NanoFATE
Nanoparticle Fate Assessment and Toxicity in the Environment

NEWSLETTER
AUTUMN 2012 /#4

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our work

The NanoFATE project has:

- Composed maps showing where and when nanoparticles may cause risk in Europe
- Looked closely at how long it takes for nanoparticles to leave the soil
- Developed a Café Scientifique (with ENNSATOX) where you can meet us

About NanoFATE

editorial

by Coordinator Claus SVENDSEN

Our puzzle pieces are coming together

Welcome to the fourth Newsletter from the NanoFATE project. In this issue you will learn how we tackle our research challenge – assessing environment risks of engineered nanoparticles. We hope you'll gain insight into the methods we use and how the different scientific tasks fit together to gain knowledge from the whole project greater than the sum of its parts.

First we'll introduce our seemingly "mission impossible", and how the NanoFATE project partners are banding together to conquer their daunting tasks. We'll then show how our risk mapping experts are drawing together results from our project (and others in the EU NanoSAFETY cluster) to show what we have found out so far and where our enquiries should lead us next.

The health of our environment is dependent upon tiny organisms such as bacteria, algae and soil invertebrates. These fix and cycle the nutrients essential to maintaining the habitats they live in. By studying how nanoparticles can affect these creatures we can predict how the release of these products could affect our environment. Read about how our colleagues are improving understanding of just what happens when nanoparticles and invertebrates get together in soil.

Finally, learn where to meet us in North America and the UK this year.

We hope you find these insights enjoyable and informative, if you do please pass our Newsletter along to your colleagues.

CLAUS SVENDSEN

Intro: Is it a "Mission Impossible"?

A COLLECTION OF VERY SMALL PARTICLES is conveyed across a vast countryside by a series of unwitting agents who do not know each other, do not know what they are carrying, and use different conveyances on a route only very roughly traced in advance. You do not know precisely how large the collection is, where or when the journey will start nor just how long it will take, nor how these particles will choose to disguise themselves.

YOUR MISSION, SHOULD YOU CARE TO ACCEPT IT, is to follow that collection no matter where it goes. You will watch where and when particles stop moving, or are picked up by a new series of couriers. You will figure out whether the particles stay just as they are, or transform, and how quickly, and why, and whether they become more or less dangerous. You will assess whether small animals coming into contact with the particles are at risk, immediately or next month or next year. You will observe whether the particles behave any differently than much larger versions of the same stuff. Finally, you will report, with maps, sharp photos and impeccable data, in the most faithful and reliable way to concerned authorities and a sceptical public.

THE PARTICLES CANNOT BE SEEN by the naked eye or by a classical microscope; released by the tonne, they measure 20, 30 or 50 billionths of a meter... You have 48 months to complete your mission, and the ability to call upon 12 international partners each renowned in their own widely differing field, and often almost ignorant of the other partners' expertise.

IF YOU ACCEPT THIS MISSION, your team will deploy an astonishing range of methods to follow these tiny particles and assess their fate and their impact. You will alternate state-of-the-art sensors that you will tune to detect unfamiliar nanoparticles. Indeed you will produce your own particles, characterise them and tag them and distribute them to your partners, to be certain that you learn to follow and observe them exactly. You will sprinkle the test particles into environmental media, determining the effective exposure and effects upon worms, woodlice and other tinier squiggly things. You will contact industries, attempting to learn enough about their production schemes to calculate the future amounts of the particles that may spread and accumulate throughout Europe.

YOU AND YOUR SENIOR COLLABORATORS WILL ACCOMPLISH THIS in one tenth of your total working time, relying also on the brains and enthusiasm of 14 young researchers who will make this mission the centre of their scientific quest...

WELCOME TO NANOFATE

NanoFATE Integrative Risk Mapping

Contact Component Leader **Andrew Johnson** (NERC CEH) to learn more

From black boxes ...

