Benchmarking and passive dosing/sampling in fish BCF experiments

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The BETTER-BCF project

- measure fish BCF in the lab
- reduce the cost and the number of animals
- use OECD 305 as the point of reference
- financed by Cefic LRI

Innovative Techniques

- *Passive dosing* to maintain constant concentrations in the dissolved phase
- *Passive sampling in fish* to allow kinetics to be followed in an individual fish rather than a succession of individuals
- *Internal benchmarking* chemicals to improve method precision

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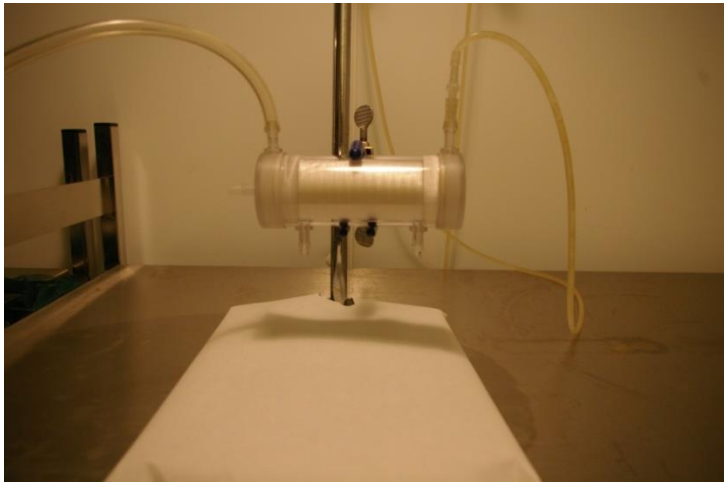
Study Chemicals

	Abbreviation	log K_{OW}	BCF (literature) (L kg^{-1})
2,3,4-trichloroanisole	ANI	3.95	920
p-diisopropylbenzene	ISBE	4.9	3200
musk xylene	MUSK	4.9	6810
chlorpyrifos	CHPY	4.77	1750
4-n-nonylphenol	NP	6.19	1200
2,4,6-tri-*tert*-butylphenol	BUFE	6.55	20000
pentachlorobenzene	PENT	4.6	5700
2,5-dichlorobiphenyl	PCB		
hexachlorobenzene	HCB	5.7	27000
p,p'-DDT	DDT	6.9	25000

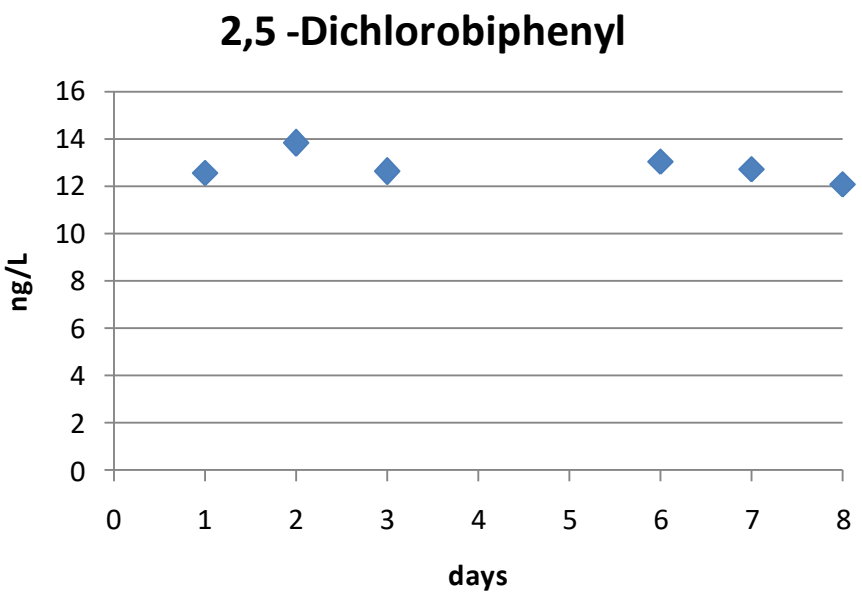
Passive dosing system

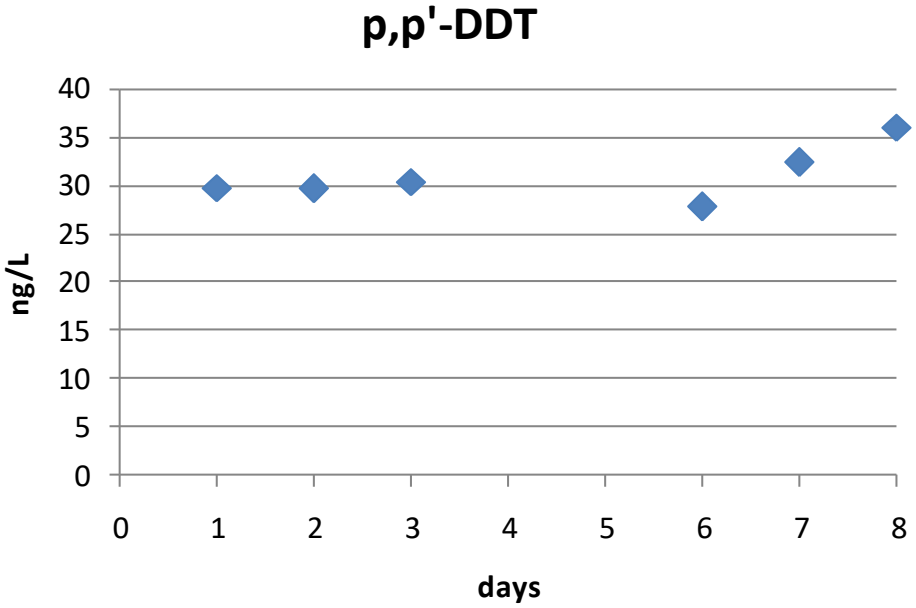
(developed with Philipp Mayer, DMU)



