NanoMILE's spring in Antalya (NanoTox2014)

By Benoît Hazebrouck (EU-VRi), Iseult Lynch (University of Birmingham) with the NanoMILE consortium (speakers and poster authors)

It is not yet the harvest season, but after just one year of existence, NanoMILE blossomed in the warm atmosphere of Antalya, with no fewer than 10 posters as well as 2 oral presentations delivered at NanoTox 2014. In addition, NanoMILE had its month 12 meeting in Antalya immediately prior to the NanoTox2014 congress, with partners participating in some lively discussions and making decisions for future activities, such as a joint workshop with NanoSolutions on high throughput screening as part of our Month 18 meeting in Copenhagen.

We present here an overview of these contributions as a snapshot of the progress made during the 1st year of NanoMILE. All 8 technical WPs of NanoMILE were represented, showing that exciting progress is being made in all aspects of the project. The posters can be downloaded here.

Poster 1: ***Isaac Ojea-Jiménez*** (Joint Research Centre, JRC) and his colleagues performed **surface engineering of fluorescent silica nanoparticles for biolabelling applications**. They successfully functionalized SiO_2 nanoparticles with a variety of ligands via a simple, fast and biofriendly process while preserving the main properties of the particles, i.e. good size distribution, morphology and stability. For that, they used a regrowth procedure in water and exploited epoxysilane chemistry as a convenient and versatile route for activation of the silica surface. This work relates to **WP2** of NanoMILE: particle synthesis & functionalization. (see image at end).

Oral 1: ***Bernd Nowack*** (Swiss Federal Laboratories for Materials Science and Technology, EMPA) spoke about his groups work on release and environmental exposure of nanomaterials, and included some highlights from the activities regarding ageing of MNMs in products. A key aspect of NanoMILE is the focus on testing aged MNMs in addition to the pristine ones, and we have postulated that ageing will reduce the complexity of MNMs via reduction of the wide variety of surface chemistries to a simpler subset, primarily oxides and sulfides. This work relates to **WP3** of NanoMILE: Life cycle evolution of MNMs.

Poster 2: ***Katrin Volkmann*** (Karlsruhe Institute of Technology, KIT) and her colleagues presented a **high throughput screening assay for *in vitro* testing of MNM cytotoxicity**, The assay was implemented using 10 of the NanoMILE particles. Development of a high throughput *in vivo* assay using zebrafish embryos adapted from the new OECD Test No. 236 is under way. First results show high similarities between toxicity of the MNMs *in vitro* and *in vivo*. Finally, KIT will develop a screening platform and carry out systematic toxicity screening for up to 100 MNMs by combining high throughput *in vitro* testing in cultured cells and *in vivo* whole organism exposure of zebrafish embryos with high content imaging and image analysis. This work relates to **WP4** of NanoMILE: Development of a screening platform for MNMs.

Poster 3: ***Kirsten Gerloff*** *(Joint Research Centre, JRC) and her colleagues described the concept of* ***Adverse Outcome Pathways as a tool for understanding and analysis of nanoparticle-induced liver toxicity.*** An AOP is a conceptual construct that portrays existing knowledge concerning the link between a Molecular Initiating Event and an Adverse Outcome, by capturing the sequential chain of causally-linked Key Events (KEs) at different levels of biological organisation. Nano-specific AOPs would support structuring and organising the fast-increasing amount of existing knowledge on mechanisms of NP-induced toxicity and inform study developments. The application of this concept to nanotoxicology is proposed, and opportunities and challenges are pointed out on the example of a nano-specific liver-AOP. Information derived from the NanoMILE project will directly feed back into future AOP developments. To this end, a wide range of MNMs is used to detect KEs such as mitochondrial damage, apoptosis induction and cell toxicity in differentiated human hepatic HepaRG cells. This work relates to WP4 of NanoMILE: Development of a screening platform for MNMs.

Poster 4: ***Phil Vincent*** (NanoSight / Malvern Instruments Ltd, MIL) and his colleagues applied **Nanoparticle Tracking Analysis (NTA) for Time-resolved, reproducible Nanoparticle Characterisation in biological and environmentally relevant media** for a range of complex sample types. NTA allows simultaneous measurements of nanoparticle size distribution and concentration and as such provides detailed information on nanomaterial characteristics throughout their lifecycle. An NTA Round Robin has developed protocols and SOPs exhibiting proven increases in repeatability and reproducibility of particle size measurements throughout the entire NanoSight instrument range independent of operator.