Engineered nanoparticles (ENPs) used in consumer products enter the environment in a variety of ways. Fuel additives, for instance, are spewed into the atmosphere in vehicle exhaust and ENPs then settle onto the earth and natural waterways. Nano products in cosmetics and treated textiles are sluiced into waste water when skin or clothes are washed.

What volume of ENPs will eventually end up in the European landscape? What risk do they then represent to life, health and the environment?

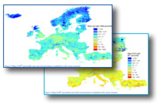
Assessing that risk is a complicated task. Traditionally, to perform risk assessments, scientists model the environment as a series of *black boxes*: one box for soil, a separate one for water... These modeled environments are largely symbolic and bear little resemblance to a real location. The risk assessment looks at what might go into the box (for instance, a pollutant) and what might come out (damage to someone or something's health). In a black box model, what actually goes on "inside the box" is of lesser importance. And the possible interactions and relationships among these different "boxes" or environmental compartments are not taken into account. While understandably the black box approach simplifies a complex equation, the actual *cumulative* risk that is due to particles present in waterways *and* deposited in soils may not be reflected in the final result.

Enter NanoFATE and its integrative approach. A broad and skillfully constructed set of methods is applied to follow a designated type of ENPs on a realistic voyage. On this voyage, ENPs pass out of consumer products into waste water, and eventually to rivers and agricultural land and thence into small living organisms. The safety of this tiny wildlife is then assessed experimentally – seeking to learn whether and how nanoparticles may impact vital biological processes. Coordinated efforts by twelve partner institutions let us build up a more complete picture of what happens inside the black box – and moreover, what is actually going on between boxes.

Is it new? What is it good for?

While some similar risk mapping work has been done outside NanoFATE, our maps show a novel level of detail – which will continue to grow as knowledge arrives from other parts of the project. New and important: our maps demonstrate that potential risk from ENPs is not the same across the extent of Europe. NanoFATE maps show fine-grained spatial variability, pointing up the exact geographical regions where ENPs are more likely to accumulate in the environment. Such information can help risk managers and regulators allocate their attention and resources more wisely.

> *"NanoFATE will culminate in a [highly] informed and realistic model integrating ENP fate, availability, accumulation and toxicity at all stages of the post-production life cycle. We have already started generating risk maps facilitating both communication and decision making on both regional and European levels."*
> *Andrew Johnson, Component leader, in* International Innovation *(August 2012)*

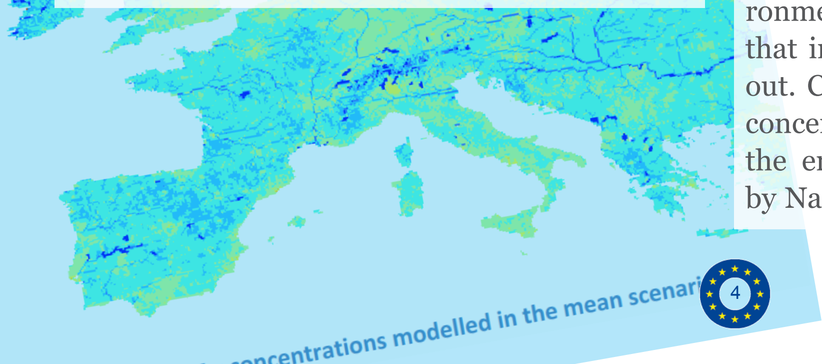


… to bright maps

Among the many subtasks performed in NanoFATE, **risk mapping** is a particularly integrative activity that allows us to model the environment to a greater degree of complexity. The black box approach can say whether organisms *are* or *are not* at risk; **NanoFATE tells *where and when*** this is so. Our maps indicate just *where* in Europe the tiny soil- or water-dwelling animals we study may be subject to greater or lesser stress from ENPs. As well, NanoFATE has set out to map *seasonal variations* – revealing how tested organisms may be impacted during rainy or dry periods.

