ENPRA Risk Assessment of Engineered NanoParticles

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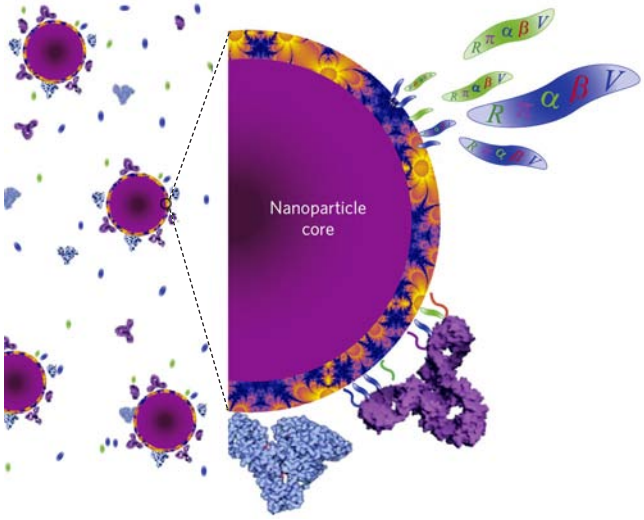

Environmental, Health and Safety Impacts of Nanoparticles

European Observatory on NanoSafety

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Schematic representation of competitive adsorption of small molecules and proteins onto the surface of nanoparticles. Reprinted with permission from Nature Nanotechnology 5, 671 (2010). Copyright 2010 Macmillan Publishers Ltd.
To see also: "North Carolina State Univ.: Sampling the surface of nanomaterials with molecular probes" on page 5.

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Environmental, Health and Safety Impacts of Nanoparticles

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Introduction

The third meeting of the European Observatory on NanoSafety (EONS) has been organized in Paris on October 6, 2010. The present document summarizes the presentations and discussions held during this event.

*About the **E**uropean **O**bservatory on **N**ano**S**afety*

The European Observatory on NanoSafety (EONS) is a collective initiative launched in 2009 by the Observatory for Micro&Nanotechnologies (OMNT) and the European consortium ENPRA (Risk Assessment of Engineered NanoParticles). Every 6 months, EONS meetings bring together experts in environmental health and safety issues related to nanoparticles and nanomaterials (including OMNT experts, partners of the ENPRA project and invited key scientists) and provide them the unique opportunity to collectively review and comment the latest research progresses of the domain. Topics addressed by the panel cover the full scope of 'NanoSafety' including detection and characterization of nanomaterials, toxicology, ecotoxicology, risk assessment and risk management as well as normative and regulatory aspects. Proceedings of the meetings are published by the OMNT.

Detection & Characterization

North Carolina State Univ.: Sampling the surface of nanomaterials with molecular probes

Reviewed by E. Burello

Predicting nanoparticle interactions at the nano–bio interface

When nanomaterials come into contact with biological systems, their surface gets covered by (macro)molecules which display a high affinity for the material's surface. This interaction forms the solid-liquid interface, which determines the behaviour of nanomaterials in biological systems. In order to identify and control the properties of such interfaces, **Jim Riviere** and colleagues of **North Carolina State University** reported an approach called the biological surface adsorption index (BSAI). Such method consists of measuring the adsorption of different molecular probes on the surface of a nanomaterial, each probe representing a unique profile of molecular forces involved in the interaction with the nanomaterial. The adsorption coefficients are then expressed as a function of 5 solute solvation descriptors calculated on the molecular probes: such descriptors represent the lone-pair electrons, the dipolarity/polarizability, the acidity/basicity and the London dispersion forces. The regression coefficients obtained are finally used as descriptors for the nanomaterials.

The method proposed is one of the first examples in literature aiming at predicting the physicochemical properties and behaviour of nanomaterials in complex, biological systems (Figure 1). Moreover, the BSAI approach has the advantage of being applicable to any kind of nanomaterial, opening up the possibility to compare different nanomaterials.

