ENPRA Newsletter – Issue 4

Newsletter Contents

ENPRA news & events

- **Second ENPRA consortium meeting : mid-term progresses *(February 10-11, 2011 - Venice)***

ENPRA partners held their **2nd consortium meeting on February 10th and 11th at the University Ca'Forcasi in Venice** (IT), during which the mid-term progress of the project was presented.

ENPRA mid-term meeting *(Venice - February 11-12, 2011) - Top left*: Antonio Marcomini (Univ.Venice); *Top right*: ENPRA partners; *Bottom left*: Lang Tran (IOM), ENPRA coordinator; *Bottom right*: Keld A. Jensen (NRCWE) and Giulio Pojana (Univ. Venice).

An important step has been achieved in *WorkPackage 3* (*WP3)* with the completion of the **primary characterization** of the 10 different engineered nanoparticles (ENP) included in the project. A protocol for nanoparticle dispersion has also been validated and distributed to all partners. This dispersion protocol has been submitted to the OECD WPMN sponsorship program.

In *WP4*, **in vitro** experiments have been conducted in order to establish **dose-response relationships** for the various ENP. Results obtained so far showed very **coherent data among partners for the 5 different test systems** (pulmonary, hepatic, renal, cardio-vascular and developmental systems), with a higher toxicity observed for zinc oxide and silver nanoparticles over the other types of nanoparticles (carbon nanotubes and TiO_2).

As for the **in vivo approach** (*WP5*), crucial progresses have been made with the fulfilment of a **kinetics study** with the benchmark TiO_2 nanoparticles to assess the time course of the ENP distribution across key target organs. Investigation of **in vivo dose-response relationship** has been initiated: **intratracheal instillation studies** have been performed in order to determine the toxic effects after acute exposure for the full panel of ENP. The data have been analysed and dose-response relationships have been established for a range of endpoints.

For *WP6* **'risk assessment and modelling'**, several reviews have been performed including i) a report on the applicability of available computational tools for the prediction of ENP ADME, ii) a review of available computational approaches that can be used for the prediction of toxicity of ENP, and iii) a review of physico-chemical data on ENP available in the literature and evaluation of their pertinence with respect to ENPRA molecular modelling strategy. The generic Physiologically-Based-Pharmaco-Kinetics/Pharmaco-Dynamics (**PBPK/PD) and exposure modelling** built in collaboration with the NANOMMUNE project will be soon optimized with data collected from the ENPRA in vivo experiments. *WP6* partners have also started to build a **Weight of Evidence** (WoE) approach for ENP risk assessment, based on the review of the existing literature. The model will then be implemented with the experimental data obtained by the consortium.

During these first 2 years of the project, ENPRA partners have established an efficient **EU-US collaboration** (*WP2*) by promoting material, protocols and data exchanges between transatlantic partners. ENPRA actively contributes to the **dissemination of the knowledge** to the nanosafety community through a number of events organized in *WP7* (including 2 stakeholder workshops and 4 expert panel meetings), and through its contribution to several international nanosafety networks and

committees such as the OECD WPMN sponsorship program, the NANOhub platform, the NanoImpactNet network and the Nanosafety Cluster.

- **4th EONS meeting *(March 31, 2011 – Brussels)***

On **March 31st**, ENPRA partners and French experts from the Observatory on Micro and Nanotechnologies (OMNT) met in **Brussels** (BE) met at the Université Catholique de Louvain (UCL) for the **4th expert panel meeting** of the **European Observatory on NanoSafety (EONS)**. Launched within the ENPRA *WP7 "Dissemination strategy to maximize impact"*, the EONS meetings provides experts with the opportunity to collectively discuss the latest Nanosafety research progresses. Participants have presented and commented on a selection of recent publications in nanomaterial environmental, health and safety.

As invited speaker of the meeting, **Fritz Krombach** from the University of Munich (DE) gave a state-of-the-art presentation on the "**Fate and effects of nanomaterials in the microvasculature**".

Proceedings of the meeting (4th EONS report) have been published by the OMNT, excerpts of which can be accessed via this link. Summary excerpts of previous EONS reports are available on the ENPRA website.

Fourth EONS meeting *(Brussels-March 31, 2011).* Invited speaker: Fritz Krombach from the University of Munich (*bottom left*).

