Installation of unique fractionation instrument in the EnForce lab.

Dear EnForce partners!
The recruitment of personnel is now almost complete. We have recruited skilled researchers to all positions except for a post doctor in bioinformatics and this is ongoing. Here is a presentation of the newly hired researchers connected to EnForce.

Maria Larsson, postdoctoral research fellow

Maria has been hired as a research fellow in analytical chemistry. Her research area is environmental chemistry, focusing on identification, exposure and receptor-mediated mixture effects (cocktail effects) of organic pollutants. Maria is combining chemical analysis and mechanism-specific bioassays for characterization and risk assessment of contaminated soils, dust and other complex mixtures. A special focus is on polycyclic aromatic compounds. Maria is the lab director of the bioanalytical laboratory at MTM Research Centre and the project leader of the PAC project in EnForce.
Anna Rotander, postdoctoral research fellow

Anna Rotander has been hired as a research fellow in analytical chemistry. Her research has been focused bioanalytical studies of soils and sediments, PFAS studies, occurrence of microplastics in Swedish lakes and on studying the association between persistent organic pollutants (POPs) and plastic litter in ocean gyres. Anna will work mainly with microplastics research.

Ulrika Eriksson, postdoctoral researcher

Ulrika Eriksson has been hired as a post doctor in analytical chemistry. Her research area is per- and polyfluorinated alkyl substances in the environment. At her current position as post-doctor at EnForce, her research is focused around method development of fractionation procedures for polycyclic aromatic hydrocarbons, applying an effect-directed approach for risk assessment in environmental compartments such as contaminated soil, water, sediments, and also recycled materials.

Liem Nguyen, post doctoral researcher

To assess the effects of metals to environments and living organisms, total concentration of metals is, in many cases, not significant. Therefore, I am interested in metals speciation. I have conducted studies for elucidating biogeochemical forms of mercury in soil and water environments. I also developed several analytical methods for characterization of thiol which is an important factor that control speciation of mercury. Currently, I am involved in a project in which we are studying the impacts of housing construction activities at a legacy heavy metals contaminated site to proximity inhabitants and the aquatic ecosystem.

FangFang Chen, post doctoral researcher

My research area is environmental and analytical chemistry focusing on per- and polyfluoroalkyl substances (PFASs), including their sources, fate and distribution in the environment, wildlife and human exposure. I also worked with other class of ionogenic organic contaminants (IOCs) such as pharmaceuticals and personal care products (PPCPs) focusing on bioaccumulation mass balance models to examine the bioaccumulation and tissue distribution mechanism of IOCs in fish.
Rebecca Bülow, laboratory technician

Rebecca has been hired as a laboratory technician within EnForce. She is mainly working with cell culturing with the bioassay panel and with PAC analysis, screening samples from the different projects.

Greta Nilén, PhD student

My name is Greta Nilén and I started as a PhD within the EnForce project in August 2017. I have a background in Biology and Environmental toxicology at Uppsala University. My research at Örebro University will focus on mixture toxicity with the main aim to investigate the toxic responses of mixtures of pollutants and integrate the results into risk assessment. I am performing both in vitro and in vivo tests with cell lines and zebrafish embryos. Perfluorinated alkyl substances (PFASs) are of major concern since they are persistent and able to enhance the toxicity of other contaminants and one objective of the project is to develop a novel bioanalytical tool for measurement of PFAS contamination.

Ivan Titaley, post doctoral researcher

I recently finished my PhD in Chemistry at Oregon State University working under the supervision of Dr. Staci Simonich. My primary research concerns analysis and measurement of polycyclic aromatic hydrocarbons (PACs) and the degradation products in various environmental matrices using gas chromatography coupled with mass spectrometry (GC/MS). I also incorporated computational chemistry into my research projects. At EnForce, I work closely with Dr. Maria Larsson and Dr. Magnus Engwall for the PAC-Risk project. We analyze the presence of PAC in various matrices and assess their potential human health implications using effects-directed analysis. Outside the lab I enjoy watching basketball and soccer, listening to classical music, and cooking!