NanoFATE risk maps pull in information from all parts of the project: the gradually growing understanding of just how many ENPs are likely to enter actual environmental compartments, what changes these engineered particles undergo when they stay in water or soil; to what extent small organisms uptake ENPs, and how their health is affected. To construct the maps, this information is combined with an analysis of the actual geography and waste treatment infrastructure of Europe, and year-round behavior of waterways and climate. As background work, a total of 132 maps was produced (one per month detailing the past 11 years). Finalized NanoFATE maps display the European locations where ENPs will be apt to gather, and highlight spots where concentrations may reach a level that could trigger health impacts in certain small soil- and water-dwelling species.

As our project advances, the maps will build in more and more precise information about how *predicted environmental concentrations* of ENPs are likely to affect these species over time: weeks, months or years.



The minds behind the maps

NanoFATE's **Integrated Environmental Risk Assessment** team – responsible for developing risk maps – is headed by **Andrew Johnson** at NERC CEH. Nine project partner institutions help to address these overall NanoFATE objectives: predict levels of ENPs in water and soil; show how those ENPs become "available" to penetrate and possibly affect organisms; and finally, work out a complete and readable picture of the risk introduced by product-based ENPs, integrating information about where they end up in the environment, how available and how toxic they are to a range of small organisms.

STEP ONE Estimating concentrations of ENPs in the environment

Within that scheme, the risk mapping task involves first finding out what are the *likely or expected concentrations* in European surface water and soils of selected ENPs. In our example, these are particles of nano silver - for instance, those woven into the fabric of clothes to provide antibacterial protection, or nano zinc oxide used as a reflector in sunscreens. (All other nano silver from other uses is included in our calculations as well.) Nanoparticles would be released into waste water when for instance that clothing or a vacationer's skin is washed - waste water which then moves through sewage treatment, with the ENPs ending up in sludge or released to waterways.

NanoFATE partners first looked into existing estimations of how much silver or zinc are lost from such products. NERC CEH researchers then pinpointed likely patterns and locations of consumer use, and gathered extensive information on the year-round behaviour of the environmental systems (rivers, agricultural land) that in the end would absorb the ENPs washed out. Combined with existing predictions of how concentrated these nanoparticles will become in the environment (collected from the literature by NanoFATE partners at Faust + Backhaus and

U. Gothenburg), the result was a finely detailed prediction of just how much nanomaterial would be found where, and at different times of the year.



Richard Williams, Virginie Keller, and Egon Dumont work together with Andrew Johnson at NERC CEH to model where ENPs go once they have washed out of consumer products and passed, in waste water, through sewage treatment.

Virginie looks at the large proportion of washed out ENPs captured during waste water treatment. These remain in sludge which is subsequently spread as fertilizer across agricultural land. Virginie, an environmental modeler, identifies just where the ENPs end up on the ground, working at a country scale.

Egon, also an environmental modeler, looks at the much smaller proportion of ENPs that do not stick in sludge, but instead are released into surface waterways after treatment. The calculations take into account how sewage treatment plants are distributed across the land, how large a human population they each serve, and which waterways are connected to the plants. A picture is built up of the likely route of released ENPs through the river network and where they may eventually be found in lakes, rivers and wetlands. Drainage of rainwater from catchment areas of the land into rivers is figured in as well.

Egon and his colleagues have modeled how great a volume of liquid will be found in water bodies during rainy or dry seasons. When there is more water, ENPs will be less concentrated in surface waters; at dryer times of year, we can expect to find higher concentrations of ENPs in surface water, especially close to human population centers. So far, this modeling has been run for the whole of Europe, and a report details results for three UK rivers and three continental European river catchments. Maps for nano zinc oxide and nano silver can be seen on our website.

The dynamic models produced by NERC CEH are very sophisticated, taking into account a wealth of factors to predict environmental concentrations of ENPs in both soil and water. The black box is left far behind.

STEP TWO Comparing with concentrations that would harm organisms

In a second step, NERC modelers ask whether the predicted environmental concentrations of ENPs are likely to cause harm to organisms in the water and soil. Again, they keep in view the different seasons of the year: during dryer summer months in Europe, when rainfall is lower and concentrations of ENPs potentially greater, overall temperatures are higher as well and wildlife in soil and water are growing and reproducing. The tiny organisms may be most vulnerable at this period.

