NYHETSBREV FRÅN ENFORCE

Newsletter nr 3, 8/10 2018.

Dear EnForce partners!

The department is now full of skilled researchers from all over the world. Scientific results are being produced and some publications are seeing the light of day. We are still recruiting people within or connected to EnForce. The metabolomics lab is now up and running as well as all the new instruments. The senior researchers in EnForce are all very active, pulling in grants of different sizes. We are getting samples from our partners, but we are very interested in getting more soil samples. These can be both from contaminated or clean sites. Please contact Maria Larsson (maria.larsson@oru.se) as soon as you have something interesting to send to us. Starting from 2019, we will send out biannual reports, which is a more fitting name for the current format. We will also distribute a shorter biannual newsletter in Swedish, starting next year. Early next year, the planning for phase 2 of EnForce will begin, including updating the research agenda and formulating new contracts with all participating companies. These need to be in place from 2020.

New EnForce members

Mio Skagerkvist is our new lab technician for the PFAS-risk project; he has worked with us since June 1st, 2018.

Swapnil Chavan has been hired as a postdoctor in bioinformatics. He will focus on developing a high content method for screening and categorization of morphological changes in cells exposed to environmental toxicants. He will also work in a bioinformatics project under the guidance of Professor Tuulia Hyötyläinen.

Professor Åke Bergman has a formal affiliation as guest professor at MTM since April 2018. He is working on strategic issues, helping with large grant applications and as a senior advisor in EnForce.

Dr Ernesto Alfaro-Moreno has joined EnForce as a guest researcher from Swetox, starting in October. His expertise is on local and systemic effects of inhaled particles and the role of size and composition.

Infrastructure

APGC/UPLC-Xevo XS-TOF-MS

The APGC/UPLC-Xevo XS-TOF-MS has been successfully installed in September. Researchers may use the system with target screening, suspect screening and non-target analysis.

Mixture Risk project

Aquatic toxicology

Within this project the investigation of fish embryo toxicity of the binary mixtures of the selected compounds (**arsenic, bisphenol S, PCB 126, permethrin, benzo(a)pyrene, and PFOS**) are being finalized. Beside that each compound was tested alone first, because it turned out that either no literature data was available or the data was produced under different conditions as recommended by the related OECD guideline 236. For the concentration tested, bisphenol S and permethrin did not show acute toxicity; however, for the other four compounds concentration-response depending toxicity were determined. Binary mixtures of these compounds showed either concentration addition or synergistic effects. For example, we used single concentrations of PFOS and BaP when 25% of the embryos died after 96 h (LC25). The mixture of the two LC25 caused 100 % death already after 24 h. Therefore, the **combination of PFOS and BaP showed a clear increase of the acute toxicity. In addition, the mixture showed specific sublethal effects which did not occur for the single exposure.** Next steps will be the investigation of ternary mixtures.