The **next EONS meeting** is to be held in Paris on **October 13, 2011**. During this meeting, **Martin Hassellöv** from the University of Gothenburg (SE) will give a presentation on the "Detection and characterization of nanomaterials in environmental media".

- **Second JRC/ENPRA Stakeholders' workshop *(May 10-12, 2011 – Somma Lombardo)***

Organized by the **Nanobiosciences Unit** of the **Joint Research Centre** (JRC), **Institute for Health and Consumer Protection** (IHCP), in co-operation with the FP7 ENPRA consortium, the workshop "**Challenges of regulation and risk assessment of nanomaterials**" was held at the Hotel Hilton Garden Inn in **Somma Lombardo (IT) on May 10-12, 2011**.

2nd JRC/ENPRA stakeholder workshop *(Somma Lombardo – May 10-12, 2011)*

Aimed at informing stakeholders of the **latest regulatory developments** and the most **recent scientific advances in nanomaterial risk assessment**, the event gathered over 80

participants from more than 20 countries including key scientists from academia, representatives of the European commission services, regulatory agencies (ECHA, EFSA, US-EPA), research organisations, environmental and workers' protection institutes, industry, as well as NGOs and press.

While progressive adaptation of the regulation is required for ensuring safe development and use of nanotechnologies, the JRC/ENPRA workshop provided participants with a unique platform of exchanges to learn, share and discuss the most recent progresses in regulation and risk assessment of nanomaterials. Among up-to-date research findings, preliminary results of the ENPRA consortium were revealed in exclusivity.

The **agenda**, with links to the **presentations** of the workshop is available via this link. A **full report** of the meeting will soon be published by the JRC and available to download on the IHCP website.

Focus article – ENPRA approaches for risk assessment of engineered nanoparticles

In *Work Package 6* (*WP6*), ENPRA partners are currently developing various approaches to implement a **risk assessment for engineered nanoparticles** (ENP). By combining data from different sources (literature, ENPRA US partners, EU databases and projects…) with experimental outputs from the ENPRA project, *WP6* partners are building mathematical models that will enable to predict nanomaterial behaviour in terms of exposure, dose-response effects, structure/activity; taken into account current uncertainties, their work will help to evaluate the potential health risk of ENP. On-going researches performed at the **Joint Research Centre (JRC)** and at the **University of Venice (UniVe)** respectively are further detailed in the following interviews.

- **The nano-QSAR modelling approach**

Dr. Enrico Burello is introducing the **computational quantitative structure-activity relationship (QSAR)** model developed in the **JRC's Computational Toxicology and Modelling research group**.

Why is modelling a necessity for ENP risk assessment?

Enrico Burello: A comprehensive understanding of the relationships between the properties and the behaviour of nanomaterials in the environment and in biological systems is mandatory for assessing the risk of nanomaterials. Toxicological tests, however, are time-consuming and resource-intensive, which is why researchers are developing computational methods to predict the behaviour of nanomaterials in biological systems. Such predictions would allow researchers to streamline and prioritize toxicological tests on real nanomaterials.

What kind of mathematical approach is developed in your laboratory?

EB: In the **Computational Toxicology and Modelling research group** at the **European Commission's Joint Research Centre**, we are developing **Quantitative Structure-Activity Relationship (QSAR)** models to predict the toxicity of nanomaterials. A QSAR is a statistical model that relates a set of structural parameters of a chemical compound to its biological activity. These parameters, which are called descriptors, are typically related to the steric and electronic properties of a compound, and can be determined empirically or by computational methods, whereas activities include chemical measurements and biological assays.

How are you specifically applying QSAR modelling to ENP?

EB: Although the QSAR methodology is well known and extensively used in the areas of drug discovery and chemical toxicity, its application to model the behaviour of nanomaterials requires new ideas to account for the novel properties of this class of compounds. The shift from classic to nano-

QSAR currently relies on both theoretically and experimentally derived descriptors, and the solutions adopted for modelling are diverse[1]. This option reflects not only the heterogeneity among classes of nanomaterials, but also their diverse behaviour when encompassing different length scales.

