NanoFATE
Nanoparticle Fate Assessment and Toxicity in the Environment

NEWSLETTER
WINTER 2013-14 /#5

- Our work
- Our advice
- Our findings
- We were here
- Our people
- Our reports / Meet us here

Visit www.nanofate.eu

our work

NanoFATE replies to 5 Big Questions:

1 What are the effects of coatings on fate, behaviour and toxicity of NPs?

2 Can metal nanoparticles act through a different mechanism and/or be more toxic than the ionic forms?

3 How to prove / analyse whether nanoparticles are present in tissues or media?

4 When is a 'case by case' risk assessment of nanoparticles necessary?

5 Is the accuracy of today's risk assessment for 1st generation NPs sufficient to assess risk?

Find the answers

editorial

by Coordinator Claus SVENDSEN

At the Diamond Light Facility, Aug. 2013

The Foundations are Laid

Welcome to the fifth Newsletter from the NanoFATE project. As we near completion in March 2014 our results are attracting interest from a wide array of stakeholders. European Member state environmental authorities such as Defra, international organisations like OECD, and the academic and regulatory communities have all used NanoFATE results to inform the debate on sustainable use of nanotechnologies. Approaches and methods developed through NanoFATE and our sister NanoSafety Cluster projects are orienting tomorrow's nanotechnology research through the Horizon 2020 programme.

This Newsletter tells how NanoFATE helps to lay the foundations of future nanotechnology-focussed ecotoxicology work by providing insight into fate and toxicity processes. We have tested a tiered approach to characterising and tracking ENPs during key processes. We have related the physiochemical properties of receiving waters to the fate of ENPs in freshwater systems. NanoFATE has more than doubled the species coverage of toxicity data for silver and zinc oxide ENPs, demonstrating the effect of confounding factors such as media pH and co-exposure to organic contaminants, as well as how bioavailability and aging processes affect the scale of influence. The internal fate of ENP metals within organisms and the mechanistic basis for toxicity have been studied. All of these aspects further parameterize NanoFATE's improved fate and distribution models and resulting risk maps.

The 5 Big Questions shown at left are deceptively simple - they go to the heart of research issues significant for ENP regulation. To reply, we've distilled our work into Advice Notes that will help regulators understand our major findings. Our NanoFATE library lets you access these along with the detailed reports and journal papers. And if you want to learn more in a direct setting, **please join our free Workshop and Training School "*Putting environmental realism into nanosafety assessment*" (4-7 March 2014, Birmingham)**!

One of NanoFATE's most rewarding features is our strong emphasis on the training of young scientists. The first of our 9 PhD students, Pauline Waalewijn-Kool, successfully defended her thesis; well done Pauline, and the best of luck for those to follow!

Please pass along our Newsletter to interested colleagues – and accept our best wishes for a happy and productive 2014.

CLAUS SVENDSEN

our advice

NanoFATE has identified **Five Big Questions** current today in ecotoxicological risk assessment for engineered nanoparticles (ENPs). Replies to these questions are the concentrate of NanoFATE methodological development and testing.
The resulting **Advice Notes** are useful for researchers but also for regulators. They point the way to further research, and flag issues that regulators should attend to when setting assessment guidelines and regulatory practice.

Lee Walker, NanoFATE Project Manager, put together our Advice Notes on the basis of NanoFATE findings – as summarised in the next pages of this Newsletter.

Luisa Doughty, part of the Coordination team, will be happy to field your enquiries regarding the Advice Notes and provide high-resolution images and figures generated from the project. Contact Luisa at NanoFATE@ceh.ac.uk

*Here are the **Five Big Questions** with associated rules-of-thumb. Choose the Advice Notes best for you!*

Q1 *What are the effects of coatings on fate, behaviour and toxicity of NPs?*

- Advice note 1 – NanoFATE has demonstrated a long lasting effect of coating on the behaviour and toxicity of zinc oxide (ZnO) NPs in regard to soil invertebrates.
- Advice note 2 – NanoFATE has demonstrated (confirmed) a need for case-by-case consideration of coating effects on NP behaviour and toxicity.

Q2 *Are there examples of metal nanoparticles acting through a different mechanism and/or being more toxic than the ionic forms?*

- Advice note 3 – NPs and ions have different toxicokinetics – leading to differing internal levels of metals. Ionic Ag is more toxic than AgNP based on mass calculations.
- Advice note 4 – NanoFATE has demonstrated that the protective genes, biochemical pathways and sequestration mechanisms invoked by organisms exposed to ionic metals also are employed during NP-based exposures, but importantly that NP-based exposures do lead to additional effects and mechanisms being observable too.

