NanoFATE
Nanoparticle Fate Assessment and Toxicity in the Environment

NEWSLETTER
SPECIAL 18-MONTH ISSUE
WINTER 2012
– UPDATED EDITION –

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our work

NanoFATE progress in the first 18 Months:

While work has begun on all nine of the main NanoFATE S&T objectives, the working objectives addressed in the first 18 months of NanoFATE can be summarised as follows:

- Source, produce and fully characterise the commercial ENPs and match the tagged version of ZnO as closely as possible. (Delivering Main Obj.1)
- Establish particle behaviour in the pure ecotox media to be used and at higher than environmental concentrations (to enable hazard assessment studies). (Working towards Main Obj. 3, 4 & 5)
- Establish acute and chronic toxicity (to enable selection of relevant doses for the progression into the work in environmentally relevant media conditions. (Working towards Main Obj. 5, 6, 7 & 8)
- Develop initial simple assumption-based fate models and estimate worst case environmental concentrations (Working towards Main Obj. 2 & 8).

About NanoFATE

editorial

by Coordinator Claus SVENDSEN

18 Down, 30 to Go

Welcome to this special issue Newsletter following our 18-month review. It was a pleasure for me and the Work Package leaders to present the progress of NanoFATE to the reviewers, and receive comments like: "*The overall pace of work during the period under report has been impressive*", "*The project has been carried out in a very focused and satisfactory manner, and for this the consortium deserves commendation.*", and "*Excellent progress has been made in each Work Package during the period under report*". We would therefore like also to share an overview of the project today with you in this newsletter.

So please find in this newsletter summaries of our progress on our primary objectives, along with scientific updates and highlights from the RTD Work Package leaders. Work has progressed well, as highlighted at the left of this page, and NanoFATE is now moving from the initial phase of characterizing particles, their fate and environmental hazard in standard systems to working in environmentally realistic media and concentrations.

During the first period NanoFATE has provided knowledge transfer at several levels. In 2010 we ran the successful training course and workshop on environmental fate and ecotoxicology of engineered nanoparticles (ENPs).

In September 2011 NanoFATE partners co-organised and presented at a NanoSafety cluster meeting on the environmental fate and ecotoxicology of ENPs with collaborators from Ennsatox and NanoRetox. This meeting targeted integration of stakeholder knowledge requirements, technical limitations and state of the art results and research plans of NSC projects, highlighting a series of joint recommendations.

In January 2012 I was happy to lecture on the Ennsatox "Nano Winter Training School". This was a laboratory-based training event on: synthesis, characterisation, ecotoxicity, hazard and risk assessment of engineered nanoparticles nanomaterials, and attended by early career scientists PhD students, Post-Docs, industry and regulators.

CLAUS SVENDSEN CEH (NERC)

NanoFATE

our special highlight
Taking our science to stakeholders

EU NanoSafety Cluster:
Environmental Fate and Ecotoxicology Meeting

On 22 Sept. 2011 **NanoFATE**, Ennsatox and NanoReTox organised an *Environmental Fate and Ecotoxicology Meeting*

at the Natural History Museum in London, UK, in conjunction with the *6th International Conference on the Environmental Effects of Nanoparticles and Nanomaterials*, The Royal Society, London, 19-21 Sept. 2011

Chaired by Claus Svendsen (CEH), NanoFATE

Much work has been carried out in the area of safety testing and hazard assessment of engineered nanoparticles and engineered nanomaterials in human health with relatively little being done to address safety and hazard of these materials in aquatic and soil ecosystems. European-funded FP7 projects are addressing this shortfall.

Our meeting brought together the environment-related projects of the NanoSafety Cluster (NSC) as well as regulators and industry representatives to discuss how Environmental Risk Assessment and Regulation of «nano» should progress. Read the Report to learn about stakeholder needs, research gaps, and which EU projects are covering these needs and gaps.

FP7 Project members, regulators and industry stakeholders presented and discussed the following areas:

- **Introduction to FP7 NanoSafety Cluster Projects with an Environmental Context:** What aspects does each project cover?
- **Environmental Load and Fate Assessments:** Current knowledge and issues faced in estimating nanoparticle sources and predicting realistic environmental concentration.
- **Ecotoxicology and Characterisation in Experimental Work – Techniques Review:** Experiences so far in terms of preparing, maintaining and characterising realistic nano exposures in environmental media. Is there a minimum set of characterisation needed?
- **Characterisation of NP in Environmental Matrices and Tissues:** Characterisation techniques applicable for nanoparticles in environmental matrices and tissues.
- **End Users/Stakeholders Session – Needs & Wishes:** What do industry and regulators require from the research community?