In this context, optimal progress could be achieved by very close collaboration between modellers and experimentalists, and research should focus on both aspects of a QSAR study: the generation of nano-specific theoretical descriptors and experimental test data. This in turn requires an effort to implement different knowledge pieces into one single multidisciplinary framework.

How are you going to exploit the ENPRA outputs to implement your nano-QSAR model?

EB: In the ENPRA project we are starting to build a **conceptual framework**, which is **based on the structure-property-activity paradigm**. The framework, making explicit **links between the structure, physicochemical properties and biological activity of nanomaterials**, is used to **identify toxicity related descriptors** as well as rationalize the behaviour of nanomaterials in complex biological systems in terms of mechanistic insights. This approach would allow mapping the knowledge advancements and gaps in the field, streamlining research in the risk assessment of nanomaterials and harmonizing the work and competences of different research areas, from computational chemistry to analytical chemistry, toxicology and medicine into one single research strategy.

Conceptually, the framework is centred on a number of phenomena which describe the ways a nanomaterial can elicit an adverse effect towards living systems, including **reactivity, biopersistence, macro-molecule adsorption, mobility and leaching**. For each phenomenon we are both looking for activity/toxicity related descriptors as well as identifying potential modes of action and their corresponding toxicological test.

Can you provide examples of structure-property-activity relationship that are applying for ENP?

EB: A well-known example is represented by the **toxicity paradigm of asbestos like structures**: biopersistent materials, with high aspect ratio structures can induce frustrated phagocytosis which in turn can produce chronic inflammation and finally lung cancer. This information shows the links between structure, property and activity of a class of nanomaterials and indicates what kind of physicochemical properties and related endpoints could be measured to determine the adverse effect of needle-like nanostructures.

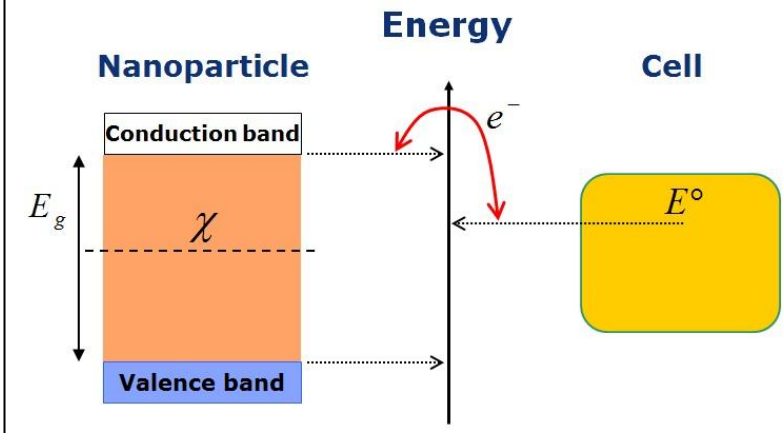

Figure 1: Schematic representation of electron transfer mechanism in the generation of oxidative stress by oxide nanoparticles (e.g. is the bandgap of a nanomaterial and χ its electronegativity; E° is the redox potential of a biological reaction).

Another paradigm can be represented by the **reactivity of oxide nanoparticles**: these materials can have catalytic properties which in turn affect the redox balance of cells and cause oxidative stress. We recently developed a model on the generation of oxidative stress by oxide nanoparticles[2]. The model uses reactivity descriptors to calculate the energy structure of nanoparticles and predicts their oxidative stress

[1] Burello, E., Worth, A. QSAR modeling of nanomaterials. WIREs Nanomedicine and Nanobiotechnology, 2011;3:298-306.

[2] Burello, E., Worth, A. A theoretical framework for predicting the oxidative stress potential of oxide nanoparticles. Nanotoxicology, 2011;5:228-235

potential by comparing their conduction and valence band energy levels with relevant redox potentials of biological reactions occurring inside cells. If these two energy levels are comparable, then electrons can be transferred and the oxide nanoparticle, which acts as a catalyst or an electron donor/acceptor, is responsible for unbalancing the cellular redox state (see Figure 1).

By including several other toxicity related descriptors for metal oxides we built a **multidimensional property space** for this category of materials. Then, we used these descriptors to group 50 oxide nanomaterials with a classification tree algorithm and Principal Component Analysis. Both methods group in a similar manner the materials according to the formal charge of the cations forming the oxide. This descriptor-based grouping is consistent with trends in toxicological data recently published in the literature, and tells us that oxide nanoparticles' toxicity is essentially related to the formal charge on the cation.