Toxicology results from other NanoFATE teams are needed here. The NERC CEH team compare predicted ENP concentrations from step one, with the concentrations that have been shown in NanoFATE experiments to have the potential to affect the growth, lifespan or reproduction of a range of tiny animals native to soil or water.

On the resulting risk maps all this information can be displayed at once: where, across Europe, the ENPs are likely to be at any time of year, and whether their concentration falls above or below a level that would affect the health of studied organisms.

Learning from tiny wildlife about ecosystem health

How do scientists figure out if a pollutant may disturb the healthy balance in the environment around us?

For instance, how can we assess whether a chemical contaminant in the soil is hazardous for ecosystem health? One way is to look at how that chemical's presence affects the health of small organisms that live in soil. Environmental toxicologists can do that by studying soil-dwellers' growth, survival rate and ability to reproduce when exposed to a contaminant. These tiny wildlife are used like canaries in a mine – signalling to us the possible presence of hazard before ecosystems are affected.

As engineered nanoparticles (ENPs) become more and more commonly used in consumer products, regulators and decision-makers need to learn whether these particles are safe, or whether they could make our environment less healthy. An early step in this learning process will be for scientists to perform complete hazard assessments – investigating in a systematic way the relationship between environmental contaminants and effects on ecosystem health.

NanoFATE contributes to this learning in several manners: looking at the health of certain small wildlife when selected ENPs are placed in their environment; but also, checking whether the standard methods of testing are adequate for assessing ENPs. Can the well-established procedures be applied with these new particles and still produce reliable answers?

This article focuses on soil-dwelling organisms, particularly those called springtails, but NanoFATE also looks at other organisms that live in soil, or algae, bacteria, molluscs and crustaceans living in fresh or sea water. All the chosen organisms are common candidates for ecotoxicology studies because large populations of these tiny wildlife are common across the planet. This global presence suggests that they are significant actors in many ecosystems. And these populations' wide extent across the Earth suggests that general scientific conclusions about their safety may be valid in all hemispheres.

Learning from tiny wildlife about ecosystem health

NanoFATE is collecting basic information that will help improve scientific methods for assessing whether nanoparticles are hazardous to soil-dwelling organisms.

In particular, NanoFATE will help show whether classical procedures, good for assessing both ionic and "bulk" (larger than nano-sized) metals, can be applied as well to "nano" forms of the same metals. That means, showing whether the same things happen when zinc oxide (ZnO) for instance – which may reach the environment in particles in the micrometer range – arrives instead in nano-sized particles measuring just billionths of a meter across.

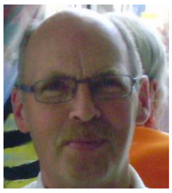
To meet these objectives, NanoFATE builds experiments in environmentally realistic conditions – mimicking situations that can really take place in nature. The research is carried out in NanoFATE Component 2, which is led by Dr. Kees van Gestel (VU University Amsterdam, VUA) and involves 9 NanoFATE partner institutions.

NanoFATE Component 2: "Ecotoxicology and Bioavailability"