Q3 *What is a workable way of proving / analysing whether nanoparticles are present in tissues or media (cf. EU Definition)?*

- Advice note 5 – Given the right resources we have the methodology to find NPs inside tissues. Some of the identified particles are of an environmental rather than a manufactured origin.
- Advice note 6 – Within *in vivo* environmental studies, differences in the internalised forms and distributions of metals between NP- and ionic-based exposure can be indicated based on differences in functional endpoints, before proceeding to mechanistic or NP localisation demonstration.

Q4 *Given that particles differ in their properties, what determines if particles can be grouped for risk assessments, and when is a case-by-case approach necessary?*

- Advice note 7 – NanoFATE has demonstrated that for zinc oxide (ZnO) particles, properties like coating and size do importantly differentiate the ENPs in terms of their fate and toxicity.
- Advice note 8 – ENPs can be treated as "the same" if properties fall within the range seen in already investigated ENPs.

Q5 *Is the accuracy of today's risk assessment for 1st generation NPs sufficient to assess the risk that these materials may pose?*

- Advice note 9 – The following components are needed to improve risk assessment:
  - Good data on production volumes and use patterns
  - Broader selection of well characterised test particles in sufficient amounts
  - Assessment of uncertainty in scenarios and estimation of risk quotients
- Advice Note 10 – For both zinc oxide and silver the risk assessment for ionic forms would most often be adequate for ENPs.

our findings

NanoFATE has delivered or focussed on the following tasks, building throughout the project towards environmentally realistic risk assessments:

- *Source, produce and fully characterise the commercial ENPs, matching a tagged version of ZnO as closely as possible.*
- *Establish particle behaviour in pure ecotox media, and at higher than environmental concentrations.*
- *Establish acute and chronic toxicity to enable selection of relevant doses.*
- *Develop initial simple assumption-based fate models and estimate worst case environmental concentrations.*
- *Train all staff cross discipline, and disseminate to other EU projects and stakeholders.*

Read below about our progress in each work package.

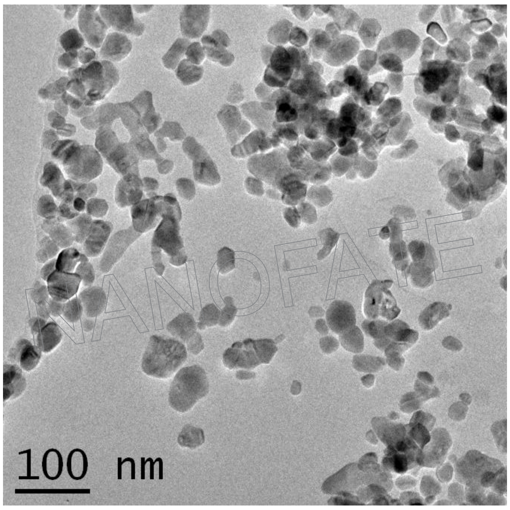
Visit our NanoFATE Library to download reports and summaries, identify peer-reviewed articles, and obtain our Advice Notes. You can also view our images and request high-resolution non-watermarked copies for use with citation.

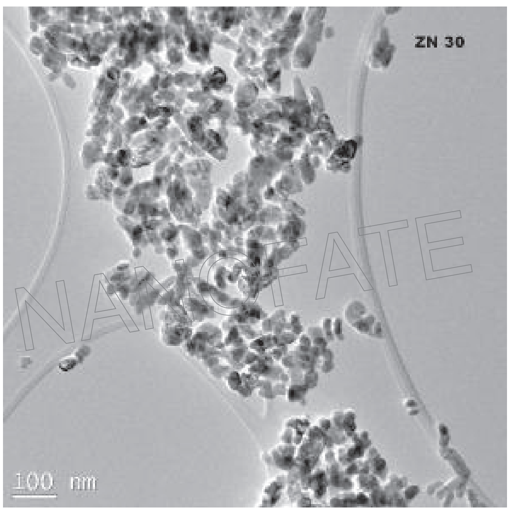
WP 1 Characterisation and tracking of ENPs during processes involved in fate and toxicity

The final set of high-quality, well-characterised and consistent NanoFATE commercial particles are:

- 30nm Nanosun ZnO particles from Microniser in Australia, with matching tagged ZnO ENP by IHPP, with some work on BASF z-cote and z-cote HP1 ZnO
- Amepox 3-8nm Ag ENP and a 50nm Ag ENP from NanoTrade
- CeO2 as Antaria fuel additive and a polishing agent from Umicore.