What are your plans for the coming months?

EB: In future research work we plan to further expand the list of descriptors and theoretical models, and explore their relationship and applicability with **toxicological data produced within** the **ENPRA** project. In particular we are also focusing on the **definition of intrinsic and extrinsic physicochemical properties**, taking into account their relevance in determining toxicity pathways. Extrinsic properties, which result for example from the formation of the hard and soft protein corona around nanoparticles, play a key role in determining the absorption, distribution, metabolism and excretion of nanomaterials and in general determine the interactions that take place at the nano-bio interface.

- **A novel Weight of Evidence methodology for ENP risk assessment**

In the following interview, **Dr Andrea Critto** and his PhD student **Danail Hristozov** are presenting the novel approach for nanoparticle risk assessment developed in the **Department of Environmental Sciences, Informatics and Statistics** at the **University of Venice** (**UniVe**).

What is your main objective within WP6?

Andrea Critto: Within the ENPRA *WP6*, a novel methodology for risk assessment (RA) of ENP is being developed. Its main goal is to **effectively estimate the risks of ENP for human health even in cases when the uncertainty is high**. The procedure is not intended to be a preliminary risk screening or research prioritization tool, but a methodology to quantitatively assess the risks of ENP and thus inform adequate regulatory decisions.

Which kind of data are you going to integrate in your RA procedure?

AC: We will weigh and integrate **all available occupational and consumer exposure data** from the **NANEX**, **NANOSH** and **NANOINNOV** projects with the effects data produced in ENPRA into risk indices for the ENPs considered as case studies in ENPRA. For this purpose the **Weight of Evidence (WoE) approach** supported by Multi Criteria Decision Analysis (MCDA) techniques will be used.

What are the specificities of your RA approach?

AC: The procedure needs to demonstrate **high accuracy** in the assessment of risks for human health even if the available input data are scarce and the uncertainty is high. In the context of the paucity of nano environmental, health and safety (EHS) data, the capacity to operate with limited information input is crucial for the successful application of the methodology. Our approach will **estimate uncertainty** related to different aspects and data input and how it is propagated through the aggregation procedure up to the final results in order to ensure that the risks are estimated in a robust and sound manner.

We are designing a **highly flexible methodology** that allows to handle different types of data (i.e., both experimental and predicted) and scenarios. Considering the enormous variety of 'nano-applications', it is necessary to apply a universal approach, covering a broad spectrum of uses and exposure scenarios (ES) and applicable for both consumer and occupational risk assessment.

Another specificity of the approach resides in the inclusion of **expert judgment** through an Expert Elicitation (EE) process. EE is the synthesis of opinions of experts on subjects where high uncertainty is present due to insufficient or conflicting data. It would play a crucial role in the assessment of risks of ENP since it allows filling some of the present critical knowledge gaps. Nonetheless our approach is being developed **in compliance with the basic REACH requirements** in order to ensure broad acceptance of the approach (from regulators in addition to scientists). It will **support regulators** in prioritizing ENP both for further research or testing and for **risk management** purposes.

What are the successive steps developed in your methodology?

Danail Hristozov: The ENPRA approach is based on a conceptual framework, which takes into account the basic principles of the REACH Chemical safety assessment (CSA) for industrial substances. Similarly to the regulatory CSA scheme, the ENPRA conceptual framework for ENP consists of five inter-related phases: 1) Problem Formulation; 2) Exposure Assessment; 3) Hazard Assessment; 4) Uncertainty Characterization; altogether, these steps enable 5) Risk Assessment.

The **Problem Formulation** is a systematic planning step that identifies the major elements to be considered within the study. In this phase, the assessor formulates and specifies the goals and the scope of the assessment. The nanomaterial is introduced, the system boundaries (e.g., occupational or consumer exposure) are defined and the system components (e.g., sources) are identified.

The second phase of the ENPRA conceptual approach is the **Exposure Assessment** (EA), which includes (1) the characterization of one or more occupational or **consumer exposure scenarios** (ES); and (2) the calculation of an **exposure index** (EI) using the WoE approach.

