# EnForce newsletter 5

1/11 2019

## Introduction

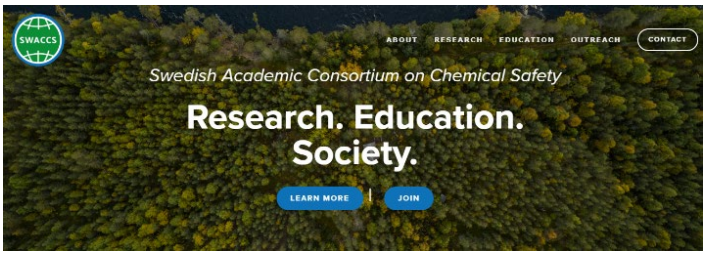
EnForce is now approaching half time and all four projects are active. The recruitment intensity is now going up and we have just recruited Ulf Norinder from Swetox as an affiliated professor in computational toxicology and machine learning and Bei Wang, a new researcher in the PFAS risk project. In addition, we are recruiting a new cell culture technician, and a new PhD student for the PAC risk project. The research in the projects has been quite active and many publications are being published.

In September Christine Schönlau presented her thesis "Microplastics in the marine environment and the assessment of potential adverse effects by plastic-derived chemicals". One important conclusion from her thesis is that microplastics do not absorb and transport environmental toxins in seas and lakes as much as previously feared. According to the work of Christine, microplastics which are just a few millimetres thick, do not transport toxic substances in seas and lakes as well as researchers once believed. However, there are still many unknown factors, like the potential role of nanoplastics as a vector for toxic chemicals and as a toxicant in itself.

EnForce is also generating several new applications. One example is the KKS Synergy application "Cleaning of stormwater from environmental contaminants and microplastic pollution" managed by Steffen Keiter. The vision, aims and expected impact of this highly applied project are
1) to develop and design technical solutions and strategies to minimize the risk resulting from pollutants and microplastic transported by stormwater from roads,
2) to create and provide a test strategy to identify and assess the potential risk for human and environmental health caused by hazardous compounds in storm water, and,
3) to manage and recycle the filter materials as well as elements and materials origin from stormwater as a resource in the context of a circular economy.

In May, EnForce and Swaccs organized the Åke Bergman Symposium "From science to action for a chemical safe world." At this well attended meeting world leading researchers in the area of chemicals and risk gave great presentations. Professor Juliette Legler from Utrecht University was one of the speakers. She was looking ahead what a safe world would look like in terms of chemicals – a world where both humans and animals are protected from harmful chemicals. Her talk was titled "Vision for a chemical safe world".

In September 9-12, EnForce members Maria Larsson, Ivan Titaley and Ulrika Eriksson organized and hosted the International Symposium on Polycyclic Aromatic Compounds (ISPAC). At this well attended international meeting, researchers from 18 countries met at Örebro University to present new results and discuss PACs in an environmental risk perspective. There was a clear consensus that the PAH 16 list needs to be expanded, since so many other PACs contribute to the total risk of PACs.


Several EnForce members are highly involved in organizing the Swedish Academic Consortium on Chemical Safety (SWACCS). Developed from Swetox, SWACCS is a cooperation between scientists at 13 Swedish universities which offers academic support to the pursuit of a chemical-safe world. All companies in the EnForce group are invited to connect with SWACCS in order to find ways forward to achieve this goal. Contact Åke Bergman, Magnus Engwall or Ingrid Ericson Jogsten for more info.

Currently, we are working hard planning for phase 2 of EnForce, which includes producing a scientific and organizational report and letters of agreement regarding collaboration and in kind with all participating companies. This process is progressing very well.

Do not forget that news about EnForce are also on Twitter!

