ENPRA Newsletter – Issue 6

Newsletter Contents

ENPRA News & Events

- **Third ENPRA/JRC stakeholder workshop**

As part of the dissemination strategy of the ENPRA project (WP7), the **3rd ENPRA stakeholder workshop** was organized as a Joint Dissemination Event with the FP7 projects NEPHH, HINAMOX, NANOPOLYTOX, and in collaboration with the **Joint Research Centre (JRC)** Enlargement and Integration Programme. This major international event on '**Safety issues and regulatory challenges of nanomaterials**' took take place in **San Sebastian, Spain** on **May 3-4, 2012**.

Third ENPRA/JRC stakeholder workshop *(San Sebastian, Spain – May 3-4, 2012)*

Amongst stakeholders, the expert in nanosafety of the European Commission, Giorgios Katalagarianakis, and the adviser to the Dutch government in matters of nanosecurity, Tom van Teunenbroek, were present. During the event, results from four FP7 projects on nanosafety (Hinamox, Nanopolytox, NEPPH and ENPRA), as well as state of the art and recent developments in the legislation and regulations in the EU and the world concerning nanomaterials, were presented. The symposium provided participants with the opportunity to share knowledge and experiences about the critical issues specific for the risk assessment and life cycle assessment (LCA) of nanomaterials in a regulatory context. It also allowed identification of the needs and challenges for policy making and regulation of nanotechnology based materials.

A summary report of this 3rd ENPRA/JRC stakeholder symposium will be published on the JRC-IHCP website. A report from the previous ENPRA/JRC stakeholder workshop is available at http://ihcp.jrc.ec.europa.eu/past_events_workshops/joint-jrc-nano-enpra-2011.

- **Year-3 ENPRA consortium meeting**

On **May 22nd and 23rd 2012**, the **University Paris Diderot (FR)** hosted the **year-3 ENPRA consortium meeting**. Gathering delegates from the 15 European partners involved in ENPRA, this 2-day meeting was the last checkpoint of the consortium. Partners presented updates from each workpackage and discussed the remaining steps before the end of the project (October 2012).

In particular, the meeting provided partners with the opportunity to precisely plan the Round Robin testing, an important final milestone of Workpackage 4. Partners examined and refined the experimental conditions selected for this inter-laboratory approach designed to i) assess the *in vitro* toxicity of selected nanomaterials (in standardized conditions) and ii) to evaluate the reproducibility of the protocol (see the following focus article on WP4 for further details).

Year-3 ENPRA consortium meeting - *University Paris Diderot, Paris (FR) - May 2012*. *Left:* ENPRA partners and project officer, Nicolas Segebarth (*foreground)*; *Right*: The ENPRA coordinator, Lang Tran (IOM).

The **final ENPRA meeting** will be organized as a large dissemination event and will take place in **Edinburgh** (UK) on **September 19-20, 2012**. Further details (exact location, program and registration, etc) will be soon available on the ENPRA website.

- **Sixth EONS meeting**

ENPRA partners and French experts of the Observatoire des Micro & Nanotechnologies (OMNT) met at the sixth meeting of the **European Observatory on NanoSafety (EONS)** on **May 24th**, in **Paris** France. For this last expert panel meeting organized within the dissemination strategy of the ENPRA project, **Pr. Patrick Couvreur** (*Université Paris Sud, FR*) was invited to present state of the art on "*Multifunctional nanomedicine for therapeutic and diagnostic*". Through a number of illustrative examples, Pr. Couvreur addressed the great potential for nanotechnologies to overcome current limitations of conventional drug delivery, especially for cancer therapies.

A selection of studies and reviews from the recent nanoEHS literature was then presented by the panel of experts and collective discussions were developed around the latest research progresses.

Sixth EONS meeting *(Paris, May 24, 2012)*. ENPRA partners and OMNT experts *(left)*; Pr. Patrick Couvreur *(right)*.

Among topics of interests, the panel highlighted studies on the potential presence of non-intentional nanomaterials in consumer products, on the environmental behaviour of nanomaterial-based commercial products as well as a recent approach for the safety assessment of nanomaterials in food products.

A **summary report** of this meeting (6th EONS report) has been published by the OMNT and a PDF version of the full report can be accessed via this link. Summary excerpts of previous EONS reports are available on the ENPRA website.

Focus article - In vitro hazard assessment of engineered nanomaterials: Update from WP4

*Workpackage 4 is responsible for the in vitro component of the ENPRA project and is led by **Vicki Stone**, **Professor of Toxicology at the Heriot-Watt University** (Edinburgh, UK). In the following interview, Prof Stone and her PhD student, **Ali Kermanizadeh** present an overview of the progresses achieved by the 11 partners involved in WP4 over the last 3 years.*

1- Can you remind us the main objectives of WP4?

