Bioaccumulation Assessment: Evolution or Revolution?

(summary of the ECO 14 project)

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Aims of the project

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

Innovative Techniques

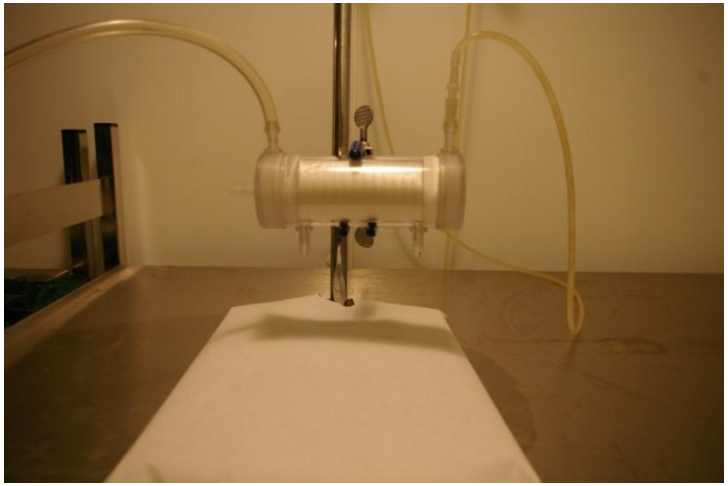
- *Passive dosing* to maintain constant concentrations in the dissolved phase
- *Passive sampling in fish* to allow kinetics to be followed in an individual fish rather than a succession of individuals
- *Internal benchmarking* chemicals to improve method precision and information content

Study Chemicals

	Abbreviation	log K_{OW}	BCF (literature) (L kg^{-1})
2,3,4-trichloroanisole	ANI	4.1	470
p-diisopropylbenzene	ISBE	4.9	2600
musk xylene	MUSK	4.4	10000
chlorpyrifos	CHPY	5.27	1750
4-n-nonylphenol	NP	6.1	900
2,4,6-tri-*tert*-butylphenol	BUFE	5.3	20000
pentachlorobenzene	PENT	5.18	16200
2,5-dichlorobiphenyl	PCB	5.22	
hexachlorobenzene	HCB	5.73	27000
p,p'-DDT	DDT	6.9	30600

