_3, NiO, CeO_2, Fe_2O_3}

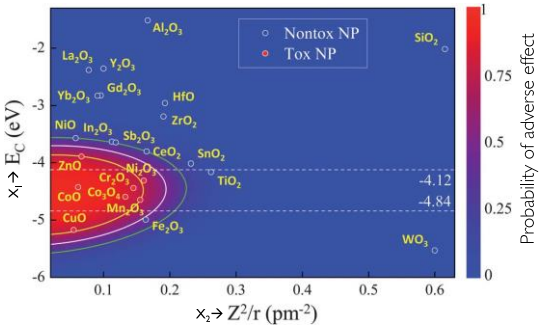


Figure 3. SVM-based toxicity model for metal oxide eNPs.

To 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).

5 Progress and Outcomes to date

The main results achieved after the first 18 month include:

1. Development of a federated database system for storing nanosafety data in ISA-TAB Nano compliant format. The data management system also includes a web-based ISA-TAB Nano validator.
2. Preliminary library of metal and metal oxide nanoparticles synthetized and fully characterized. The library includes: CuO, Co3O4, Sb2O3, TiO2, WO3, ZnO, Mn3O4, Fe3O4, MgO, Al2O3, SiO2 and Pd.
3. Validation of two approaches for the development of nanodescriptors. The first method is based on the modeling of the electronic properties of basic building block of metal oxides and their subsequent use to infer the properties of larger structures. The second, based on molecular dynamics simulation, allows the fast computation of about 40 size-dependent nanodescriptors including constitutional, potential energy, coordination numbers and lattice energies.
4. Development of a web-based tool for the integrated analysis of genes and pathways of organisms exposed to nanoparticles.
5. Biological characterization of the eNP library in (2) using algae (P. subcapitata), protozoa (T. termophila), bacteria (E. coli, V. fischeri, S. aureus). Results showed that CuO and ZnO were the most toxic to all tested organisms, most likely due to dissolution.

Current results have been disseminated in different international conferences as well as in a number of peer-reviewed publications.

Publications (first 18 months):

Liu R, France B, George S, Rallo R, Zhang H, Xia T, Nel A, Bradley K, Cohen Y. (2014) Association Rule Mining of Cellular Responses induced by Metal and Metal Oxide Nanoparticles. *Analyst*, 139: 943-953

Ivask A, Juganson K, Bondarenko O, Mortimer M, Aruoja V, Kasemets K, Blinova I, Heinlaan M, Slaveykova V, Kahru A. (2013) Mechanisms of toxic action of Ag, ZnO and CuO nanoparticles to selected ecotoxicological test organisms and mammalian cells in vitro: A comparative review. *Nanotoxicology*, Early Online: 1-15

Gómez S, Fernández A, Granell C, Arenas A. (2013) Structural Patterns in Complex Systems Using Multidendrograms. *Entropy* 2013, 15(12): 5464-5474

Liu R, Hassan T, Rallo R, Cohen Y. (2013) HDAT: web-based high-throughput screening data analysis tools. *Computational Science & Discovery*, 6: 014006

Liu R, Zhang HY, Ji ZX, Rallo R, Xia T, Chang CH, Nel, AE, Cohen Y. (2013) Development of Structure – Activity Relationship for Metal Oxide Nanoparticles. *Nanoscale*, 5(12): 5644-53

Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A. (2013) Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review. *Archives of Toxicology* 87(7): 1181-1200.

6 Expected Impact

MODERN will contribute to the assessment of eNP effects and to the reduction of animal testing by **developing methods** for inferring toxicity of eNP via the integration of *in vivo/in vitro* studies **and in silico models**. These two elements will serve in the

long-term as the basic building blocks of a predictive framework that will guide the production of a new generation of safe-by-design eNPs. The research carried out in MODERN addresses the key research challenges of the NMP.2012.1.3-2 call and will impact several of the areas stated in the work programme and in the Nanosafety Strategic Research Agenda with the following contributions:

Development of new nanodescriptors and QNPR models from the computational characterization of molecular structure and physicochemical properties of eNPs.

Data-driven and *in silico* models of the effects produced by eNPs on biological systems (aquatic and terrestrial ecosystems) and development of eNP categories that will impact the nanosafety community by providing a comprehensive dynamic repository of eNP toxicity information, including not only toxicological endpoints but also mechanistic information on the nano-bio interactions.

Establishment of synergies with other FP7 and Horizon2020 projects as well as relevant national and international initiatives in Nanosafety.

Signature analysis to identify key molecular-level responses (genes, proteins and pathways) to establish early-detection mechanisms of potential long-term effects due to the exposure to low eNP concentrations.

MODERN will also contribute to the reinforcement of the international dimension of European research and collaboration between industry, researchers, environmental agencies, authorities (at Member State and European level) and international standardization bodies. To support governance in nanotechnology, MODERN will contribute to the development of an overarching strategy for risk management with the purpose of supporting EU regulatory bodies, agencies and authorities responsible for making informed decisions.

7 Directory

Table 1 Directory of people involved in this project.

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