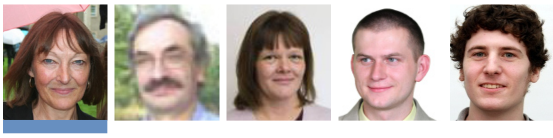
Read the Report prepared by Karen Steenson and Dave Arnold (SETAC), Ennsatox with C. Svendsen.

our people and their work progress

Recognize these faces? Our Work Package Leaders were introduced in our first Newsletter and our Young Scientists (along with Supervisors) in our last one. Both letters are available at the News section of our website. Read here about the strides made in the first 18 months of our four-year project. Summaries and public reports can be found online (see our calendar).

WP1

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



In the first 18 months the major deliverables here related to provision of stable well characterised particles for the remaining project partners. A large range of commercial ENPs were characterised and assessed to ensure material was suitable for planned experiments and without significant batch to batch variation issues and that tagged particles had similar attributes to the untagged material. Consequently the final set of NanoFATE particles has been selected:

- The main ZnO particles are 30nm Nanosun from Micronisers in Australia, with matching tagged ZnO ENP by IHPP, with some work on BASF z-cote and z-cote HP1 ZnO
- Partner Amepox provided 3-8nm Ag ENP and a 50nm Ag ENP comes from partner NanoTrade.
- CeO2 will be the Envirox or Antaria fuel additive and most likely a polishing agent from Umicore.

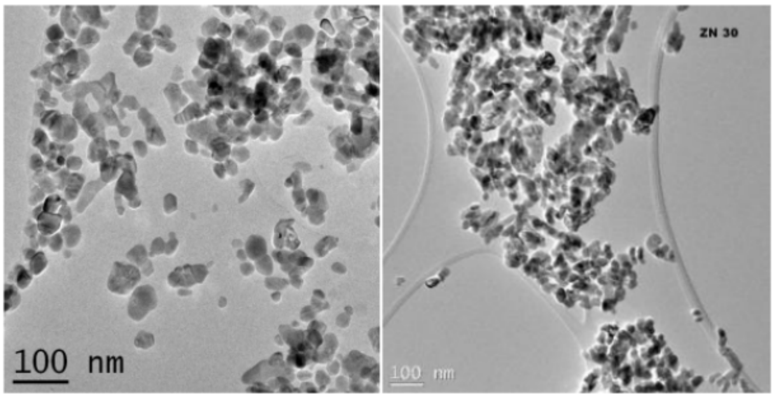


Figure 1.1 TEM micrographs of 30nM ZnO (test particle Zn10 supplied Microniser) and Co doped (10 wt%) particles Zn30 (supplied by IHPP).

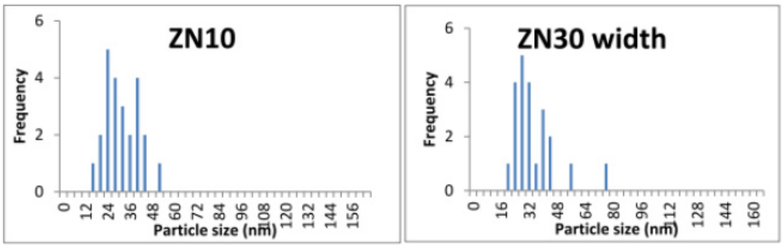
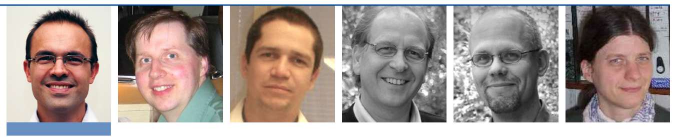


Figure 1.2 Particle size distribution from TEM micrographs 30nM ZnO (test particle Zn10 supplied by Microniser) and Co doped (10 wt%) particles Zn30 (supplied by partner IHPP).

WP2

ENP environmental behaviour and fate modelling



We have identified and prioritised specific properties that need principal consideration during the development, adaptation and validation of environmental fate models for nanoparticles (D2.1).

