

ENPRA Newsletter – Issue 3

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ENPRA News & Events

- **3rd EONS meeting**

The **3rd expert panel meeting** of the **European Observatory on NanoSafety (EONS)** was held in **Paris (FR)** on **October 6, 2010**. Organized by the Observatory on Micro and Nanotechnologies (OMNT), this series of meetings is an initiative launched within the **ENPRA consortium**. Aimed at reviewing recent trends and research progresses in nanomaterial environmental, health and safety impacts, EONS meetings involve a panel of experts including ENPRA partners and French experts from the OMNT.

At this meeting, ENPRA partner **Dr. Wim de Jong** from the **Dutch Institute of Public Health and the Environment (RIVM, NL)** offered a keynote presentation on **“In vivo kinetics and tissue distribution of solid nanoparticles”**.



Third EONS meeting (Paris-October 6, 2010)

Experts then presented and discussed a selection of articles from the recent literature dealing with studies on nanoparticle detection and characterization, risk assessment and nanotoxicology. Proceedings of the meeting (3rd 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](#).

- **ENPRA in vivo studies : Report on nanoparticle kinetics studies**

An important step towards the objectives of the ENPRA in vivo approach has been achieved via the release of an internal report entitled **“Kinetics study with radio-labelled nanoparticles”** by **WP5** partners from the **Helmoltz Zentrum München German research Centre (HMGU)**.

As outlined by **Dr. Wolfgang Kreyling** unit leader at HMGU, this report summarizes the results from the first 18 months of the project. In order to assess the biological fate of Engineered NanoParticles (ENPs), the organ distribution of **radio-isotopically-labelled gold and titanium dioxide nanoparticles** has been determined after inhalation, intra-tracheal instillation and intravenous injection in a rodent model. The main conclusions of the study indicate that small fractions of ENPs taken up by lungs can cross the lung membrane and reach the blood stream. ENPs then accumulate in various organs in a size dependent manner (smallest ENPs show highest accumulations in target organs).

While these results on time-course and tissue distribution of ENPs across the key target organs will **help to validate** current **in vitro tests** developed in the ENPRA WP4, kinetics will also provides an important **input for** the physiologically-based pharmacokinetic/dynamic (**PBPK/PD**) **modelling** to be undertaken by partners from WP6.

As a first output from the consortium, based on the experimental approach used for this kinetics study, an **article** detailing the methodology for the preparation of radio-labelled nanoparticles for rodent inhalation studies has been recently published by **Dr. Kreyling** and collaborators, in the **Journal of Nanoparticle Research**. For additional details on this article, please [click here](#).

- **Calendar of future ENPRA events**

- **4th EONS meeting**

The next panel meeting of the **European Observatory on NanoSafety** is to be held on **March 31st** in the premises of ULC's partners at the Louvain Centre for Toxicology and Applied Pharmacology (LTAP) in **Brussels (BE)**. **Dr. Fritz Krombach** from the **Ludwig Maximilians University of Munich (GE)** will give a state-of-the-art presentation on "**Fate and effects of nanomaterials in the microvasculature**". Proceedings of the meeting will be available in the 4th EONS report (due for publication June 2011).

- **Next ENPRA annual meeting**

ENPRA's **mid-term consortium meeting** will take place on **Thursday 10th and Friday 11th February 2011** at the **University Ca' Foscari, Venice (IT)**. Discussions will concentrate on work package progresses and plans for Year 3 of the project. Specific round up sessions will be dedicated to discussions around *in vitro* (WP4) and *in vivo* (WP5) activities.

- **2nd annual Stakeholder workshop**

The **second stakeholder ENPRA workshop "Challenges of Regulation and Risk Assessment of Nanomaterials"** will be held in **Somma Lombardo**, near the Lake Maggiore (IT) from the **10th to 12th May 2011**. Organized by the Nanobiosciences unit of the **Institute for Health and Consumer Protection** of the **Joint Research Centre**, the workshop will involve about 100 invited participants, from Industry, Government, NGOs, and Academia.