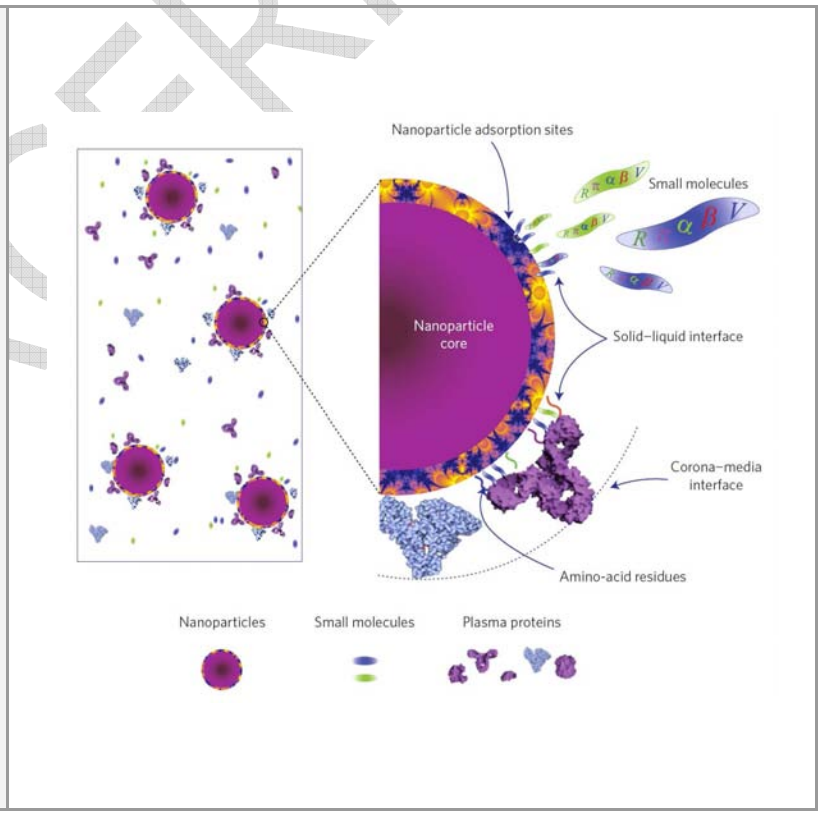

Figure 1: Competitive adsorption of small molecules and proteins onto the surface adsorption sites of nanoparticles.
Left: in a physiological environment, nanoparticles are exposed to different proteins and small molecules.
Right: the competitive adsorption of small molecules (upper) and the amino-acid residues of proteins (lower) on a nanoparticle. The orange ring on the nanoparticle with blue irregular shapes represents the adsorption sites that are not uniformly distributed on the nanoparticle surface. Small molecules with known molecular descriptors [R, p, a, b, V] can be used as probes to measure the molecular interaction strengths of the nanoparticles with small molecules and biomolecules.
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One direct application of the BSAI approach is to predict the adsorption of small molecules onto nanoparticles, which is a critical process for nanomaterials in biological and environmental systems. The method could also be used to correlate with the membrane interaction and biodistribution parameters (such as absorption rate, distribution coefficient and extent of cellular uptake) to develop physiologically based pharmacokinetic models. In this context, the model is limited to describe the adsorption of small molecules and its use for predicting the adsorption of larger and

more complex macromolecules is not straightforward. Complex phenomena not taken into account by the model and which occur when a macromolecule adsorbs on the surface of a nanomaterial (e.g. the structural changes that a protein undergoes when it gets into contact with a nanomaterial's surface), might play a fundamental role in the adsorption of polymers and proteins.

The method, however, is still useful for grouping nanomaterials which display similar surface properties. Using such assumption, one can expect that nanomaterials with similar surface properties will likely display similar adsorption profiles and therefore will form a similar protein corona.

Another relevant issue that has not yet been considered is the determination of the surface pattern of a nanomaterial, for example the density of surface charge or crystal defects, which control the binding mode with large structures such as biomembranes.

"An index for characterization of nanomaterials in biological systems" ; X.R. Xia, N.A. Monteiro-Riviere, J.E. Riviere : ***Nature Nanotechnology*** **5**, 671 (2010).

EONS12-10-1

EXCERPT

Univ. of Edinburg: Metal oxide inflammatory footprints

Reviewed by J. McLeish

Variability of the immuno-inflammatory responses elicited by metal oxide nanoparticles

There is a perceived need for rational toxicological (hazard) assessment of the large number of untested NP and their variants (size, derivatisation, composition, etc.), but there is also strong ethical and financial pressure to carry out such toxicological testing using in vitro approaches. The findings presented here by the **University of Edinburg** sound a note of caution on the reliance on purely in vitro testing regimes and strongly implies the usefulness of in vivo models, since the variability in the type of inflammatory responses, its tempo, and the ultimate types of pathology seen could never have been predicted, nor detected by current in vitro assays.