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

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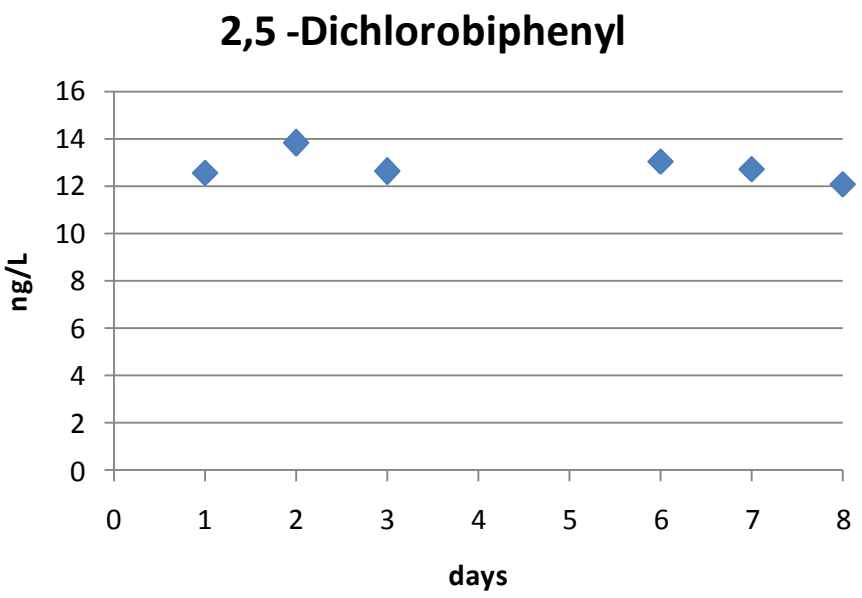
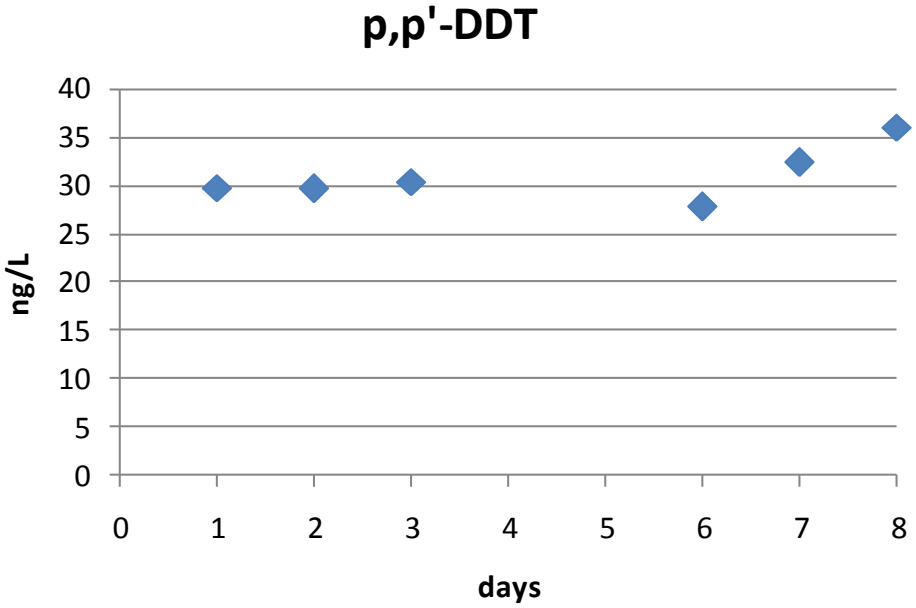
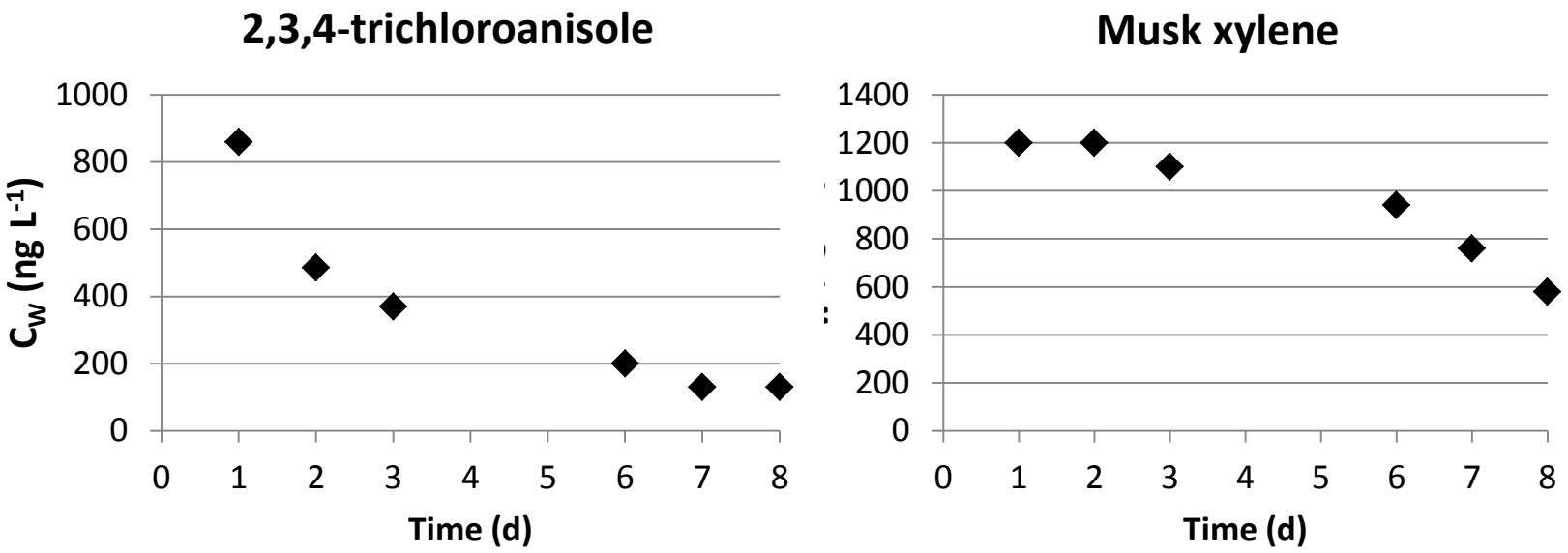
Passive Dosing of Water





