Report EnForce 2018

26/4 2019

EnForce has now been in action more than two years. The four projects are all active, and the microplastics project has been strengthened with Anna Rotander, who now leads the microplastics project. The recruitment intensity is now lower, but during the fall we will probably recruit two postdocs and one PhD student. The research activities are following the research agenda, which has three themes: Techniques and Tools, Molecules and mechanisms and Hazard assessment, risk reduction and dissemination. In the first theme Techniques and Tools some highlights are that we are developing sampling techniques such as POM and SPMD for sampling of soil, biochar. We have also worked on validation of different marine microplastics sampling techniques. In addition, we now have a very nice set of bioreporters that have been validated. Further, the GC-based fractionator is now being used with soil extracts with post seeding using our bioreporter panel. Some preliminary tests with zebrafish embryos (FET) have been performed with really interesting and promising results. This could become a very interesting rapid in vivo test for detection of unknown toxicants in the environment. In February Eurofins and EnForce organized a seminar on contaminated areas. The fully booked seminar attracted more than 80 participants and included presentations from 12 researchers in EnForce. EnForce and Örebro University will arrange the 26th International Symposium on Polycyclic Aromatic Compounds (ISPAC) 9-12 September in Örebro. To learn more, go to www.oru.se/english/schools/science-and-technology/conferences/ispac-september-9-12-2019/.

New EnForce members

Anna Rotander is a postdoctoral research fellow and heads the microplastics project. Anna studies sources and occurrence of microplastics in Swedish fresh water lakes. She is also interested in development of proper methodologies for monitoring of microplastics in the environment.

Felicia Fredriksson is our new PhD student partly for the PFAS-risk project; she has joined us since November 1st, 2018. She is studying the fate and transport of “side-chain copolymers” from Scotchgard products. We would like to know if these “side-chain copolymers” contribute, by how much, to the unidentified organofluorine in Swedish sludge samples. She is now developing analytical methods using LC-MS/MS, LC-QTOF-MS and combustion ion chromatography (CIC) to measure these “side-chain copolymers” and conducting experiments to understand the fate and transport of these products.

Infrastructure

The huge amounts of data being produced in EnForce has prompted us to initiate development of a laboratory information management system (LIMS). A first beta-version of the new LIMS-system, currently under development by Matus Kuchalik, has now been released to our internal reference
An agile approach is applied in the development process, with continuous delivery of functionalities and modules. The LIMS system will enable for easy, secure and efficient management of projects, samples, and data handling. Other upcoming features includes among others centralized handling of lab consumables ordering and booking systems.

Mixture Risk project
Aquatic toxicology
The tests to investigate embryotoxic and teratogenic effects of the single compounds and binary mixtures of arsenic, bisphenol S, PCB 126, permethrin, benzo(a)pyrene, and PFOS are completed. The next step is to investigate ternary mixtures with a focus on combinations with PFOS. Particularly, PFOS showed a strong impact on the toxicity of the other compounds. In addition, we found that the toxicity of PFOS is increasing strongly after 96 hours and after 120 hours of exposure the toxicity of PFOS increased fivefold (Fig. 1); thus, results based on 96 hours of exposure may lead to an underestimation of the hazard potential of PFOS.

![PFOS (120 och 96 h)](image)

**Fig. 1:** Fish embryotoxicity of PFOS after 96 and 120 hours of exposure.

The investigations of the different mixtures on behavioral alterations are ongoing. In addition, the interlaboratory study on neurotoxicity and behavior is completed, and the report is already submitted to the coordinator at RWTH Aachen University, Germany. Within this study, we showed that the provided samples (diazion, diazoxon and a sediment extract) altered the behavior of fish embryos using 10 consecutive vibrational stimuli. The protocol to investigate of such behavioral alterations by pollutants was developed during a master project within the EnForce Laboratory. The results of these investigations will be published this year.
Fig. 2: Boxplots of the travelled distance [cm] as function of the treatment after 96 h exposure to diazion, diazoxon and the sediment extract (sample 3) under light conditions. SC= Solvent control; NC= Negative control; C5- C1= Exposure dilution series. The red line connects the median values from each treatment (each Boxplot). Asterisks indicate the different significance levels: p<0.05 = *; p<0.01 = **; p<0.001 = ***; Blacklined circles show single values.