- Two manifolds connected by 16000 silicon tubes
- Loaded with a methanol solution of the chemicals
- Chemicals partition into silicon
- Methanol replaced by water
- Chemicals partition out of silicon into water

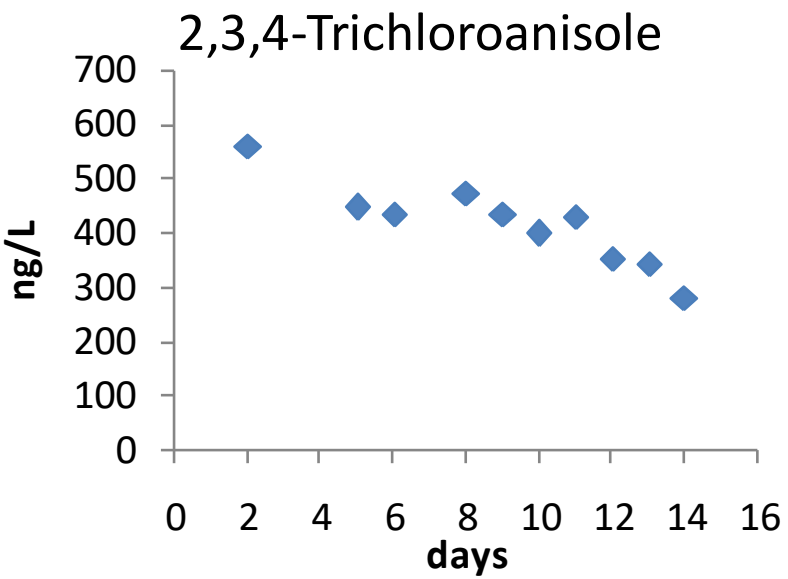
Passive Dosing of Water

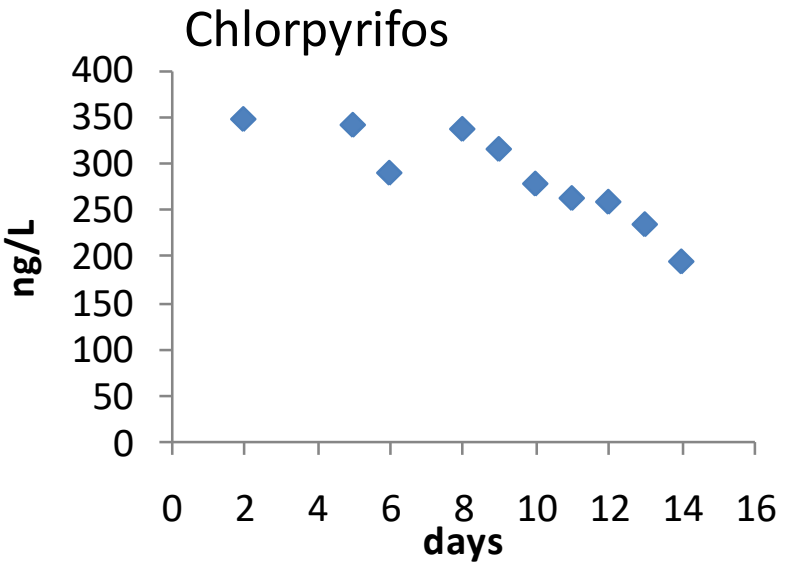