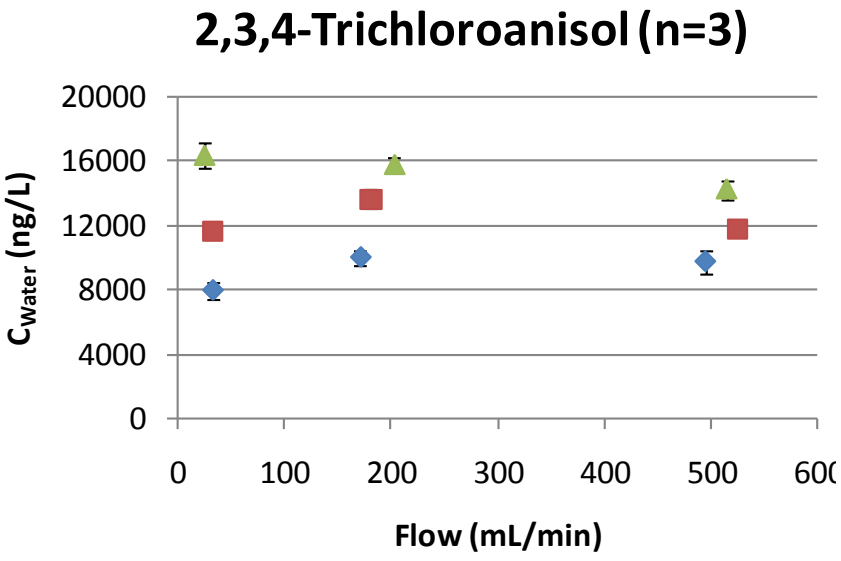
- constant exposure concentrations of hydrophobic chemicals

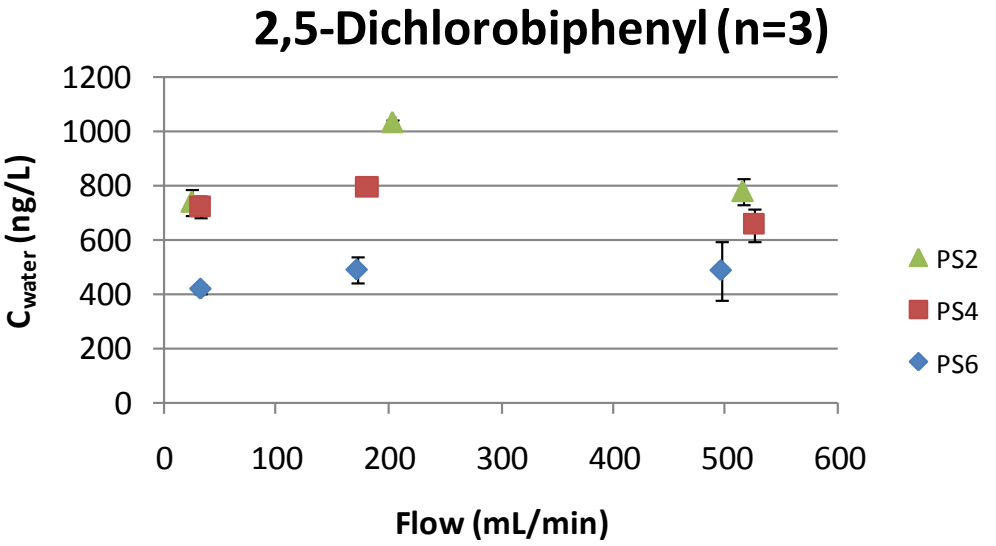
Passive Dosing of Water



- 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

Adolfsson-Erici et al., Chemosphere 86, 593-599

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Evolution or Revolution?

