Mixture Risk - Development of an effect-based chemical risk assessment strategy for sites contaminated with complex mixtures

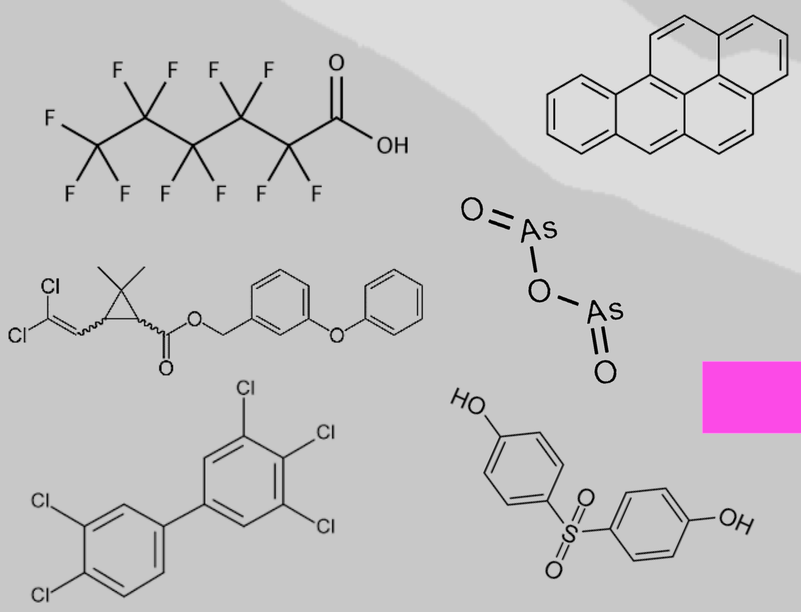
Nilén G.[1]*, Holmes B.[1], Larsson M.[1], Scherbak N.[1], Engwall M.[1], Keiter S.[1]
[1]Man-Technology-Environment Research Centre (MTM), Örebro University, Sweden

*Contact email: **greta.nilen@oru.se**

Aims

- Investigate the toxicity of mixtures of environmental pollutants
- Increase knowledge regarding molecular and mechanism-specific effects of mixture toxicity
- Develop a vertebrate test to quantify PFAS contamination
- Integrate results into risk assessment of contaminated sites
- Communicate the results with industry partners and stakeholders

Mixture toxicity



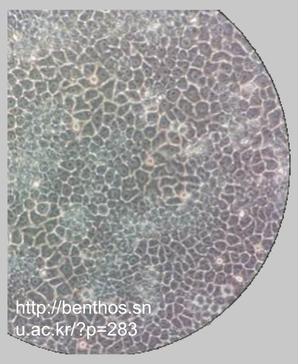
Materials & Methods

Test systems:

Cell lines with luciferase reporter gene (H4IIE and U2OS) and zebrafish embryo acute toxicity test (FET, OECD no. 236).

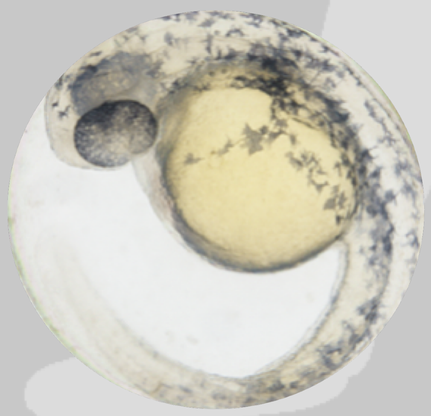
Endpoints for *in vitro* test system:

- Estrogen receptor activation/inhibition
- Androgen receptor activation/inhibition
- Aryl hydrocarbon (Ah) receptor activation/inhibition
- Peroxisome proliferator-activated receptor activation/inhibition
- Genotoxicity
- Cytotoxicity
- Oxidative stress



Selection of endpoints for *in vivo* test system:

- Malformations
- Detachment of tail
- Lack of heartbeat
- Hatching time
- Behavioral alterations



