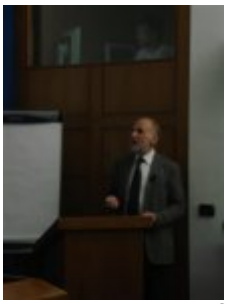
Report from the Health and Environmental Safety of Manufactured Nanomaterials Workshop, hosted by JRC Ispra

Sheona Peters

On the 20th February 2009, a workshop on the Health and Environmental Safety of Manufactured Nanomaterials was hosted by the European Commission Joint Research Centre (JRC) in Ispra, Italy. This workshop which followed two other important nanotechnology meetings at the JRC (NAPIRAhub and ENRHES), brought together around 30 leading nanotechnology experts for discussion on the latest progress and remaining uncertainties in the field of nanotechnology health and environmental safety. The morning seminar was co-chaired by Dr. Hermann Stamm and Dr. Birgit Sokull-Klüttgen, both members of the Nanobiosciences Unit within the Institute for Health and Consumer Protection at JRC Ispra.

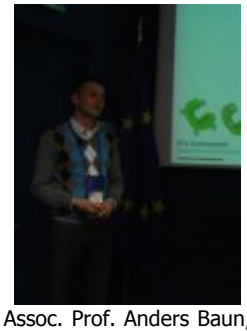

Dr. Hermann Stamm of the JRC presents to attendees.

Dr. Hermann Stamm, Head of the JRC's Nanobiosciences Unit, began the seminar with an introduction to the structure of the JRC, emphasising that the central aim of the JRC is to provide customer-driven scientific and technical support to community policy making. Within this core structure, the Institute of Health and Consumer Protection (IHCP) applies scientific expertise to a number of policy driven domains which are relevant to protection of the interests and health of consumers in the framework of EU legislation on chemicals, food and consumer products. Dr. Stamm highlighted that nanotechnology is an area of particular importance within this context and provided an overview of the extensive nanotechnology activities currently taking place at IHCP. On-going activities include the development of appropriate test methods for nanomaterials, nanotoxicological studies, radiolabelling of nanomaterials, nanotechnology exposure assessment and risk/safety assessment. For further information on the JRC's nanotechnology activities, follow this link.

Professor Vicki Stone, Professor of Toxicology at Edinburgh Napier University's Applied Research Centre for Health, Environment and Society, gave the first invited talk of the day. This focussed on recent research attempting to relate the physicochemical characteristics of nanoparticles to their behaviour in biological systems. Professor Stone outlined the key physicochemical characteristics of nanoparticles which are of importance in determining their biological activity, namely shape, size, surface area, charge, solubility, crystal structure, composition, strength and electrical conductance. By making reference to key studies undertaken in collaboration with her research group, Professor Stone highlighted aspects of the now expanding body of scientific evidence which is attempting to link these

physicochemical characteristics to the biological behaviour of particles and nanoparticles, including toxic effects such as inflammation and reactive oxygen species production. Professor Stone suggested that knowledge and analysis of such relationships would potentially allow Quantitative Structure Activity Relationships (QSARs) or Ranking Technologies (e.g. DART) to be constructed for nanoparticles, a major milestone in terms of understanding the toxicological risks of nanomaterials.

Anders Baun, Associate Professor and Head of Innovation at the Department of Environmental Engineering, Technical University of Denmark (DTU), provided an overview of his research in the area of the ecotoxicity of engineered nanomaterials. His work surrounds the central hypothesis that nanoparticles may act as carriers of co-existing environmental contaminants and thereby influence their uptake and toxicity.


Assoc. Prof. Anders Baun, DTU

When assessing the influence of C_{60} on the ecotoxicity and genotoxicity of xenobiotic organic chemicals (substances foreign to an entire biological system), Assoc. Professor Baun presented results which indicated an increase in the algal toxicity of **atrazine** in the presence of C_{60} nanoparticles. However, there was no observed change in the toxicity of **methylparathion** to algae or water flea *Daphnia magna*, in the presence of C_{60}nanoparticles.