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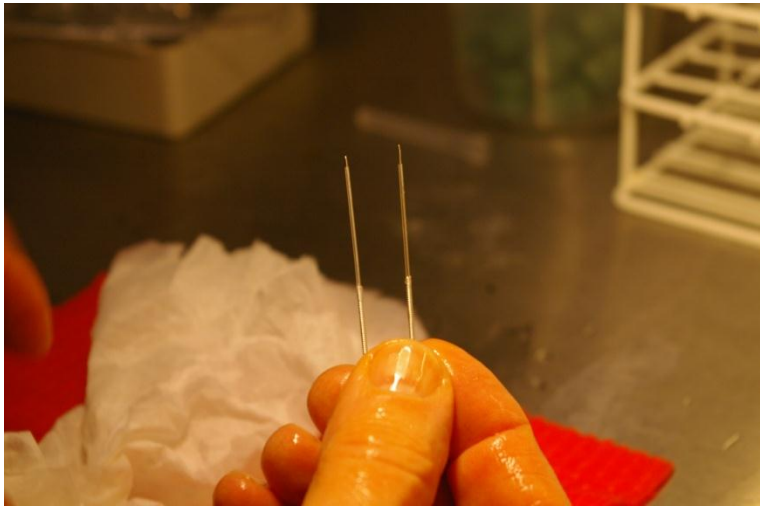
Evolution, not Revolution

