

# Promoting the use of physics/chemistry-based materials modelling in assessing nanotoxicity for health and medicine

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Goal: Preparing an operational proposal for the next EMMC Road Map Meeting about 2018-2020 on May 20th 2016 (<http://emmc.info/>).

## 1. STATE-OF-THE-ART IN MODELLING NANOTOXICITY

Nanomaterials (NM), i.e. materials with at least one dimension in the nanoscale range, are currently produced in large amounts and large number of variants, which differ in chemical composition, size, shape as well as chemical surface modifications. Thousands of new nanomaterials are entering the market annually while even more are at the development stage. In many cases, the risks of personal or environmental exposure to these materials are unknown or poorly understood. For most original NMs of market value material characterization data are routinely collected. These are often referred to as *intrinsic NM properties (i.e. synthetic identity)*. However, NM properties can change during the life cycle (e.g. by degradation) or NM may, upon release for the matrix, experience non-intended interactions, especially when they come into contact with biological matrices or the environment. Therefore a similar number of system-dependent properties, also referred to as *extrinsic properties (i.e. biological identity)*, have to be collected as well, to allow for toxicity prediction. Both intrinsic and extrinsic properties influence the fate and the effects of NM on cells or tissues [1].

Nanotoxicity modelling aims to predict the health risks associated with the use of NM. Risk is defined as the probability that exposure to a hazard will lead to a negative consequence for the organism, or more simply, Risk = Hazard × Exposure [2]. Hence modelling nanotoxicity is generally based on two types of models:

- Exposure models
- Hazard models

*Exposure models* aim to predict how NM propagate and evolve in the environment, including environment-induced aggregation and formation of complexes with environmental substances and biomolecules, and hence NM may harm human health and/or wildlife. In addition, physiologically-based pharmacokinetic models (PBPK) are used to predict the actual dosage of NMs in a particular organ or tissue. *Hazard models* are intended to predict what happens when NMs come in contact with living organisms. Hazard models can include three different layers:

1. Modelling how electronic, atomic, and molecular structure and composition determine the *nano-descriptors*, which summarize the most relevant information about NM, including the properties of concern (materials modelling). These are usually physics and chemistry based models;
2. Modelling how the nano-descriptors are correlated with most significant *biological endpoints*, namely those specific biological events, which are supposed to trigger or produce adverse effects (biological event modelling). This modelling is often done at the statistical level;

3. Modelling how one or more endpoints are significant markers *adverse events* for living cells in contact with NM (computational systems biology). This modelling is done at the level of mass-balance equations, ODEs and statistics.

## 2. MOVING FORWARD MODELLING TOXICITY IN THE NEXT 10 YEARS

It is generally accepted that the established procedures in safety assessment are very time and cost intensive and often involve animal testing. Most of currently existing cheaper alternatives based on *in vitro* or *in silico* tests do not possess sufficient predictive power to be used for regulatory purposes. A systematic understanding how NM properties (intrinsic and/or extrinsic) are related to specific biological and environmental fate and effects is still lacking or only emerging [1]. This kind of knowledge is necessary to identify the NM properties of concern and enable categorization or grouping of NM with similar properties and/or bioactivity profiles, which is prerequisite for the development of targeted models [1].

Therefore, in order to reduce the costs of testing and the need for animal testing in the next ten years, it is fundamental to improve the *mechanistic understanding of toxicity*. In particular, the goal is to elucidate the molecular basis of the biological activity of NMs. On the other hand, the understanding of bio-nano interactions would also allow for designing NMs with specific biological activity (i.e. safe/toxic-by-design NM). We envision a development of computer-assisted design of safe NM, optimized for specific applications in health and medicine over the next decade.

### 2.1 Systematic exploration of nano-descriptors for hazard predictions by materials modelling

Multiple nano-descriptors of interest can be screened with existing modelling tools, starting from basic ones like chemical composition of the core and coating, size, shape, mass density, but including also complex ones like conduction band gap, ionization potential, dissolution rate, hydration energy and surface energy. Materials modelling based on the physics and chemistry of NM will allow clarifying how the relevant nano-descriptors depend on electronic and atomic properties of NM in a systematic way (step 1: from electronic/atomic details to nano-descriptors, see section 1). Interaction of NMs with biological macromolecules (nucleic acids, proteins, lipids, carbohydrates) can also be predicted for some materials via available computational tools, providing additional nano-descriptors and hints on the possible mechanisms of NM toxicity. Yet, a quantitative description of the bio-nano interface is not currently possible for arbitrary materials/ NM properties. Open questions also remain on how such nano-descriptors and mechanistic insight can be linked to current toxicity models. At the moment, most quantitative toxicity models rely on purely statistical correlations between the NM properties and the toxicity endpoints. Using in depth characterization of NMs with modelling tools, we will be in a position to step forward from the black-box-type statistical approaches and formulate a new paradigm in nanotoxicology – a mechanism-aware NM toxicity screening. An action coordinating material modellers and toxicologists is required to improve the hazard models by establishing the relationships between materials properties and biological activity. On the other hand, materials modelling can enhance the predictive power of statistical toxicity models, which include also statistical regressions and systems biology, by providing the relevant nano-descriptors and mechanistic insights. Progress in the mechanistic understanding of toxicity is crucial for future development of materials that are safe-by-design.