The **Hazard Assessment** (HA) step involves the evaluation and integration of the available data, regarding the intrinsic physicochemical characteristics of a substance and its toxicological effects. Just like in the EA, the HA in ENPRA involves the WoE approach that will help to organize the available data delivered in the project into Lines of Evidences (LoE) and indicators in order to calculate indices, representative of the inherent hazards of the investigated ENP.

Uncertainties are usually present in all steps of a RA. Their consideration is important in the estimation of risks because they may directly impact the results of the assessment. Proper uncertainty analysis is crucial for a robust, reliable and adequate risk assessment. The REACH CSA Guidelines suggest the use a tiered approach to uncertainty analysis, starting with basic qualitative characterization and continuing, if appropriate, with more detailed deterministic and probabilistic evaluations.

Practically, how are you going to integrate the ENPRA experimental data in your model?

DH: The ENPRA risk assessment methodology is designed to accommodate all the data delivered in the project. The hierarchical structure of the WoE approach makes it very flexible and applicable to virtually any dataset, including the ENPRA one. For each LoE scores can be calculated after normalization of related indicators on a common scale 0-1 and then aggregation of their values. These sub-LoE scores should be then integrated to obtain exposure and effects indices. After aggregating them (taking the uncertainties into account) a final **risk index** will be derived. MCDA is an effective tool for these purposes, taking also into account expert judgment. Some occupational/ consumer exposures can be acute and others: repeated or continuous. To account for the type of exposure, it is

also important to distinguish between acute and (sub-) chronic effects. This is foreseen in LoE hierarchy, which allows for calculating indices, considering the type of effect for each exposure route.

In the coming months we will extend our conceptual approach to a mathematical model and will apply it to the panel of ENP considered as case studies in the ENPRA project in order to estimate their occupational and consumer risks.

The development of this novel Weight of Evidence approach for ENP risk assessment has been performed By Danail Hristozov and Dr. Andrea Critto, in collaboration with ***Dr. Stefania Gottardo*** *(left) and* ***Pr. Antonio Marcomini*** *(right) in the Department of Environmental Sciences, Informatics and Statistic at the* ***UniVe****. The group recently submitted its research for publication in Nanotoxicology: "Hristozov D., Gottardo S., Critto A., Marcomini A. Risk assessment of engineered nanomaterials (ENMs): available data and existing approaches (submitted)"*

Upcoming events

You will find below announcements of a selection of future nano EHS events.

- **5th International Conference on Nanotechnology – Occupational and Environmental Health**

The **5th International Symposium on Nanotechnology, Occupational and Environmental Health** (NanOEH) will take place in **Boston, MA (US)** on **9-12 August 2011**. The meeting will provide a high quality of professional presentations to scientists and engineers who wish to promote and communicate the interaction between technical advances and societal, occupational and environmental impacts in the field of nanotechnology research. This international symposium will be the fifth in a series recognized for the high technical quality of its biennial conference. The goal is to bring researchers and practitioners together to share the latest knowledge on nanotechnology-specific risks to occupational health and the environment and assessing how to reduce these potential risks.

Three parallel sessions are planned with the following subject areas:

- Nanoparticle toxicity and related topics (toxicity screening techniques, biomarkers of exposure, epidemiology, etc.).
- Nanoparticle occupational health and safety (worker exposure assessment, exposure control techniques, good practices, fire and explosion, personal protective equipment, etc.).
- Nanoparticle environmental release and exposures, and responsible development (societal, ethical and policy issues, regulations, life cycle assessment, etc.).

For more information, please click here.

- **Eurotox Paris 2011 – Safety Evaluation: A Translational Science**

The **47th Congress of the European Societies of Toxicology** will be held at the Palais des Congrès (Porte Maillot, **Paris**, **France**) from the **28th to the 31st August 2011**.The organizing committee is pleased to announce an exciting innovative congress, with sound scientific presentations covering a wide range of topics representing the latest scientific and regulatory developments.

The conference includes a theme on "Nanomaterials" as well as 2 specific sessions on "Safety and risks of engineered nanomaterials" and "Nanotechnology safety concerns revisited".

For more information, please click here.