Why are these chemicals of particular environmental concern?

Atrazine is a widely used herbicide which acts as a potential endocrine disruptor and carcinogen.
Methylparathion is a pesticide which disrupts the nervous system and is highly toxic to non-target organisms.
Phenanthrene is a type of polycyclic aromatic hydrocarbon and potential carcinogen.
Pentachlorophenol has, in the past, been used as a herbicide, insecticide, fungicide, algaecide and disinfectant, and is associated with carcinogenic, renal, and neurological effects.

Studies monitoring the uptake and accumulation of **phenanthrene** in *P. subcapitata* algae and *D. magna* indicated in both species that presence of C_{60}nanoparticles increased toxicity of phenanthrene, suggesting that sorbed phenanthrene is bioavailable. Conversely, the toxicity of **pentachlorophenol**towards algae and daphnia was found to decrease after addition of C_{60}nanoparticles. Uptake and excretion rates of phenanthrene, however, were not significantly affected by addition of C_{60} nanoparticles. C60 nanoparticles also appeared to have no effect on *D. magna* mobility. Thus, overall these results indicated that no specific interaction exists between either the C_{60} nanoparticles and chemical used, or the C_{60} nanoparticles and the biological species.

In conclusion, these studies provided evidence that environmental behaviour is highly dependent upon both the nature of the chemical and the nature of the nanoparticle, although Assoc. Prof. Baun did stress that further research is required to fully understand the results and mechanisms of the interactions. In addition, he noted that, although a structured research programme is yet to be developed, literature assessments indicate that research in the area of nano-ecotoxicology is steadily increasing, serving to highlight the growing level of concern within the scientific community surrounding the potential environmental toxicity of nanomaterials.

Dr. Christoph Klein, who leads the action on Nanotechnology of the IHCP at JRC Ispra, closed the first session with an overview of the NAPIRAhub development and its role in nanotechnology policy support. The aim of the NAPIRAhub database is to provide an information management platform for the collection, exchange and analysis of nanotechnology data and methodology. Through collating existing data on manufactured nanomaterials relating to human health and environmental exposure, toxicity and risk/safety assessment, it is envisaged that the NAPIRAhub will provide a tool to collect, share and analyse relevant data. This is a necessary pre-requisite to address the issue of nanosafety in an integrated approach, suitable for regulatory use. It is planned that the NAPIRAhub will be complemented by a set of suitable reference nanomaterials and the development of integrated testing strategies (discussed further by Dr. Rob Landsiedel later in the seminar). The NAPIRAhub will also serve to identify current information gaps and future data needs in the field of nanotechnology. Dr. Klein highlighted the current lack of continuity between the information produced from research projects and the information required for sound policy making in this area. It is hoped that implementation instruments such as the NAPIRAhub will help resolve this issue, encouraging collaboration and a more coherent, focused approach to future nanotechnology research programmes. It is intended that data held within the NAPIRAhub will be updated in accordance with publication of relevant output from other major nano EHS schemes, such as the OECD WPMN . Updating is supported by JRC-IHCP's Nanotechnology Scientific Expert Network NAPIRA, which consists of experts from science, industry, governmental, NGO and international bodies across the fields of material science, human health, environment, and IT-systems toxicology. Currently, the NAPIRAhub is publically available on request through the network.


Dr Christoph Klein, IHCP

The second session was opened by Dr. Rob Aitken, Director of SAFENANO and of Strategic Consulting at the Institute of Occupational Medicine (IOM). Dr. Aitken provided an overview of IOM's work into environmental, health and safety aspects of nanomaterials (nano-EHS), outlining some of the key drivers of UK nano-EHS activity and highlighting IOM's role in filling the gaps between academia, industry, regulators and consumers.