Breanne Holmes, post doctoral researcher

My research background centers on environmental endocrine disruption using both biological and chemical analyses. The focus of my post-doctoral research is the application of bioreporter assays to help prioritize contaminants of concern. In collaboration with analytical chemists, the toxicity of nuclear receptor mediated toxicity, inflammation and stress response pathways is being characterized from chemical mixtures found in soil and sediment samples.
**Tim Sinioja, PhD student**

I attained my bachelor’s and master’s degrees in chemistry with a focus on environmental forensics after graduating from Örebro University. Since summer 2017, I’m a Ph.D. student at Man-Technology-Environment research centre (MTM). My main research areas are exposomics and metabolomics. I perform comprehensive analysis of human exposures using techniques based on high resolution mass spectrometry, as well as a data analysis and modelling of chemical, clinical and toxicological data.

**Maria Björnsdotter, PhD student**

A wide range of chemicals are being used in a variety of consumer products in today’s society and it is not clear how the majority of them will affect human health and the environment. Recently, concern has been raised regarding ultra-short chain per- and polyfluorinated alkyl substances (PFASs) due to contamination of ground- and drinking water in Sweden as these chemicals are being more commonly used in consumer products and have a potential for adverse effects on human health and the environment. My research is aimed on studying the occurrence and fate of these chemicals in the Swedish environment with a focus on Lake Vättern. For this purpose, I am measuring the levels of PFASs, including the ultra-short chain PFASs in environmental samples by using supercritical fluid chromatography coupled with tandem mass spectrometry.

**Dawei Geng, post doctoral researcher**

I started working as a postdoctoral researcher at Man-Technology-Environment Research Centre (MTM) since December 2016. I received my PhD in chemistry in September 2016. My main research interests are human exposure and environmental studies. Special focus is on exposome studies and metabolomics. Currently my research is the development of novel comprehensive methodologies for halogenated persistent organic pollutants as well as identification of unknown compounds in complex biological (mainly human) samples using advanced mass spectrometric tools.

**Rudolf Aro, PhD student**

My research project aims to investigate the current human and environmental exposure levels to unidentified PFASs. This will help to better understand how these compounds are released to the environment and their sources. The final stage is to identify these unknown substances. My background is in metrology, traceability and uncertainty analysis.
Viktor Sjöberg post doctoral researcher

My research background is on element mobilization from materials such as steel slag. My post-doctoral research focuses on the use of waste materials as sorbents for valuable as well as toxic elements. An important part of my research is to better understand the mechanisms of sorption and element speciation.

Infrastructure

We have acquired several instruments from Agilent and Waters. From Agilent these include an Agilent 4210 Microwave Plasma-Atomic Emission Spectrometer (MP AES), an Agilent 8900 Inductively Coupled Plasma Mass Spectrometer (ICP-MS/MS) and an Agilent 1260 HPLC.

The Agilent 4210 MP AES is well suited for analysis of all major metals in solution and equipped with a sample introduction system made of Teflon it is also possible to analyze solutions containing several percent hydrofluoric acid. Detection limits range from ng/l for lithium to some 50 ug/l for actinides. The system can analyze liquids ranging from water to pure oil. The instrument is intended to be used as standalone as well as a detector for HPLC.

The Agilent 8900 is a triple quadrupole mass detector ICP capable of detection of most elements in the ng/L-pg/L range with high precision. The triple quadrupole design allows for efficient interference removal and the instrument is capable of using several gases in the reaction cell e.g. oxygen, hydrogen, ammonia and helium. Due to the design of the triple quadrupole unwanted side reactions in the reaction cell are almost completely avoided and very clean spectra can be obtained. The instrument is intended to be used as standalone as well as a detector for HPLC. As a detector for SEC-HPLC (size exclusion chromatography), it will be possible to determine the major element content of e.g. humic and fulvic acids and proteins due to the instruments very low LODs. As a standalone instrument the reaction cell allows for detection of elements that previously has been extremely difficult to quantify with quadruple ICP-MS due to severe interferences, including phosphorous, sulfur, arsenic and selenium.
From Agilent we have also acquired a GC/MS that has been modified to an automated gas chromatography based fraction collection system. The modification was done by DaVinci Laboratory Solutions. The GC fractionator is currently being used to develop methods to separate PACs and for fractionation of microplastics extracts. It has a lot of potential for the EDA work that is done in the PAC project.

We have also acquired a high content imaging system (In Cell 2200) for fast, sensitive, and flexible wide-field cell imaging. Using this system we are able to detect structural and functional changes on a cellular level as effect of contaminants. It will be highly valuable in elucidating mechanisms if action for various PACs and PFAS compounds.