- **ESF Research Conference Nanocarbons 2011**

Organized by the European Science Foundation, the conference **Nanocarbons 2011 "Carbon Nanotubes and Related Materials: From Physico-Chemical Properties to Biological and Environmental Effects"** is to be held at the Hotel Villa del Mare in **Acquafredda di Maratea, Italy on 6-11 September, 2011**.

The general aim of this conference is to give the different actors of carbon nanotubes (CNT) research (physicists, chemists, biologists) the proper level of knowledge required to discuss with the other participants and understand each other correctly. The conference will therefore propose courses accessible to scientists of different fields, as well as more specialized courses in each domain of interest, including the most recent advances on the subject. The program will focus on four general topics:

- Synthesis & characterization of CNT,
- CNT chemistry
- Health effects of CNT
- Environmental effects of CNT.

For additional information and registration, please click here.

- **International Conference on Biological Responses to Nanoscale Particles**

An international conference on "**Biological Responses to Nanoscale Particles**" organized by the Priority Programme (SPP1313) of Deutsche Forschungsgemeinschaft (DFG) and the University of Duisburg-Essen (UDE) is to be held in **Essen, Germany on September 11-15, 2011**. Contributions to the following topics are invited:

- Interactions of NPs with biomolecules and membranes
- Mechanisms of cellular uptake and intercellular trafficking
- Biological impacts of NPs
- Synthesis and characterization of NPs
- In-vivo and in-vitro imaging and diagnostic techniques
- Biodegradation of NPs G. Life cycle analysis and risk assessment.

For more information, please click here.

- **6th International Conference on the Environmental Effects of Nanoparticles and Nanomaterials**

The **6th International Conference on the Environmental Effects of Nanoparticles and Nanomaterials** is to be held in **London**, **UK** from **Monday 19th to Wednesday 21st September 2011**. This meeting is the sixth international meeting on this topic following the success of the 5th meeting held in Clemson, USA, last year. The venue for 2011 will be the prestigious **Royal Society building** in London, UK.

The conference will focus on the following topics:

- Chemical and physical properties of manufactured or natural nanoparticles and other nanomaterials in the environment;
- Fate, behaviour, interaction and biogeochemistry;
- Toxicological and ecotoxicology;
- Effects of nanomaterials on microbes, plants, animals, and ecosystems;
- Detection, measurement and bioassays for nanosubstances;
- Environmental Risk Assessment, life cycle analysis, modelling and human health;
- Environmental and industrial applications of nanotechnologies;
- Horizon scanning for new materials and knowledge transfer on effects;
- Regulation, legislation, policy and public perception of nanotechnology.

For more information, please click here.

- **3rd Nanosafety School: Understanding Human Health Effects and Environmental Impacts of Engineered Nanomaterials**

The **Third Nanosafety Autumn School** "Understanding Human Health Effects and Environmental Impacts of Engineered Nanomaterials" will take place at the Ca' Foscari Palace, **Venice (Italy)** from **October Sunday 2nd to Friday 7th 2011**. Organized in the frame of the FP7 MARINA and NANOVALID projects, the School will focus on the emerging trends in the nano environmental, health and safety (EHS) research area and at the same time it will update the state-of-the-art on the scientific knowledge and technical tools for an integrated risk assessment of nanotechnologies. It will highlight the best practices and approaches for physicochemical characterization, (eco) toxicity testing, exposure, risk and lifecycle assessment of ENMs. It will provide an interactive learning environment and direct access to key experts from Europe and the United States.
For more information and registration, please click here.

- **SENN2012 : International Congress on Safety of Engineered Nanoparticles and Nanotechnologies**

The NANODEVICE project partners and the Finnish Institute of Occupational Health organize the "**International Congress on Safety of Engineered Nanoparticles and Nanotechnologies**" to be held on **28–31 October 2012 in Helsinki**, **Finland**.

The goal of the SENN2012 Congress is to summarize and share the latest knowledge on the safety of engineered nanomaterials and nano-related technologies. The emphasis is on producing solutions to the safety challenges related to engineered nanomaterials and nanotechnologies. Another aim is to enable commercial opportunities for the safe use of these materials and technologies.

The Congress will provide a forum for reporting and demonstrating findings, methods, tools, and approaches to safety and health at workplaces using nanoparticles and nanotechnologies. The plenary and free communication sessions will be designed to facilitate interaction between participants and presenters.

For more information, please click here.