

ENPRA Newsletter – Issue 2

Newsletter Contents

Feedback from the latest ENPRA events	2
- First ENPRA Stakeholder workshop	2
- Second EONS meeting	3
- ENPRA annual Project Meeting & Year-1 progress	3
Focus article: <i>in vitro</i> and <i>in vivo</i> approaches for hazard assessment of engineered nanoparticles – The ENPRA approach	4
Upcoming events	7
- Nanomaterials and Worker Health: Medical Surveillance, Exposure Registries, and Epidemiologic Research	7
- NanoImpactNet Workshop 2010	7
- 2nd Nanosafety Autumn School	7
- NanoSafe 2010	7

Feedback from the latest ENPRA events

- **First ENPRA Stakeholder workshop (14-15 April 2010)**

The **first ENPRA stakeholder workshop**, organised in collaboration with the **European Commission's Competent Authorities Subgroup on Nanomaterials (CASG-Nano)** was held at the Joint Research Centre in Ispra (Italy) on the 14th and 15th of April. The workshop, entitled "**Early harvest of research results on Nanosafety**" aimed to present and discuss state-of-the-art knowledge on Nanosafety in order to provide support on implementation of the European chemicals legislation, REACH.



Chairs and speakers of the 1st ENPRA Stakeholder workshop at the Joint Research Centre, Ispra.

The workshop gathered over 90 participants including key scientists from the Nanosafety community, industrialists, representatives of the European Commission, OECD, EFSA, ECHA, and of environment and worker protection organizations. Its talks were delivered by international experts on the key research areas of Nanosafety including characterization, exposure assessment and environmental and health impacts of nanomaterials. Representatives of several European consortiums were invited to present feedback from FP6 as well as recently started FP7 projects.

To close the workshop, **3 breakout sessions on risk assessment of nanomaterial in REACH** were organised on:

- (i) measurements characterisation and categorisation,
- (ii) exposure measurement and mitigation and
- (iii) toxicity and ecotoxicity of nanomaterials. These working groups addressed nano-specific as well as common issues for nanomaterials and "traditional" chemicals and provided recommendations to RIP-oNs 2 & 3.

For more information, follow this link to access [the final meeting programme and presentations](#).

On April 14th, two additional closed sessions were held in conjunction with the workshop:

- i) a meeting of the **NanoSafety Industrial Colloquium** ('Strategic NanoSafety Group'), aimed at deciding upon the next steps to shape and support to provide to the future NanoSafety Research Agenda; and ii) a meeting of the [EU Nanosafety Cluster](#), a DG RTD NMP initiative set up to maximise the synergies between the existing FP6 and FP7 projects and addressing all aspects of

Nanosafety. Finally, on the 15th April, a parallel case-studies workshop also took place organised by the [REACH Implementation Plan Projects RIP-oN 2 and RIP-oN 3](#).

The next ENPRA stakeholder workshop will be organized in spring 2011. Further information will be posted on the ENPRA website in due course.

- **Second EONS meeting (13 April 2010)**

In conjunction with the ENPRA/CAGS-Nano stakeholder workshop, the 2nd meeting of the **European Observatory on NanoSafety (EONS)** was held at the Joint Research Centre in Ispra on April 13th 2010.



Launched in November 2009, EONS is a joint initiative from the ENPRA consortium and the Observatory on Micro & Nanotechnologies (OMNT). Gathering key experts on NanoSafety, including ENPRA partners, OMNT experts and invited leading scientists, EONS meetings are aimed at performing a scientific watch on environmental, health and safety issues related to nanotechnology.

Second EONS meeting at the Joint Research Centre, Ispra.

To commence the second EONS meeting, a state-of-the-art presentation on **Environmental impacts of Nanotechnologies** was given by **Dr. Jérôme Rose**, CNRS research director at the **CEREGE** (France). Using several research initiatives from the Franco-American collaborative group iCIENT (international Consortium for the Environmental Implication of Nanotechnology) as illustration, Dr. Rose's talk outlined the current knowledge in the relationship between environmental fate and the potential toxicity and ecotoxicity of nanomaterials.