In parallel to the different in vivo studies with zebrafish embryos, we started testing of mixture toxicity using the zebrafish liver cell line ZFL. Test concentrations are selected based on test with the zebrafish embryos. Both test system in vivo and in vitro, will be conducted to investigate mixture
toxicity of selected environmental samples that are already chemically characterized by the other EnForce projects. These samples will be investigated using an ultra-performance liquid chromatography (UPLC) system connected to a fraction manager. By that, we are able to fractionate sample extracts with a high resolution directly into 96-well plates. The fractionated samples will be tested for embryotoxicity, teratogenicity and alterations in the behavior of zebrafish embryos, representing a novel approach for effect directed analysis. Preliminary tests for background toxicity and investigations using fractions of a selected soil extract were successfully conducted.

Moreover, we started to select different genes for toxicity pathway analysis. The selected genes should represent typical responses of the chemicals selected within project one. Literature work and data of the DanTox project (previously conducted in Germany and coordinated by the leader of project one) built the basis for the gene selection. A specific qPCR chip will be assembled from the selected genes. Besides the possibility to understand the different mechanisms underlying mixture toxicity this chip may also be used for screening and monitoring of environmental samples, as it covers several important toxicity pathways altered by priority pollutants typically found on contaminated sites.

Publications


Workshops and conferences


Blanc M, Hyötyläinen T, Scherbak N, Keiter SH: Immediate and long-lasting effects of environmental chemicals in the Zebrafish Liver (ZF-L) cell line and subsequent unexposed passages. SETAC YES meeting, Ghent, Belgium, 2019.


PAC Risk project

- The bioreporter assays obtained from Biodetection Systems have been put to use to assess a variety of environmental samples. Extracted deployed microplastic samples have been analyzed for their activity with the anti-Androgen, Estrogen, Thyroid, and Pregnane X receptors, which contributed to one article in development. Dust samples from homes and childcare centers around Sweden have also been analyzed for their activities in the anti-Androgen, Estrogen, and Thyroid pathways, with analyses of their activity for anti-Thyroid and Pregnane X receptor signaling ongoing. Finally, preliminary analyses of soil samples that were extracted and fractionated using the GC-MS fractionator have been done to test their activity with the estrogen pathway. These effect-directed analyses will continue as more soil samples are prepared, using the full battery of bioreporter assays. We have two student bachelor projects studying car tires powder and granulates from RagnSells with different extractions methods to see which effect the extraction methods and size of granulates have on the efficiency and reproducibility of the analytical result. All extracts are analyzed with GC/MS for analysis of occurrence and concentrations of PACs and our bioreporter panel for measurement of biological activity.

- We are currently extracting all collected soil samples ("background soils" and contaminated soils provided by EnForce partners) for total initial concentrations with our ASE method to study occurrence of PACs, source specific PACs and potential toxic biological effects (Figure 1). The ASE method seems to work good even for several OH-PAHs.

- The developed LC/MS analysis of OH-PAHs seems very promising.

- GC-fractionation of soil extracts with post seeding using our bioreporter panel are ongoing. Also some first tests with zebrafish embryos (FET) have been performed with really interesting and promising results.

- Field study in Rydahl. Soil sampling (10 samples) and deployment of 18 POM-samples in water for one month was performed during the autumn 2018 by Golder. Chemical analysis and bioreporter analysis of the field POM and soil samples have been finished and the results are now evaluated. Lab studies with POM stripes (soil-POM slurry) are ongoing for the measurement of pore water concentrations of PACs and bioreporter activity of available fractions in soils.

- Bioreporter panel analysis of deployed micro plastic samples. Low response in the bioreporter assays.
Figure 3. Results from bioreporter analysis of soil extracts from seven PAC-contaminated sites and PACs profile in selected soils.

Manuscripts


High throughput analysis of 90 polycyclic aromatic compounds (PACs) and metabolites in soil – accelerated solvent extraction with in cell clean up. Titaley I & Eriksson U, et al. Manuscript.


Workshops and conferences
Maria attended the CSME conference in Nancy, France, in November and presented results from the PAC Risk project “Characterisation of PAHs and Polar PACs at Contaminated Sites – Combining in Vitro Reporter Gene Assay and Chemical Analysis”

Ivan will present orally at ICCE in Thessaloniki, Greece (16-20 June).