With regards to the above goal, the question of data quality is central for material characterisation and screening *in silico*. Provision of necessary data quality requires standardization of methods for data sharing and data cross-validation. In addition, some of the underlying physical/chemical models for materials must be tailored for the specific interaction with biological matter. In particular, the current challenges are:

- lack of appropriate force fields for molecular simulation of interface properties: most of the existing force fields are derived for homogeneous systems and neglect the interface effects; hence advanced approaches are needed for describing interfaces, including quantum effects;
- lack of hydrophilicity/hydrophobicity data for NM surfaces, including their coatings: these properties are essential as most relevant interactions occur in aqueous media and involve competition between water and biomolecule adsorption;
- lack of data on protein/lipid adsorption on NM including quantum effects;
- lack of efficient codes to treat long-range molecular (van der Waals) interactions in heterogeneous systems;
- need to close large time- and lengthscale gaps between the necessary molecular details and the phenomena of interest: while material and biomolecule specificity requires an inclusion of atomistic and even quantum effects at the scale of Ångstroms and nanoseconds, the biomolecule interaction with NMs spans tens of nanometres and minutes; hence systematic coarse-graining methodologies are needed to transfer the specificity to the relevant scales.

As a meaningful example of how important these computational details are, it is worth to mention that, very likely, the most important effect influencing the uptake of NM by the cell and consequent transport is determined by the protein corona, which is extremely sensible to the interfacial properties of NM.

## 2.2 Improving aggregation studies for exposure predictions by materials modelling

Controlling the exposure is much more difficult and expensive than controlling the hazard. For example, controlling inhalation by humans and wildlife is problematic, because NM release in terms of aerosols and their aggregation is far easily controlled in the production processes. Multi-scale simulation approaches, in which the output of more detailed simulations is used as an input for coarse-grained simulations, need to be developed in order to predict NM aggregation. These approaches could be used also for studying NM transport across lipid membranes on experimentally relevant time and length scales is another relevant application. Hence *physics/chemistry-based materials modelling will improve the accuracy of exposure models by providing a better understanding of NM aggregation in different environments.*

From the perspective of environmental fate and transport, materials models can provide reliable input information (e.g., aggregation/agglomeration, sedimentation, solubility, partition coefficients) for multi-media modelling.

## 3. IMPACT OF MATERIALS MODELLING ON ENVIRONMENTAL AND HUMAN HEALTH

The use of NMs with enhanced properties facilitates the development of novel nano-enabled products and applications, which are expected to have a high impact in many EU industrial sectors. However, the increased presence of NM in consumer products and the transformations across their life cycle results in higher exposure and significant environmental and human health impacts. Therefore, it is fundamental to ensure the safety of all NM and nano-enabled products before they enter the market and arrive to consumers and the environment.

The development of accurate and reliable material models is fundamental for achieving the above objective since models can contribute to the discovery of the mechanisms that govern the interactions of NMs with elements in their surrounding environment. Materials models will also play a key role for the development of intelligent testing strategies for NMs, which will be cost-effective and will require less *in vivo* testing thus contributing to the 3R (replace, reduce and refine) objective. Specifically, the use of physics/chemistry-

based material models linked with exposure and hazard models will set the basis for the development of future computational nanosafety assessment frameworks that will facilitate the design of new NMs with precise property/bioactivity profiles. Further linking the above models with process simulators and process optimization systems will close the loop for the computer-aided design and production of NMs with properties and activity optimized for specific applications.

Materials modelling will clearly impact environmental and human health by:

- **Ensuring the safety of all newly produced NMs.** The development of novel NMs for all sorts of applications (e.g., energy storage, catalysis, water treatment) requires ensuring their safety from the environmental and human health viewpoints. Materials models will be key, not only for the design of novel NMs with specific properties, but also to identify and control the NM features that result in significant environmental and biological impacts. The integration of material models with exposure and hazard models will facilitate the development of safe NMs by including nanosafety constraints in its computational design. More specifically, a progress in modelling NMs and bio-nano interactions will facilitate faster definition of NM toxicity mechanisms, hazards and risks. Knowledge of the mechanism of action of each NM will enable identification of biomarkers for exposure and health effects. It will reduce the need for assessing morbidity and mortality and define new strategy for the assessment endpoints based on bio-nano interactions. Quantitative description of NMs will enable “safer by design” approaches, tailored to stakeholders’ needs (modellers, industry and regulators). A systematic study of interactions between the NMs and all the building blocks of biomolecules (BM) will enable a prediction of the outcome of interaction of arbitrary key molecules with the NM and the content of NM-BM complexes for any NM with known physicochemical properties. By scanning main groups of engineered NMs, one will be able to identify the NM properties that might be responsible for causing a particular toxic effect and lead to a particular adverse outcome, and thus should be modified or avoided. This will provide means of grouping and read-across characterization of NMs and enable development of materials that are safe by design.
- **Opening new ways for diagnostic and treatment of disease.** Personalized medicine is expected to be one of the biggest paradigm-shift in the future medicine. In this context, nanomedicine – mainly based on the use of nanoparticles for theranostics- is expected to play a determining role. Hence predicting the impact of different dose schedules on healthy and diseased bodies, cross-interactions among different nanodrugs and planning for administering drugs will be essential. With this regards, materials modelling may provide the right connection between nanodrug design (already done in some cases by materials modelling, at least as preliminary screening) and biological events. Material models can contribute to link structural and physicochemical features of nanodrugs with their diagnostic use and therapeutical effects (e.g., maximizing the killing of damaged cells while minimizing the effect on healthy cells). This goal is extremely challenging because the interaction of nanodrugs with each other and with the functioning of an organism is extremely complex, and predicting this interaction goes well beyond the approach relying on nano-descriptors. We would need to look at specific drug-protein interactions and then to develop a computational systems biology approach.
- **Reducing significantly (and ultimately avoid) animal experiments.** Estimates for the needs of animals for *in vivo* testing exceed what is considered reasonable in any modern society, where particular emphasis is given towards the need to reduce reliance on animal models. In some arenas

(such as cosmetics) particular rules eliminate the potential for animal testing. Materials modelling at the level of bio-nano interactions and PBPK should form the basis of a future screening strategy to predict the health effects resulting from exposure to NMs. Development of *in silico* toxicological tests will provide alternatives and replacements to support the 3Rs policy (Replacement, Reduction, Refinement) for minimizing the use of animals in toxicological research.

Materials modelling will clearly impact beyond environmental and human health by:

- **Clarifying organic-inorganic interactions.** The methodology for modelling bio-nano interactions will have wide implications for other fields like food science, pharmaceuticals, and medicine, where organic-inorganic interactions are important. Clearly the toxicity or otherwise of inorganic particulate food additives is of great significance to the industry and our methodology will be able to address this issue but the methodology for modelling protein/inorganic interfaces will have much wider impact. For example, in development of effective antifouling surfaces with applications ranging from water purification, biomedical implants, medical materials and products, surgical equipment and protective apparel, biosensors, textiles, food packaging, coatings in marine and industrial equipment. These models will offer significant insight into protein fouling of inorganic membranes both for medical applications such as filtering of body fluids but also in general for any filter in a wide range of industries where protein is present (biotechnology). Predicting the fate of nanoparticles at biological interfaces obviously impacts pharmaceuticals and the delivery of pharmaceuticals through NM vectors as in nanomedicine but is also of crucial importance in understanding and innovating for imaging technology, which rely on nanoparticle contrast agents. Any medical or industrial process which involves NMs or which involves biological / inorganic interfaces in the widest sense, will be impacted either directly through the use of the nanotoxicity predictive software or indirectly by the simulation methodologies.
- **Improving life-cycle analysis of all nano-enabled technologies.** Whilst in no way diluting the importance of nanosafety issues of consumer-oriented products, there is quite clearly a longer-term aspect of science to be addressed. Thus, the key issues of where nanoparticles go, and their interaction with living matter is a long-term question, which will have implications for life-cycle analysis of all nano-enabled technologies, including those never intended for contact with living systems. As the NMs present in consumer products and NMs used in medicine most likely share the same systemic pathways, the modelling of NM uptake and distribution kinetics will influence the biomedical research.

Overall, materials that are safe-by-design will contribute to boost the competitiveness of EU industry by providing a more cost-effective toxicity assessment and by attracting more users that will favor them over other NMs.

Besides the above mentioned fields which will benefit from materials modelling in assessing toxicity, it is important to highlight that this field is genuinely multi-disciplinary, and thus offers the opportunity to promote highly multi-disciplinary training for young scientists, including biologists, physicists and materials modellers.

Materials modelling for nanotoxicity would be the ideal challenge for exploring the full potential of the next-generation high performance computing (HPC), with the framework of the PRACE program (DG CONNECT).

## References

- [1] Andrea Haase, *EU Roadmap Nanoinformatics 2020*, NanoSafety Cluster (<http://www.nanosafetycluster.eu/>), (2016).
- [2] David Ropeik, *Risk*, New York, USA, (2002).