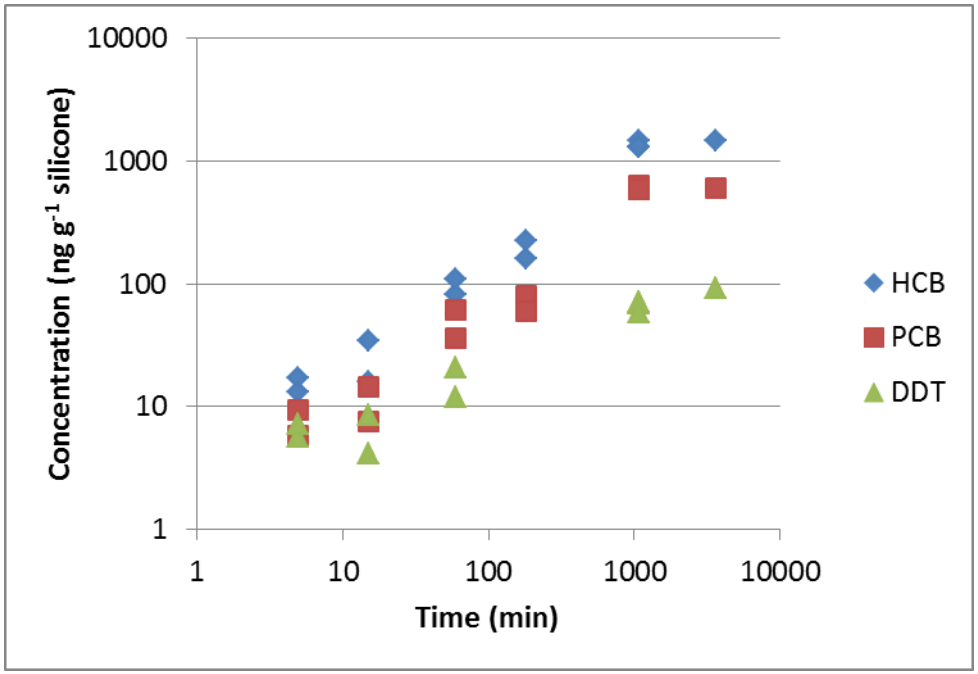
Passive Sampling in Fish



Silicon tubing on needle

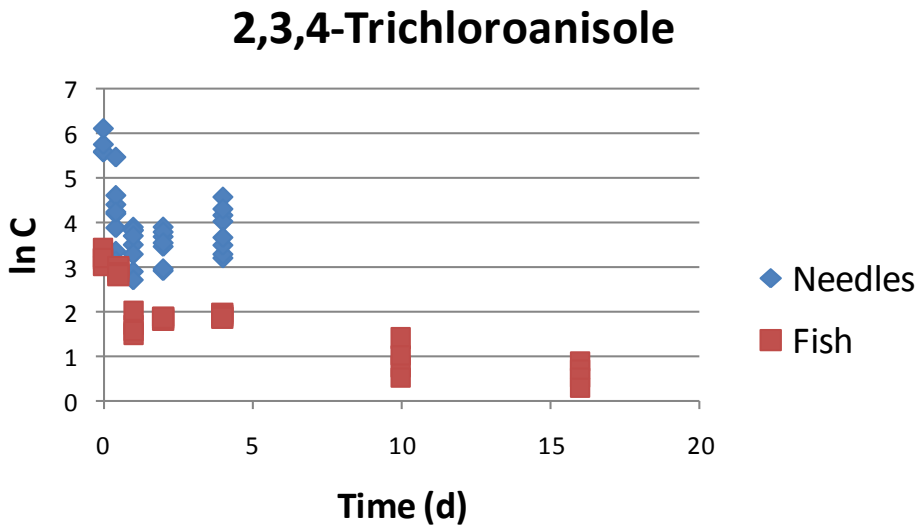
Passive sampling in rainbow trout

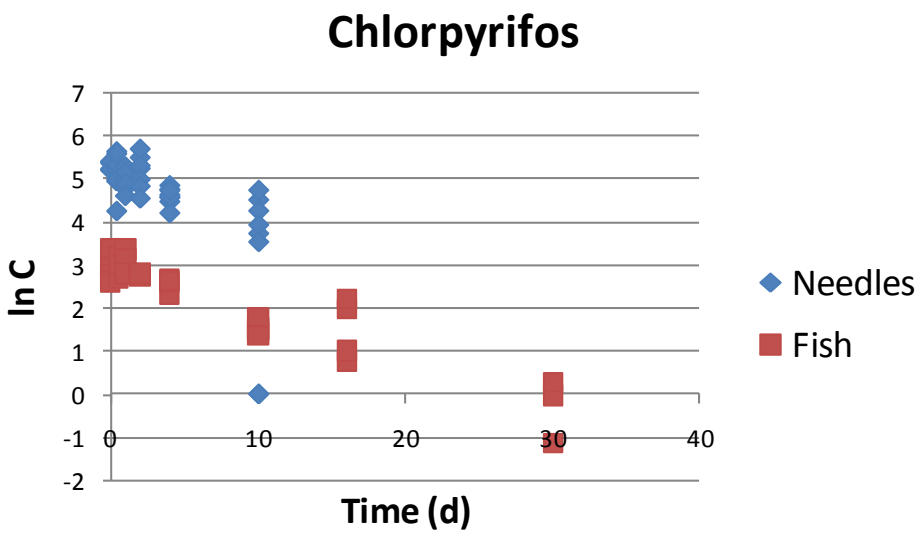
Chemical accumulation in sampler vs. time of exposure