The last 18 months have focused on characterisation of ENPs in all the used exposure media and some natural media. The dissolution rates of ENPs (ZnO, co-doped ZnO and Ag) not only depended heavily on the water and or media involved, but particularly for zinc oxide on the particle morphology and coating with e.g. high aspect particles dissolving more readily than equiaxed particles. Dissolution rates for ZnO were slower than generally reported elsewhere and our small Ag particle persists for weeks in aqueous media. In soil the dissolution rates appear even longer and on the order of months.

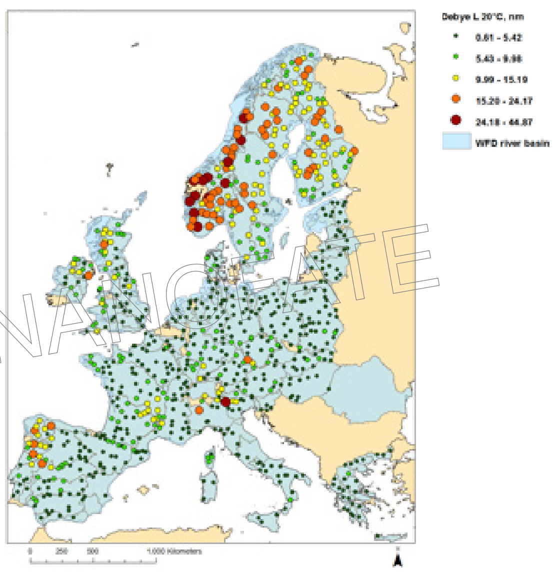




From WP1 - Transmission Electron Microscope (TEM) micrographs of 30nM zinc oxide (ZnO) and cobalt (Co) doped (10 wt%) particles (supplied by IHPP). The Co doped particles have been used within the NanoFATE project to study bioavailability and toxicokinetics of ZnO in biota. (TEM generated by UOXF.DJ)

WP 2 ENP environmental behaviour and fate modelling

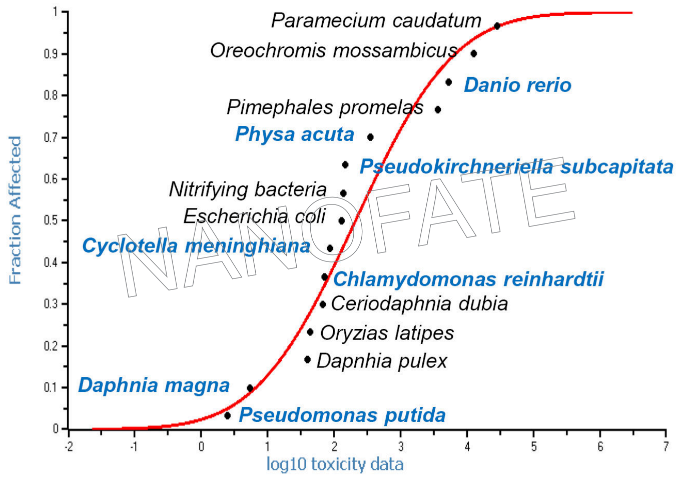
We identified and prioritised specific properties that needed principal consideration, then developed the initial basic environmental fate models. Numbers on CeO_2 deposition in soils, and nano ZnO and Ag in effluent and sludge from sewage treatment, were supplied to serve decision making and exposure design. We then worked to parameterize the ENP environmental fate modelling with information on dissolution, agglomeration (homo- and hetero-), sedimentation of agglomerates, and aging (transformations). Kinetic models building on colloid theory are especially promising and have been developed to deliver fate predictions at the EU scale. Dissolution is an especially important process to study for silver and zinc oxide, overshadowing other processes in governing the fate of these ENPs.



From WP2 - Geographic distribution of Debye lengths for each sampling point in the stream water dataset. The points are coloured according to the thickness of their Debye length with red indicating a thick Debye length. (UGOT)

The Debye length is a generic colloidal stability parameter, which provides a relative measure of NP persistence in river waters. In the geographic distribution of Debye lengths it is apparent that in certain drainage basins (e.g. Scandinavian countries and northwest Spain, some regions of the Alps and Scotland) the stability and persistence are high, while by contrast basins in England and most of continental Europe are characterised by low stability. The Debye length measure does not take into account the effects of natural organic matter, but it is noteworthy that the same drainage basins that are characterized by greater Debye lengths, also have high concentrations of natural organic matter.