- constant exposure concentrations of hydrophobic chemicals

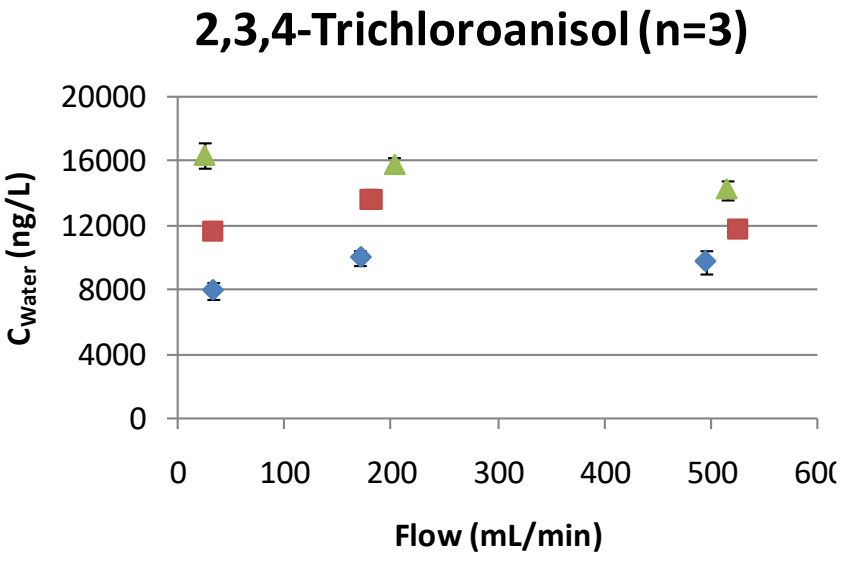
Passive Dosing of Water

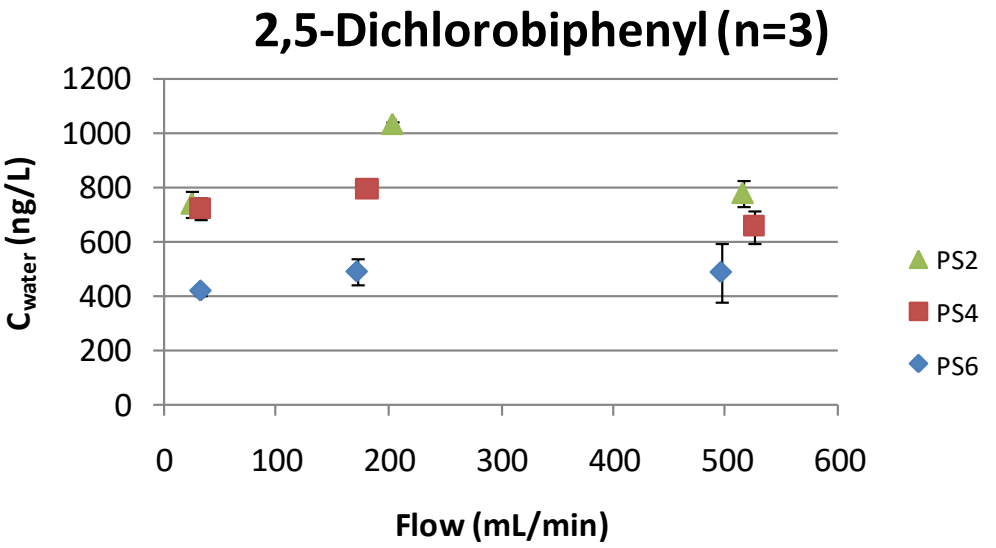




- Depletion of hydrophilic chemicals over long exposures

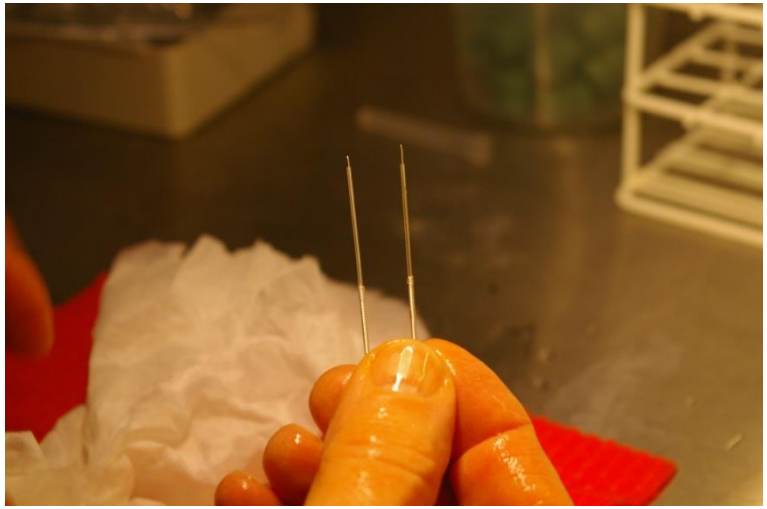
Reproducibility and Influence of Water Flow Rate