We have, based on this, developed the initial basic fate models (with WP6) and supplied the initial worst case environmental concentration estimates serving to inform decision making and exposure design in WP 3 & 4 (M 2.1 and D 2.2). In addition we have developed analytical procedures to assess the dissolution and agglomeration behavior in environmental and biological media, and developed single particle ICP-MS for detection of silver nanoparticles in e.g. waste water.

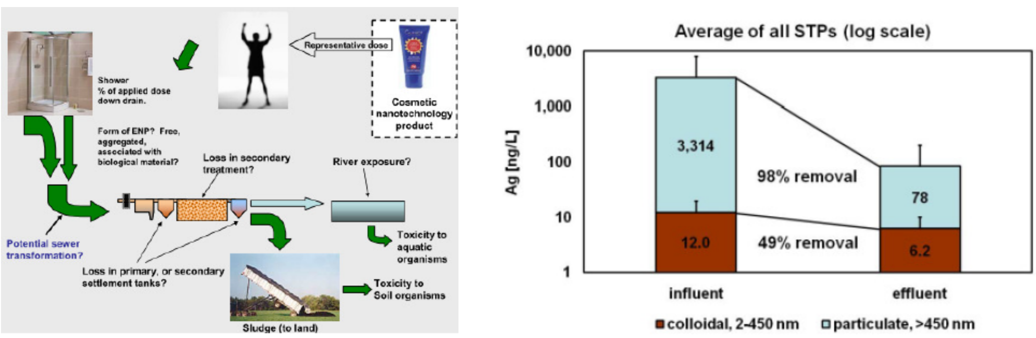
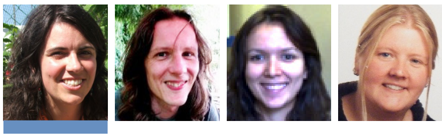


Figure 2.2 Removal of colloidal (2-450nm) and particulate (>450nm) Ag size

WP3

ENP Ecotoxicology



We adapted standard ecotox protocols, principally adjusting properties of test media, media renewal frequencies and soil and food spiking methodologies, to ensure relevant and homogenous exposures of nanoparticles during toxicity testing. With the implementation of these improved protocols, most of the exposures needed for the hazard assessment have been completed (except for CeO2). This information has allowed progress into the chronic testing phase and also some work to deliver data for WP4 on bioavailability drivers, WP6 for the Risk Assessment and samples for WP5.

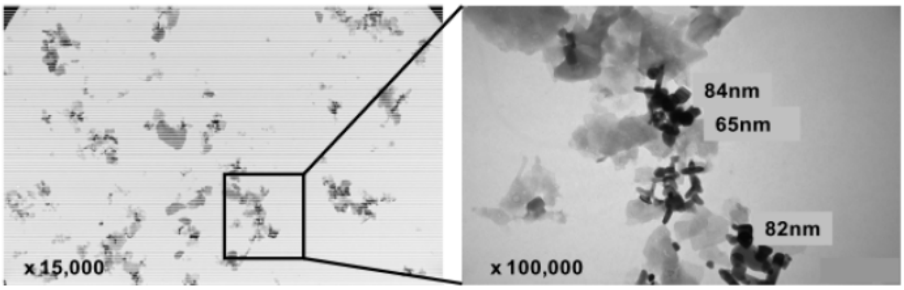


Figure 3.1 TEM images of ZnO-NP in spiking solution, with small aggregates bound to DOM (VUA Partner 2).

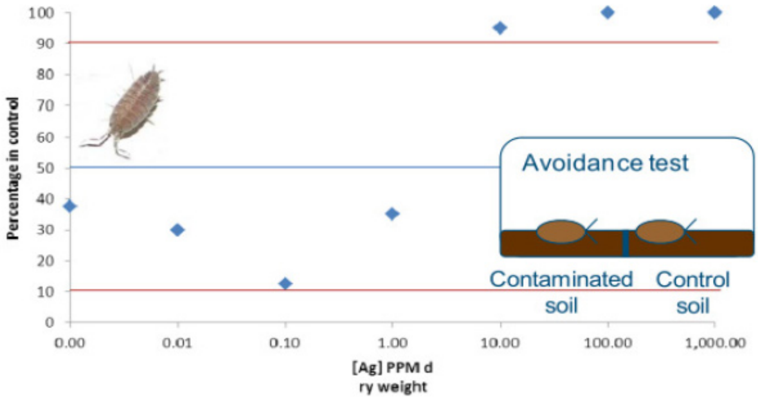


Figure 3.2 Avoidance of woodlouse Porcellionides pruisonus to exposure to iionic Ag EC50 (48h) 8.8 mg Ag/kg dry soil, being two orders of magnitude more sensitive than mortality with 7 and 14 day LC50 being 726 and 398 mg Ag/kg dry soil, respectively. Matching Ag NP exposures are currently underway (UAVR Partner 4).