## New EnForce members

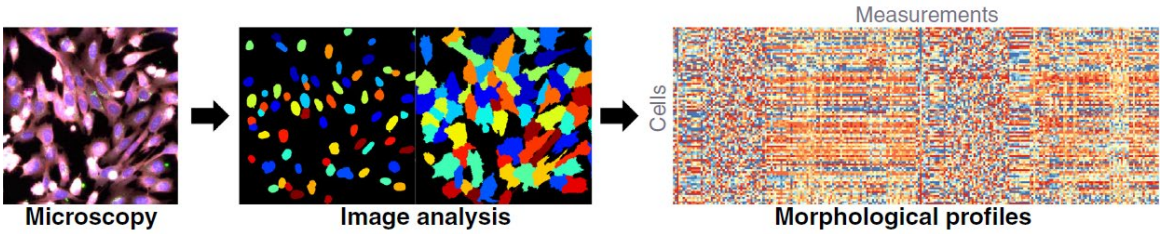
Professor Ulf Norinder has been appointed as affiliated professor at MTM/EnForce. Ulf Norinder received his M.S. in Chemical Engineering and Ph.D. degree in Chemistry from Chalmers University of Technology (CTH). From 1985 – 2014 he worked as computational chemist, senior principal scientist and research fellow in the pharmaceutical industry (Karo Bio AB, AstraZeneca AB and H. Lundbeck A/S) and from 2015 - 2018 as senior research specialist at Swetox Södertälje (Karolinska Institutet). His areas of expertise include computer-assisted drug design and pattern recognition with special emphasis on multivariate data analysis and machine learning. In EnForce Ulf will work with computational methods needed in development of new bioassays based on image analysis of cell cultures (Cell painting assay) and support other research groups at Örebro University.

A new researcher, Dr. Bei Wang, will join the PFAS-risk project in November, 2019. She will focus on PFAS characterization in environmental samples particularly on method development for novel PFAS and total oxidizable precursor (TOP) assay in different environmental matrices to estimate the levels of unidentified PFAS precursors. Dr. Wang obtained her PhD degree in Civil and Environmental Engineering from the University of New South Wales, Sydney, Australia. She had a position as research fellow in Nanyang Technological University in Singapore and a postdoctoral researcher position at Karolinska Institute (KI).

## Mixture Risk project

Among other things, the Mixture Risk project is working with development of EnTox – Chip: At present we develop a molecular chip that covers various physiological pathways in fish. Each of these pathways are represented by selected genes. Changes in the expression of these genes are known to cause adverse effects; for example, developmental alterations of the heart, eye or spinal cord. Gene expression changes are caused by different specific environmental pollutants. Therefore, the specific response of the EnTox-chip will allow us to predict certain environmental pollution in various samples (e.g. soil, sediment, water).

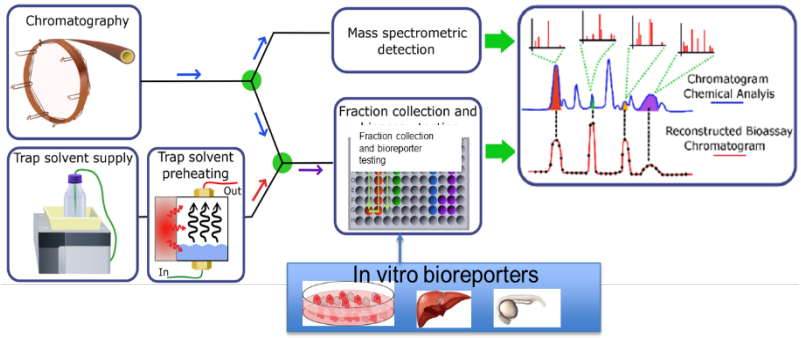
Another novel bioassay under development is the Cell painting assay. This is a high content morphological phenotype screening of cells. It employs six fluorescent dyes that reveal eight cellular components or organelles. The idea is that the cellular phenotype (appearance in microscope images) is related to chemical exposure and we are using deep learning methods like convolutional neural networks to analyse high content images of cells exposed to different chemicals. Cellular phenotypes (morphological profiles) after exposure to known chemicals can then be compared to phenotypes resulting from exposure to unknown chemicals from environmental sample fractions of complex extracts. This assay will be a great tool to discover novel environmental toxicants.