Results

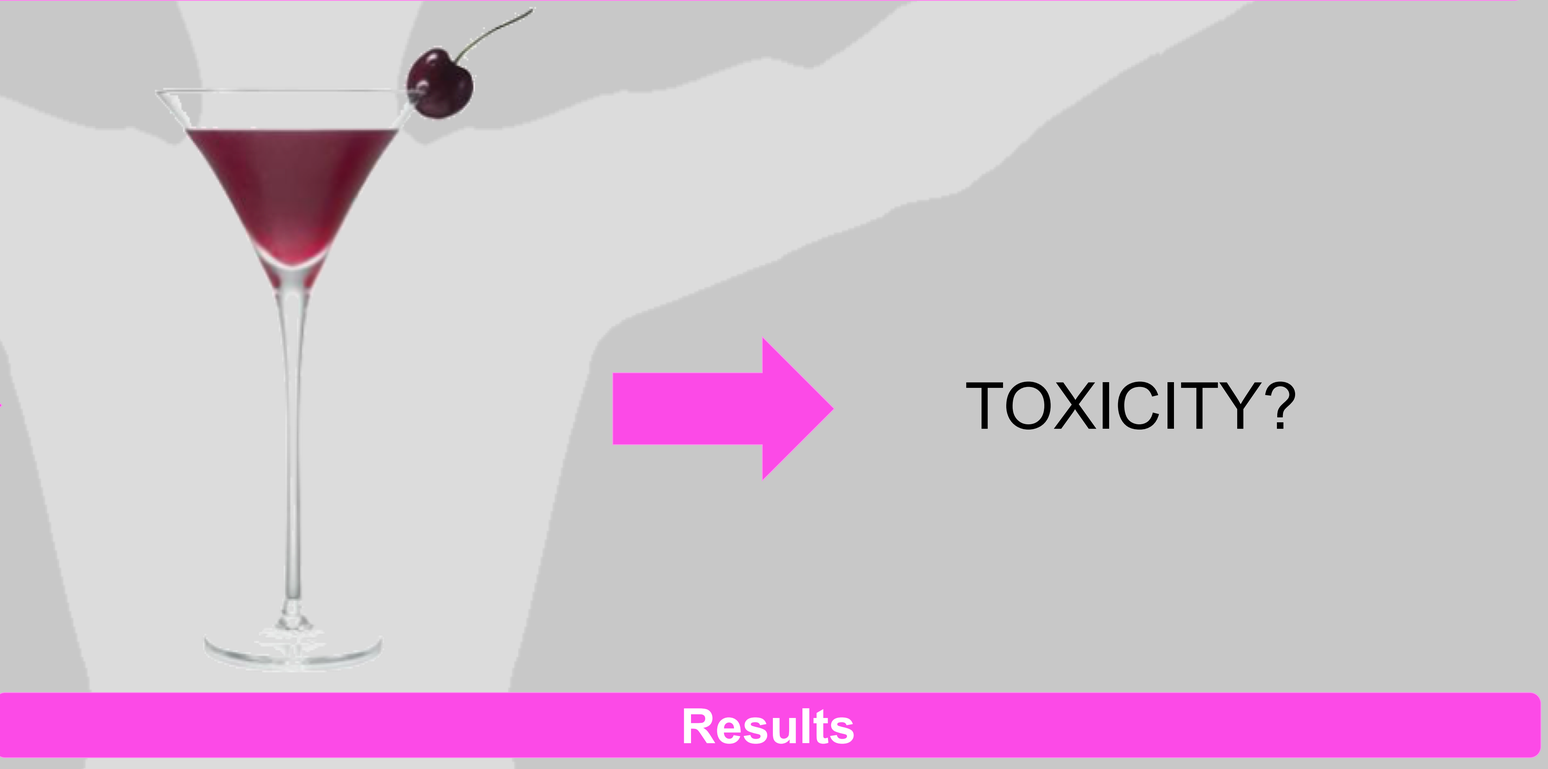


Figure 1. The highest concentration of an equitoxic mixture (LC_{25} + LC_{25}) containing PFOS (96 %) and BaP (4 %) caused 100 % mortality already after 24 hours (left). In addition, the mixture affected the activity of the larvae (right) in light (yellow/orange) and darkness (blue). A mixture of PFOS (4.9 mg/L) and BaP (0.2 mg/L) resulted in a lower activity in darkness, a higher activity in light and no difference between activity in darkness and light.