- C_{water} independent of water flow rate
- Between unit reproducibility ok

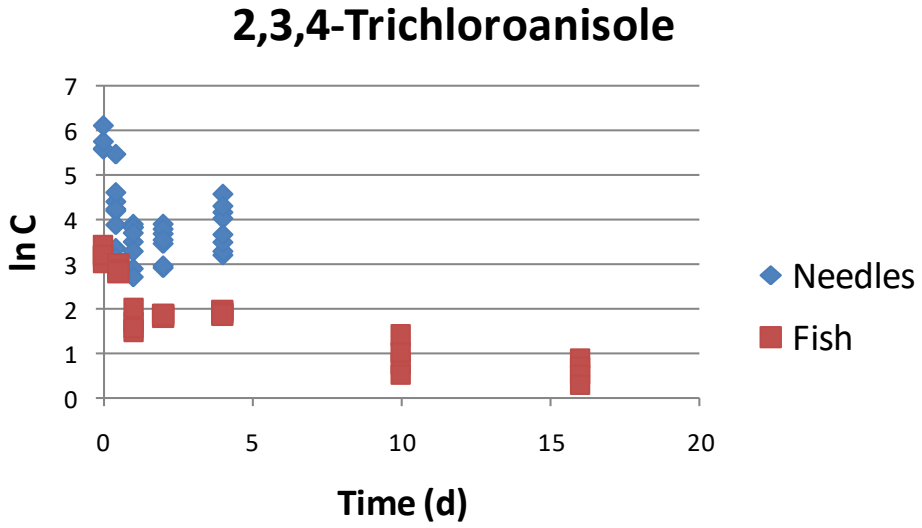
Passive Sampling in Fish

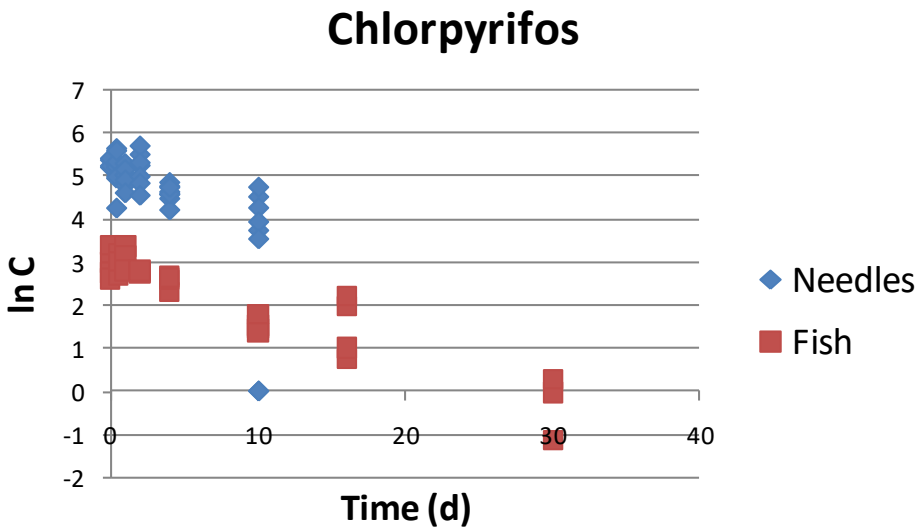


Silicon tubing on needle

Passive sampling in rainbow trout

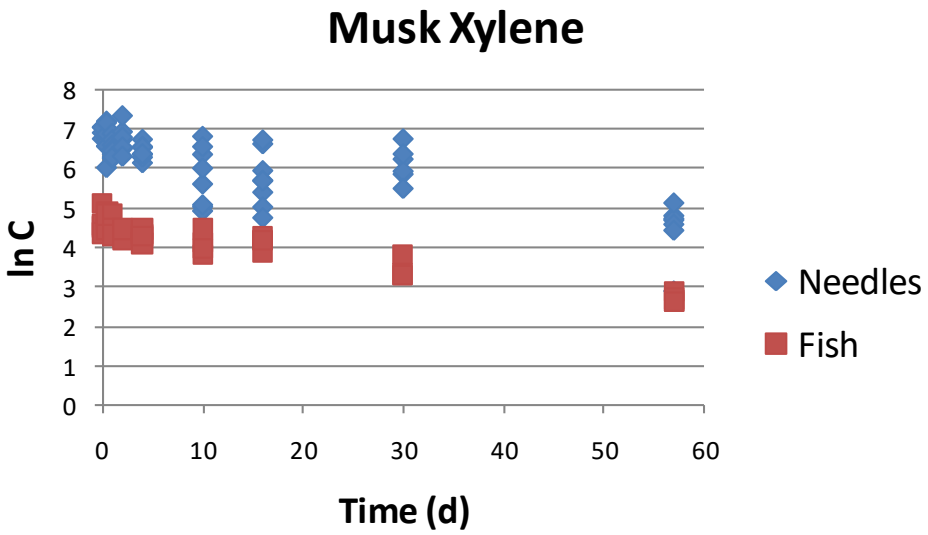
Comparison of passive dosing and analysis of whole fish homogenate during a depuration experiment

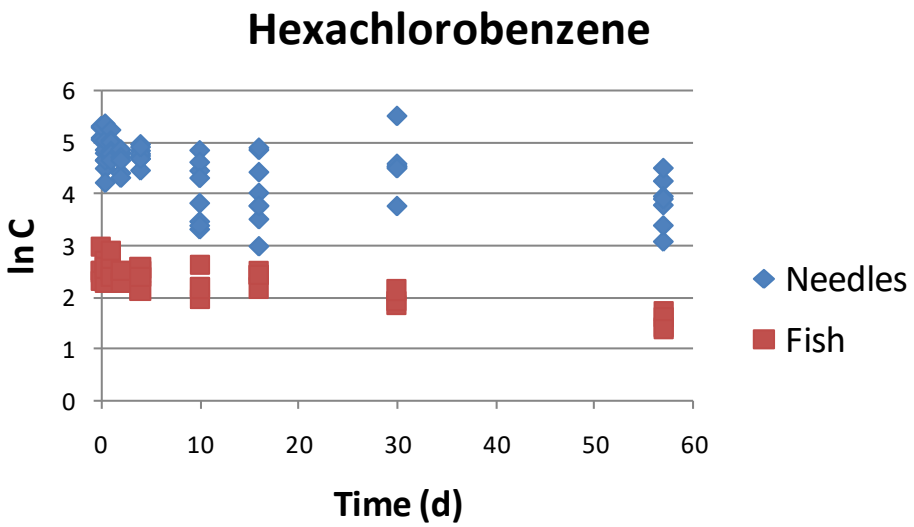




- in-tissue samplers and whole body homogenates track each other
- in-tissue sampling less sensitive