## PAC Risk project

We have received around 90 background soils and contaminated soils from the EnForce partners from 2017 until August 2019. These samples have been extracted using the developed ASE method and characterized by chemical analysis and H4IIE-luc bioreporter analysis. Next we will test a selection of soil extracts in the other bioreporter assays. The bioavailable concentrations of PACs can be assessed with the POM method, and we have done this on a number of soils.

Currently, we are performing effect directed analysis (EDA) using high-resolution GC or UPLC fractionation and H4IIE-luc analysis on soil extracts. The results show a large unexplained toxicity, probably caused by unknown compounds in the soil. During November and December we will perform additional tests with GC-fractionation and zebrafish embryos (FET).




*The principle behind the GC based EDA.*

We have developed a method for semi-quantification of several alkylated PAHs in soils using relative retention times. This will enable us to include more alkylated compounds in the analysis. Results from the EDA indicate that a number of alkylated PAHs and larger molecular weight PAHs are bioactive in the H4IIE-luc assay, and thus Ah-receptor agonists.

During autumn/winter 2019 we are evaluating the chemical and bioreporter results with the aim to increase our understanding about which PACs are frequently present in soil; source specific PACs, their availability/mobility in soil and potential toxic biological effects.

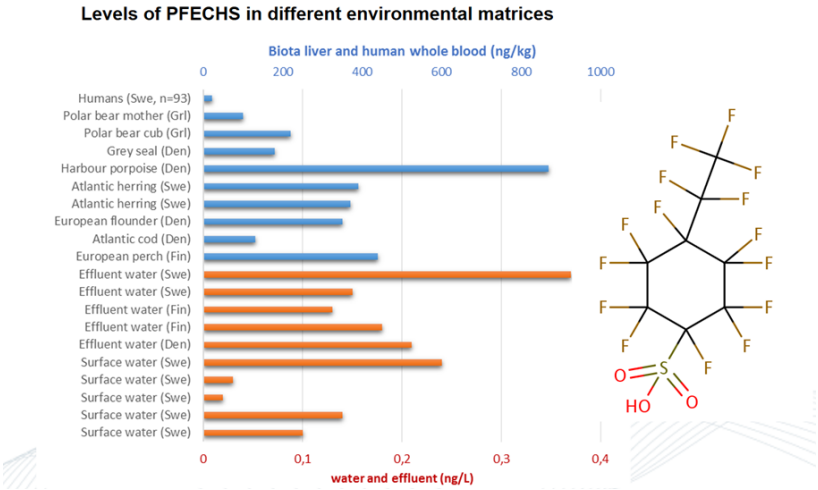
During summer we also studied car tires granulates using chemical and H4IIE-luc bioreporter characterization. We found very interesting results with a large portion of Ah-receptor activity that could not be explained by the quantified PACs. This indicates that the car tire granulates contain many unknown AhR agonists. We are planning to study this more using chemical and bioreporter assay screening of additional granulates.

## PFAS Risk project

In the last six months, our researchers were quite active in participating in different national and international meetings. A number of visiting scholars from Asia and Europe came to our lab to improve the understandings on characterization of PFAS in different samples and identify the unknown organofluorine in the samples. In August, a number of researchers from Örebro University attended the 39th International Symposium on Halogenated Persistent Organic Pollutants, Dioxin 2019, Kyoto Japan and gave presentations on PFAS focusing on analytical development for measuring and detection of novel PFAS including polymeric-based PFAS in the environment and human matrix as well as understanding the potential pathway of PFAS transfer from aquatic to terrestrial environments. Our PhD student Maria Björnsdotter won the Best student presentation award. Some highlights from selected presentations are provided below:

**WIDE-SPREAD CONTAMINATION OF PERFLUORO-4-ETHYLCYCLOHEXANESULFONATE (PFECHS) IN THE NORDIC COUNTRIES**

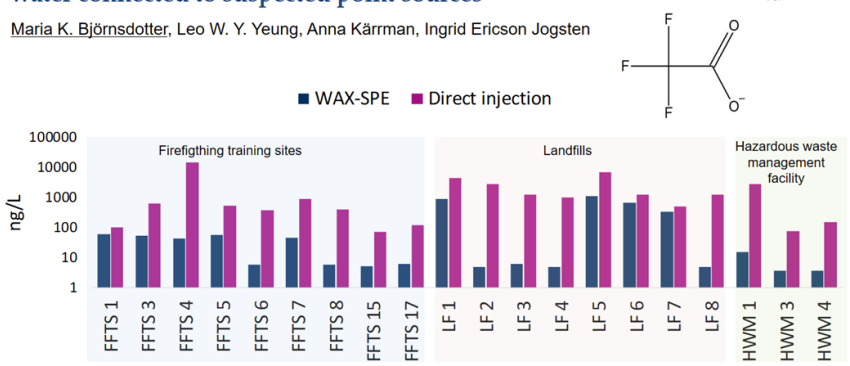
Kärrman A., Aro R., Eriksson U., Wang T., Yeung L.W.Y.



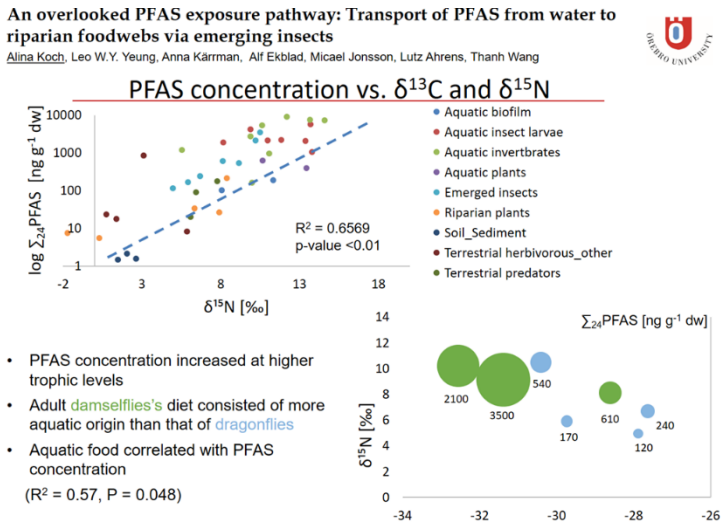

- PFECHS has been reported as an anticorrosive agent in hydraulic fluids
- Possible source is aviation activity
- PFECHS was detected in surface water, WWTP effluent, fish, marine mammals, polar bears, and human blood.
- Other emission sources than aircraft fluids is possible.

**Direct injection analysis by supercritical fluid chromatography coupled to tandem mass spectrometry of trifluoroacetic acid in water connected to suspected point sources**

Maria K. Björnsdotter, Leo W. Y. Yeung, Anna Kärrman, Ingrid Ericson Jogsten




- Trifluoroacetic acid (TFA) is highly persistent and a polar perfluoroalkyl acid
- Found globally (precipitation, surface water, groundwater, air, soil, vegetation)
- Atmospheric degradation of hydrofluorocarbons (HFCs) and hydrochlorofluorocarbons (HCFCs)
- Not removed by currently used water treatment methods (e.g. GAC filters)
- The extraction of TFA from water by WAX-SPE is pH and matrix dependent.
- Analysis by direct injection was an adequate method for measuring TFA in water samples
- Firefighting training sites, landfills and hazardous waste management facilities may be relevant sources for TFA in the environment.




- Is there a lateral export of PFASs from the freshwater system to the riparian zone?
- Study site: Kallinge
- One pathway of PFASs are ecological subsidies such as emerging aquatic insects to the riparian food web
- Link between emerged aquatic insects to riparian predators should be investigated.

## Microplastics project

We are currently running a project funded by the Swedish EPA that aims at characterizing and source track microplastics in the Svartån water system, mainly focusing on the contribution of Örebro city. This work is done using a high volume pump (20000 L/h). This yields sampling of two size fractions (50 and 300 µm). In the project we are also validating the use of a new smaller flexible pump, capacity 8000 L/h, that can sample smaller bodies of water compared to the big pump (for example in WWTP effluent streams). We have found that Svartån contains a large variety of microplastic particles. We are also planning a similar project in the river Lidan in Lidköping together with Jordnära AB using the same methods.

*Selected microplastic particles from Svartån downstream of Örebro.*