WP4

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

In deliverable D4.1 we collected, databased and critically reviewed all available information from the literature. This identified which environmental factors have the greatest proven effect on the bioavailability and toxicity of nanoparticles to organisms living in soil and water. Based on this initial bioavailability trials testing for pH, organic matter and cation effects have been designed. The tests on pH effects on ZnO ENP toxicity to different soil organisms are currently run within WP3. Figure 4.1 shows, as an example of this work, how soil pH affects Zn dissolution from ZnO ENP over time. In WP4 also additional long-term (12 months) exposures addressing the ageing ZnO ENP have been set up. Figure 4.2 shows the Zn dissolution from ZnO ENP with time: at low soil concentrations pore water concentrations of Zn increased with increasing soil concentration but at high concentrations the concentration of Zn in the pore water decreased again suggesting that aggregation and agglomeration processes inhibit dissolution.

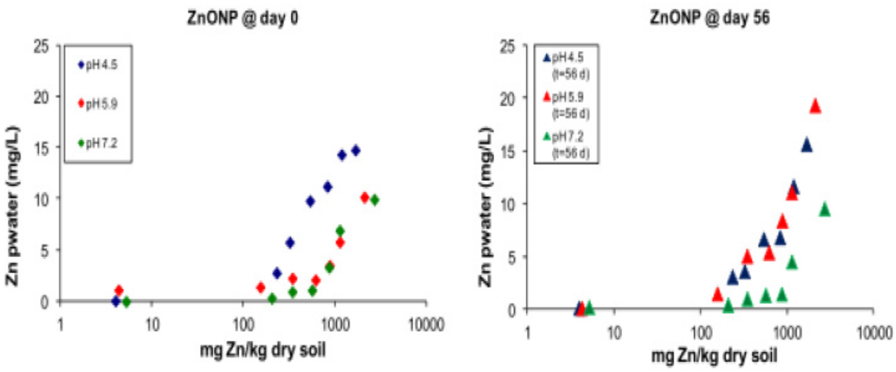


Figure 4.1 Total Zn concentration in pore water (extracted by centrifugation and filtered to <450nm) from 3 pH adjusted soil (pH 4.5, 5.9 & 7.2) dosed with a concentration range of Nanosun 30nm ZnO ENP at day 1 and after 56 days (NERC & VUA, partners 1 & 2).

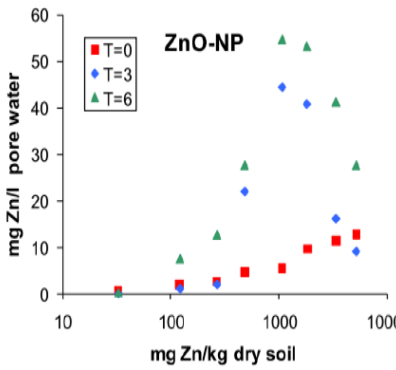
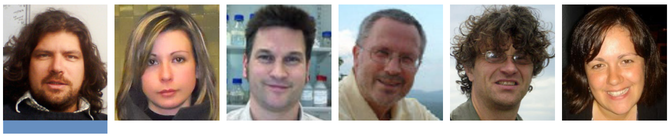


Figure 4.2 Total Zn concentration in pore water (extracted by centrifugation and filtered to <450nm) from Lufa 2.2 soil dosed with a concentration range of BASF z-cote at time zero, 3 months and 6 months (VUA, Partners 2).

WP5

ENP toxicokinetics and toxicodynamics



WP5 underpins the mechanistic effects of nanoparticles in biological systems employed by consortium members undertaking ecotoxicology experiments in entire NanoFate project. Our work will allow a better comprehension of modes of action of metal based nanoparticles to project partners and help to widen the societal acceptance for the newly developed technologies to stakeholders by reducing uncertainty.