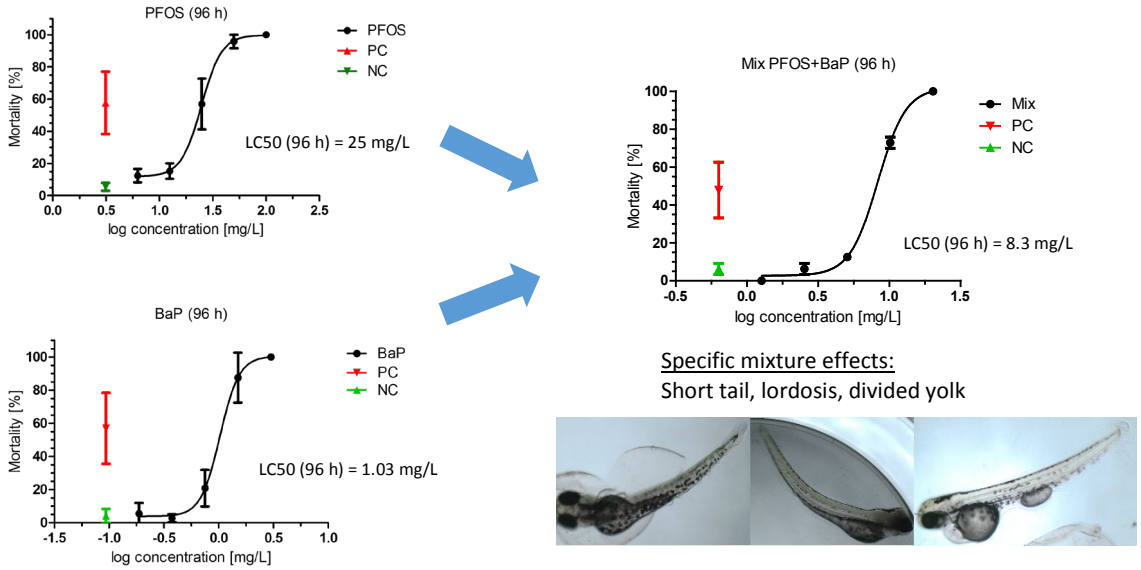


Figure. The mixture of PFOS and BaP increase the overall toxicity using an exposure concentration combining the LC25 values of each compound. In addition, the mixture caused specific teratogenic effects which were not observed for the single compounds.

In addition, we started to investigate alterations in the **behavior of zebrafish embryos** for each compound and their mixtures. Changes in behavior can evidently result from neurotoxic effects after exposure to pollutants. These alterations can be measured as **swimming activity, positive rheotaxis, locomotive behavior, preference and avoidance**. Present research focusses mostly on dark-light challenges. That means fish embryos are exposed to subsequent changes of light and darkness, and during this different conditions the distance they travel during a specific time will be measured. Under regular conditions, darkness cause anxiety and increases the traveled distance. The addition of chemical exposure can alter this typical behavior. **BaP caused hypoactivity in the highest concentration in darkness. That means they were less anxious. In contrast, PFOS and permethrin caused hyperactivity.** For permethrin this was measured in light and darkness, while PFOS is causing hyperactivity only in darkness.

Beside the investigation of such dark-light challenges mechanical stimuli can alter the behavior of a fish as well. There are two different systems belonging to the sensory system of zebrafish to detect mechanical changes: (1) the lateral line can measure motions in the surrounding water, close to the organism and can detect e.g. pray or predators, and (2) the inner ear can detect particular motions caused e.g. by gravity, or soundwaves. However, studies on such mechanical stimuli and the impact of chemicals on this sensory systems are scarce. **Therefore, we developed a test design measuring the impact of chemicals on this sensory systems in fish embryos.** The test uses a tapping device which is function like a small hammer causing a vibrational stimulus for the fish embryos. The test design was already successfully tested with two chemicals. The development and testing was part of the master project of Norina Pagano and will be published in an international per-reviewed journal. Moreover, the test design will be used to contribute to an inter-laboratory organized within the NORMAN Network by the RTWH Aachen University, Germany.

At current, we discuss and decide on the selection of bioreporter tests to be used for the mixture toxicity testing of the selected compounds but also for environmental samples. The latter will be selected based on the chemical characterization by the other EnForce projects and the collaborating partners.

Publications 2018

Meyer-Alert H, Ladermann K, Larson M, Schiwy S, Hollert H, Keiter SH (2018): A Temporal High-Resolution Investigation of the Ah-Receptor Cascade during Early Development of Zebrafish (Danio rerio) using PCB126 and β-Naphthoflavone. Aquatic Toxicology in press.

Beiras R, Bellas J, Cachot J, Cormier B, Cousin X, Engwall M, Gambardella C, Garaventa F, Keiter S, Le Bihanic F, López-Ibáñez S, Piazza V, Rial D, Tato T, Vidal-Liñán L (2018): Ingestion and contact with polyethylene microparticles does not cause toxicity on marine zooplankton. Journal of Hazardous Materials 360, 452-460.

O'Donovan S, Mestre NC, Abel S, Fonseca TG, Garcia AR, Ilharco LM, Carteny CC, Groffen T, Blust R, Cormier B, Keiter S, Bebianno MJ (2018): Ecotoxicological effects of organic contaminants adsorbed to microplastics in the clam Scrobicularia plana. Frontiers in Marine Science, section Marine Pollution 5 (143), 1-15.

Pittura L, Avio1 CG, Giuliani ME, d'Errico G, Keiter SH, Cormier B, Gorbi S, Regoli F (2018): Microplastics as Vehicles of Environmental PAHs to Marine Organisms: Combined Chemical and Physical Hazards to the Mediterranean Mussels, Mytilus galloprovincialis. Frontiers in Marine Science, section Marine Pollution 5 (103), 1-15.