The workshop's goals are: to inform the stakeholders of the latest scientific progress in the field of nanoparticles risk assessment produced within the ENPRA project; to present and discuss developments in the last year in the field of legislation and regulatory context in EU and beyond; to gather relevant information from invited experts and projects for use in the regulatory development and implementation; and finally to enhance networking among experts.

To fulfil these goals, the first day of the workshop will be devoted to regulatory development and implementation. A discussion about risk assessment and life cycle assessment tools applied to nanomaterials in relation to regulatory issues will be also included. The second and third days include scientific sessions about characterization and detection, exposure, and (eco)toxicity. Results of ENPRA project as well as from other studies will be presented. At the end of each session there will be a podium discussion involving presenters, experts and other delegates attending.

In the selection of speakers and topics, much emphasis has been put on stimulating regulatory issues, through discussion of new assessment and management methodologies, and comparison with practices of countries outside European Union (e.g. USA). From the scientific viewpoint, we expect the presentation of concrete results, taking into account realistic conditions (e.g. exposure scenarios), and in some cases discussing nanomaterials currently on the market (e.g. testing a form of nano-TiO₂ used in sunscreens).

Proceedings of the workshop will be published by the JRC, details of which will be provided on the ENPRA website when available.

Focus article: Characterization of Engineered NanoParticles (ENPs) – The ENPRA approach

Work Package 3 (WP3) “Hazard Identification: Characterization of the Physico-chemical Properties of Engineered NanoParticles (ENPs)” is dedicated to the physico-chemical characterization of the nanomaterials used in the ENPRA project. In the following interview, our WP3 leader, **Dr. Keld Alstrup Jensen** from **National Research Centre for the Working Environment (NRCWE, DK)** and **Dr. Giulio Pojana** from the **University of Venice (UniVE, IT)** discuss the challenges of ENPs characterization and provide details on ongoing research performed in WP3.

Today, a wide range of nanomaterials is developed and already incorporated into commercialized products. On which specific nanomaterials does the ENPRA project focus?



Dr. K A Jensen: Six ENPs were chosen from the list of nanomaterials included in the **OECD WPMN sponsorship programme**. These materials comprise NM101 (Titanium dioxide: TiO_2), NM110 (zinc oxide: ZnO), NM111 (silane-coated ZnO : silane- ZnO), NM300 (dispersed silver particles: Ag), NM400 (Pyrogenically carbon coated multi-walled carbon nanotubes: PC-MWCNT) and NM402 (multi-walled carbon nanotubes: MWCNT). These materials have been provided by the [European Commission-Joint Research Center-NANOMaterials repository](#). The

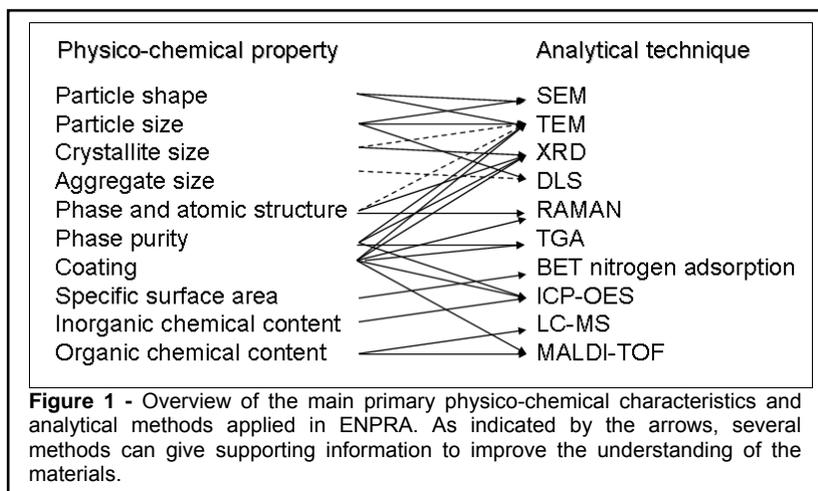
JRC is responsible for the **harmonization** and the **sub-sampling of the nanomaterials** which enable to reduce the risk of experimental variation related to potential inhomogeneity in the test materials.