Dr. Aitken emphasised the key role of the SAFENANO initiative in addressing the potential risks to the environment and human health from nanomaterials, through providing independent and impartial information and consultancy services. Another aspect of IOM's work lies in reviewing and interpreting existing knowledge. Dr. Aitken highlighted the recent work undertaken by the IOM in this area, including DEFRA funded projects ' CELL PEN ', (a study to identify the physico-chemical factors controlling the capacity of nanoparticles to penetrate cells), ' HARN ' (a review to assess whether high aspect ratio nanoparticles should raise the same concerns as do asbestos fibres) and ' EMERGNANO ' (a worldwide merging evidence review to assess the extent to which key research objectives have been met), as well as ongoing EC project ' ENRHES' (Engineered Nanoparticles: Review of Environment, Health & Safety).

Dr. Aitken also emphasised IOM's contribution to the prioritisation of research needs and the provision strategic advice to Government through, for example, the DEFRA funded ' REFNANO ', whose aim was to provide a priority list of candidates for inclusion in a set of reference materials to support the measurement, toxicology and risk assessment of engineered nanomaterials in the UK. Finally, Dr. Aitken outlined IOM's work towards developing and implementing good practice for nanotechnology, with examples of recent

work including the production of a BSIGuide for Safe Handling & Disposal of Engineered Nanomaterials and technicaldevelopment of 'AssuredNano', the UK's first Nanomaterials Accreditation Scheme.

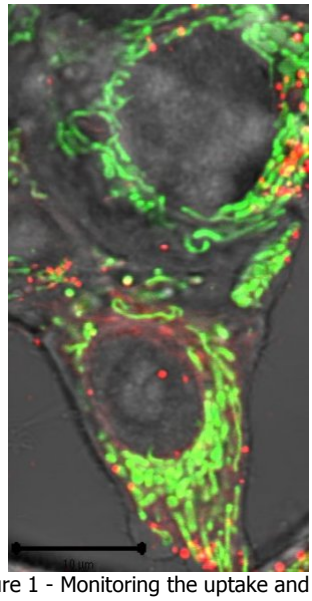


Figure 1 - Monitoring the uptake and intracellular fate of 20 nm fluorescent polystyrene nanoparticles (red) in HepG2 liver cell lines. Dr. H. Johnston, Napier University.

Dr. Lang Tran, Director of Quantitative Toxicology at the IOM, continued the session with an overview of EU FP6project ' PARTICLE RISK' . The aim of this project was to acquire a bank of five nanoparticles potentially generated by NEST (new emerging science and technology) and assess the health risk from exposure to these materials through air or the food supply with a work programme, integrating in vitro experiments, in vivo models of healthy/susceptible individuals and exposure/risk assessment. Dr. Tran highlighted that this project was instrumental in laying down the rationale and work programme strategy that has been applied to several FP6 and FP7 projects since. Dr. Tran summarised the main findings of 'PARTICLE_RISK' with relation to the observed liver effects (Figure 1), biokinetics, in vivo pulmonary effects, genotoxicity and platelet aggregation effects of the five studied nanoparticles (gold, single walled carbon nanotubes, fullerene C60, quantum dots and carbon black), concluding with a description of the, then novel, exposure and risk assessment strategy that was formulated within the project.

The penultimate presentation of the workshop was provided by Dr. Rob Landsiedel, Head of the Short Term Toxicology Department at BASF SE . Following a brief discussion of the potential behaviour and adverse effects of nanomaterials in the human body as compared to larger-sized particles and dissolved molecules, Dr. Landsiedel outlined a proposal for a concern-driven testing strategy for nanomaterials, whereby the selection of appropriate test methods would be based upon initial health based concerns; this would include basic concerns (e.g. skin and eye irritation, short-term inhalation etc.), cellular/nano-specific concerns (e.g. inflammatory and fibrogenic effects) and biokinetics. Dr Landsiedel pointed out that in order to achieve this, it is necessary to build up a toolbox of validated in vitro and in vivo testing methods based on OECD or other standard test methods. These methods need to be adapted to fit the specific properties of nanomaterials (e.g. preparation and characterisation of the test items). Where no validated in vitro method is yet available, confirmatory in vivo studies may be necessary. Based on the properties of individual nanomaterials and the available information, an adequate testing method should be selected from the toolbox to address specific concerns. Dr. Landsiedel also highlighted the importance of facilities such as the NAPIRAhub in the development of agreed testing strategies, through allowing sharing and gathering of the required data. These agreed testing strategies will ultimately be of vital importance in achieving the much needed rigorous safety evaluation of nanomaterials.