This review was followed by a **round table** providing participants the opportunity to present and discuss, selected research progresses in nanotoxicology, risk assessment and risk management.

The 2nd **EONS report** summarising the panel discussions was published in June 2010. **Excerpts from EONS reports** are available on the [ENPRA website](#). The next EONS meeting will be held early October in Paris.

- **ENPRA annual Project Meeting & Year-1 progress**

The **first ENPRA consortium meeting** was held on **May 31st, 2010 in Edinburgh, UK**. The meeting was one of the four satellite events hosted by the **Nanotoxicology 2010 Conference** (2-4 June). Over 25 partners from the 15 European institutions involved in the project were present to discuss research progresses and to coordinate future work package activities.

ENPRA Year-1 progress

A large part of ENPRA's 1st year was focussed on carefully setting up the experimental protocols and at coordinating partners' activities in order to optimize the output of the project.

An important step in the initiation of the work was achieved with the **distribution of the 9 engineered nanoparticles (ENP) samples** to all partners including US collaborators and the **development of common dispersion protocols**.

For the experimental approaches, ***in vitro* and *in vivo* protocols** are now **established** and ready to be tested with ENP. Finally, in relation to improving and supporting the risk assessment process, a **mathematical model (PBPK: Physiologically-Based-Pharmacokinetics)** aimed at describing the exposure-dose-response relationship is being developed **in collaboration with US partners** from the **NIOSH**.



First ENPRA consortium meeting (Edinburgh, UK)

Following the annual meeting, on June 1st, an **Exploitation Strategy Seminar** proposed by the **European Commission** and facilitated by Dr. Mauro Caocci, gathered **ENPRA** members together with partners from other 2 other FP7 projects: **InLiveTox** and **NANOMMUNE**. The purpose of this session was to create a constructive discussion among the partners and to help them to identify potential exploitable results from their projects.

[Focus Article: *in vitro* and *in vivo* approaches for hazard assessment of engineered nanoparticles – the ENPRA approach](#)

While the market for nanotechnology-based products is in constant increase in Europe and worldwide, assessment and management of potential risks associated with nanomaterials is essential to ensure long term growth and sustainability of nanotechnologies and to provide consumers confidence that products developed are safe.

The ENPRA project aims at developing an approach for the risk assessment of engineered nanoparticles (ENP) using *in vitro*, *in vivo* and *in silico* models to assess the hazard of ENP and then combines the results with an assessment of workplace and consumer exposure of these materials for a rigorous final assessment of the potential health risk.

In the following interview, ENPRA partners from the **National Institute for Public Health and Environment (RIVM)** present details on the *in vitro* and *in vivo* approaches that are currently developed within the different workpackages (WP) of the consortium in order to provide relevant data for risk assessment.

Can you briefly remind us the main objectives of the *in vitro* approach developed in WP4?



Dr. Flemming Cassee: The work in this WP is directed towards hazard screening and gaining insight in the biological mechanisms on how ENP can induce toxic effects. All ENP will be tested in a large variety of test systems (i.e. cell cultures) with emphasis on the lung, heart, liver, kidney and blood as target organs. In addition, the effects on the developing embryos will be assessed. The key mechanisms are oxidative stress, inflammation and genotoxicity.

By combining cell systems and endpoints more than 150 dose response curves will be generated and corresponding **Benchmark Concentrations (BMC)** will be used for risk assessment in WP6. In addition **round robin testing** will be performed to assure that effects that are observed can be replicated in other laboratories. The *in vitro* WP will also produce a range of **standardized protocols**.

What kind of results is expected from the *in vivo* experiments in WP5?