An additional four TiO_2 samples were selected for the study: A small 10 nm- (NRCWE-001) and a large 100 nm-size rutile TiO_2 (NRCWE-004), of which subsamples of the small-size TiO_2 were chemically modified to achieve a positive (NRCWE-002) and negative charge (NRCWE-003). This titanium dioxide ENP series was established to specifically address potential for different toxicity due to differences in crystalline polymorph (anatase vs. rutile), particle size (10 vs. 100 nm rutile) and surface charge (positive vs. negative rutile). Identification and selection of these two industrially available ENPs was a much larger challenge than originally anticipated. In fact, we had to establish a larger pre-selection analysis to identify suitable candidates. In this process, our selection criteria were based on calculations of phase compositions and crystallite sizes from powder X-ray diffraction analysis and the apparent peak size dispersed in acidic water as determined by dynamic light scattering.

To ensure relevance of the studies undertaken, the **ENPs studied in ENPRA are among the most used particulate nanomaterials on the market**. Most particle types were already identified in the project application. Moreover, supporting the **OECD sponsorship programme** also ensures **high relevance of the test materials**. It is well-known that titanium dioxide, zinc-oxide and silver are currently among the most used ENPs in the world; they are incorporated into many different types of products including paints and lacquers, sunscreens, photocatalytic cleaning surface coatings, pesticides, antibacterial coatings etc. Multi-walled carbon nanotubes have numerous potential applications and have high potential to become a so-called ‘high-volume chemical’. Products such as sports goods, electronics and antistatic coatings with carbon nanotubes (CNTs) are already available on the market. Textiles, paints, polymers and other composite materials containing CNTs are also being developed or at near-market stage.

Can you explain why good characterization of nanomaterials is important for risk assessment?

KAJ: We are still in the “learning phase” in particle and especially ENP toxicology. First of all, it is crucial to carefully determine the **primary characteristics** of the ENPs in order to assess the properties of the test materials and to validate that ENPs truly are the anticipated ones. For example, two different industrial ENPs may be sold as rutile TiO₂: the first may be pure TiO₂ while the other may be chemically modified by Aluminium (Al), Zirconium (Zr) and Silicon (Si) and coated with an organic surface layer resulting in a TiO₂ content of only 80 wt%. Clearly, both materials are not the same and may have different dispersability, chemical reactivity and solubility due to their different surface characteristics. Second, a **multi-parametric physico-chemical analysis (Figure 1)** is essential for identifying potential relationships between the different physico-chemical properties and specific toxicological endpoints. Together, these characterization efforts will help to evaluate the comparability of test results obtained from different nanotoxicological studies.



In toxicological tests, particles and ENPs in particular, may not be homogeneous and evenly distributed in the medium. Hence, ENPs cannot be characterized in the same way as classical soluble chemicals. An ENP is to be considered a sizeable entity with surface and volume-related properties, which depend on its chemical composition and on the specific arrangement of the atoms. **Size** is the most commonly used descriptor for ENPs, but the actual size distribution (including “micron” fraction) and the corresponding dry or wetted **specific surface area (SSA)**, are important parameters. Size and SSA are related to the scalable surface effects that may drive, at least in part, ENP toxicity.

The importance of **atomic arrangement** in silica polymorphs has been known for decades, but has only recently been suggested for nano-size TiO₂. In addition, particle **aggregation**, **agglomeration** and **shape** are expected to play a specific role on their toxicological effects. There is a well-established fiber-paradigm, from which the risk of fibrous asbestos and man-made fibers can be predicted. This parameter may be extended to insoluble nanofibrous and tubular nanomaterials. However, a major work effort is underway to clearly identify the potential thresholds for this large class of nanomaterials. Challenges are still ahead, not only in field of toxicological studies, but also in the development of analytical techniques and procedures to perform validated bulk analysis of compositional and dimensional parameters of CNTs.

