SNAPFISH – Searching for refined *in vitro* approaches to predict bioconcentration in fish

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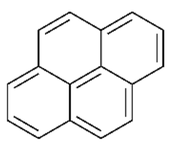
Introduction

The ecotoxicological assessment of chemicals requires information about their physical-chemical characteristics. Regarding hydrophobic organic substances, information about their biotransformation and potential bioaccumulation is important because they can accumulate along the food chain. Besides the direct determination of steady-state bioconcentration factors (BCFs) in fish (e.g., via OECD TG 305), these data can also be obtained by the use of *in vitro* depletion methods to minimize the use of live animals in the context of the 3R-targets (replace, reduce, refine). The aim of the present study is to obtain *in vitro* metabolic rates of hydrophobic organic compounds and to analyze the enzymatic turnover of these chemicals. Partitioning-based dosing methods are being applied to improve the exposure control of low dissolved concentrations of the test substances and to avoid additional organic solvents which could affect the enzymes.

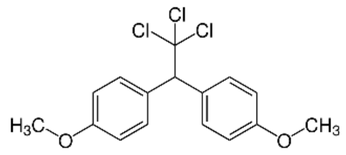
Material

Hydrophobic organic model compounds

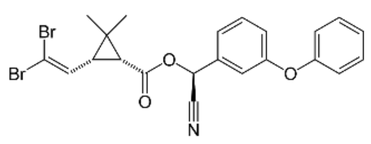
Pyrene
CAS 129-00-0
log K_{ow} = 4.88



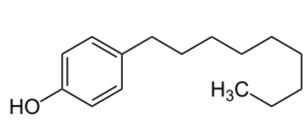
Methoxychlor
CAS 72-43-5
log K_{ow} = 5.08



Deltamethrin
CAS 52918-63-5
log K_{ow} = 6.2



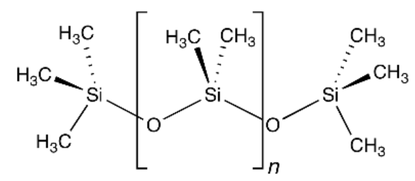
4-n-Nonylphenol
CAS 104-40-5
log K_{ow} = 5.76



+ 4-8 further compounds

Passive dosing

Polydimethylsiloxane
(PDMS)
CAS 63148-62-9



Biological material

Rainbow trout

→ S9 fraction
→ Hepatocytes (HEP)
→ (perfused liver, planned)

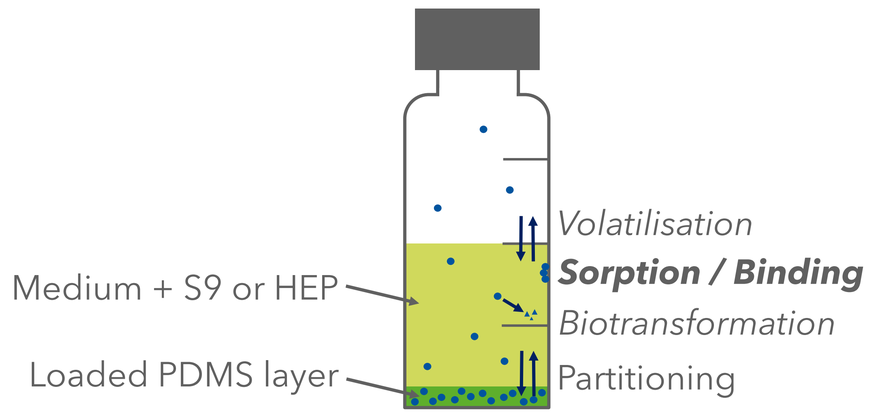
Methods

Biodegradation

- Passive dosing from PDMS layer at the bottom of the vial
- Based on OECD guidelines 319a (HEP) and 319b (S9)
- Test duration 4 h, 6 timepoints
- No pre-equilibrium
- Compared to spiked medium without passive dosing
- Heat-inactivated S9 used as negative control

Analytics

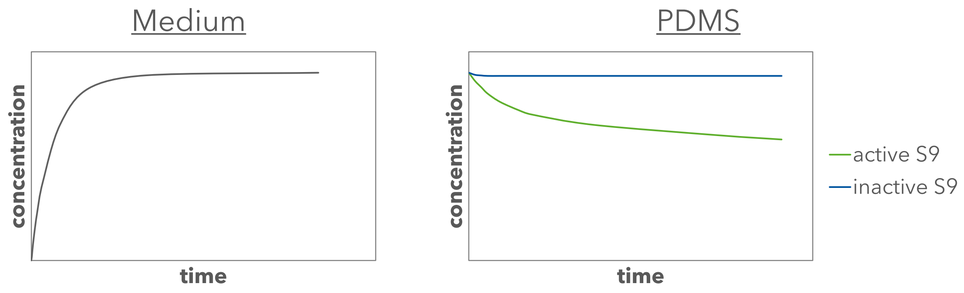
- Extraction of remaining compound from PDMS with methanol
- GC-MS to measure depletion of compound in PDMS layer



Test setup in HPLC vials and potential processes of loss of test compound (cursive).

Experimental plan

- First experiments with S9 and pyrene
- Partitioning from PDMS layer takes place so that a constant concentration in the medium is ensured
- Based on the depletion from PDMS the metabolic rate can be determined
- Schematic diagram of the compound concentration over time in the medium (see left figure, active and inactive S9 should show same results) and the PDMS (see right figure)



Further expected outcomes in future experiments

- Comparable *in vitro* metabolic rates in S9, HEP and perfused liver
- Equilibrium partitioning measurements to S9, HEP and blood plasma
- Testing existing polyparameter linear free energy relationships for predicting equilibrium partitioning of neutral organics to S9, HEP and blood plasma
- Investigations to determine which fractions (dissolved and/or matrix-bound) are available for biotransformation
- Accounting for slow desorption and binding effects during *in vitro-in vivo* extrapolation
- Identify key parameters leading to uncertainties in BCF prediction

Outlook

The determined metabolic rates can be used for *in vitro-in vivo* extrapolation to predict steady-state BCFs and to improve existing BCF prediction models. Perfused liver experiments will provide metabolic rates that can be used as an intermediate step in the extrapolation. A refinement of the models would result in more reliable BCF estimates and hence an improved predictability of the environmental risk of hydrophobic chemicals regarding bioaccumulation in fish.

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