Bour A, Haarr A, Keiter S, Hylland K (2018): Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. Environ Poll 236, 652-660.

Workshops and conferences

S. Keiter as representative (2018): Knowledge gaps for the ecotoxicological assessment and modelling of microplastics in the sea – Conclusions of the EPHEMARE project. ECHA Workshop on Microplastic, Helsinki.

S. Keiter as representative (2018): The European Project EPHEMARE - Ecotoxicological effects of microplastic in marine ecosystems. Renare Mark - Framtida problem, Seminarium i Östersund.

N. Pagano, M. Blanc, G. Nilén, H. Hollert, S. Keiter (2018): Behavior of Zebrafish to disturbance through tapping. Annual meeting of SETAC Europe, Rome, Italy.

G. Nilén, B. Holmes, M. Larsson, N. Scherbak, M. Engwall, S. Keiter (2018): Mixture Risk - Development of an effect-based chemical risk assessment strategy for sites contaminated with complex mixtures. Annual meeting of SETAC Europe, Rome, Italy.

M. Blanc, N. Scherbak, S.H. Keiter (2018): Persistent organic pollutants alter expression patterns of epigenetic factors in the zebrafish liver (ZF-L) cell line. Annual meeting of SETAC Europe, Rome, Italy.

Biogeochemistry project

This sub-project of the Mixture risk project is run by Dr. Liem Nguyen. His questions focus on elucidating **removal mechanisms and removal efficiency of hazardous metals using industrial waste materials e.g. steel slag**. His first project with the title "Sorption mechanism of arsenate on stainless steel slags obtaining from scrap metal recycling" has been submitted to Journal of Hazardous Materials as research article. The study highlighted the importance of arsenate chemical speciation for the adsorption, and contribution of the precipitation and adsorption to arsenate removal were identified and validated.

Ongoing plans are to further study the removal of more hazardous metals including Cu, Zn, Cr, Cd, Pb by steel slag, and the application of steel slag as filter material for treatment of industrial wastewater.

Pilot scale for treatment of heavy contaminated soils by steel slag. The samples will be sent by industrial partners.

Manuscripts

Sorption mechanism of arsenate on stainless steel slags obtaining from scrap metal recycling.

PAC Risk project

The bioassay panel is established and new and old PACs have been tested in the assays and their relative potency (REP) values calculated. Many of the tested PACs have shown to be weak inducers of the **thyroid, androgen and estrogen receptors**, but showed a higher activity in the **PXR assay** (Pregnane X receptor activation). These studies also indicate that **anti-androgen pathways** are targets for a variety of PAH structures. The PACs are also tested for Ah receptor activity in the PAH-CALUX and mutagenic potency in the P53 CALUX assay. Similar to the H4IIE-luc assay, the PAC-CALUX bioreporter is also measuring Ah receptor active compounds, but the potency of the compounds or sample extract is related to the potency of benzo[a]pyrene, generating benzo[a]pyrene equivalents. The PAH CALUX is a rapid 4 hour assay, which enables detection of low molecular weight PACs, not measured in the H4IIE-luc assay.

The **GC fractionation method** of PACs is further developed to increase the resolution of fractionation. High Ah receptor effects (H4IIE-luc assay) were shown for many of the fractions after fractionation of PAC-contaminated soil extracts into 96-well plates by using the GC-fractionator

system. **Directed by the bioactive fractions, instrumental GC-MS analysis is currently performed to identify the bioactive compounds in the fractions. At the same time method development of post seeding of the new cell lines into fractionated 96 well plates is performed.**

In the chemical analytical part focus is on development of analysis of novel PACs. A number of new PACs including high molecular weight PAHs, **azaarenes, 5- and 6-methyl chrysenes and oxy-PAHs** have been added to the target list of GC-MS analysis. **Non-target GC-HRMS analysis for identification of novel PACs and also LC-MS analysis of PAH metabolites (OH-PAHs) are under development**.

A lot of work has been put on developing the **ASE method with in cell clean up**. To have a good method that is able to extract out the total amount of all PACs from soil samples with good recoveries is really important. The method development was currently finished and extraction of all collected soil samples will start during the fall. Before starting the extractions we are now homogenizing and sieving all soil samples that have arrived.