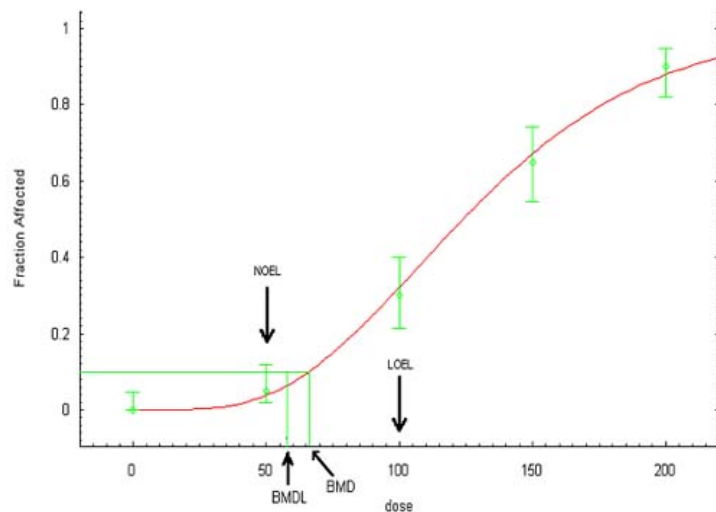


Dr. Ilse Gosens: In WP5, the toxicity of 9 different ENP will be established. For this, dose-response curves will be determined. From these dose-response relationships, the benchmark dose can be derived that can will used for risk assessment.

The **benchmark dose (BMD)** method is seen as a powerful statistical tool for risk assessment with certain advantages over the more **traditional NOAEL (no observed adverse effect level) determination**. It makes use of the whole dose-response curve and is therefore not restricted to the test doses. It also takes into account uncertainty in the data, where the **benchmark dose low (BMDL)** is the lower confidence limit of the value.

The BMD information will be combined with the **kinetics data of TiO₂** to see to which organs the particles go after administration via the lung and how long they stay there. Together with the *in vitro* data this will serve as input for the risk assessment.

Benchmark Dose Lower Bound vs Benchmark Dose vs NOEL



The biological effects of ENP will be evaluated in different target systems by focusing on several endpoints. What are the overlapping aspects between *in vitro* and *in vivo* results?

IG: Similar endpoints will be analysed in relevant tissues and cell models, i.e. lung inflammation *in vivo* will be determined by the cytokine response in the bronchioalveolar lavage fluid, whereas different lung cell types will be tested for cytokine response *in vitro*. For genotoxicity, this will be measured in the liver as well as *in vitro* in different liver cell types.

What has been done to make the results of *in vitro* and *in vivo* comparable?

IG: The same protocol for dispersion of the nanoparticles will be used both *in vitro* and *in vivo*. This includes mouse serum as a dispersant for both work packages and the same preparation of the particle suspensions. In most *in vitro* tests, acute effects will be determined as well as acute *in vivo* after 24 hours next to longer term responses after 28 days.

In the design of the *in vivo* studies, multiple dose-groups with a smaller amount of animals instead of less dose-groups having larger amounts of animals have been made. This is more useful for the benchmark dose method and approaches more the *in vitro* set-up where a whole dose-range is tested

as well. Similar endpoints have been chosen *in vitro* and *in vivo* and a check has been done whether there was enough overlap between the endpoints for inflammation, oxidative stress, genotoxicity and fibrogenicity. Most of the time, the same scientists are involved in performing the *in vitro* and *in vivo* endpoint analyses.

How do you plan to extrapolate *in vitro* and *in vivo* data for risk assessment modelling in WP6?



Dr. Jos Bessems: Basically and in theory, there are two main approaches to perform *in vitro in vivo* extrapolation (IVIVE) for risk assessment.

The first one is the **empirical approach**, meaning that *in vitro* as well as *in vivo* data for a sufficient amount of ENP are put in a graph (*in vitro* data on the x-axis, *in vivo* data on the y-axis). If the underlying experiments have been performed according to equal (preferably standardised) protocols, the differences in outcome, preferably ***in vitro* BMCs'** and ***in vivo* BMDs'** are only due to physicochemical differences in the (nano)particles. For this approach, *in vitro* and *in vivo* data on many ENP are necessary. If the correlation works well, the result is a **Quantitative Structure Activity Relationship (QSAR)-like** algorithm, i.e. a **Quantitative Property (*in vitro*) Property (*in vivo*) Relationship (QPPR)**. This means that for future risk assessment purposes for these kind of ENP, the ENP should be tested *in vitro* (providing a BMC) where subsequently, the QPPR could be used to predict the BMD from the BMC. Importantly, the ENP to be assessed using IVIVE, should be within the applicability domain of the QPPR. The predicted BMD could be used to compare with the exposure estimate to provide a risk estimate.