WP 3 ENP ecotoxicology

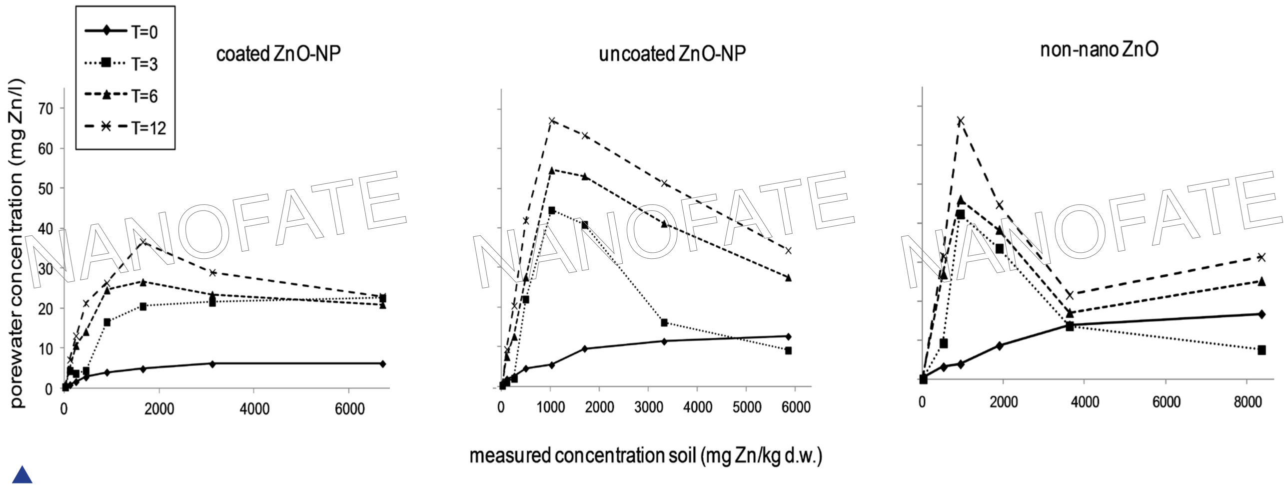


From WP3 - Species sensitivity distribution (SSD) for toxicity of nano-silver particles in freshwater species based on NanoFATE generated data (blue) and those available from other studies (black). NanoFATE has doubled the number of toxicity values and expanded the taxonomic range available for these distributions. (UAVR, NT, Amepox and UGOT)

We developed improved standard ecotoxicological exposure protocols, adjusting properties of test media, media renewal frequencies and soil and food spiking methodologies, to ensure relevant and homogenous presentation of ENPs during toxicity testing. These protocols were shared outside the project with researchers in Europe and the USA. Exposures needed for the hazard assessment were completed and we moved into the chronic testing phase. Data on bioavailability drivers were delivered. Samples have been archived and sensitive endpoint data were collated for use in PNEC assessment. Work is well progressed on co-exposure of CeO_2 and organic contaminants, and on UV radiation combined with ZnO ENP exposures in selected organisms. Enough data have been produced to add or improve almost 50% of the species data points used in the NanoFATE Species Sensitivity Distribution derivation.

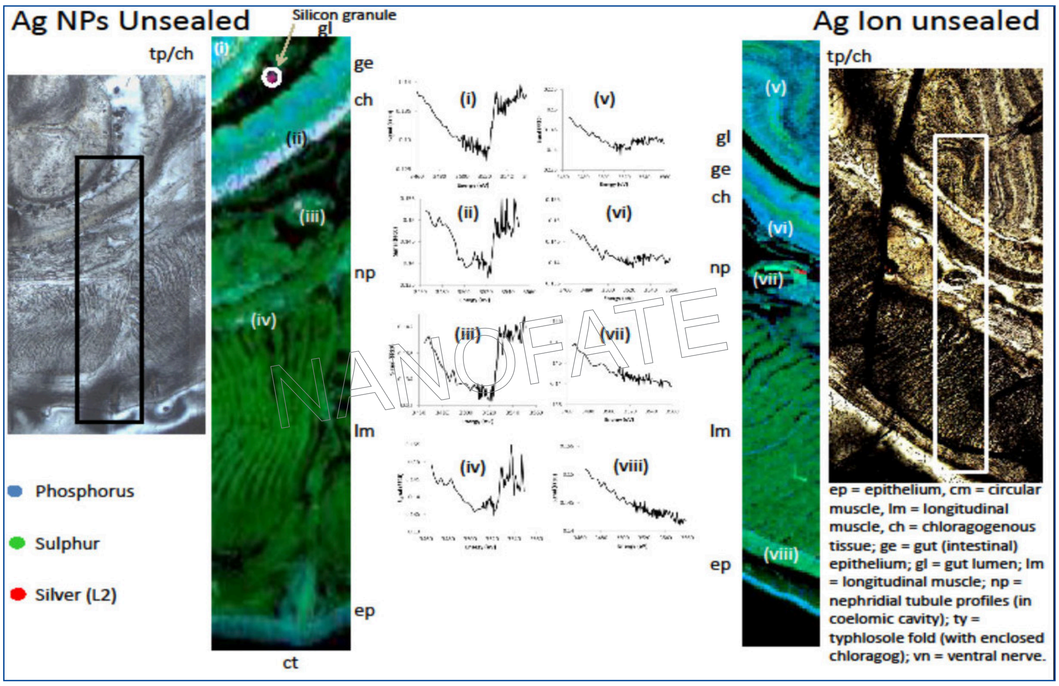
WP 4 ENP bioavailability - relations between soil and water chemistry and particle properties