Comparison of passive dosing and analysis of whole fish homogenate during a depuration experiment





- in-tissue samplers show greater variability

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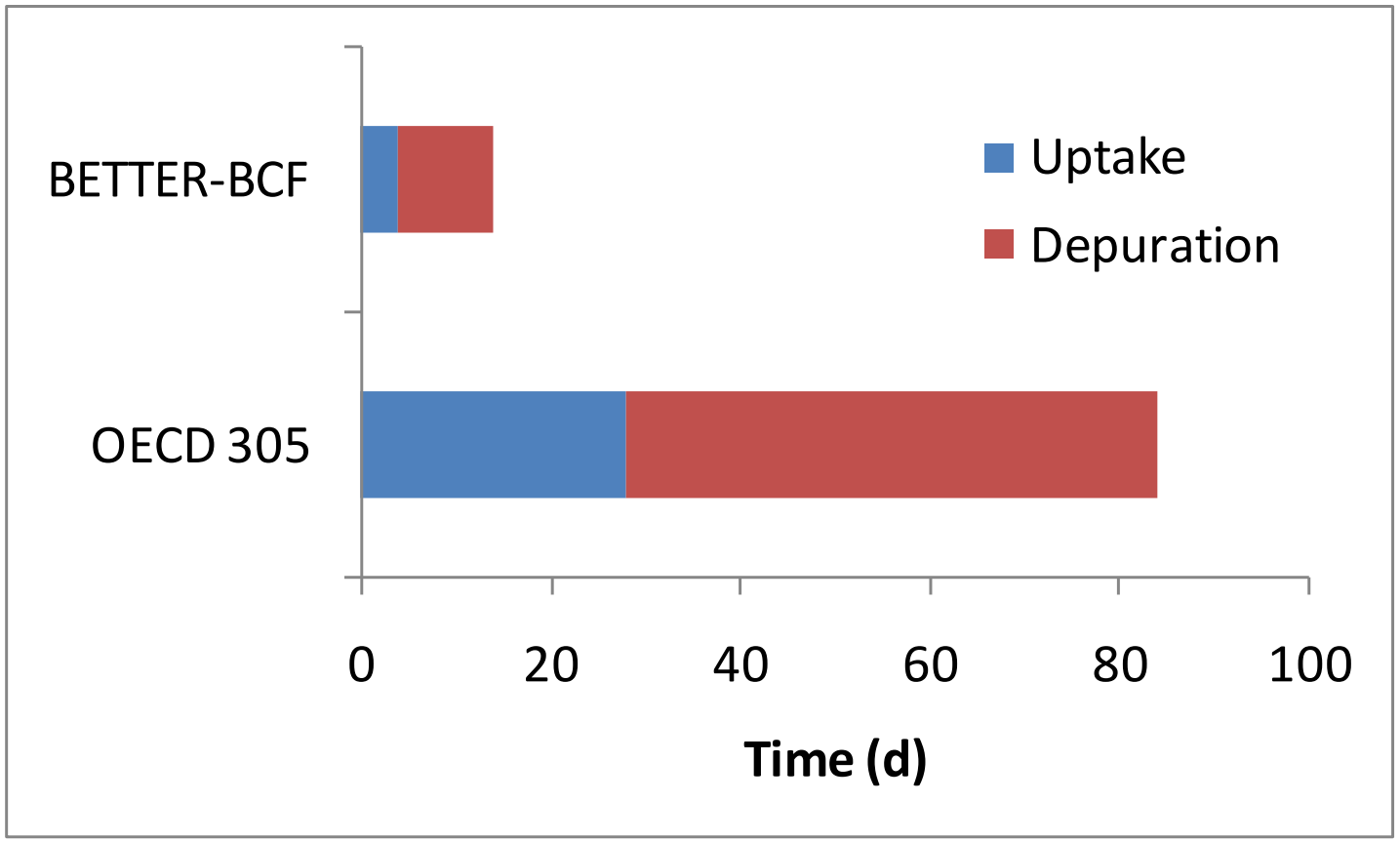
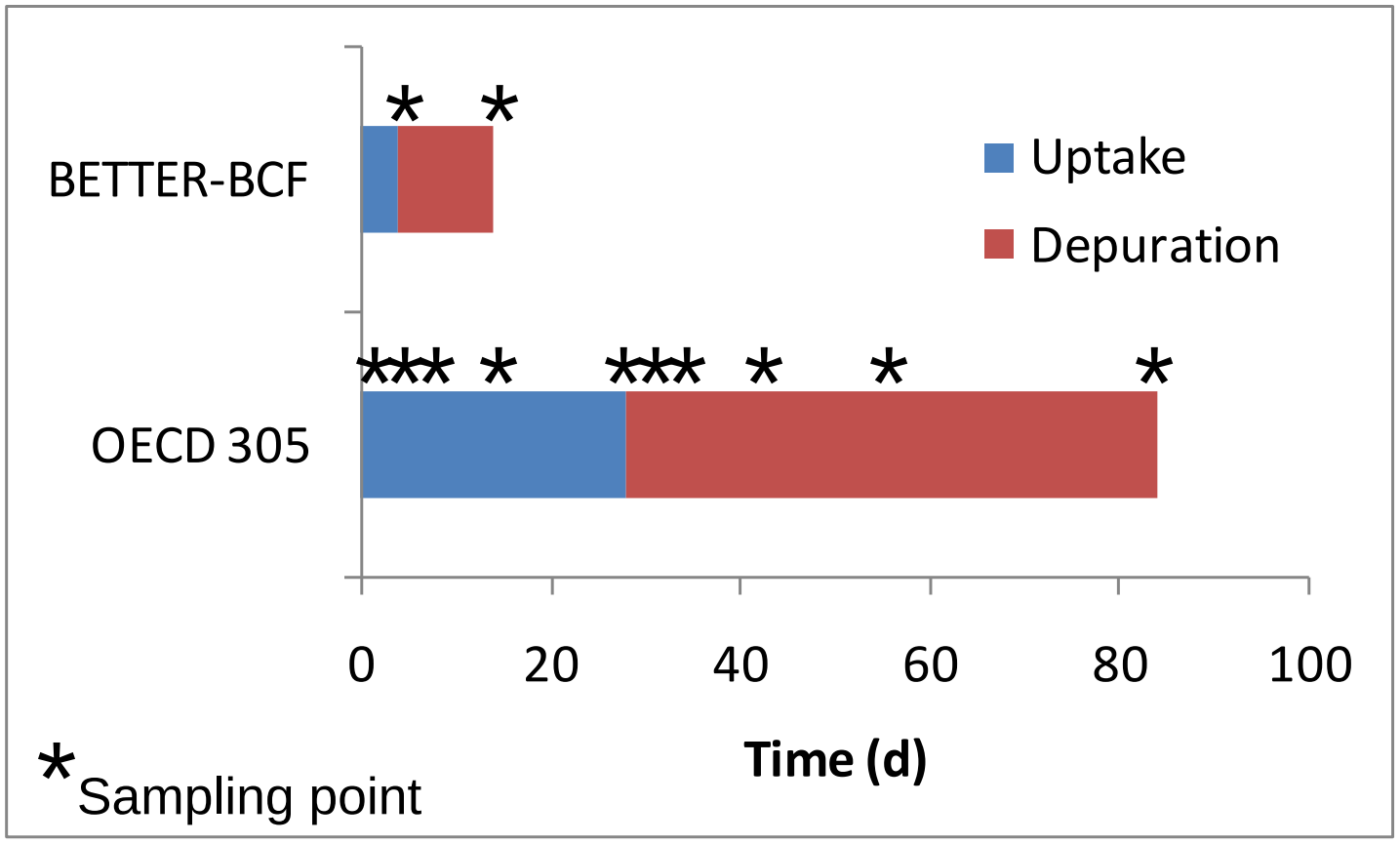
The BETTER-BCF Protocol and Benchmarking