Vicki Stone: **Phase I** of the WP4 assessed dose-response relationships for the 10 ENPRA nanomaterials (NMs) ***(Table 1)*** in a range of cell types that represent 5 systems, namely the lungs, cardiovascular system, liver, kidney and reproductive systems. The dose response relationships initially focused on cytotoxicity, but also encompassed indicators of inflammation, oxidative stress and cell specific responses. It was important to conduct extensive dose response relationships in order to provide sufficiently detailed data to allow derivation of values suitable for risk assessment.

Phase II investigated the mechanistic endpoints in more detail using a smaller panel of 6 nanomaterials (NM 110, NM 111, NM 300, NM 400, NM 402 and NRCWE 002), chosen after assessment of phase I *(see **Table 1** and **Figure 1**)*.

Phase III has just started and will develop a Round Robin based on one cell type and assay endpoint in order to provide an assay system that might be suitable for high throughput screening in future.

NM	NM code	Average size (nm)
TiO_2	NM 101	7 nm
ZnO	NM 110	100 nm
ZnO	NM 111	130 nm
Ag	NM 300	< 20 nm
MWCNT	NM 400	30 nm / 5 µm long
MWCNT	NM 402	30 nm / 10 µm long
TiO_2	NRCWE 001	10 nm
TiO_2	NRCWE 002	10 nm
TiO_2	NRCWE 003	10 nm
TiO_2	NRCWE 004	94 nm

Table 1 : List of NMs investigated in the ENPRA project, with the original source codes and nominal sizes.

2 - A large number of partners are involved in WP4, each of them testing NMs on different target systems and focusing on different endpoints. A good coordination between partners was necessary to ensure the reproducibility and comparability between experiments, and great efforts have been made to set up standard operating procedures. How did you proceed to harmonize your protocols?

VS: Step 1 involved a detailed dissection of the description of work to generate a series of tables which clearly stated the cell types to be investigated and a selection of the endpoints that could be

conducted for each cell culture system. The kick-off meeting and a teleconference were used to generate a plan of work, with deadlines, and to agree cytotoxicity methods that would be used across all laboratories. The tables were circulated amongst partners to allow partners to indicate the cell types and endpoints for which they would be responsible.

This had two outcomes, firstly identification of teams of researchers who could generate **standard protocols** for each endpoint to be shared amongst the ENPRA partners and subsequently with the research community via **NanoImpactNet**. Therefore by around month 6, a range of standard protocols were available to the consortium. This approach also allowed identification of potential gaps within the proposed work. No such gaps were identified.

3 - One of the first steps of the work plan was to establish dose-response relationships for the different ENMs. What did you learn from these experiments?

Ali Kermanizadeh: The dose response data generated by different partners in WP4 of the ENPRA project was collected in a table in the form of LC50 values. This table clearly demonstrated that despite the use of different cell lines and media for each system (pulmonary, cardio-vascular, hepatic, renal and developmental) the ENPRA NMs could be divided into a highly toxic (Ag and ZnO) and low toxicity (TiO2 and MWCNT) group. Furthermore this data allowed the identification of a sub-lethal dose range for each target system to investigate the molecular/cellular mechanism driving the toxicity of the NMs chosen in this study. With respect to the *in vivo* studies in WP5, this data was used to choose the materials that could be tested further with repeated exposures.

4 - A tremendous amount of work has been done by the different partners with the analysis of the impacts of the various NMs on the 5 target systems. Can you summarise the main conclusions of these studies?

AK: The first observation has already been provided above; the division of the particles into high toxicity group (Ag and ZnO) and a low toxicity group (MWCNT and TiO_2) that was common across different cell types and different laboratories *(see NM-induced hepatic cytotoxicity on **Figure 2**, top panel)*.

For the ***in vitro*** **hepatocyte model** (*see NM internalization in **Figure 1**)* the silver particles elicited the greatest level of cytotoxicity (24 hr LC50 – 2 µg/cm^2). The silver was followed by the uncoated ZnO (24 hr LC50 - 7.5 µg/cm^2) and coated ZnO (24 hr LC50 - 15 µg/cm^2) particles with respect to cytotoxicity. The ZnO NMs were found to be about 40-50% soluble which could account for their toxicity. The LC50 was not attained in the presence of any of the other engineered NMs (up to 80 µg/cm^2). All NMs significantly increased IL8