Broad-scale critical literature reviews on soils and waters examined data quality and identified which environmental factors have the greatest proven effect on the bioavailability and toxicity of ENPs to organisms living in those media. Bioavailability trials testing for pH, organic matter and cation effects were implemented, plus additional long-term (12 months) exposures addressing ageing. A database of results for ENP exposure across soil and water types was completed along with reports on ENP property-effect relationships to address confounding effects on bioavailability. Main findings include: Zn toxicity in all cases was highest at lower soil pH; coated ZnO ENPs were more toxic to springtails than uncoated ones; in soil ZnO ENPs and non-nano ZnO particles only slowly released (months) Zn ions, with Zn ion release being highest at intermediate exposure levels and reduced at high exposure levels; ageing of ZnO ENPs increased Zn concentrations in pore water, but reduced the toxicity for both nano ZnO, non-nano ZnO and ZnCl2; release of Zn ions into soil pore water was much slower for coated ZnO ENPs than for uncoated ones.

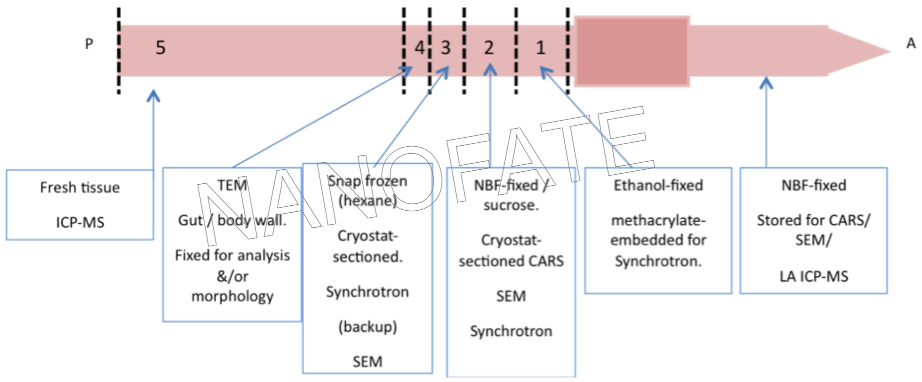


From WP4 - Zinc concentrations measured in soil pore water (mg Zn/l) as a function of total zinc concentrations in Lufa 2.2 soil (mg Zn/kg) freshly spiked with coated ZnO ENP (left), uncoated ZnO ENP (middle) and non-nano ZnO (right) (T=0) and after three (T=3), six (T=6) and twelve months (T=12) of equilibration. (VUA)

From WP5 - A compendium of images, Ag fluorescence maps, and Ag (L-edge) XANES spectra derived from alcohol-fixed methacrylate-embedded sections of mouth-unsealed earthworms exposed either to Ag ENPs (left panels) or Ag ions (right panels). Note that a discernible, albeit very noisy, Ag L-edge was recorded in the typhlosole chloragocytes (tp), chloragogenous tissue (ch), nephridium (np), and longitudinal muscle (lm) of the earthworms exposed to Ag ENPs but not in the earthworms exposed to Ag ions. (NERC, UOXF.DJ, NT, CU, and Amepox)



WP 5 ENP toxicokinetics and toxicodynamics



From WP5 - Schematic of the NanoFATE sampling and preservation protocols to secure multiple samples from each individual earthworm suitable for multiple analysis techniques. NanoFATE has developed and applied these techniques to a range of biological samples. (UOXF.DJ, UNIPMN, and CU)