WP5 has a focus on toxicokinetics and toxicodynamics of ENPs into biological systems. The WP5 workshop session in Portugal (Jan 2011) developed a practical and workable sample handling and preservation system to enable the success of NanoFATE tissue banking. Since then, WP5 scientists have started developing a "Tiered Approach" to look at ENPs and their effects into biological tissues at different resolution levels. This includes both cost effective screening techniques as well as sophisticated ones i.e. synchrotron radiation emission and transcriptomics to provide detailed information on uptake, distribution, speciation, metabolization and excretion of exogenous nanomaterials.

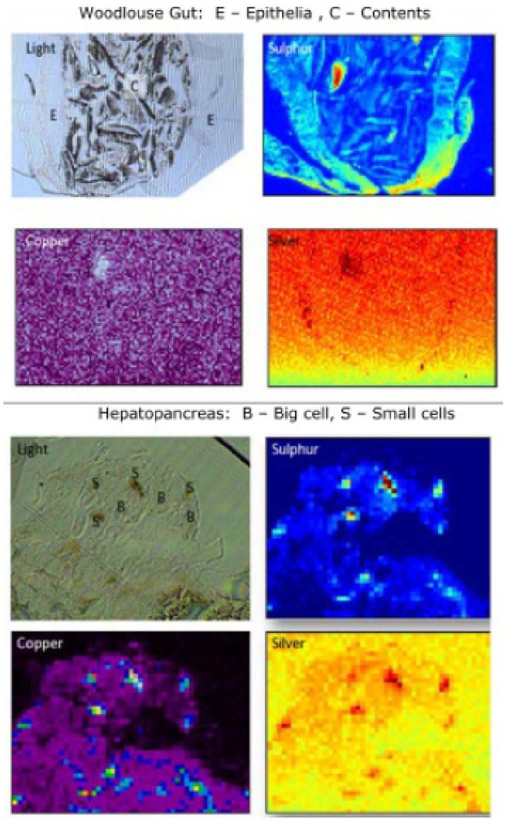


Figure 5.1 Atomic distribution of S, Cu and Ag in woodlouse tissues exposed to silver ENP evaluated by means of microfocus x-ray spectroscopy (DIAMOND Synchrotron, UK). The results showed co-localization of Ag, Cu and S which may suggest a possible complexation of Ag with either inorganic or organic sulphur compounds such insoluble crystal granules of metallothioneins. Each pixel in the image is 5x5µM.

Just with the aim to track nanoparticles and their speciation into biological tissues, a few samples have been run at Diamond Light Source synchrotron science facility in UK (Fig. 5.1). This study will provide a test bed for development of other more accessible techniques for nanoparticles effects characterization.

Furthermore, WP5 deals with microarrays and -omics approaches which usually generate large amount of information with a single analysis. Therefore, a data structure suitable for the development of a relational database has been agreed and designed. This will host the information obtained under NanoFATE allowing later cross-particles, -effects, -species comparisons. The format has been kept flexible to enable adaptation to the Nanosafety Cluster database.