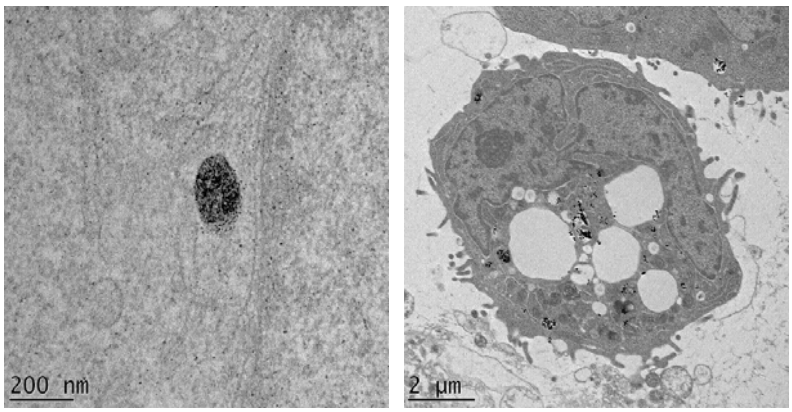

Figure 1: Transmission Electron Microscopy (TEM) images showing internalisation of silver (NM 300, left panel) and titanium dioxide (NRCWE002, right panel) by C3A hepatocytes 4 hr post exposure to 5 µg/cm^2 of each NM.

production *(see **Figure 2**, bottom panel)*.

Meanwhile no significant change in TNF-α, IL6 or CRP was detected. Urea and albumin production were measured as indicators of hepatic function. These markers were only altered by the coated and uncoated ZnO, which significantly decreased albumin production.

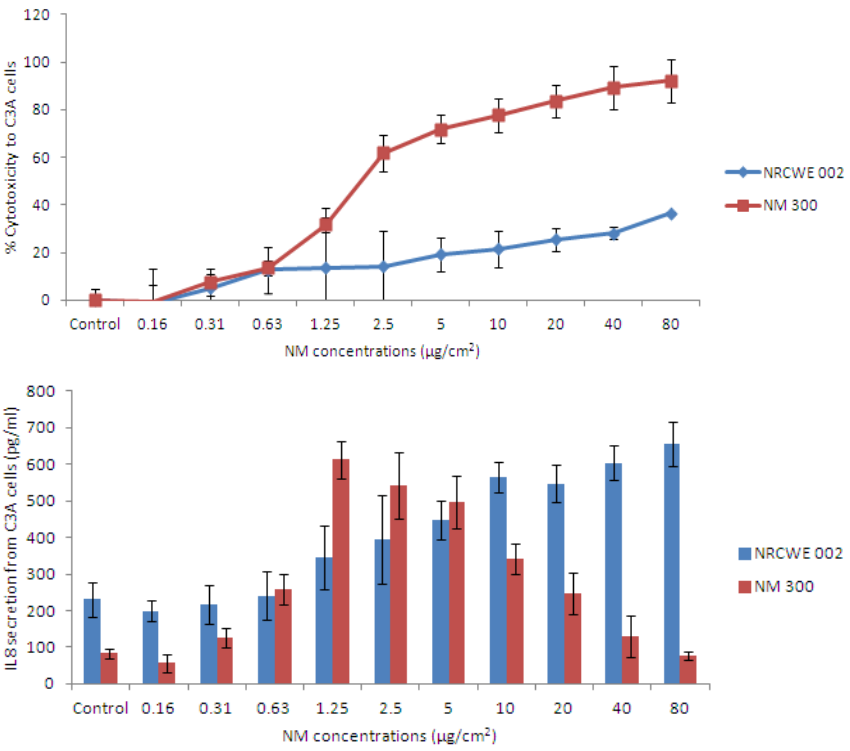

Figure 2 : Cytotoxicity (top panel) and IL-8 production (bottom panel) by C3A cells in the presence of silver (NM300, in red) or titanium dioxide (NRCWE, in blue) NMs. Cells were exposed to NMs for 24h with cytotoxicity measured via WST-1 assay.

Overall the WP4 findings suggest that in addition to the cytotoxicity observations, the sub-lethal particles were not without effect in terms of oxidative, pro-inflammatory and genotoxic effects, although genotoxicity was generally low. There appear to be differences between the cell types with respect to their sensitivities to the sub-lethal effects of the particles and differences between the particles. A deeper analysis of the data in other work packages of the project is required to pick out a more detailed picture of how the particle properties relate to their impacts on different cell types.

The first ENPRA results on the impacts of NMs on the hepatic systems have been compiled in research articles. One article has already been accepted for publication in **Nanotoxicology**[1], while 2 additional articles have been submitted for publications[2,3]. Further publications detailing the studies on the other target systems are currently in preparation.

5 - WP4 final experiments are now in progress and partners are running the Round Robin testing. Can you explain the objectives and the expected outcomes of these inter-laboratory assays?