Within NanoMILE, protocols for assessment of evolution of dispersion quality in complex biological and environmental solutions in a time-resolved manner are being developed. This work relates to **WP5** of NanoMILE: MNM interactions with biomolecules and environmental factors.

Poster 5: *Annette Piechulek* (Leibniz Research Institute for Environmental Medicine, IUF) and her colleagues showed that **Silica nanoparticles induce premature aging phenotypes in the soil nematode *Caenorhabditis elegans*.** In the NanoMILE project, *C. elegans* is used as a screening platform for environmentally relevant nanomaterials such as CeO_2, Ag and ZnO. MNM bio-interactions and effects on organismal aging are investigated. The research team showed that fluorescently labeled silica nanoparticles are taken up by the nematode's intestinal system and the reproductive system. Furthermore, silica nanoparticles induce a premature onset of insoluble protein aggregation and a reduction of pharyngeal pumping that represent distinct aging phenotypes on the molecular and behavioral level. This work relates to **WP6** of NanoMILE: MNM bioavailability & biological effects in vitro/in vivo (ecotoxicology).

Poster 6: *Rhys M. Goodhead* (University of Exeter, UEXE) and his colleagues investigated a **novel fish model, *Xenotoca eiseni*, to study the maternal transfer of MNMs and their biological effects on developing offspring**. *X. eiseni* shows direct maternal nutrient provisioning to developing embryos and live bearing. In a pilot study, the bioavailability, uptake, biodistribution and maternal transfer of nano versus bulk sized Ag MNMs was assessed in *X. eiseni*. Initial findings show transfer of Ag MNMs into the larvae and partitioning to many organs, especially the liver. Ag MNMs (or Ag+ derived from these particles) appeared to be more bioavailable than the bulk counterpart. Full scale studies are under way, considering bulk sized Ag MNMs and nano Ag MNMs with different surface coatings. This work relates to **WP6** of NanoMILE: MNM bioavailability & biological effects in vitro/in vivo (ecotoxicology).

UEXE's Fish Model Xenotoca eiseni *(UEXE Poster 6)*

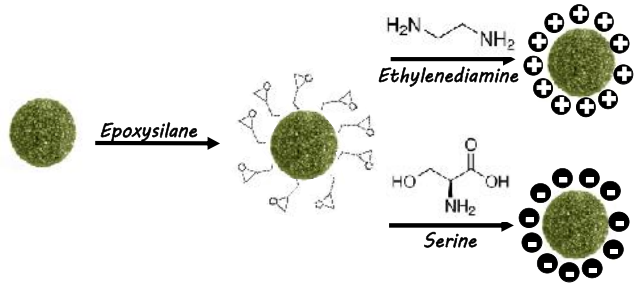
Poster 7: *Susan Dekkers* (National Institute for Public Health and the Environment, RIVM) and her colleagues determined the **effect of a series of nanomaterials on inflammasome activation**. Inflammasomes are intracellular protein complexes that upon sensing danger signals can initiate inflammatory responses, resulting in interleukin-1 beta (IL-1β) or interleukin-18 (IL-18) induction. CeO_2 and SiO_2 NMs caused a clear dose-related increase in IL-1β production that was dependent on NLRP3 and ACS, indicating NLRP3 inflammasome activation. Micro-sized CeO_2 particles and TiO_2 or ZnO NMs did not cause a dose-related increase in IL-1β production, suggesting that this inflammasome activation depends on the size and chemical composition of the particles. For both SiO_2 NMs the increased IL-1β production observed at the highest dose levels was accompanied by a pronounced decrease in viability. This decreased viability was NLRP3- and ASC-dependent, suggesting pyroptosis (programmed and inflammatory cell death) might occur beyond a certain threshold. This work relates to **WP7** of NanoMILE: MNM biokinetics and toxicity testing *in vitro/in vivo* (toxicology).

Poster 8: *Mark R. Miller* (University of Edinburgh, UEDIN) and his colleagues studied the **translocation of inhaled gold nanoparticles and accumulation in atherosclerotic plaques**. Using high sensitivity ICP-MS in complementary animal and human studies, they demonstrated that inhaled / instilled gold nanoparticles (5 nm diameter) can translocate from the lung into the blood. Particles are cleared by the liver and urine, however, the nanoparticles selectively accumulate in atherosclerotic plaques in ApoE knockout mice. The results suggest that inhaled nanoparticles may localise at sites of vascular inflammation which are likely to be susceptible to their harmful effects. This model will subsequently be applied to the panel of NanoMILE particles. This work was not funded by NanoMILE, but will directly be further used in **WP7**: MNM biokinetics and toxicity testing *in vitro/in vivo* (toxicology).