- accumulation sampler, not equilibrium sampler
- sampling time fixed at 2 h

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

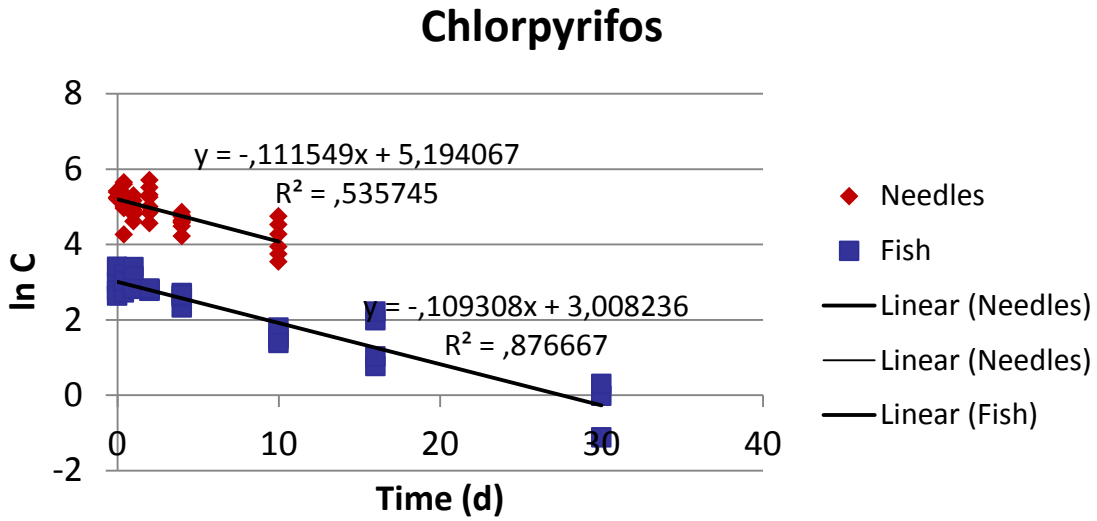




- in-tissue samplers and whole body homogenates track each other
- in-tissue sampling less sensitive

Adolfsson-Erici et al., Chemosphere 88, 62-68

Elimination kinetics measured with passive dosing vs. analysis of whole fish homogenate



- good agreement between the rate constants (slopes)
- passive dosing less sensitive

Adolfsson-Erici et al., Chemosphere 88, 62-68

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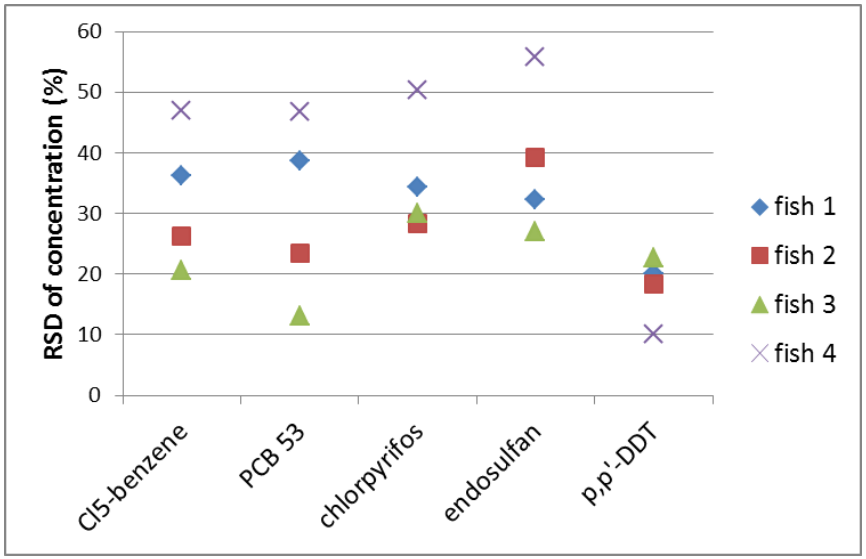
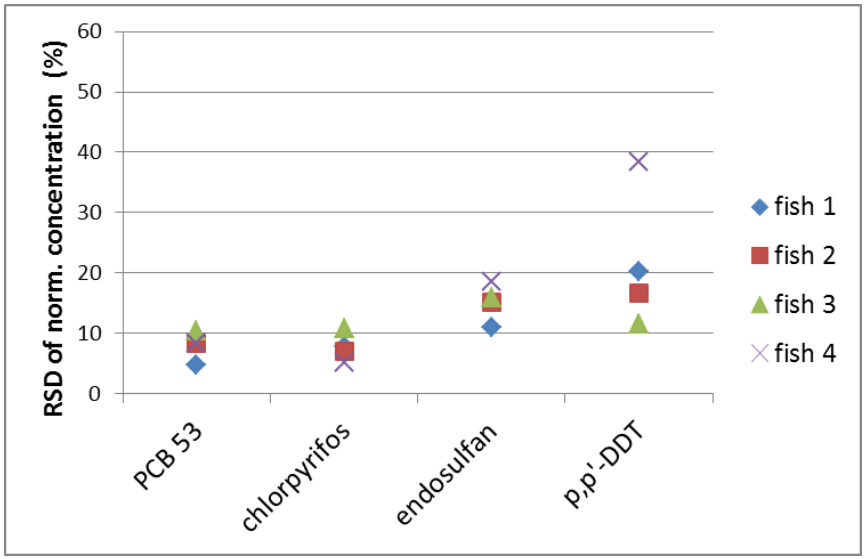
Evolution, towards Revolution?

