In-vitro biotransformation of hydrophobic chemicals by fish liver enzyme fractions: a dosing approach using molecular carriers

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1. INTRODUCTION

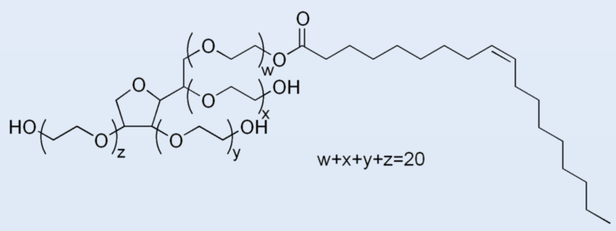
- Intrinsic metabolism rates are a crucial parameter for the evaluation of toxicity and bioaccumulation potential of a chemical.
- *In-vitro* assays that use liver enzyme fractions (S9) for biotransformation studies have been developed as an alternative to whole animal tests [1].
- Challenge: defined substrate supply of hydrophobic test chemicals *in-vitro*. Sorption and binding reduce substrate availability; low aqueous solubility necessitates co-solvent for dosing which may adversely impact metabolic activity

Aim of this study

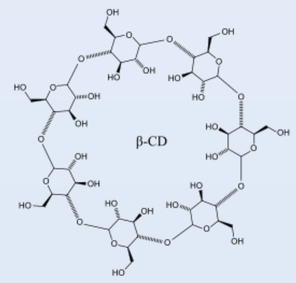
- **delivery of hydrophobic test substrate to fish liver S9 by molecular carriers**

2. MATERIAL & METHODS

Carriers



Polysorbate 80 (Tween 80) – nonionic surfactant: compatible with S9 in the test: 0.1 g/L



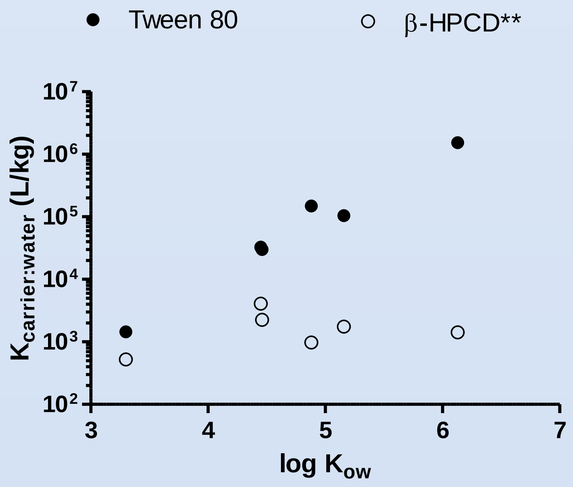
β-Hydroxypropyl-cyclodextrin (β-HPCD): in the test 1 g/L

Carrier loading with PAHs

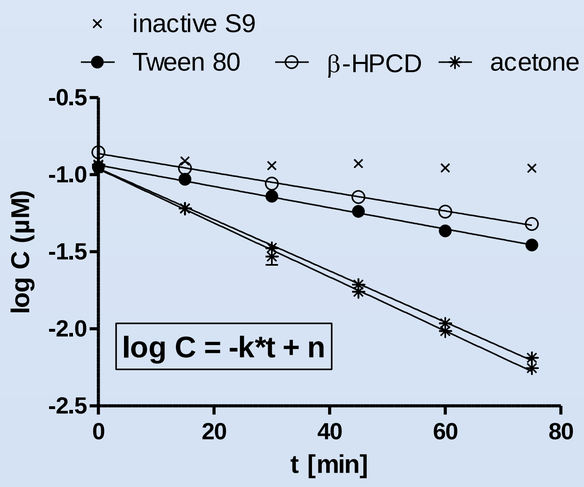
- preparation of micro-crystals by solvent-evaporation and re-dissolution in concentrated aqueous carrier solution

3. RESULTS

(1) Capacity of carriers for PAHs



(2) Biotransformation rates (k): e.g. anthracene



(3) Biotransformation rates as function K_{ow}

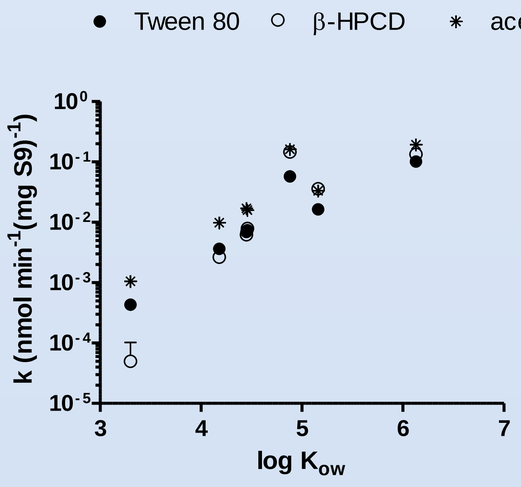


Figure 1:

- **partition ratios ($K_{carrier:water}$) reveal that capacity of Tween 80 > β-HPCD for PAHs**
- **PAHs likely partition into Tween 80**
- **inclusion complex formation of PAHs with β-HPCD not only controlled by hydrophobicity of the PAHs**

**HPCD-data taken from ref [2]. $K_{HPCD:water}$ for BaP estimated from HPCD loading recovery.

Figure 2:

- **biotransformation rates (k) are smaller when substrate is supplied with carrier (exception: fluoranthene with β-HPCD)**
- **S9 enzymes operate against the freely dissolved concentration, which is likely decreased by carriers**

→ free fractions of PAHs are currently being determined

Figure 3:

- **biotransformation rates correlate with K_{ow} (!)**
- **same trend when substrate is supplied by molecular carriers**

→ lipophilicity-selective enzyme activity may be a generalizable feature of PAH metabolism by fish

4. CONCLUSIONS

- Tween 80 and β-HPCD can be used as delivery vehicles for hydrophobic chemicals
- binding capacity of carriers is crucial for dimensioning and controlling substrate availability
- further comparison, biotransformation may need to be normalized to freely dissolved concentrations
- biotransformation of more hydrophobic PAHs is faster

5. PERSPECTIVE

- determine binding/partitioning behavior of PAHs toward carriers in the presence of S9
- investigate other carrier molecules, e.g. BSA
- molecular carriers are promising dosing vehicles also for other *in-vitro* assays

References

1. Nichols, J.W. et al. (2009) Bioaccumulation assessment using predictive approaches. Integ. Env. Assess. Manag. 3, 3-17.
2. Gouliarmou , V. et al. (2012) Measuring binding and speciation of hydrophobic organic chemicals at controlled freely dissolved concentrations and without phase separation. Anal. Chem. 84, 1601-1608.

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