- B-BCF, like OECD 305, based on 1 compartment first order BCF model (k_1/k_T model)

Test Design – Time Savings



Test Design – Fish Savings



Benchmarking: The key to simplification

- Measure relative bioconcentration, not absolute!
- Introduces internal standards for whole procedure

For test chemical x and benchmark chemical bm

i. Normalize C(x) to C(bm) in fish and water

ii. Depuration phase: calculate normalized k_T from normalized C_{fish} and k_T(bm)

iii. Uptake phase: calculate normalized k_1 from normalized C_{fish} and C_{water} plus Δt, correct with k_2

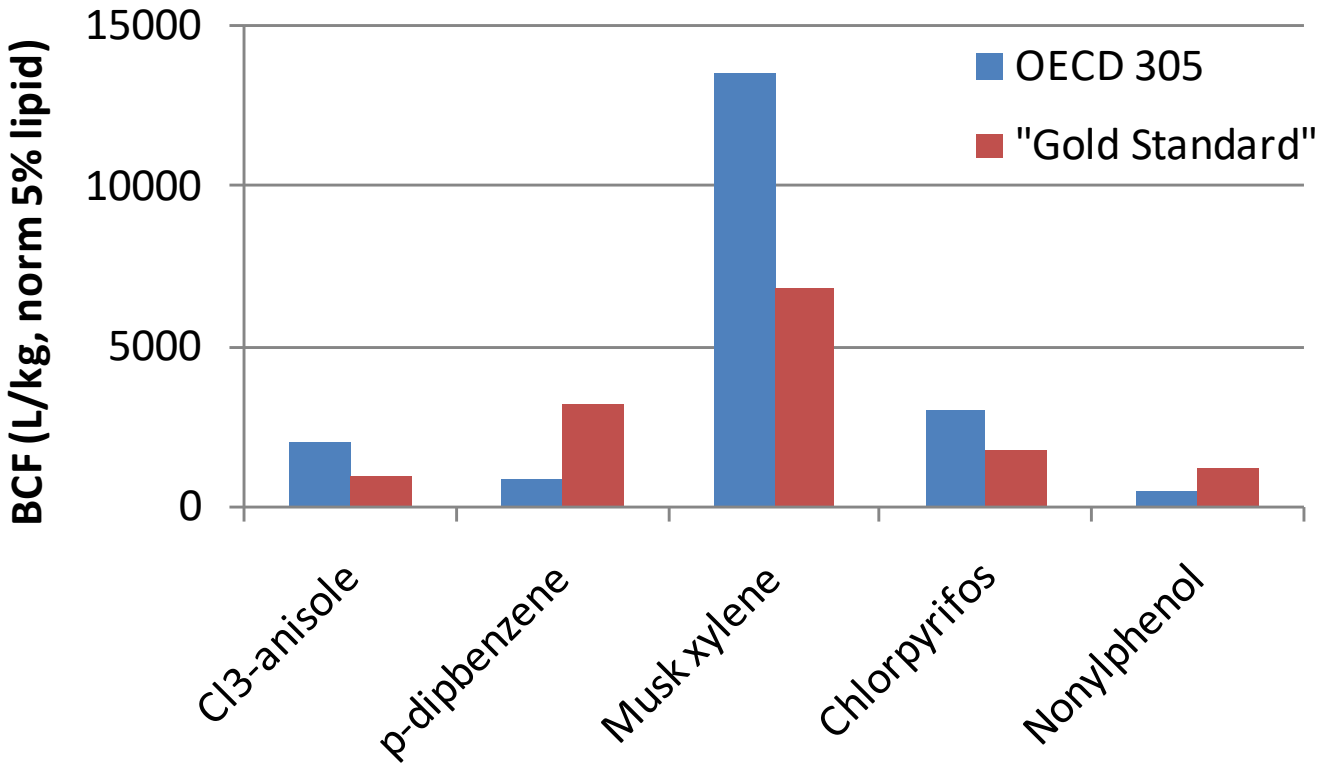
iv. $BCF_{norm} = k_{1\text{-norm}} / k_{T\text{-norm}}$

v. $BCF(x) = BCF_{norm} \times BCF(bm)$

Experiments

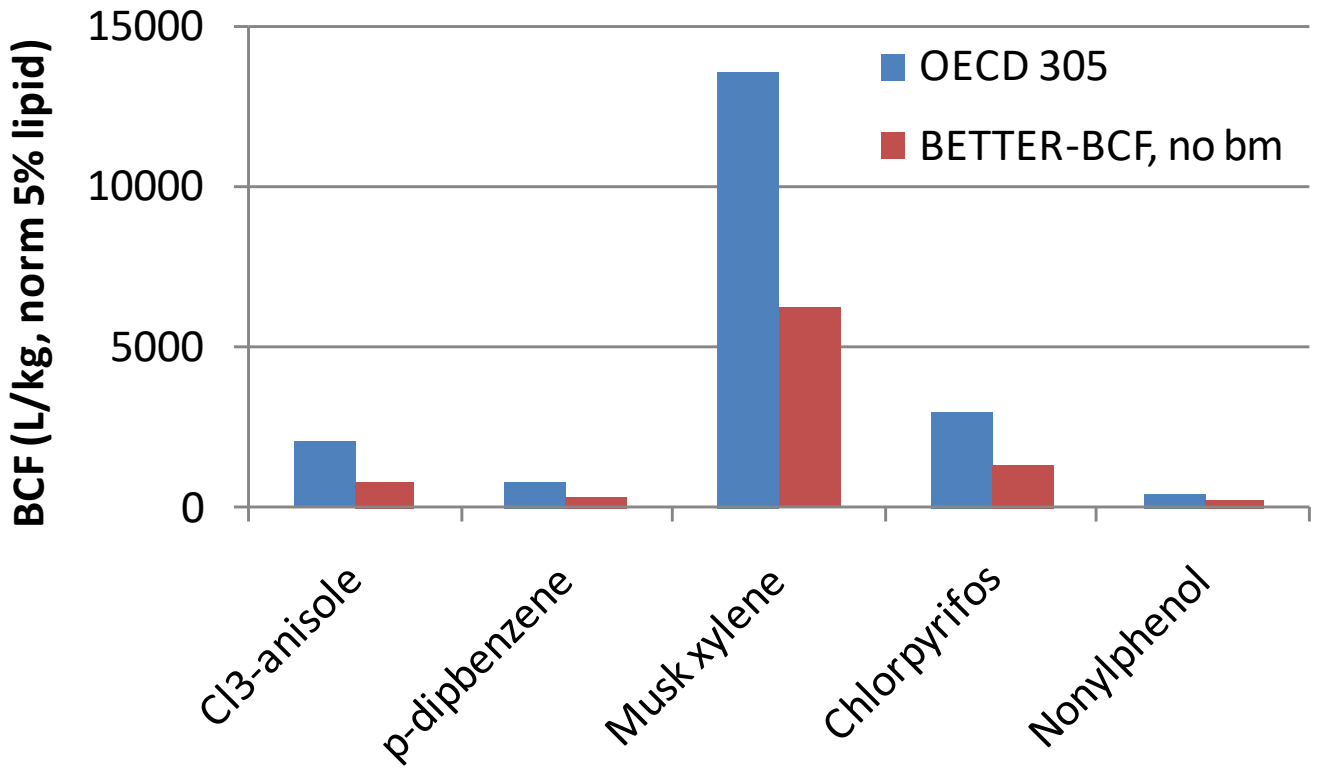
- Running OECD 305, exposing the fish simultaneously to the 10 chemicals
- Running the BETTER-BCF method, exposing the fish simultaneously to the 10 chemicals
- Comparing the results