Influence of benchmarking on variability of sampler results

Raw data



Normalized to Cl5-benzene

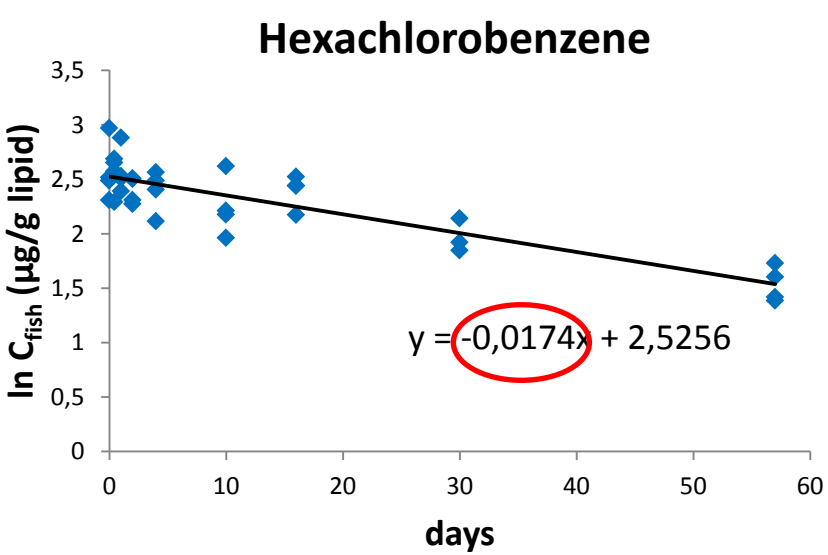


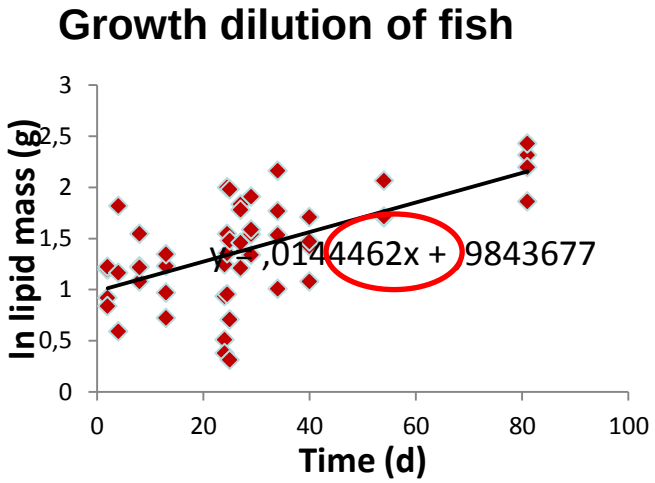
- benchmarking can reduce variability a lot!