From Waters, several instrument have been purchased. One mass spectrometer, Xevo G2-XS QTof, connected to gas chromatographic systems will be used for screening for new drivers of toxicity. This system can also be connected to an instrument for separation of analytes using supercritical carbon dioxide. As a complement for existing instruments we are installing a Xevo TQ-S micro, which a triple quadrupole mass spectrometer. This will increase our throughput capacity in the analytical laboratory. Both these mass spectrometers can be connected to different chromatographic systems to cover all types of contaminants.
We are also working on setting up a laboratory information management system, a so called LIMS that is a database will be used to collect and keep track of all the data being generated within EnForce. This is quite a challenging task since the data has many different forms and is of different type.

From Biodetection systems and AcceGen Biotech we have acquired bioassays based on the CALUX technology for different mechanisms of toxic action. They will be able to detect compounds that act via the AR-, DR-, PXR, ERα-, FXR and TR receptors. In addition, the p53 CALUX assay will enable detection of genotoxic compounds. These bioassays will be central in the search for novel toxicants.

The behavior of an animal is often an early marker for toxicological effects. The zebra fish research group has therefore acquired Daniovision, which is a complete system designed for high-throughput tracking of zebrafish larvae. Larvae activity and movement can reveal information on stereotypic and epileptic behaviors, circadian rhythmicity, motor control, movement disorders, neural development, and more. This system will be used to monitor effects of toxicant mixtures on zebrafish development. Since the development of vertebrates follow the same general pattern, results from this research can be a good indicator of risk also for higher animals.
Mixture Risk project

This project is run by Steffen Keiter, and Magnus Engwall, Stefan Karlsson, Viktor Sjöberg, Liem Nguyen and Greta Nilén are also involved. The mixture studies that have just started include combination studies of the toxicity of arsenic, bisphenol S, PCB 126, permethrin, benzo(a)pyrene and, PFOS. The effects of these mixtures are being studied in several in vitro and in vivo assays. The later are based on zebrafish investigating embryotoxicity, morphology, teratogenicity and behavior. Other effects will be studied using genomics and metabolomics methods. Also, the new high content imaging system will be used to study effects on the cellular level. In addition, the mixtures will, starting in March be tested in the in vitro bioassay battery. The overall purpose of the mixture studies is to generate more knowledge about how mixtures of compounds may interact and affect the cumulated risk in the environment. Based on earlier studies we have reason to believe that PFAS compounds for instance may enhance or reduce the toxicity of other compounds.

There is also a plan for a collaborative project with the Norman network, a European Network of reference laboratories, research centers and related organizations for monitoring of emerging environmental substances. It concerns a pilot study of sewage effluents using biotests and is a preparation for a large grant application within EU. Enforce will contribute with several bioassays in this project.

Together with Jordnära Miljökonsult AB is EnForce currently investigating a former porcelain deposit in Lidköping. The aim of the project is to trace and evaluate the contamination of the area and to identify the sources. Örebro University and EnForce focuses mainly on the occurrence of inorganic contaminants such as heavy metals e.g. cadmium and lead. Today the site is used for recreation but in a near future, it is intended to become a residential area. At some locations exceptionally high metal loads have been found. However, it is unclear how available the metals are and no information about their speciation is available, which is studied in a part of the project. Depth profiles of solid material have been collected and there is a groundwater monitoring program running, using newly installed as well as older wells all over the site. The outcome of the project will provide information that can be used to evaluate whether transformation of the area into residential can be done with or without remediation. Furthermore, its potential threat to the nearby lake Vänern can be estimated and probably also the lakes impact on the mobilization of and transport of metals from the site.

If you have questions about the Mixture Risk project, contact Steffen.Keiter@oru.se.
PAC Risk project
This project is run by Maria Larsson, and Magnus Engwall, Monika Lam, Breanne Holmes, Ivan Titaley and Ulrika Eriksson are also highly involved. Currently a lot of focus is on establishing the bioassay panel. Breanne Holmes is in charge of this process. Also, a lot of work is put into screening soil samples from various sites for their PAC content and for their activity in the DR-CALUX bioassay. This is mainly done by Rebecca Bülow and Maria Larsson. A list of PACs is compiled by Breanne and Ivan based on what has already been tested in the Tox21 project and there seems to be many data gaps for responses in the bioassays we are setting up. When the final list of PACs is established, the most relevant individual PACs will be tested in all the bioassays in order to establish relative potency factors (REPs). These can then be used for potency balance calculations that will be helpful in directing us towards the most important drivers of toxicity in the soil samples. At the same time, method development for analysis of novel PACs is being done by Ivan.