RagnSells is planning two ex-situ remediation projects of PAC contaminated sediment/soil during the fall. We are going to do sampling before, during and after treatments to study the changes in availability, bioactivity in bioreporter assays and PAC-profiles including metabolites during treatment.

Soil and sediment samples have been arriving from EnForce partners during summer and fall. **However, we are very interested in getting more soil samples.** These can be both from contaminated or clean sites. Please contact Maria Larsson (maria.larsson@oru.se) as soon as you have something interesting to send to us.

Maria is going to attend the CSME conference in Nancy, France, in November and present results from the PAC Risk project “Characterisation of PAHs and Polar PACs at Contaminated Sites – Combining in Vitro Reporter Gene Assay and Chemical Analysis”.

We are developing a new bioassay, called **cell painting.** In this assay, we can detect very small changes in the morphology of cells exposed to different toxicants. By this approach we will be able to develop a library of morphological profiles in cells exposed to different chemicals with known mechanisms of action. Using this library we then will be able to see if novel or unknown toxic compounds give similar effects in the cells. Thereby we can determine their mechanism of action. In addition, the cell painting assay will provide an excellent tool for search of non-target effects. This information will be an important first step in elucidating mechanisms of action of unknown toxicants. To achieve capacity to screen thousands of cellular images from this assay automatically, we are developing image recognition software with neural networks and artificial intelligence technology.

Manuscripts

Concentrations and source characterization of polycyclic aromatic compounds (PACs) in soil from a railway area. Larsson M, et al. Manuscript.

Characterization of polycyclic aromatic compounds (PACs) and their mobility in soil using non-target screening. Titaley I, et al. Manuscript.

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.

PAC toxicity and structure-activity studies with the AR, ER-alpha, and TR-beta receptors. Holmes B, et al. Manuscript.

Workshops and conferences

Maria Larsson, Monika Lam, Patrick van Hees, John Giesy and Magnus Engwall. Occurrence and availability of PACs and total AhR agonists in contaminated soils –Combining in vitro reporter gene assay and chemical analysis with passive sampling and column leaching. Poster presentation at Setac, Rom, 2018.

Ivan A Titaley, D. McCauley-Walden, O. M. Ogba, P. H.-Y Cheong S. L. Massey Simonich. Environmental chemistry and exposure assessment: analysis, monitoring, fate and modelling. Poster presentation at Setac, Rom, 2018.

PFAS Risk project

Leo Yeung has taken over the PFAS-risk project from Anna Kärrman since May 1st 2018. If you have any project ideas, please feel free to contact him (Leo.Yeung@oru.se).

Activities

Golder Associate AB: Providing **groundwater and soil** samples from mostly contaminated sites for a complete PFAS analysis including EOF;
Swedavia: Providing **water** samples after treatment to evaluate the effectiveness of the water treatment technique;
Ragnsell: Performing leaching test on **contaminated soil** collected by Sweco; providing samples after water treatment (Chromafora) to perform a comprehensive evaluation of removal of PFAS in contaminated water.
Sweco: Providing **contaminated soil** samples to Ragnsell for leaching test and landfill leachate samples for our on-going projects.
Jordnära: Providing **groundwater samples** to understand the hydrogeological movement of PFAS and metals in a contaminated area in Lidköping.
Niras: Providing **sediment core** samples to understand the deposition of PFAS in lakes; setting **rain collection** station in Stockholm city to understand the importance of wet precipitation of PFAS in Stockholm city.
Eurofins: Contributing to total **oxidizable precursor (TOP) assay** to understand the levels of unknown identified PFCA and PFOS in contaminated soils

Regarding toxicity studies, we are testing per- and polyfluoroalkyl substances (**PFAS) exposures in the HepG2 human liver cell** line for metabolomics studies and as preliminary work to be linked to cell painting assays using the high-content screening system. These studies are expected to give a more complete picture of the activity and target pathways of interest, as well as the interaction between pathways, for these common environmental pollutants.