OECD 305 versus “Gold Standard”



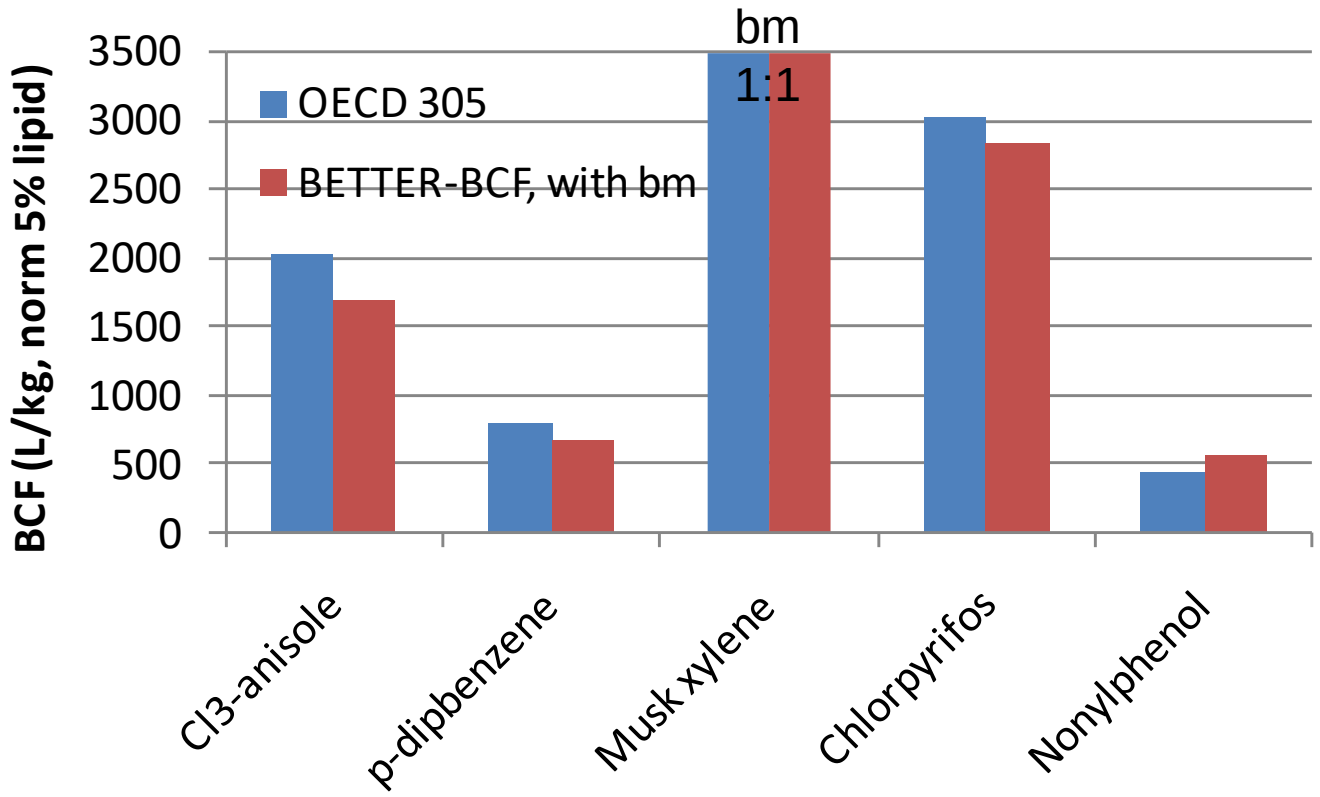
- precision of OECD 305 poor

OECD 305 versus BETTER-BCF without benchmarking



- BETTER-BCF performs poorly

OECD 305 versus BETTER-BCF with benchmarking



- benchmarking gives BETTER-BCF excellent agreement with OECD

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Conclusion

A better BCF test is possible!

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Thanks to benchmarking which largely eliminates sources variability in BCF determination arising from:

- Exposure conditions (e.g. temperature)
- Fish characteristics (e.g. ventilation)
- Sampling & analytical error (e.g. lipid determination)

Acknowledgement

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