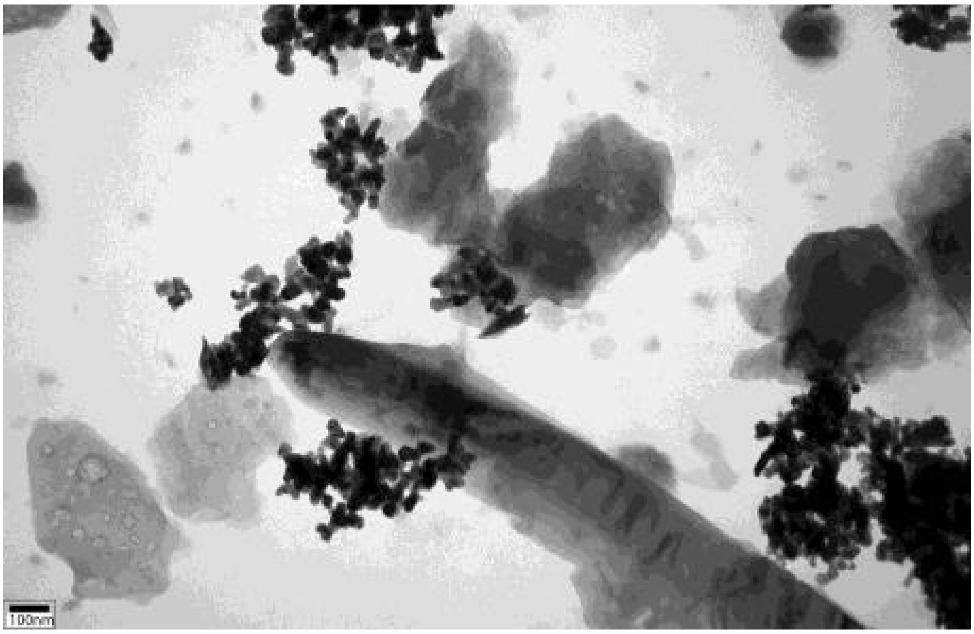
This project component focuses on assessing the ecotoxicity of selected ENPs. Research looks at:

- how toxic ENPs are to various soil and water-dwelling organisms,
- how the properties of the soil and water - and of the ENPs themselves - affect their toxicity,
- how quickly ENPs will enter an organism from the environment,
- what happens to them once they are inside (toxicokinetics),
- and exactly what effects ENPs have on cells within the organism (toxicodynamics).

Learn more on our website about the NanoFATE Work Packages 3, 4 & 5 involved in this part of the research.



Contact Component Leader Kees van Gestel (VUA) to learn more



ZnO ENP agglomerates bound to dissolved organic matter in a soil extract.

Zinc oxide might reach the environment in various forms and sizes (ranging from a single molecule to large particles).
NanoFATE compares:

- *mid-range "bulk metal" or non-nano particles, measuring a few one-millionths of a meter across (that's a millimetre divided by 1000)*
- *and nanoparticles, measuring a few billionths of a meter (a millimetre divided by 1,000,000 - one thousand times smaller than typical non-nano particles).*

Getting her hands dirty

Pauline Waalewijn-Kool is a PhD-level researcher at VUA, working under Kees van Gestel's supervision. She is running a whole series of laboratory studies that give insight into how selected ENPs behave in natural soil, and what influences the way they will affect small wildlife. One of Pauline's experiments investigates fate and effects of ENPs weeks or months after a given quantity has been placed in soil. After this period of time, what proportion of the metal-based ENPs will remain bound to the solid matter in soil? How much metal has been released from the ENPs to dissolve into the "pore water" (the tiny pockets of water between soil particles)? How long does this dissolution process actually take?

Such questions are important for hazard assessments, because soil-dwelling organisms have different manners of drawing their livelihood from the dry and wet parts of soil. Earthworms, for instance, absorb nutrition through their skin but also through swallowing soil (therefore they can be exposed to metal in both these ways). Pauline's work concerns six-legged creatures called springtails, which uptake mainly from pore water through their ventral tube. For springtails, the issue will not be not how great a concentration of metals-based ENPs remains in the dry matter, but instead, *what is the amount of metal ions that may become available by dissolving into pore water.*

When introduced into the environment, metals in the form of particles tend to release their ions: this is called dissolution. The rate of dissolution depends on several factors, like particle size, the coating materials and the type of metal, as well as properties of the environment (such as the acidity of the soil). Metal ions generally are considered to be more toxic than the particulate metal forms.

Results obtained by Pauline showed that nanoparticles and non-nano ZnO follow the same pattern of dissolution – but the nano-sized particles take much longer (> 1 year) to fully dissolve in soil than expected from studies performed in water.
This is important information: it means that if we want to be accurate in judging the safety of these nanoparticles, we will have to visit soil that has aged for a longer time – more than six months, or even one year.