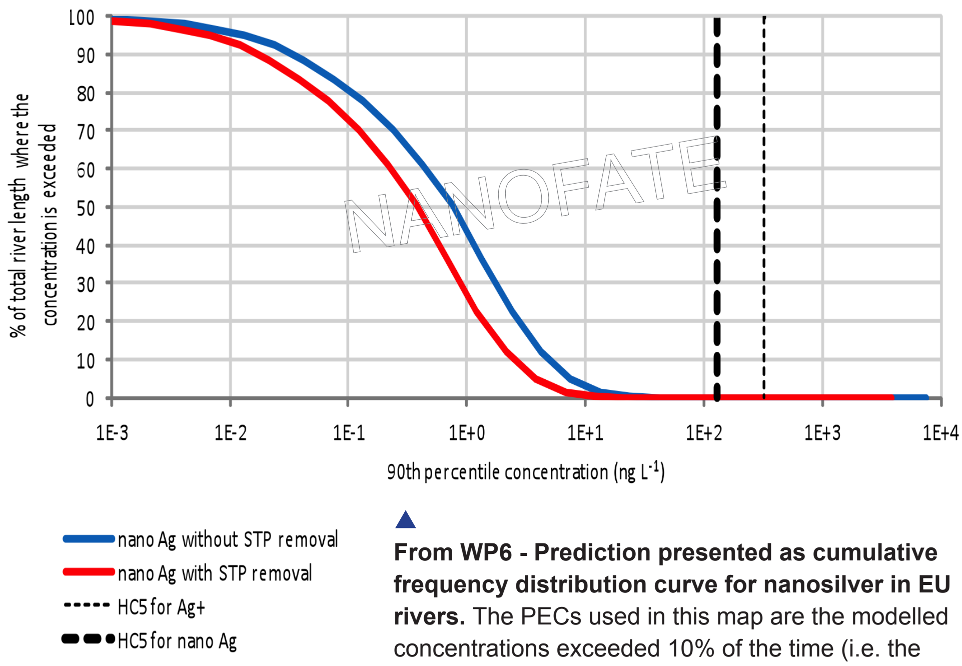
NanoFATE tissue banking has successfully relied on our practical and workable sample handling and preservation system.

We have made the most of our technical abilities (and tissue samples) by applying a tiered approach to tracking of ENPs in tissues, ensuring that the high-end expensive low throughput techniques are applied only to samples where we have evidence ENPs may be present. We have developed knowledge of signatures of possible ENP tissue damage by running samples with an eye to biological markers of ENP and dissolved metal effects.

An agreed data structure allows later cross species comparison, with a flexible format to enable adaptation to the NanoSafety Cluster database when agreed. Toxicokinetic studies were completed for ENPs in soil and aquatic invertebrates. *In situ* characterisation of ENP uptake, using the UK synchrotron at Diamond Light Source and the CARs facility at Exeter (via QNano TA), has provided a highly characterised test bed for development of other more accessible (mainly EM based) techniques.

Extensive use of the QNano facilities will help complete these studies, which so far added gene expression data for nematodes and daphnia via microarrays, while 2nd generation sequencing advances our earthworm transcriptomics. Further efforts addressed uptake kinetics of ENPs in daphnia, mussels, collembolan and earthworms.

WP 6 Integrated risk assessment



From WP6 - Prediction presented as cumulative frequency distribution curve for nanosilver in EU rivers. The PECs used in this map are the modelled concentrations exceeded 10% of the time (i.e. the 90th percentile concentrations). The dashed lines show the most protective environmental cutoffs (HC5 values) for nano and ionic silver. (NERC)

We started out by estimating ENP production and product incorporation, based on a review of peer-reviewed as well as grey literature (reports from R&D projects, reports to governmental authorities, etc.) on production volumes of the three NanoFATE ENPs. Our initial fate and distribution models (from local to EU-regional scale) produced very informative PEC maps – albeit very conservative as based on high estimates. Updated production estimates improved the models and were combined with newly developed protective environmental concentrations cutoffs predicted from species sensitivity distributions. Preliminary modelling suggests current predicted levels of nano Ag in water and nano ZnO in soil are unlikely to exceed thresholds of concern for aquatic or soil species.

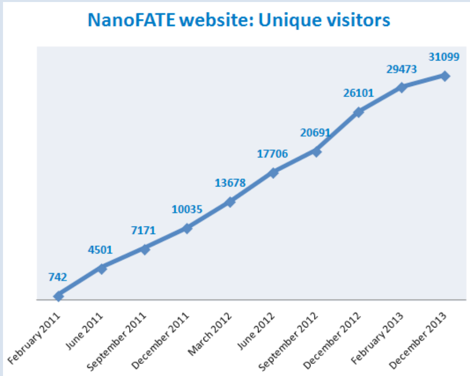
WP 7 Dissemination and training

As the New Year starts, NanoFATE counts 18 peer reviewed articles, 3 in press and another 30+ under preparation. preparation. Strides were made in engaging regulators including a NanoFATE presence at the 2nd Regulatory Review Workshop in Brussels, contributions to the OECD Working Party on Manufactured Nanomaterials, a bespoke ECHA workshop in the first quarter of 2014, and Advice Notes described in this Newsletter. Our latest meetings and workshops are mentioned below, and this Newsletter includes invitations to upcoming events.