Trophotaeniae of larval X.eiseni 4 weeks post fertilisation. These prominent structures absorb maternally derived nutrients from a surrounding fluid matrix *(UEXE, Poster 6).*

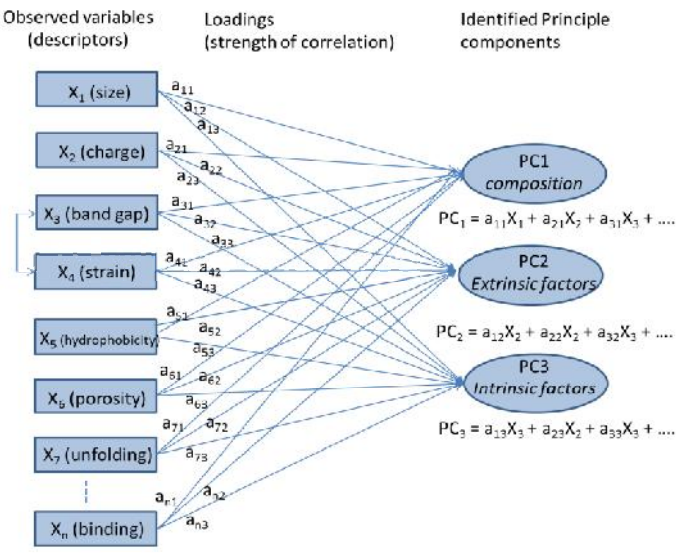
Poster 9: ***Jana Keller*** (BASF) and her colleagues studied the **pulmonary effects and biokinetics of CeO_2 nanoparticles in 5-day, 28-day and 90-day rat whole-body exposure studies**. This NanoMILE work is combined with a chronic and carcinogenicity long-term inhalation study (OECD TG 453) in rats, currently being performed within the EU-project NanoREG. Bronchoalveolar lavage fluid (BALF) analysis and histopathology of the respiratory tract were performed. The lung retention and clearance kinetics up to a post-exposure period of 129 days in the 28-day study were analysed by ICP-MS. Inhalation of aerosols containing 5 and 25 mg/m³ (but not 0.5 mg/m³) CeO_2 was associated with high CeO_2 retention in the lungs, translocation of the particles to the regional lymph nodes and a retarded lung clearance already after 5-days of exposure. These exposure conditions caused inflammation in the lung and findings in the draining lymphoid tissue. The 5-day inhalation study relates to **WP7** of NanoMILE: MNM biokinetics and toxicity testing *in vitro/in vivo* (toxicology).

Oral 2: ***Francesco Falciani*** (UoB / University of Liverpool) spoke about **a systems biology approach to understand toxicity** which starts from the identification of important molecular components and aims to infer the underlying structure of the molecular networks connecting these components coupled with application of advanced statistical modelling and network inference techniques for the development of mechanistic biomarkers of environmental pollution. Within NanoMILE, this approach is being utilised to identify adverse outcome pathways associated with MNMs: using statistical or machine learning approaches, each of the individual 'omic datasets can yield expression signatures that identify barcode-like responses to the MNMs, as already shown successfully for chemicals. This work relates to **WP8** of NanoMILE: Systems biology approaches to reveal mechanisms of MNM activity.



Surface modification of SiO_2 nanoparticles (JRC, Poster 1)

Poster 10: ***Iseult Lynch*** (University of Birmingham, UoB) and her colleagues proposed a **novel strategy for grouping of nanomaterials based on key physicochemical descriptors as a basis for safer-by-design NMs**. The approach identifies interlinked physicochemical descriptors, and on this basis overarching descriptors (axes or principle components). It can be used to correlate physico-chemical descriptors with toxicity, and to disentangle the relative contribution of a specific parameter to the different principle components. As an example, three principle components are used to fully describe each NM: composition, the intrinsic (inherent) properties of the NM, and extrinsic properties (interaction with media, molecular coronas etc.). The next step will be to link the principle components to adverse effect outcomes and develop a predict model using factor analysis. This work relates to **WP9** of NanoMILE: Data integration/QPARs, risk assessment, safe MNM designs.



Schematic illustration of use of PCA as applied to determination of primary descriptors of NM toxicity (UoB, Poster 10)

As results reach maturity and are published in peer-reviewed journals, links to the publications will be posted on the NanoMILE website, so please check-back regularly. You may also subscribe to the NanoMILE newsletter on the NanoMILE website: this newsletter will inform about NanoMILE's activities and results.

http://nanomile.eu-vri.eu