Örebro University will arrange 26th International Symposium on Polycyclic Aromatic Compounds (ISPAC) 9-12 September in Örebro.
PAC Project-Chemical analysis update (GC)
The accelerated solvent extraction (ASE) with in-cell clean-up method has been successfully established through the collaborative work between Ulrika Eriksson and Ivan Titaley. We then tested the extraction method using certified reference material and the results are within the certified value. We also compared our results with the results obtained from a previous inter-laboratory study and the values are within the range of values obtained from other types of extraction method. Furthermore, our extraction method was also capable of extracting oxygenated PAHs (OPAHs) and azaarenes, two groups of PACs that are often not screened, with smaller deviations than other extraction techniques, suggesting the reliability of our extraction method. We used the extraction method to analyze PACs from three different soil samples and the results will be included in the manuscript that discusses the extraction technique, which is currently being written. The results will also be presented at an international conference in June (e.g., International Conference of Chemistry and the Environment 2019). For our next step, we have identified a group of soil samples from EnForce partners that will be extracted in order to obtain the background risk due to PACs in Sweden as outlined in one of the research goals of the EnForce project.

At the same time, a workflow to identify unknown chemicals in soil samples through the use of non-targeted screening is currently being developed. For this research goal, we rely on the high resolution instrument GC-Q-Exactive-Orbitrap Mass Spectrometry. Currently, the workflow is in the final step of confirming a tentative list of unknown chemicals that have been detected by the instrument. Once the workflow is established, and validated, we will apply the method to help identify chemicals found in the fractions that have positive response in our battery of bioassay reporters.

An upcoming student work project involves the analysis of PACs from car tire samples that have been provided by one of the EnForce partners. Our goals are twofold: 1) to apply our new extraction method (i.e., ASE with in-cell clean-up) to analyze the occurrence of PACs in car tire, including OPAHs and azaarenes; and, 2) to screen for other PACs that may be present in car tires, beyond the list of eight compounds identified by the EU.

Finally, the PAC group would also like to remind everyone to participate in the 26th International Symposium on Polycyclic Aromatic Compound (ISPAC) meeting, which will be held at Örebro University on September 9-12, 2019. This meeting will bring worldwide speakers with expertise in various research areas related to PACs. More information about the meeting can be found on the website: https://www.oru.se/ISPAC2019.

PFAS Risk project

PFAS-risk
In last newsletter, our results showed substantial increases in PFCA concentrations in 10 contaminated soil samples collected from a PFAS contaminated area after total oxidizable precursor (TOP) assay indicating the presence of significant amounts of PFCA precursors. We were also interested to know if there were any precursor compounds leached from the contaminated soil. In view of this, 6 soil samples from the 10 contaminated soils were selected for the leaching test; additionally, one groundwater was analyzed for TOP to reveal any precursor in the samples. Some findings were presented in Vårmöte 2019 – Vatten och sediment, Nätverket Renare Mark, March 20-21, 2019, Arlanda airport, Sweden: Are available evaluation methods for PFAS-contaminated areas sufficient? (Platform presentation)
3. Are there any precursor compounds of PFOS or other regulated PFAS in the contaminated sites?

- Figure on the left: significant increases in PFAS concentrations were observed in some soil samples (i.e., 1, 2, 3 and 7) after TOP assay.
- Figure on the right: identified precursor compounds (6:2 and 8:2 FTSA, highlighted with the red rectangles) and some unidentified precursors were oxidized to different PFCAs (PFHpA, PFHxA, PFPeA, PFBA) leading to increases in PFCA concentrations.

3. Are there any precursor compounds of PFOS or other regulated PFAS in the contaminated sites?
Figure on the left: significant increase in PFAS concentrations were observed in some of the leachates after TOP assay.

Figure on the right: identified precursor compounds (6:2 and 8:2 FTSA) and some unidentified precursors were oxidized to different PFCAs (PFHpA, PFHxA, PFPeA, PFBA) leading to increases in PFCA concentrations.

- Significant increases were observed for some of the PFSAs and PFCAs indicated the presences of precursor compounds in the contaminated groundwater.

### Concluding Remarks

Is PFOS the major compound in the contaminated sites? And how much of PFOS or other regulated PFAS* are present in the contaminated sites?

- Sites and locations dependent

Are there any precursor compounds of PFOS or other regulated PFAS in the contaminated sites?