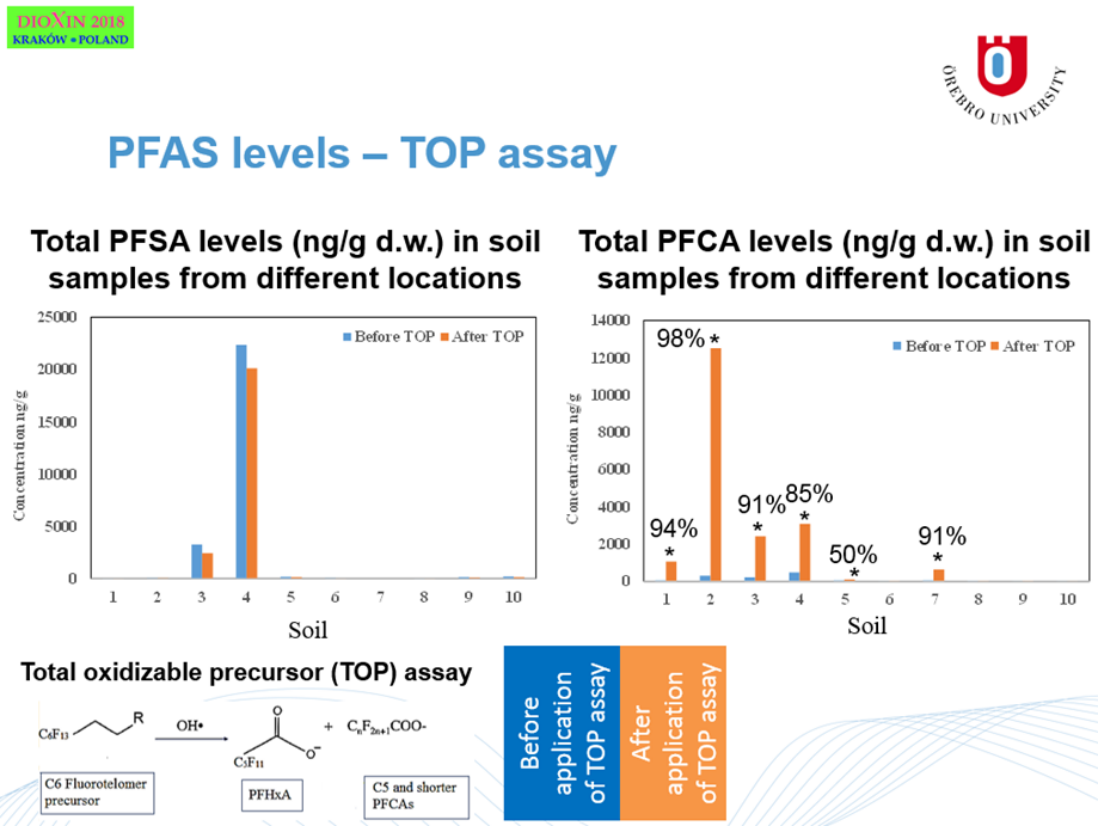
Findings highlight

Title

Yeung LWY, van Hees P, Karlsson P, Söderlund L, Filipovic M: Total fluorine, extractable organofluorine, per/polyfluoroalkyl substances and total oxidizable precursor assay on contaminated soil.

Abstract

The Swedish Geotechnical Institute (SGI) derived guideline values for assessing contamination status for different land uses for PFOS. For sensitive land use (e.g., residential), the guideline value is 0.003 mg PFOS/kg dw in soil; for less sensitive land use (e.g., industrial) the guideline value is 0.020 mg PFOS/kg dw. What would we miss if only PFOS is considered when assessing contaminated soil? Total oxidizable precursor (TOP) assay may be used to reveal the presence of any PFOS or PFCA precursor compounds. Ten soil samples were collected from a PFAS contaminated area; not much differences in PFOS/PFSA levels with/without the application TOP assay onto the samples; however, substantial increases in PFCAs were observed after TOP assay, indicating that significant amounts of PFCA precursors are present in contaminated soil.