Several additional parameters may be important. Many of them have already been listed in the [OECD's WPMN project](#) on the **Safety Testing of a Representative Set of Manufactured Nanomaterials**. However, official procedures and protocols for their determination are not always available. Moreover, not all analytical methods can be applied to all ENPs and their analysis depends on the judgment of the analytical expert. Therefore, **careful characterization is a critical step for at least the early phase in ENPs risk assessment**. Within the ENPRA project, efforts of WP3 will be devoted to these open issues.

On which aspects of characterization do you specifically concentrate in WP3?



Dr. Giulio Pojana: Specific care of WP3 is devoted to the correct evaluation of obtained results, since the same parameters can be acquired with different techniques, each being optimized for specific ENPs. In addition to a set of basic properties commonly defined as “**primary**” **characterization** (size, chemical structure, size distribution etc.), specific efforts of WP3 are currently devoted to the evaluation of the “**secondary**” **characterization**. At this level, we concentrate on the state of **agglomeration** of ENPs dispersed in **various aqueous environments** (stock dispersion, water at various pH values, mediums for *in vitro* and *in vivo* toxicological experiments, biological fluids, etc) and on the **quantification of ENPs in biological samples**. This latter step requires further development and validation of new methods and techniques.

Secondary characterization of ENPs is generally strongly underestimated in nanotoxicology, although data can provide very valuable information on how ENPs actually behave in living systems and therefore help understand their biological effects.

Can you give examples of techniques used in WP3 for the determination of ENP properties?

GP: Some ENP properties can be investigated by means of different methods and techniques, which are often complementary in the information they can provide. WP3 is applying more than one technique in order to compare and give more confidence in obtained results (**Figure 1**). **Powder X-ray diffraction (XRD)** analysis and Rietveld refinement has been conducted at the National Research Centre for the Working Environment (NRCWE) to calculate the phase purity and nanocrystallite sizes of selected ENPs. The physical size and particle size distribution has been investigated at the University of Venice (UniVE) by both Scanning (**SEM**) and Transmission (**TEM**) **Electron Microscopy** for ENPs in their **powdered form** (**Figure 2**). These techniques can give valuable information on crystallite, agglomeration processes in powdered form, size distribution, as well as on shape of particles. **Dynamic Light Scattering (DLS)** can provide additional information on **size**

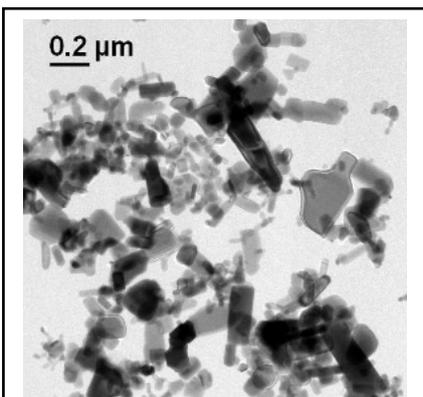


Figure 2 - TEM picture of ZnO nanoparticles (in its powdered form), showing aggregates/agglomerates of euhedral primary crystallites

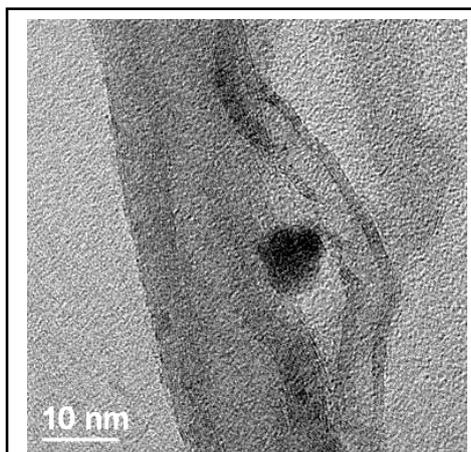


Figure 3 - TEM picture of a two entangled MWCNT associated with an iron (dark spherical particle) catalyst nanoparticle

Scattering (DLS) can provide additional information on **size distribution in various media**, such as MilliQ water (water that has been purified and deionized to a high degree), biological media, as a function of pH or in the presence of other components, such as salts, proteins, surfactants etc. This type of experimentation is currently performed at the NRCWE.