- Precursors of 6:2 or 8:2 fluorotelomer-based compounds were shown to be present
- These precursor compounds might leach from the soil and contaminate the groundwater

### Publications

Alina Koch, Rudolf Aro, Thanh Wang, Leo W.Y.Yeung (in press) *Towards a comprehensive analytical workflow for the chemical characterisation of organofluorine in consumer products and environmental samples.* *Trends in Analytical Chemistry*
Our recent review summarizes and discusses eight analytical methods for organofluorine (OF) analysis, which offer detection limits suitable for consumer products and environmental samples. Direct sample analysis of OF only applies to some techniques on consumer products, whereas others require sample pre-treatment or concentration before measurements. Comparison between methods for OF analysis were found to be difficult because of different selectivity (between OF and fluoride), sensitivity and type of samples (bulk, extract, surface) analysed. A top down approach for the comprehensive assessment of OF is proposed, where OF/extractable OF is first measured, followed by target analysis to obtain unquantifiable OF concentrations using the mass balance approach. For further identification of unquantifiable OF, approaches such as total oxidizable precursor assay, suspect and non-target screening are briefly discussed.

Presentations

Leo W.Y. Yeung
Towards a total PFAS exposure assessment in human sera?

EnForce Forskningsseminarium med Tema Förrorene Områden, January 22, 2019, Stockholm, Sweden
Leo W.Y. Yeung
Analytical methodology for PFAS contaminated soil – What do SLV11, TOP and EOF/TOF tell?

Patrick van Hees
PFAS case study – Precursors in soil and fluor budgets

Mattias Bäckström
Sorption of PFOS isomers on solid phases as a function of aqueous chemistry

Miljödirektoratet ligger på “Arkiv”, March 6-7, 2019, Oslo, Norway.
Eirik Aas, Patrick van Hees, Marko Filipovic, Patrik Karlsson, Linn Lindblom, Leo Yeung
PFAS and Precursors in soil and leachates - How can total oxidizable precursor (TOP) assay reveal the unknowns?

Vårmöte 2019 – Vatten och sediment, Nätverket Renare Mark, March 20-21, 2019, Arlanda airport, Sweden
Leo W.Y. Yeung, Patrick van Hees, Patrik Karlsson, Crystal Ho, Lydia Söderlund, Marko Filipovic
Are available evaluation methods for PFAS-contaminated areas sufficient? (Platform presentation)

Marko Filipovic, Maria Pettersson, Ulf Mohlander, Sara Holmström, Katrin Holmström, Patrik Karlsson, Patrick Van Hees, Anna Kärrman, Leo W.Y. Yeung
Fördelning av poly- och perfluoralkyl substanser (PFAS) i vatten och sedimentkärnor i Mälaren-Kan sedimentkärnor användas som ett verktyg för att identifiera när i tiden PFAS-utsläppen började i Stockholm?(Poster presentation)

The Fifth International Symposium on Bioremediation and Sustainable Environmental Technologies, April 15-18, 2019, Baltimore, Maryland, USA
Marko Filipovic, Patrick van Hees, Patrik Karlsson, Leo W.Y. Yeung
Total fluorine, extractable organofluorine, per/polyfluoroalkyl substances and total oxidizable precursor assay on contaminated soil (Platform presentation)
Microplastics project

In January, we submitted the final report of the Ephemare project to investigate ecotoxicological effects of microplastics (MPs) in marine ecosystems. This project represented a multidisciplinary consortium of 14 Partner Institutes from Belgium, France, Germany, Ireland, Italy, Norway, Portugal, Spain, Sweden, and microplastics experts from the UK. EnForce at Örebro University was involved in the following working packages: WP1 - Adsorption and equilibrium partition of persistent pollutants to MPs (WP leader), WP3 - Organism toxicity assessment (Task 3.4. Embryotoxicity), and WP4 - Underlying mechanism of action (Task 4.4. Genotoxicity, Ames test).

Within WP1 we were responsible to spike different MPs with two different concentration of BaP (benzo(a)pyrene), PFOS (perfluorooctane sulfonate), and BP3 (Benzophenone 3, oxybenzone), each. We also tested different sorption times to spike successfully the MPs with the chemicals. Moreover, we applied a long-term sorption test to investigate the sorption behavior of PFOS and BaP on low-density polyethylene particles. The results showed that under the conditions tested sorption processes of environmental pollutants depend on the chemical properties of the compounds, the plastic material and size but also on the sorption time suggesting rather complex and dynamic sorption, desorption and degradation processes of pollutants on MPs. The related publication is in preparation.