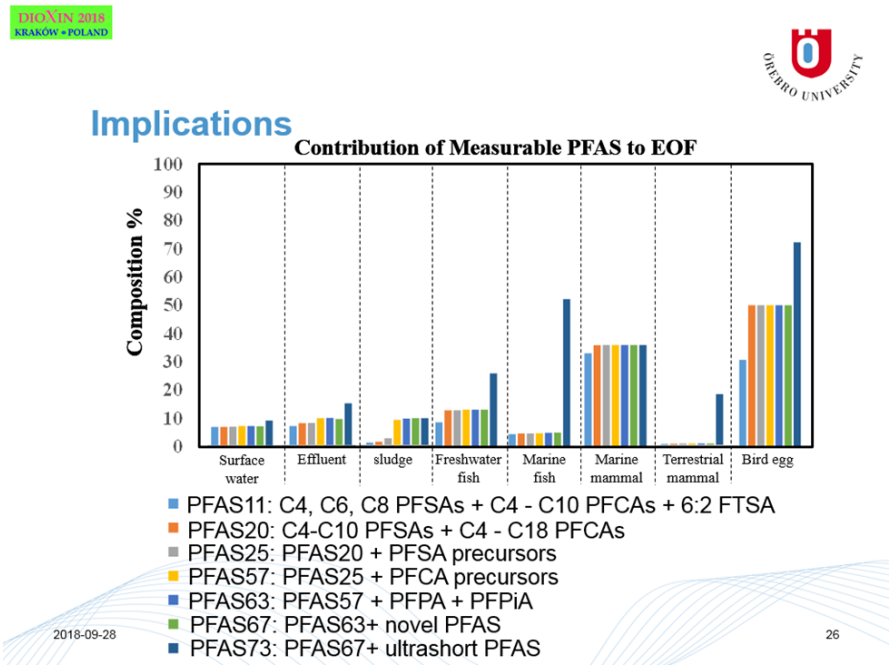
Title

Yeung LWY, Aro R, Fredriksson F, Eriksson U, Chen F, Wang T, Kallenborn R, Kärrman A: Mass balance analysis of extractable organofluorine in environmental samples from the Nordic Countries.

Abstract

Mass balance analysis of fluorine may allow better understanding on how much quantifiable/measurable PFAS account for extractable organofluorine (EOF). Recently, our team took part in a Nordic screening project looking for novel PFAS (e.g., GenX, F53B, Cyclic PFAS) and ultrashort PFAS (TFA, PFPrA, PFEtS, PTPrS) including legacy PFAS (e.g., PFCA and PFSA) and EOF. By measuring more number of PFAS may help increase the proportion of quantifiable PFAS to EOF

fraction. Our results showed that quantifiable PFAS accounted for as low as 9% in surface water samples and as high as 72% in bird egg samples, suggesting up to 92% of EOF remained unidentified.



Student thesis

Crystal Ho. The study of organofluorine analysis applied to total oxidizable precursor (TOP) assay to understand perandpolyfluoroalkyl substances (PFASs). Independent Project for Degree of Bachelor in Chemistry, 15hp. School of Science and Technology, Örebro University, 2018.

Felicia Fredriksson. Distribution of Per- and Polyfluoroalkyl Substances (PFASs) along Matrices from South Africa - using a mass balance approach. Master of Chemistry in Environmental Forensics. Independent Project for Degree of Master in Chemistry, 45hp. School of Science and Technology, Örebro University, 2018.

Jean Noel Uwayezu. Sorption of PFOS to different solid components as a function of aqueous chemistry. Master of Chemistry in Environmental Forensics. Independent Project for Degree of Master in Chemistry, 45hp. School of Science and Technology, Örebro University, 2018.

Conferences

Chen F, Eriksson U, Aro R, Yeung LWY, Wang T, Kallenborn R, Kärrman A. Screening of per- and polyfluoroalkyl substances (PFASs) and total organic fluorine in wastewater effluent from Nordic countries. (Poster presentation).

Aro R, Eriksson U, Kärrman A, Chen F, Wang T, Kallenborn R, Yeung LWY. Contamination of per- and polyfluoroalkyl substances, including novel PFASs, in wastewater treatment plant effluent from Nordic Countries. (Poster Presentation)

Koch A, Yeung LWY, Westermann S, Ericson I, Kärrman A, Wang T. Comparison of reversed-phase liquid chromatography and supercritical fluid chromatography on ultra-short chain perfluoroalkyl acids (C2-C4 PFAAs). (Poster Presentation)

Aro R, Eriksson U, Kärrman A, Chen F, Wang T, Yeung LWY: Per- and polyfluoroalkyl substance (PFAS) homologue profiles, including ultrashort-chain compounds, and extractable organofluorine (EOF) in wastewater treatment plant effluent and sludge from Nordic countries (Platform presentation)