Four metal oxide nanoparticles, cerium oxide (CeO_2), copper oxide (CuO), nickel oxide (NiO) and zinc oxide (ZnO), when instilled into the lungs of rats, promote different inflammatory responses. Time points of 24 hours and 4 weeks were used for assessing acute and chronic inflammation, where bronchoalveolar lavage (BAL) fluid, inflammatory mediators and lung histology were analysed. Results clearly show that the recruitment of neutrophils to lung tissue was driven by NiO and CeO_2, eosinophils by ZnO, and neutrophils and eosinophils by CuO at 24 hours. Additionally ZnO was found to be highly cytotoxic. At 4 weeks however NiO caused sustained inflammation and severe cytotoxicity. MIP-2 is detected in the BAL along with IFN-γ, there is an increase in recruitment of neutrophils and lymphocytes (B and T cells) to tissue, and alveolar lipoproteinosis. Granulomatous inflammation is seen in Cuo and ZnO exposed rats along with fibrosis, though cytotoxicity has been resolved. In the case of ZnO, eosinophils are still present and lung pathology shows collapsed alveoli. Interestingly, no fibrosis occurs with NiO exposed rats at this time point.

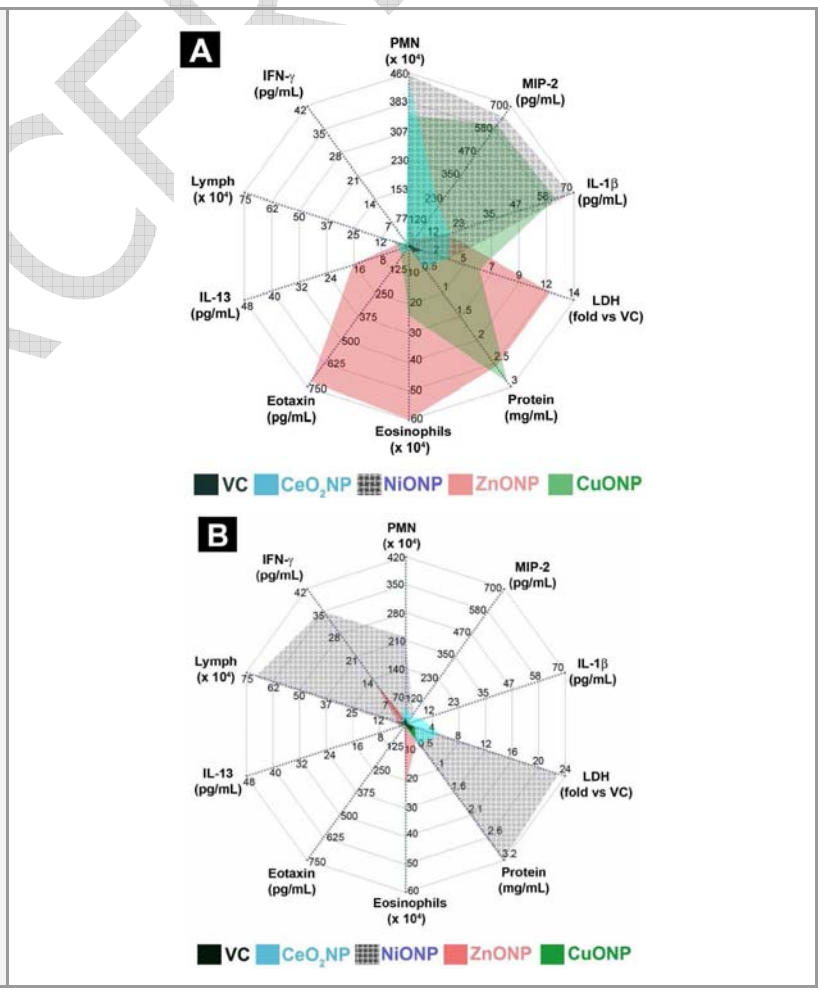

Figure 9: Vector diagrams showing trends and types of (A) acute (24 h) inflammation and (B) chronic (4 wks) inflammation after instillation of NP at 150 cm^2/rat

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These results demonstrate that it is necessary to consider information from both in-vitro and in-vivo experiments when looking at inflammatory outcomes from nanoparticles, and that no single risk assessment model can be used to manage such different potential hazards.

This is the first report that particles can produce immunologically-mediated lung injury (inflammatory footprints, see Figure 9). The metal oxide nanoparticles elicited different patterns and types of inflammation, some involving the immune system and therefore present different hazards and potential risk outcomes. It is necessary to develop in-vitro assays that can detect such differing types of immuno-inflammatory responses, specifically those involving eosinophils and lymphocytes.

It will be important to explore the individuality of nanoparticles in relation to the immuno-inflammatory responses that they generate. If more in-vitro tests are developed, key information will not be lost, and may lead to the phasing out of in-vivo experiments. It was felt that this information is important to the ENPRA consortium as more concise conclusions can be drawn with results from both in-vitro and in-vivo studies aiding the development of risk assessments that cater to each particle.