The GC fractionator was installed during the fall and has already been tested and, despite having some prototype issues, it is working well. Maria Larsson and Ivan Titaley are developing an in cell cleanup method in the ASE to be able to process many samples rapidly. They are also setting up a GC-based separation method for fractionation of as many PACs as possible. After this, the system will be run on real soil samples to direct the mass spectrometry analysis towards the most toxic compounds in the mixtures.

We also have a UPLC system for separation of more hydrophilic samples. Separation and fractionation of less polar PACs will be developed in this system by Ulrika Eriksson.

Samples are arriving from many EnForce partners at a varying pace. During fall and until now we have received soil samples for the PAC project from Golder, Sweco and Liljemarks. 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. Within the PAC project we are now having a dissertation coming up, since Monika Lam has just finalized her thesis. More on that below.

PFAS Risk project
The PFAS risk project is run by Anna Kärrman. Ulrika Eriksson, FangFang Chen, Leo Yeung, Breanne Holmes, Tuulia Hyötyläinen, Dawei Geng, Ingrid Ericson, Thanh Wang, Nikolai Scherbak and Magnus Engwall are also involved. It is divided into an analytical and a toxicological part. In the analytical part focus is on development of analysis of novel PFAS compounds, including cyclic sulfonic acids, polyethers and others, as well as efforts to expand the concept of total-PFAS analysis. So far work has been focused on developing analytical methods in the lab. We are in the process of finalizing a screening of novel PFASs and total organic fluorine in abiotic and biota samples using those methods. Sampling is also ongoing. Swedavia has planned to do sampling during the first half of 2018 in their water treatment plant for PFASs at Landvetter. For these samples, analysis of total organic fluorine, a large number of PFAS compounds and ultra-short chained PFASs will be carried out. Niras have collected sediment samples in Stockholm that has been analyzed, and next step is to collect rain water and air samples. Golder has started sampling a PFAS-contaminated site that will be studied within EnForce.

In the PFAS project we have tested the effect of PFOS and PFOA on the gene expression and metabolome of developing chicken embryos. These results have been submitted in two manuscripts “Effect of perflurooctanesulfonic acid on the liver lipid metabolism of the developing chicken embryo” and “Effects of perfluorooctane sulfonate on genes controlling hepatic fatty acid metabolism in livers of chicken embryos” and we are currently waiting for the response from the
journal editors. These manuscripts show that PFAS compounds are influencing the hepatic fatty acid metabolism.

Breanne Holmes started testing the effect of PFAS compounds on inflammation and immune cells together with researchers from the research group iRisc. In addition Breanne is starting testing of PFAS compounds in the HepG2 liver cell line to search for PFAS-specific biomarkers. This is done using metabolomics and genomics methods in collaboration with the metabolomics lab, headed by professor Tuulia Hyötyläinen.

If you have questions about the PFAS Risk project, contact Anna.Karrman@oru.se.

Microplastics
We are now including a project on microplastics within EnForce. The rationale for this is that it is a highly relevant issue within the EnForce area, with several companies interested and a long experience and ongoing research within MTM. The plan is to focus the work mainly towards development of sampling and quantification methods and on screening of Swedish samples of all relevant types. Anna Rotander will be the project leader for this project. During 2018 she will be on parental leave, but during this time questions regarding this project can be directed to Magnus Engwall or Anna Kärrman.