Within this work package, we also investigated extraction of adsorbed PFOS under simulated digestion conditions. For this purpose we developed an artificial gut fluid (AGF) simulating digestive conditions in the gut of Atlantic Cod (Gadus morhua). The AGF contains sodium taurocholate (NaTC) a vertebrate bile salt as well as 3.5% artificial sea water. PFOS-contaminated MPs were digested under the assumption that a cod take up 1.26 % food of the body weight and digestion takes 4 days. In addition, we assumed that the daily amount of food contains up to 0.3 and 1 % contaminated MPs. Under these conditions, approx. 70-80% of PFOS desorbed from the MPs; thus, PFOS is theoretically available for the organism (Fig. 3).

![Fig. 3: Desorption of PFOS und simulated digestive conditions after 4 days (t=4). The red bars shows that 70-80% of PFOS desorbed under the conditions tested.](image)

The results of the Task 3.4 within work package 3 showed that MPs contaminated with BaP, BP3 and PFOS do not cause acute toxic or sublethal effects in zebrafish embryos following the standardized
protocol of OECD TG 236 (fish embryo test (FET)). Therefore, the FET seems inappropriate to investigate acute embryotoxic effects of MPs using zebrafish. Minor alterations for the hatching rates of zebrafish embryos were observed. Hence, results indicate that sublethal toxic effects are most likely caused by pollutants associated with MPs rather than by the MPs themselves. This lends support to the view that MPs might play a role as a vector of pollutants. The results of these investigations are submitted to Frontiers in Marine Science as a joined study between Bordeaux University, IFREMER – France, Heidelberg University, and Örebro University.

For the Ames test significant mutagenic effects were observed for BaP; however, only when assuming that 100% of BaP is desorbed from MPs. By that, BaP concentrations are rather high and not representing environmental relevant levels, indicating that the Ames test seems to be inappropriate to detect genotoxic effects caused by MPs contaminated with BaP.

A report about microplastics in the big Swedish lakes was produced by Anna Rotander and Anna Kärrman: [https://www.oru.se/nyheter/mest-mikroplaster-i-tillfloden-visar-forsta-studien-av-de-stora-svenska-sjonna/](https://www.oru.se/nyheter/mest-mikroplaster-i-tillfloden-visar-forsta-studien-av-de-stora-svenska-sjonna/). The results show that the highest concentrations were found in rivers with outlets in the big lakes like Svartån and in lake Munksjön flowing into lake Vättern. The next step is to conduct source tracking.

**Articles and manuscripts**


Christine Schönlau, Florian Dubocq, Maria Larsson, Anna Rotander, Magnus Engwall and Anna Kärrman. Using a gas chromatography fractionation platform with parallel mass spectrometric detection in an effect-directed approach for the analysis of AhR active contaminants on deployed plastic pellets. Manuscript.


**Dissemination, past and future events**

Presentation på Renare Mark “Mikroplaster i svenska ytvatten” Anna Kärrman

Poster Renare Mark “Fördelning av poly- och perfluoralkyl substanser (PFAS) i vatten och sedimentkärnor i Mälaren- kan sedimentkärnor användas som ett verktyg för att identifiera när i tiden PFAS-utsläppen började i Stockholm?” Filipovic et al.
The Environmental Epigenetic Workshop was organized at MTM Research Centre and co-funded by the EnForce laboratory. The aim of the workshop is to present and discuss different environmental aspects of epigenetic alterations including current knowledge about epigenetic mechanisms, adverse outcome pathway of epigenetic alterations, data handling, and regulatory issues. Presentations were given by Carlos Guerrero Bosagna (Linköping University, Sweden), Xavier Cousin (IFREMER, France), Mélanie Blanc (Örebro University, Sweden), Juliette Legler (Utrecht University, Netherlands), Jorke Kamstra (NMBU, Norway), Joëlle Rüegg (Karolinska Institute, Sweden), Michelle Angrish (US-EPA), Oscar Karlsson (Karolinska Institute, Sweden), Philip Antczak (University of Liverpool, GB) and Miriam Jacobs (Public Health England, UK), Jana Asselman (Ghent University, Belgium). The workshop was organized by Mélanie Blanc (Örebro University (local host)), Xavier Cousin (IFREMER, La Rochelle, France), Jorke Kamstra (Utrecht University, Netherlands), Steffen Keiter (Örebro University (local host)), and Joelle Rüegg (Institute of Environmental Medicine (IMM), Karolinska Institute).