WP6

Integrated environmental risk assessment

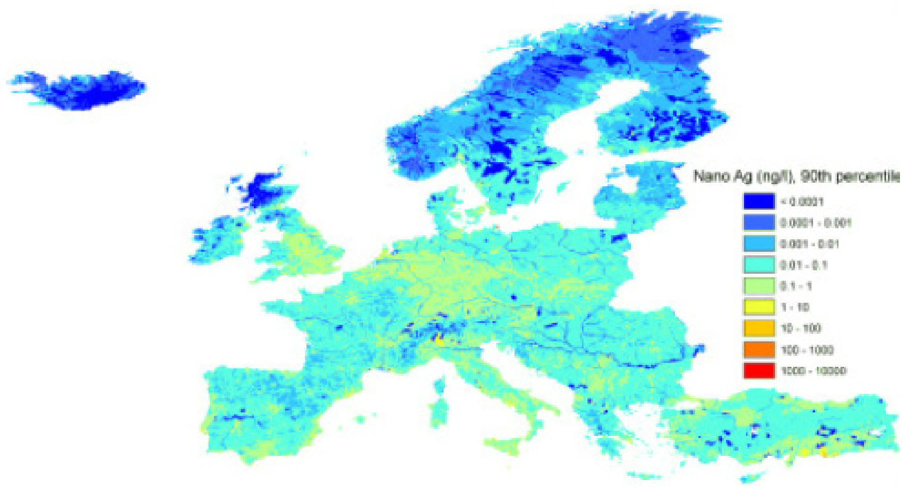


Figure 6.1 Map of 90th percentile nanosilver surface water concentrations modelled with the GWAVA model (values that would only be excedeed on 10% of occasions). Assumptions (conservative): Lowest reported removal in sewage treatment of 74% for ZnO and 49% for Ag ENPs, mean annual flows based on 40 years of rainfall statistics. At this stage the predictions are preliminary as more information will be imported on the actual behaviour of these ENPs in waste water and river water (obtained from this project and the literature). Hot spots of high predicted concentrations (>50 ng/L Ag and >200 ng/L ZnO) can be seen around such urbanised areas as north west Germany, the Low Countries, Milan, southern Spain, Portugal and south and central England.

The initial milestone for WP 6 was to assess ENP production and product incorporation estimation engaging industry directly through a survey. However, due to a poor response, the report was based instead upon a review of the peer-reviewed as well as grey literature on production volumes of the three ENPs. Based on these values initial predictions on soil and water concentrations throughout Europe have been made. For nanosilver water concentrations between 0.1 to 1 ng/L were possible with only a few areas in the 1-10 ng/L range (Fig. 6.1). These predictions will be refined as more information becomes available through the NanoFATE project.

NanoFATE

Nanoparticle Fate Assessment and Toxicity in the Environment

our calendar

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

Coming soon! Watch our [website Library](#)!
DEL 3.2
DEL 6.4
DEL 6.5

Winter 2011-2012

OUR STATUS

Over the first 18 months of the project we have been developing the materials and methods necessary to assess the environmental fate and toxicity of ENPs. We have critically assessed what factors should be included when predicting the likely environmental fate (D2.1) and bioavailability (D4.1) of ENPs. We have reviewed the current level of usage and predicted future use of ENPs (D6.1) which has informed our initial prediction of environmental levels of ENPS in soils and catchments (D6.2 & D6.3). ENPs pose new practical challenges to carrying out toxicity testing and these have been investigated with solutions developed (D3.1). These adapted methods are now being used to assess the acute toxicity of ENPs in a range of environmentally relevant taxa (D3.2).

PUBLIC DELIVERABLES

- **NanoFATE Deliverable 2.1** Summary Issues for Fate Models
- **NanoFATE Deliverable 3.1** Summary Standard Procedures Ecotox Testing
- **NanoFATE Deliverable 4.1** Summary ENP Bioavailability
- **NanoFATE Deliverable 6.1** Summary Report on perceived current and future use of silver, ceriumoxide and zincoxide Nanoparticles
- **NanoFATE Deliverable 6.2** Summary Report on predicted soil contamination levels; based on aerial or sludge deposition for each of the ENPs using the multi-media model
- **NanoFATE Deliverable 6.3** Summary Maps of Mean and Worst Case PEC for Catchments

Milestones
Public Deliverables

Click on our timeline to review past project milestones.

January 2012 — NanoFATE lectured at Ennsatox "Winter School"

NanoFATE transfered knowledge at the Ennsatox/SETAC Europe "Winter School" event offering a hands-on approach to risk assessment of nanomaterials

Led by Andrew Nelson (Ennsatox) of U. Leeds, a 3-day training winter school event (U. of Plymouth, 4-6 Jan. 2012) gave early career scientists in government and industry, recent and post-graduates a practical overview of the chemistry, biology, and risk assessment process for new nanomaterials. NanoFATE's Claus Svendsen lectured on "Environmental concentrations and fate of nanomaterials in real ecosystems - What do we know so far?" He introduced a laboratory practical on "Terrestrial Hazard: Soils and tests with soil organisms using nanomaterials" and along with organiser Richard Handy (UoP) initiated trainees to "Ecotoxicity tests with nanomaterials". [Download Winter School Course report here.](#)

Students working in the main laboratory on simple spectrophotometric methods with nanoparticles.

A group of students learning about Nanoparticle Tracking Analysis (NTA) on the Nanosight instruments.

Team photo of the students and some of the tutors in the lecture theatre.