Dr Rob Landsiedel, BASF

The seminar was closed by co-chair Dr. Birgit Sokull-Klüttgen with a presentation focusing on chemical regulation, highlighting the place of nanomaterials in the new regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (

REACH) implemented by the European Chemical Agency (ECHA). REACH is based on the principle that manufacturers, importers and downstream users must ensure that they manufacture, market or use such substances that do not adversely affect human health or the environment. Dr. Sokull-Klüttgen emphasised that, although there are no provisions in REACH referring specifically to nanomaterials, REACH does address chemical substances in all sizes, shapes or physical stage. Therefore, nanoscale substances are covered by REACH and its provisions apply.

Within discussion of the key stages in the REACH procedure, Dr. Sokull-Klüttgen highlighted that during the pre-registration phase 13 OECD nanomaterials were included. 12 out of 13 of the OECD nanomaterials have been pre-registered with a tonnage volume > 1000 tonnes per year. According to the stipulations of REACH this means these materials must therefore be registered through submission of a technical dossier to ECHA by December 2010. At volumes ≥ 10 tonnes per year, submission of a Chemical Safety Report is also required along with the registration.

Registration of a nanomaterial must include all relevant information covering the properties, uses, effects and exposure, as well as the classification and labelling and safety assessment. It was highlighted that nanomaterials having specific properties may require different classification and labelling compared to the bulk material. Some registered substances are then evaluated and may be required to be authorised if deemed to be a substance of very high concern. This also includes substances of "equivalent level of concern", which may include nanomaterials. Restrictions will then be applied to any substance which is considered to pose an inadequately controlled risk to human health or the environment. Dr. Sokull-Klüttgen highlighted that it could be useful to prioritise a small number of nanomaterials for evaluation, as this would allow any issues to be raised, discussed and solved as far as currently practical.

Dr. Sokull-Klüttgen highlighted that, although there is no specific guidance for substances in the nanoscale, 'Nanomaterials in REACH' , which presents the initial conclusions of the REACH Competent Authorities subgroup on nanomaterials, can be used by companies as a starting point to prepare their registration documents for nanomaterials. Important issues raised by this subgroup, and broadly amongst researchers, in terms of regulating nanomaterials under REACH include concerns that many substances may be produced/imported below 1 tonne per year and that information on low-volume phase-in nanomaterials will only become available in 2018. It has been suggested that all substances at the nanoscale be registered, even if produced at < 1 tonnes per year, with a reduced information set. The European Commission plans to respond to these concerns through a review of REACH in 2012. Dr. Sokull-Klüttgen emphasised that addressing such concerns requires the cooperation of industry, with the Commission asking industry to be pro-active in terms of substance registration. For further discussion on the implications of REACH for nanotechnology, please see SAFENANO's recent feature article.

The workshop continued into the afternoon with a tour of the Nanobiotechnology laboratories at JRC Ispra, which included an overview of the current nanotoxicology activities taking place within the IHCP, such as nanoparticle synthesis and characterisation and in vitro studies of nanoparticle-cell interactions. Overall, the day provided an excellent opportunity for knowledge sharing and information exchange. The presentations provided a useful insight into the current activities underway in the field of nano-EHS across Europe, particularly in relation to policy support and development.

Sheona Peters,
April, 2009