Springtails are six-legged animals less than 6 millimetres long. They are very abundant in terrestrial environments. Scientists estimate that 100,000 individuals are found per square meter of topsoil, essentially everywhere on Earth where we find soil and related habitats (moss cushions, rotting wood, etc.). They are commonly used in laboratory tests for early detection of soil pollution. Pauline's work for NanoFATE uses the species Folsomia candida*; for almost 50 years this has been a "standard" organism used in toxicity tests for both acute (short but severe) and chronic (longer) exposures to pollutants.*

"As soon as you add ENPs to any environment – whether simple fresh water or complex media such as soils – they react and modify. Whereas zinc oxide is known to dissolve into zinc ions in tens of hours in water, these data showed that the process takes over six months in soil, challenging whether the normal hazard-testing over a few weeks is adequate."
Claus Svendsen, NanoFATE Coordinator, in International Innovation *(August 2012)*

Good news for springtails...

Pauline's soil ageing study describes the slow dissolution of ZnO nanoparticles in soil. She found that zinc oxide nanoparticles remained bound up with soil and dissolved very slowly into pore water. As for the health of springtails living in the soil, **no difference between zinc oxide nanoparticles and conventional zinc oxide was found, suggesting that the small size of these metal-based ENPs does not influence their toxicity to these tiny ecological actors.**

Pauline and NanoFATE colleagues remain cautious. Her results may be extrapolated to other soil organisms beyond springtails, but the picture could conceivably be different if we looked at animals who uptake metals not from pore water but from the dry matter of soil. However, these results still give some basis for believing that ENPs, growing more common in consumer products, will not pose particular new hazards to the environment.

"The main highlight [of the full range of NanoFATE results to date] is that while our three metal-based ENPs clearly behave differently to the non-nano forms in certain cases, we are starting to gather evidence that there really is little reason to suspect that the use of ENPs will have any widespread deleterious effect in the environment."

Claus Svendsen, NanoFATE Coordinator, in International Innovation *(August 2012)*

Kool results

At the same time, Pauline gathered **important information to shape future hazard testing.** The slow dissolving behaviour she uncovered for zinc oxide ENPs means that traditional procedures for assessing the safety of soil dwelling organisms may have to be adjusted. Ecotoxicologists testing the health of springtails will be wise to employ their spiked soil not just after the usual few weeks' time applied for metals, but also after e.g. one year, to take proper account of the ongoing dissolution process. Regulators and decision makers with responsibility for environmental protection will be better able to rely on results of hazard assessments when methods are adjusted to the specific behaviour of ENPs.

NanoFATE is sharing data with other scientists and stakeholders. NanoFATE researchers present their results at conferences, and they are writing up their results for peer review and publishing in major scientific journals. Toxicity data for all the types of nanoparticles studied in our project are available and will be loaded into a detailed, searchable database to serve others.

our jargon explained

Ecosystem - A community of living organisms (plants, animals and microbes) in a network of interactions with each other and with the nonliving components of their environment (such as air, water and mineral soil).

ENP - Engineered nanoparticle. This refers to an intentionally designed and manufactured particle of nano size (between 1 and 100 nanometers, or billionths of a meter). This definition distinguishes the ENP from other nanoparticles that might be encountered in the environment, such as those naturally produced by biological and weathering processes, fragments of man-made products, or unintended by-products.

Toxicology - The study of the adverse effects of chemicals on living systems, whether they be human, animal, plant or microbe. **Ecotoxicology** focusses on non-human life. NanoFATE focusses on a selection of small organisms found in soil or water, investigating potential 'adverse effects' by engineered nanoparticles on their rate of growth, reproduction or survival.

our calendar

Click on titles to download summaries of the public deliverables produced as part of NanoFATE.