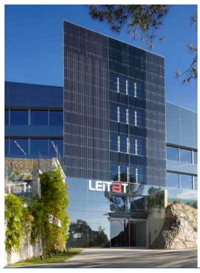
IMPACT

NanoFATE will provide robust tools, techniques and knowledge needed by stakeholders to understand and communicate risks associated with ENPs of different physical or chemical properties, including their environmental interactions and toxicity.

Visit **www.nanoFATE.eu** to download summaries, reports, Advice Notes and newsletters.



we were here



Life Cycle Analysis Workshop – Barcelona, May 2013

NanoFATE hosted a workshop jointly with NanoPolytox & NanoSustain on the "Health and Environmental Impact of Nano-Enabled Products Along Their Life Cycle".
Find the workshop report here.

SETAC – Glasgow, May 2013

NanoFATE was well represented at this major forum with 4 platform presentations and 4 posters. Project members also contributed to the SETAC Advisory group on Nanotechnology.
Find our Posters here.

8th International Conference on the Environmental Effects of Nanoparticles and Nanomaterials – Aix-en-Provence, July 2013

Claus Svendsen delivered a talk entitled "*Longterm fate and effect issues for metal nanoparticles in soils –conclusions of the NanoFATE project*". Susana Loureiro presented "*Species sensitivity distributions for silver and zinc oxidenanoparticles: using taxonomic and trait-based risk assessment approaches*".

OPEN DAY – Wallingford, September 2013

NanoFATE and sister project NanoTOES hosted a world class panel surveying the state of the art of safety assessment for engineered nanoparticles:

- Jason UNRINE (U. Kentucky) gave a US perspective on nano ecotoxicology.
- NanoFATE's own S. LOFTS & K. van GESTEL (CEH, VUA) delivered latest results on engineered nanoparticle bio-availability.
- Peter DOBSON (UOxf) told of the future of nanoparticles.
- A. JOHNSON & R. WILLIAMS (CEH) showed how NanoFATE aids integrated risk management.

Download the programme and mini summary.


Photos : Cecilia Lopez

NanoTOES researchers were given a hands-on experience of practical aspects of ecotoxicology during a worm hunt at the Blenheim Palace estate led by NanoFATE PhD students.

our people

Congratulations to Pauline Lydia Waalewijn-Kool, Ph.D!

Pauline successfully completed the first NanoFATE PhD defence on 24th of October 2013 at Vrije University, Amsterdam.

Promoters N.M. van Straalen and C.A.M van Gestel of VUA, Dr. Pauline, and a cylinder of freshly spiked natural soil

Pauline's thesis is entitled "Ecotoxicological assessment of ZnO nanoparticles to *Folsomia candida*". It focusses on the ecotoxicity and bioavailability of ZnO NP to the 3mm-long soil-dwelling organism springtail. Our Newsletter 4 reported the fate and effect studies performed by Pauline in natural soils in order to unravel the contribution of particulate and dissolved Zn to ZnO NP toxicity. Pauline's study shows that the release of toxic Zn2+ ions from the NPs into the soil pore water continues for at least one year, but this does not lead to increased toxicity. The research suggests that ZnO NP can be evaluated using the current risk assessment methodologies valid for ionic Zn. See Pauline's NanoFATE papers (2012 and 2013).

"Paranymphs" Maria Diez Ortiz (formerly CEH and VUA – now LIETAT) and Rudo Verweij (VUA, valuable technician and teacher) accompany Pauline on the big day

NanoFATE colleagues were present on 24 October: Pauline's "co-promoter" Dr. Kees van Gestel, our WP4 and Component 2 leader (Associate Professor of Ecotoxicology at VUA); Dr. Maria Diez Ortiz, and Dr. Susana Loureiro, WP3 leader.

Pauline acknowledged Kees' and Maria's great support during the lengthy and complex NanoFATE research. She had "*already used EC50 values for risk assessment, but now I know how much work is behind just one number*". Pauline appreciated the bi-weekly ecotox meetings organized by Kees at which practical issues were discussed and scientific literature was read.