Björnsdotter MK, Yeung LWY, Kärrman A, Ericson Jogsten I: Ultra-short-chain perfluoroalkyl substances (PFASs) including trifluoromethanesulfonic acid (TFMS) in environmental waters (Platform presentation)

Fredriksson F, Yeung LWY, Kärrman A, Eriksson U: Comparison of per-/polyfluorinated substances profiles and levels in bird eggs from South Africa and Nordic countries (Platform presentation)

Yeung LWY, van Hees P, Karlsson P, Söderlund L, Filipovic M: Total fluorine, extractable organofluorine, per/polyfluoroalkyl substances and total oxidizable precursor assay on contaminated soil (Platform presentation)

Yeung LWY, Aro R, Fredriksson F, Eriksson U, Chen F, Wang T, Kallenborn R, Kärrman A: Mass balance analysis of extractable organofluorine in environmental samples from the Nordic Countries (Platform presentation)

van Hees P, Filipovic M, Karlsson P, Söderlund L, Yeung LWY: What is the total budget of PFAS in contaminated soil and how does total oxidizable precursor (TOP) assay help comprehend the picture? (Platform presentation)

Microplastics project

At present, for the investigation of ecotoxicological effects of microplastics we collaborate with the group of Dr. Xavier Cousin and Dr. Marie-Laure Begout at Ifremer, La Rochelle, France. Within this collaboration, the partners in France **fed zebrafish over several month with microplastic with a daily dose of 1% of their die**t. The plastic was applied also **with adsorbed contaminants**. First results indicated for some of the contaminated microplastic particles a **reduced weight** of fish and a **decreased spawning** of females. In addition, the behavior of the fish was altered when fed with contaminated microplastic. However, data evaluations are not finalized yet.

Together with partners from Heidelberg University, Germany and Ifremer, France we prepared an interlaboratory comparison of the acute and specific toxicity of microplastic towards zebrafish embryos. The results are finished and at present we prepare the manuscript for publication in a special issue of the international journal Frontiers in Marine Science.

We also started a project about the **digestion of microplastic in polychaeta and fish**. Therefore, we will synthesize and collect digestion fluid from the lugworm (Arenicola marina) and cod (Gadus morhua), respectively. The experiments will be applied by Flora Borchert a master student from Germany and Bettie Cormier a PhD student within the microplastic project at Örebro University. The project will be also a collaboration with Prof. Ketil Hylland from the Department of Biosciences at the University of Oslo.

Beside the toxicological investigations we are also interested about the **sorption behavior** of pollutants on different types of microplastic. Therefore, we started a long-term sorption test over 6 months under laboratory conditions. At present we started the chemical analyses of the exposure medium, the plastic as well as the sorption containers. The results are expected to be submitted for publication by the end of this year.

During 2018 we will also finalize measurements of microplastics (50 um) in Swedish lakes. We are focusing in developing sampling methods and analysis methods.

Manuscripts

Christine Schönlau, Maria Larsson, Monika M. Lam, Magnus Engwall, John P. Giesy, Chelsea Rochman and Anna Kärrman. Aryl hydrocarbon receptor-mediated potencies in field deployed plastics vary by type of polymer. Submitted.

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

Renare mark seminar about Future problems Östersund 180213. Anna Kärrman, Steffen Keiter and Maria Larsson presented research from EnForce.

The Second EnForce workshop. A full day with information about the EnForce research profile was held May 4 in Örebro. Around 50 participants were there. An abstract book was produced and it can be requested from Magnus.Engwall@oru.se.

MTM Seminars. Research seminars given by guests at MTM can be viewed at https://www.oru.se/english/research/research-environments/ent/mtm/mtm-seminars/.

Posters presented at the conferences we have attended can be viewed at https://www.oru.se/english/research/research-environments/ent/mtm/posters/.

Eurofins and MTM invites everyone to a seminar about contaminated areas to be held in Stockholm 190122.
http://www.renaremark.se/event/eurofins-i-samarbete-med-orebro-universitet-bjuder-in-till-seminarium-med-tema-fororenade-omraden

Best regards,

Magnus Engwall, research director EnForce