Autumn 2012 — **OUR STATUS**

MILESTONES
- Second annual progress meeting

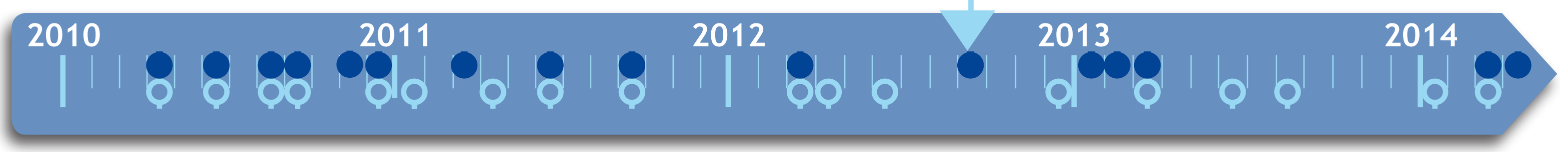
PUBLIC DELIVERABLES (PDF)

Deliverable 2.3: Summary influx estimates for ZnO and Ag ENPs: Assessment of per capita influent discharge to STWs of nano ZnO and Ag

Deliverable 3.2: Chronic ENP ecotoxicity: Comparative assessment of the ecotoxicology of pure ENPs in relation to test organisms and biological endpoints.

Deliverable 6.3: Maps of mean and worst case PEC for catchment; includes rivers in three UK regions and three Continental European river catchments.

Milestones
Public Deliverables



Some of our partners are racing through the Scandinavian environment chasing nanoparticles! Visit Martin Hassellöv and colleagues at www.nanosphere.gu.se

November 2012

meet us here

NanoFATE events

Café Scientifique Workshop:
Impact of Nanotechnology on Environmental Health

December 13th 2012 at the University of Leeds, UK

Learning lessons from past and current nano research to better plan for the future
The recently completed FP7 ENNSATOX project on nanoparticles in the aquatic environment and the ongoing project, NanoFate, raises issues concerning the potential for impacts of nanotechnology on the environment.

This one day interactive Café Scientifique-style workshop and knowledge transfer event is of relevance and interest to research, business and regulatory experts involved in nanotechnology, and, in fact, anyone who is interested in its environmental impact. It will highlight the most interesting results from the ENNSATOX and NanoFATE projects and will include the work of the Brian Mercer Award in facilitating the development of unique analytical methodologies to aid the prediction of nanoparticle behaviour at the cellular membrane level. The workshop will address the need for building capability, methods and exposure to work in environmentally realistic conditions in order to inform regulation. The ethical and societal aspects of potential impacts of nanotechnology will also be debated.

The workshop will be chaired jointly by Professor Andrew Nelson, coordinator of the EU FP7 funded ENNSATOX project and recipient of the Royal Society Brian Mercer Award, and Dr Claus Svendsen, coordinator of the FP7 funded NanoFATE project.

The event is free of charge but is restricted to 50 delegates on a first come first served basis.
To register please contact Roel Evens,
Scientific Project Manager SETAC Europe,
Email: roel.evens@setac.org / Telephone + 44 327727281.

ennsatox
NANOPARTICLES IN THE AQUATIC ENVIRONMENT

SETAC US in Long Beach

Drs Claus Svendsen, David Spurgeon and Stephen Lofts all working on NanoFATE will be attending a UK-US project meeting session at SETAC US (Nov. 2012) representing the UK arm of the Transatlantic Initiative for Nanotechnology and the Environment (TINE) project. TINE brings together expertise from Rothamsted Research, Cranfield University, CEH and Lancaster University with the following universities in the USA: Kentucky, Carnegie Mellon and Duke. Claus Svendsen will be giving a NanoFATE overview presentation in the SETAC main session.

We hope you have enjoyed participating in our NanoFATE adventure through this newsletter! Let us know whether you think we will achieve our... `Mission Impossible`

The articles were written by Claire Mays (Symlog) based on interviews with our NanoFATE scientists. The newsletter, with photos provided by the scientists, was designed by Ulysse Badorc.