The second main approach is the **mechanistic approach**, meaning that for one ENP the *in vitro* outcome (BMC) may be translated to an *in vivo* BMD using mechanistic information, i.e. information on the complete (toxico)kinetic profile of the ENP. This means that much more emphasis is placed on studying the kinetics of the ENP at stake.

Crucial in both approaches is the availability of a concentration/dose-response curve; sufficient concentrations/doses should have been tested to provide a clear-cut dose-response. Finally, it is important to remember that both approaches are still in the phase of exploration. None of them has been successfully applied to date, either in the context of classical chemicals or of nanoparticles.

How will risk assessment be strengthened with results of *in vitro* and *in vivo* workpackages? Can you give some clear examples where both WP will provide useful data?

JB: Risk assessment can be strengthened when WP4 (*in vitro*) and WP5 (*in vivo*) use the recommendations as provided from within WP6 (risk assessment & modelling). The most important issues to be taken care of are characterisation, comparable/standardised protocols and concentration/dose-response modelling. During characterisation, as many physicochemical parameters should be measured as possible. Regarding the effect assays, if the protocols used differ between the various nanoparticles, it will be unclear whether differences in output are due to the ENP or to different assay conditions. And regarding concentration-response curves, a sufficient number of concentrations/doses will be necessary in order to avoid large uncertainties in the resulting BMC or BMD.

Conclusion

Nanomaterials that will be tested within the project have now been made available to all ENPRA partners. Careful characterization of those ENP is currently under way. During this first year, partners have collectively worked on establishing adapted experimental protocols that would allow them to acquire the most relevant data for risk assessment. We now look forward for the first *in vitro* and *in vivo* experimental results in the coming months.



Upcoming events

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

- **Nanomaterials and Worker Health: Medical Surveillance, Exposure Registries, and Epidemiologic Research**

The **National Institute for Occupational Safety and Health (NIOSH)** and the **Mountain and Plains Education and Research Center** propose a conference on "Nanomaterials and Worker Health: Medical Surveillance, Exposure Registries, and Epidemiologic Research." The conference will be held **on July 21–23, 2010**, at the Keystone Resort and Conference Center in **Keystone, Colorado (US)**.

The aim of the conference is to identify gaps in information and address questions focusing on **occupational health surveillance, exposure registries, and epidemiologic research** involving nanotechnology workers.

For more information, please click [here](#).

- **NanoImpactNet Workshop 2010**

This **NanoImpactNet Workshop** is to be held in **Dublin (Ireland)** on **September 6th to 9th, 2010**. It will focus on 3 main topics:

- Hazard Assessment of Nanomaterials in Biota: Recent Advances in Methodology and Challenges Ahead;
- Impact assessment of nanomaterial - Nanomedicine and nanotoxicology, two sides of one coin;
- Standardised exposure measurements & QA

For more information, please click [here](#).

- **2nd Nanosafety Autumn School**

The second cycle **Nanosafety Autumn School** "understanding human health effects and environmental impacts of engineered nanomaterials" will take place in **Venice (Italy)** on **October 4-8, 2010**. Focusing on emerging nanosafety aspects related to human and environmental exposure to engineered nanoparticles and this School will provide the update of the state-of-the-art on scientific knowledge and technical tools available for an integrated assessment of nanotechnology products. The School is especially targeted to students, personnel from Research and Academic Institutions as well as from Industry, Governmental Agencies and Hospital Departments.

For more information, please click [here](#).

- **NanoSafe 2010**

The conference is aimed at presenting **major progresses and future trends in the domain of the safe production and use of nanomaterials**. The NanoSafe 2010 conference is to be held on **November 16-18, 2010 in Grenoble (France)**.



The topics include:

- Exposure assessment
- Characterization, Detection and Monitoring
- Nanomaterials life cycle
- Toxicology
- Environmental impact
- Nanoparticle release from consumer products
- Personal protection equipment
- Secure industrial production
- Safety parameters evaluation
- Standardization, Regulations

For more information, please click [here](#).