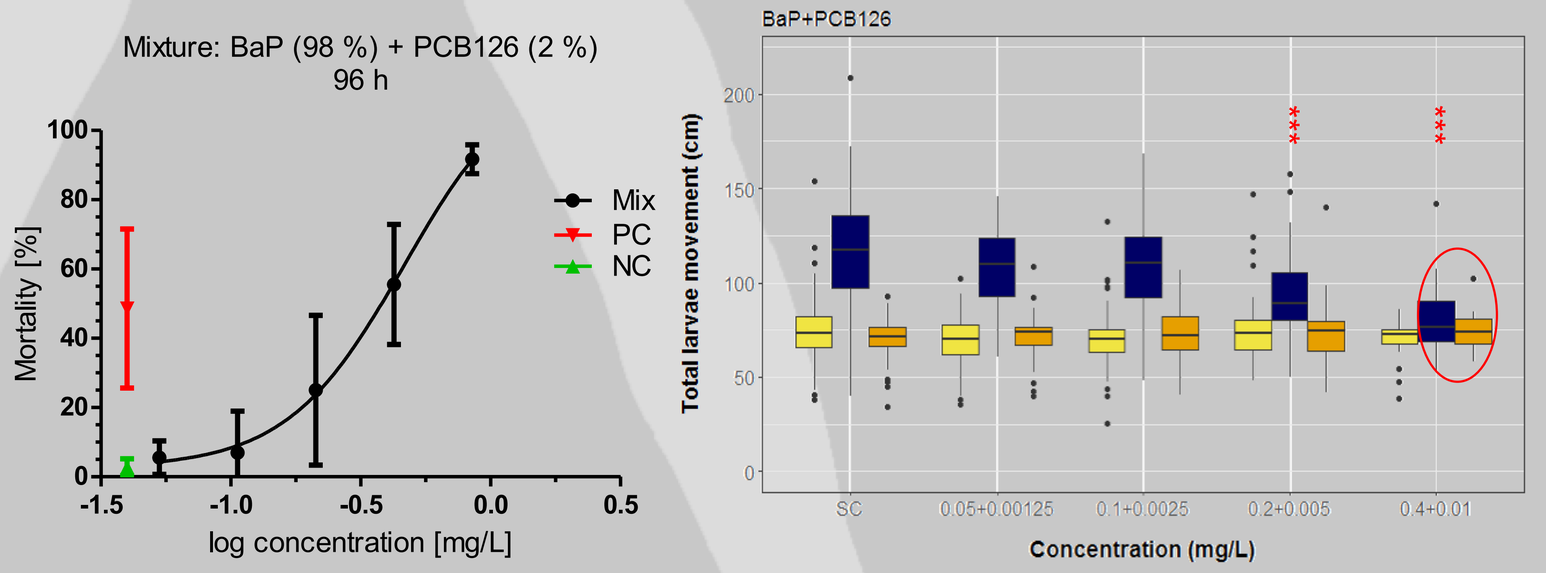


Figure 2. The highest concentration of an equitoxic mixture (LC_{25} + EC_{25}) containing BaP (98 %) and PCB126 (2 %) caused >90 % mortality at 96 hours (left). The mixture also affected the activity of zebrafish larvae (right) in light (yellow/orange) and darkness (blue). Larvae exposed to BaP (0.2 and 0.4 mg/L) and PCB126 (0.005 and 0.1 mg/L) became hypoactive in darkness. Also, the activity pattern observed in the solvent control (SC) could not be seen in the highest treatment group.

Conclusion & Outlook

Acute toxicity of BaP increases when exposing zebrafish embryos in combination with PFOS or PCB126. Higher toxicity of the mixtures might be explained by a) PFOS enhances the toxicity of BaP by affecting the uptake, and b) PCB126 increases the induction of CYPs, resulting in a bioactivation of BaP. Further investigations on mixture toxicity will include other groups of chemicals (e.g. heavy metals, pesticides and endocrine disruptors) but also other endpoints using *in vitro* bioreporter assays.

Man-Technology-Environment Research Centre
School of Science and Technology, Örebro University
SE-701 82 Örebro
Sweden