Pauline also thanked Claus Svendsen and Dave Spurgeon (NERC-CEH, UK) for their coordination of the NanoFATE project and for "*bringing PhD students together*" (Paula Tourinho came to VUA from U. Aveiro; read her 2013 paper Influence of Soil pH on the Toxicity of ZnO NPs to the Terrestrial Isopod *Porcellionides pruinosus*).

Susana Loureiro (U. Aveiro, Portugal) was on Pauline's examination committee

our reports

ISI Papers

Visit our rich library of NanoFATE publications in peer-reviewed journals.

And read our second feature article in International Innovation (Sept. 2013), explaining our methods and tools and considering "probable benefits and possible risks".

January 2014 — OUR STATUS

PUBLIC DELIVERABLES

D2.5 Research Report on Per Capita Effluent Discharge for Nano ZnO and Ag

D2.6 Research Report on Important Parameters for Soil and Water PEC Estimation: for Use in WP6 for the Assessment of Maximum Soil and Water Concentrations of nano ZnO, Ag and CeO2 and their Distribution

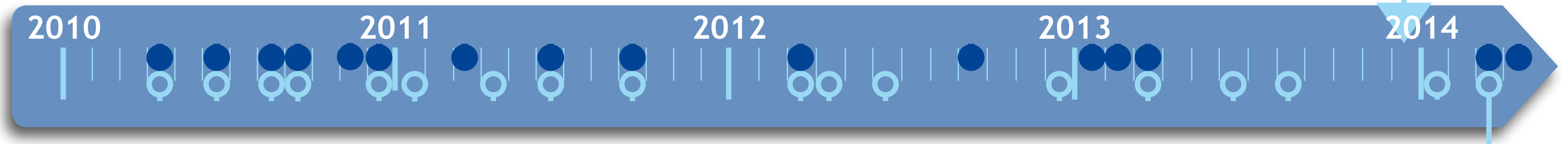
D4.3 Research Report and Associated Research Paper Addressing the Current State-of-the-art in the Analysis of ENP Property Effects on Toxicity Property-effect Relationships

D6.4 Summary report on Phylogenetic and trait based analysis of effects across species and the range of ENPs used including discussion on applicability of the SSD approach

D6.5 Briefing Note Detailing Approach for Generation of SSD Distributions; Includes CeO2, ZnO and Ag in Dissolved Metal Ions and ENP Forms

D6.6 Maps of the Regional Risk Assessment of Selected ENPs; Including Representation of PECs and Exceedances of Threshold Effect Values for a Range of Endpoints (PNECs for Taxa or Sub-groups, Biomarker Endpoints) Under Different Usage Scenarios

Milestones
Public Deliverables



meet us here

March 2014

4-7 March 2014, Birmingham UK. **Free Open Workshop & Associated Training School** on Exposure & Environmental Fate of Nanomaterials:

"Putting environmental realism into nanosafety assessment"

Learn and apply best practice for exposure, fate & hazard assessment in soils, sediments and water. **Joint meeting QualityNano, NanoFATE and NanoMILE.** See the brochure for more information or contact: NanoFATE@ceh.ac.uk

Training labs limited to 30 participants – **Register now**!

May 2014

Basel 2014
SETAC Europe

24th Annual Meeting **SETAC Europe** in **Basel**, Switzerland, **11-15 May 2014**

NanoFATE Component Leaders and other lead researchers will be present in force at the 24th Annual Meeting SETAC Europe in Basel, Switzerland, 11-15 May 2014. NanoFATE personnel co-chair 4 of the 7 SETAC Nano-sessions where the full range of NanoFATE findings will be presented and discussed by peers:

- *Environmental risk assessment of nanomaterials: open issues, pitfalls and recommendations*
  CHAIRS: Birgit Sokull-Kluettgen, Thomas Backhaus
- *Fate and effects of nanomaterials in soil*
  CHAIRS: Kees van Gestel, David Spurgeon
- *Fate and effects of nanoparticles under environmentally realistic conditions*
  CHAIRS: Claus Svendsen, Catherine Mouneyrac, Susana Loureiro, Laure Giamberini
- *Mechanistic toxicology of engineered nanomaterials: state of the art and future perspectives*
  CHAIRS: Francesco Dondero, Teresa Fernandes, Peter Kille, Stephen Klaine

Read about these sessions and topics at http://basel.setac.eu/embed/Basel/Files/Nanomaterials_all_sessions.pdf.

Newsletter writing: Claire Mays, Symlog
Design: Ulysse Badorc, www.studiobu.fr