The chemical composition (investigated at UniVE) can be also inferred by various techniques: **TEM**, coupled with the **Energy Dispersive X-Ray (EDX)** technique, can give helpful information of the presence of **particle impurities** (**Figure 3**), while **Inductively Couple Plasma**, after correct chemical digestion of the sample, can provide quantitative information on overall elemental **impurities**. Additional information on organic surface coatings and impurities, such as those present in selected CNTs, is currently being

evaluated by **Liquid and Gas Chromatography** coupled with **Mass Spectrometry (HPLC-MS, GC-MS)**. At NRCWE, CNTs have been subjected to further structural investigation by **RAMAN spectroscopy**. **Thermogravimetric analysis (TGA)** has been employed to quantify the amount of adsorbed water and organic coatings as well as to assess the structural purity of such carbon-based nanomaterials. Finally, **Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF)** spectrometry has been tested for its applicability for fast analysis of the chemical composition of organic surface coatings.

Besides complete characterization of selected nanomaterials, what are the other objectives of WP3?

GP: In addition to the physical and chemical characterization of ENPs, WP3 is working on the development of **analytical protocols** for the determination of ENPs in **biological tissues**. In the early phase of the ENPRA project, WP3 also developed a **generic test item preparation protocol** for dispersion of ENPs in serum water **prior to toxicological experiments**. For improving the exposure characterization, current work is also addressing the **stability of ENPs** toward agglomeration in biological media by DLS and analytical centrifugation (**Figure 4**). Finally, research at NRCWE is conducted to enable a multiparametric characterization of the hydrochemical reactivity, biodurability, and oxidative potential of the ENPs in synthetic biological fluids (**Figure 5**).

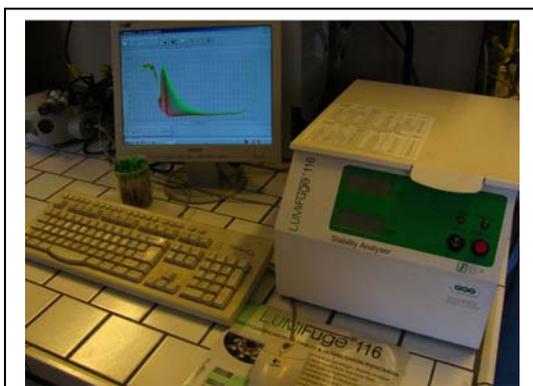


Figure 4 - The analytical photo-centrifuge at UniVE allows to give, in combination with DLS measurements, quantitative information on the stability of ENPs toward agglomeration and precipitation in various aqueous media and conditions.



Figure 5 - Set-up for stirred batch reactor analysis of the physical and hydrochemical reactivity of ENPs under controlled atmosphere, pH, and temperature conditions. The system is based on a titrator for controlling the pH and on-line monitoring of average particle size by flow-cell DLS and changes in redox potential by redox electrodes connected to a multimeter. Stable physiological test temperature is enabled by using flow-cells connected to a temperature-controlled water-bath.

A **report of the primary properties of ENPs** has been recently distributed to the consortium. Some of the results obtained so far within the WP3 will be presented in 2011, during international conferences such as the 3rd NanoImpactnet conference in Lausanne and the SETAC-Europe meeting 2011 in Milan. On January 2011, **KAJ** also presented the **ENPRA dispersion protocol as input on test item preparation methods** for consideration in the **OECD WPMN sponsorship programme**.



Additional researchers from the NRCWE involved in the ENPRA WP3: from left to right: Dr. Renie Birkedal, Dr. Ismo Koponen, Dr. Per Axel Clausen, Dr. Nicklas Raun Jacobsen, Prof. Håkan Wallin.