It is worth mentioning that the solubility of the particles may influence the pattern of toxicity and that observed effects may be due to release of ions from particles. Given more time the NiO treated mice may also develop fibrosis so that a similar pathological outcome would be seen for all 3 particles.

See also *OMNT*, *NanoMedecine*, **4**, 14, Nanomed12-10-9 (December 2010).

"Metal Oxide Nanoparticles Induce Unique Inflammatory Footprints in the Lung: Important Implications for Nanoparticle Testing" ; W.S. Cho, R. Duffin, C.A. Poland, S.E.M. Howie, W. MacNee, M. Bradley, I.L. Megson, K. Donaldson : ***Environmental Health Perspectives***, ahead of print (2010).

EONS12-10-8

EXCERPT

Risk Assessment & Risk Management

Joint Research Centre: Modelling nanoparticle-induced oxidative stress

Reviewed by C. Poland

A theoretical approach for predicting nanoparticle toxicity

The paper by **Burello** and **Worth** proposes a theoretical framework with which to predict the oxidative stress potential of oxide nanoparticles (NP). Oxidative stress is seen as a key driver of pathogenic affects such as inflammation, genotoxicity and the generation of certain pathologies such as fibrosis and it has been suggested to be an important characteristic of a NP when considering its hazard potential.

Within this study the electron transfer feasibility between an oxide NP and a biological media (such as that of a cell) was calculated by establishing the valance and conductance band edge positions, which are the relevant energy levels, of an oxide of interest. The relevant range for a biological media was estimated to be between -4.12 eV and -4.84 eV and calculated band edges of analysed metal oxides which overlap this range are likely to be able to remove or transfer electrons from the biological media. The result would be a reduction in the reducing capacity of a cell due to the utilisation of antioxidants defences such as glutathione, and/or the generation of reactive oxygen species leading to a state of oxidative stress. The result of this may be the induction of an inflammatory response by stimulation of pro-inflammatory oxidant sensitive transcription pathways such as NF-κB.

As a proof of concept, Burello and Worth calculated the conductance and valance band edges of 6 oxides with known reactivity within a biological system. Of the 6 tested (TiO_2, CuO, ZnO, FeO, Fe_2O_3, Fe_3O_4), four of these (TiO_2, CuO, FeO, Fe_2O_3) had band edges which overlaid that of the range for biological media indicating that they would be able to remove or transfer electrons from the biological media, potentially generating oxidative stress. Those which did not are still known to be reactive within the biological environment but there mode of action differs from that of the other reactive oxides. For example ZnO exerts its toxicity via the release of ZnO ions and Fe_3O_4 can be oxidised to form Fe_2O_3 which is reactive within a biological system.
This approach was further taken with a panel of 70 oxides and of which 17 had conductance bands which overlain that of the range of redox potentials of biological reactions.

The generation of a quantitative structure activity relationship (QSAR) represents the holy grail of particle toxicology. To be able to predict the toxicity of a material simply by understanding its physicochemical structure would mean that materials could be adequately accessed for hazard and risk without the need for costly and, in the case of in vivo studies, ethically questionable testing.

Whilst this study does not purport to be a full QSAR and an absence of reactivity within this theoretical method does not mean that the particle is non-toxic. For example both ZnO and NiO would not be picked out using this approach as they exert their inflammatory effects via different mechanisms from direct particle reactivity. However as a method of gaining information on a NP this study is a large step in the right direction. At very least it provides a tool for the guidance of more rational and efficient study design, avoiding ineffective random sampling or wasteful exhaustive testing.

When considering the potential risks of nanotechnology and the extraordinary range of materials available, it can be difficult to identify those materials which may require special toxicological attention. Indeed there is very much the potential for exhaustive testing for materials which are fundamentally safe or missing those materials which may cause a problem. Based on a prominent driver of particle toxicity, the study allows the modelling of the likelihood of an oxide material to be reactive in a biological media. The use of in silico methods such as the one described within this article is a real answer to the problem of 'too many particle, too little time' with regards testing and proper hazard characterisation. Also as the study is based on fundamental physiochemical properties of the analysed materials, and performs mathematical modelling based upon this, it sidesteps some of the issues such as use of unrealistic doses generating artifactual, non-representative effects sometimes seen in in vitro or in vivo toxicology. Instead the theoretical framework acts a tool for refinement when considering the potential toxic effects of a large range of oxide materials. To this end it is an important piece of work.

EXCERPT

What remains is the need for thorough testing and validation of this methodology to make it suitable for regulatory toxicology as a recognised in silico method.

"A theoretical framework for predicting the oxidative stress potential of oxide nanoparticles" ; E. Burello, A. P. Worth : ***Nanotoxicology***, early online (2010).

EONS12-10-11

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