VS: Phase III of the WP4 in the ENPRA project will concentrate on a conducting a **Round Robin** utilising the A549 cell line (human lung alveolar epithelial cells) by the WP4 partners. All partners will focus on two NMs, namely - the highly toxic uncoated ZnO NM (NM 110) at five doses (80, 40, 20, 10 and 5 µg/cm^2) and the low toxicity 10 nm TiO_2 (NRCWE 001) at the highest used in this study (80 µg/cm^2). The partners will disperse the particles in the presence and absence of serum. A total of six

[1] Kermanizadeh A, *et al.* In vitro assessment of engineered nanomaterials using a hepatocyte cell line: cytotoxicity, pro-inflammatory cytokines and functional markers. Nanotoxicology 2012 (*In Press)*

[2] Kermanizadeh A, *et al.* An in vitro liver model – accessing oxidative stress and genotoxicity following exposure of hepatocytes to a panel of engineered nanoparticles. *Submitted to Particle and Fibre Toxicology*.

[3] Kermanizadeh A, *et al.* Primary human hepatocytes vs. hepatic cell line – assessing their suitability for in vitro nanotoxicology. *Submitted to Nanotoxicology*.

groups will participate in the WP4 Round Robin for the ENPRA project with half using the same batch of cells while the other half will use different cells and passage numbers.

A great amount of time and effort has been taking to ensure all partners have the same understanding of the experimental protocol allowing for comparison between data. Although the finalisation of the protocol has been quite challenging we are now ready to start the Round Robin, with final findings expected in the middle of July 2012. We would predict very similar toxicity data from all partners in the different laboratories.

The Round Robin will therefore have multiple outputs. We have identified a protocol and cell type that will be useful for widespread use by researchers and industry when testing the cytotoxicity of nanomaterials. The Round Robin will thus provide information about the reproducibility of this protocol between laboratories and therefore its usefulness as a standardised protocol. Furthermore, the design of the Round Robin will help to verify an observation made by one partner, that serum appeared to reduce the cytotoxicity of the low toxicity materials, but enhance the toxicity of the more toxic materials. This observation was only investigated by one group, but due to the importance in terms of providing standard protocols in future, the Round Robin provides an opportunity to see whether this observation is reproducible.

Partners involved in WP4:

Upcoming events

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

- **Nanotoxicology 2012**

The **6th International Conference on Nanotoxicology** (Nanotoxicology 2012) will be held on **September 4th** (Tuesday) **- 7th** (Friday), **2012** in **Beijing, China**.

With the rapid development of nanotechnology applications, the safety assessment of nano-products has become important than ever before. The conference will hence provide a timely international forum for presentation and discussion of current and emerging sciences of all-round. Conference themes are:

- Nanotoxicology and human toxicology (NanoTOX)
- Nano Environmental Health and Safety (Nano EHS)
- Nanomedicine, Pharmacokinetics and Particokinetics (Nano PK)
- Nanobiotechnology, Nano-bio interface &Nanobiomaterials (NanoBio)
- Nano-bioinorganic Chemistry (NanoBioChem)
- Exposure scenarios and risk assessment of nanomaterials (ESRA)
- Nano-bioanalytical sciences and nanostandardization (NanoAnalysis)

For more information, please click here.

- **EU-US nanoEHS worshop 2012**

eu-us bridging nanoEHS research efforts

On **25-26 October 2012**, the Finnish Institute of Occupational Health will host a Joint Workshop between the European Union (EU) and the US, in **Helsinki, Finland**. The purpose of this second Joint Workshop is to further promote and deepen the EU-US collaboration on nanosafety research. The aim is also to develop the Communities of Research (CoR) collaboration and to bring them to a new level of activity. This Joint Workshop is aimed at administrators and policy makers, decision makers and scientists from the EU and the US.

For more information, click here.

- **SENN 2012**

SENN2012

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.

- **Nanosafe 2012**

After the success of Nanosafe 2008 and Nanosafe 2010, the next edition **Nanosafe 2012** will be held from **13th** to **15th November 2012** in Minatec, **Grenoble, France**.

The objectives of the conference will be to make available the major progresses and future trends in the domain of the safe production and use of nanomaterials. Topics of the conference are:

- 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

Deadline for abstract submission is **July 30, 2012**. For additional information, please click here.

- **Fourth Nanosafety Autumn School**

The fourth edition of the **Nanosafety Autumn School** "Understanding Human Health Effects and Environmental Impacts of Engineered Nanomaterials" will take place at **Ca' Foscari University, Venice, Italy**, from **November Sunday 25th to Friday 30th 2012**. Organized in the frame of the major EU 7th Framework Programme 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. The school 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 additional information, please click here.