Additional researchers from UniVE involved in the ENPRA WP3: from left to right: Dr. Dagmar Bilaničová, Andrea Brunelli, Prof. Antonio Marcomini (Unit leader)

Upcoming events

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

- **3rd NanoImpactNet conference: Building a bridge from NanoImpactNet to nanomedical research**

The 3rd NanoImpactNet conference, aimed at creating bridges between Nanomedicine and the NanoImpactNet network is to be held in **Lausanne (CH) on 14-17 February 2011**. The conference will focus on the following topics:



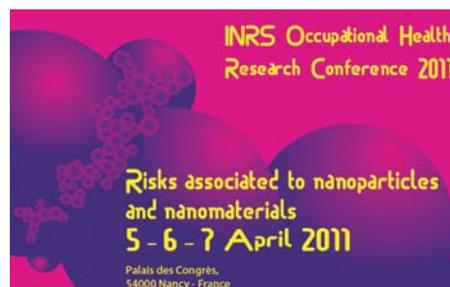
- **Session 1:** Nano-pharmacological input to the research on human and environmental impact of nanomaterials
- **Session 2:** Lessons from Nano-Immunology about the impact of nanomaterials
- **Session 3:** Human impact of engineered nanomaterials and lessons for the nanomedical field
- **Session 4:** Implications from environmental fate and behaviour research for the field of nanomedicine involving nanomaterials

Additional sessions will include i) **Stakeholder Session** “Involving stakeholders in setting research priorities”; ii) A **training school** on February 17, 2011 - “Reproducible Uptake & Quantification of Nanoparticles in vitro (and in vivo)”; iii) Symposia, discussions and brokerage sessions.

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

- **INRS Occupational Health Research Conference 2011: Risks associated to Nanoparticles and Nanomaterials**

Organized by the Institut National de Recherche et de Sécurité (**INRS**) in association with the Partnership for European Research in Occupational Safety and Health (**PEROSH**), this conference will take place in **Nancy (FR), from April 5th, to April 7th, 2011**.



It is intended to bring together researchers, experts and practitioners from different backgrounds with the aim of sharing latest knowledge and discussing research needs on the following scopes: health effect assessment, characterization of nanomaterials, exposure measurement and assessment, emission control and protective equipments, risk assessment and risk management.

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

- **SOT's 50th Annual Meeting**

The **Society of Toxicology (SOT) 50th Annual Meeting** is the largest toxicology meeting and exhibition in the world, with an expected attendance of more than 7,000 scientists from academia, government, and industry from various countries around the globe. The meeting will be held on **March 6–10 at the Walter E. Washington Convention Center in Washington, D.C (US)**.



From the Plenary Opening Lecture and featured lectures to the wide range of scientific sessions (thematic, symposium, roundtable, poster, platform sessions, etc...) and Continuing Education courses, the Annual Meeting offers an unparalleled depth of analysis in relevant toxicological issues. Among the different sessions several will be specifically dedicated to **nanotoxicology, nanomaterial characterization and nanoparticles exposure assessment**. From basic to advanced topical issues, the thematic approach provides each attendee an opportunity to learn about emerging fields.

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

- **SETAC Europe Annual Meeting - Ecosystem Protection in a Sustainable World: a Challenge for Science and Regulation**

The Society of Environmental Toxicology and Chemistry (SETAC) Europe Annual Meeting is Europe's biggest meeting on environmental toxicology and chemistry with more than 1500 presentations in parallel platform sessions and poster sessions, participants and scientific speakers from academia, business and government and a blend of scientists and practitioners, researchers and regulators all in attendance. The **21st Annual meeting** is to be held in **Milan (IT) from Sunday, 15 May to Thursday, 19 May**. A **session dedicated to Nanomaterial and Nanoparticles** will include the following topics:



- H01 - Detecting, quantifying and characterizing engineered nanomaterials in the environment and in biological systems;
- H02 - Fate and effects of nanoparticles;
- H03 - Risk assessment and risk management of nanomaterials.

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

- **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](#).