Publications and dissertations

Monika Lam is presenting her thesis “Characterization of PAH-contaminated soil with focus on bioavailability, mobility and biological activity”. Monika Lam received her Master of Science degree in ecotoxicology from RWTH Aachen in 2014. She has been a PhD student at MTM since 2014. The dissertation will take place 9 February 10.15 in Musikhögskolan (HSM) at Örebro University and everybody are welcome to this dissertation! Below is the abstract from her thesis.
Abstract
Polycyclic aromatic compounds (PACs) are common contaminants in contaminated soil of industrial areas, such as abandoned gasworks sites, gas stations and former wood impregnation facilities. The risk assessment of contaminated soil sites with PAHs are based only on the measurements of the 16 parent PAHs prioritized by the US EPA. However the traditional monitoring methodology of using chemical analysis of only a small selection of organic pollutants to determine the degree of contamination in environment matrices, like soil usually overlooks potential contaminants present in the samples. In addition, the risk assessment of contaminated areas is often still based on the total concentration of soil even though the total amount of the compounds may not relate directly to the environmental or human health risk, since usually only a fraction of the compounds can be for example taken up by organisms. In this work underlying this thesis, availability, leachability, AhR-mediated and ER-mediated activity were investigated in PAH-contaminated soil from industrial areas. The overall aim of this thesis is to develop a refined and enhanced analytical approach based on both chemical and bioassay analysis coupled to passive sampling and leaching tests that can provide a more comprehensive picture of pollution of a site contaminated with PAHs. For that, bioassay-specific relative potency factors (REPs) of PACs were determined for the H4IIE-luc bioassay detecting AhR-mediated activity, and for the VM7luc4E2 transactivation assay detecting estrogenic activity. The passive sampler polyoxymethylene (POM) and leaching tests were used to study availability and mobility of PAC in soil. Extracts of soil, POM and leachates were investigated for the estrogenic and AhR-mediated activity. REPs (61 of H4IIE-luc assay and 33 of VM7luc4E2 bioassay) were used to perform mass-balance analysis to determine the proportion of the activity accounted by the analyzed chemicals of known potency. The results showed that in all kinds of samples (total soil-concentration, POM-fraction or leachates) a great fraction of AhR- or ER-agonists remains unexplained, if assessments were based only on the quantification of PAH16. However, despite several additional PACs that were included in this thesis, unexplained fractions remains. This highlights the need of bioassays that integrates the potencies of all agonists in a sample, in risk assessment. Availability and mobility of compounds are important factors that should be considered in risk assessment. Coupling of chemical and biological analysis to passive sampling or leaching tests revealed that only a small fraction of PACs are available or leachable in soil and suggests that if assessment are based on the total concentration, the risk might be overestimated.

Titles of articles in the thesis


Paper III. Lam MM, Engwall M, Denison MS, Giesy JP, Larsson M. Polyoxymethylene (POM) is a suitable tool for effect-based hazard assessment of PAC-contaminated soil. Manuscript.

Scientific contributions 2017
Christine Schönlau, Jeroen Kool, Maria Larsson1, Magnus Engwall, Anna Kärrman. Using a GC based effect-directed approach for the analysis of AhR active contaminants on plastic litter. Poster presentation at ICCE, Oslo, 2017.
Anna Kärrman. Consequences of Firefighting foam contamination. Poster presentation at Dioxin 2017, Vancouver.
Monika Lam, Maria Larsson, Magnus Engwall AhR-mediated effects and (bio)availability of polycyclic aromatic compounds of soil with different contamination sources. Oral presentation AquaConSoil 2017, Lyon.
Maria Larsson, Monika Lam, Patrick van Hees, and Magnus Engwall1, Occurrence and leachability of PACs in contaminated soils. Oral presentation AquaConSoil 2017, Lyon.
Maria Larsson, Monika Lam och Magnus Engwall. SOILEFFECT – Utveckling av bioreporter-baserad mätning och riskbedömning av giftiga kemikalier i förorenade områden”. Poster, Renare marks vårmöte 2017.
Blanc, Melanie, Karrman, Anna, Kukucka, Petr, Scherbak, Nikolai, Keiter, Steffen (2017). "Mixture-specific gene expression in zebrafish (Danio rerio) embryos exposed to perfluorooctane sulfonic acid (PFOS), perfluorohexanoic acid (PFHxA) and 3,3',4,4',5-pentachlorobiphenyl (PCB126).” Science of the Total Environment 590: 249-257.

Second EnForce workshop
We would like to invite all profile participants and reference group members to the second EnForce workshop that will be held May 4 at Örebro University. At the workshop, results and future plans will be presented and discussed. To register, send an email to Magnus.Engwall@oru.se. More information about the workshop will follow soon.

Best regards,

Magnus Engwall, research director EnForce