Benchmarking Elimination

- OECD 305 based on 1 compartment first order BCF model (k_1/k_T model)
- Overall rate constant for elimination k_T is typically the major determinant of BCF

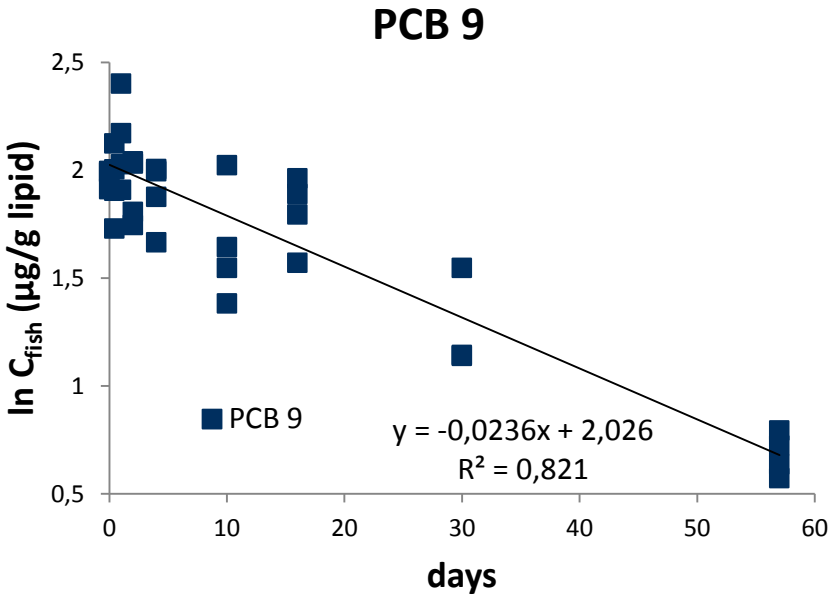
Elimination of HCB





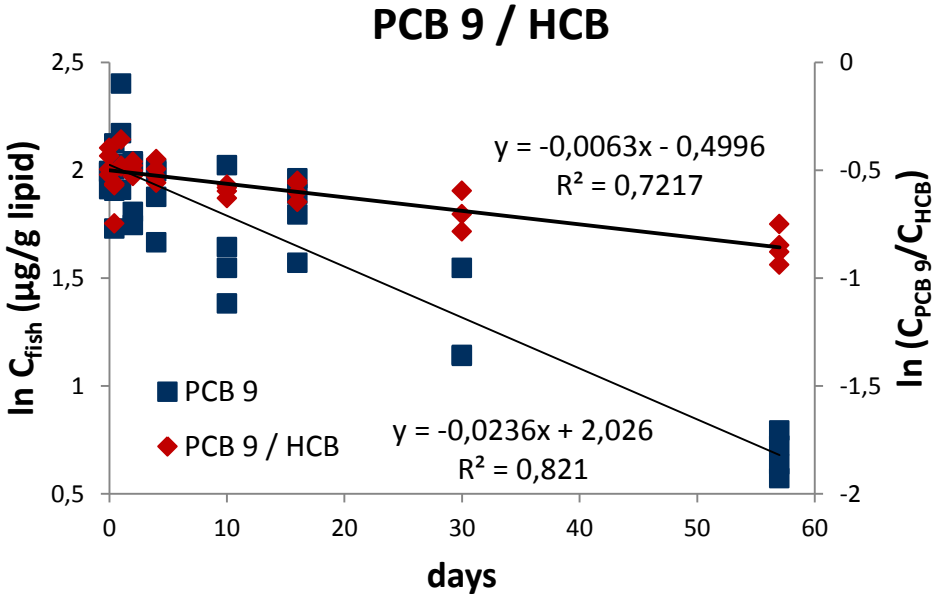
- elimination rate constant and growth rate constant similar
- BCF determined primarily by growth
- need to know BCF without growth for extrapolation to field!

Benchmark elimination against a persistent chemical (HCB)



- elimination slow (rate constant 0.0174 d^{-1} vs. 0.0144 for growth)

Benchmark elimination against a persistent chemical (HCB)



- benchmarking gives growth corrected rate constant for elimination
- data more consistent as major sources of variability eliminated

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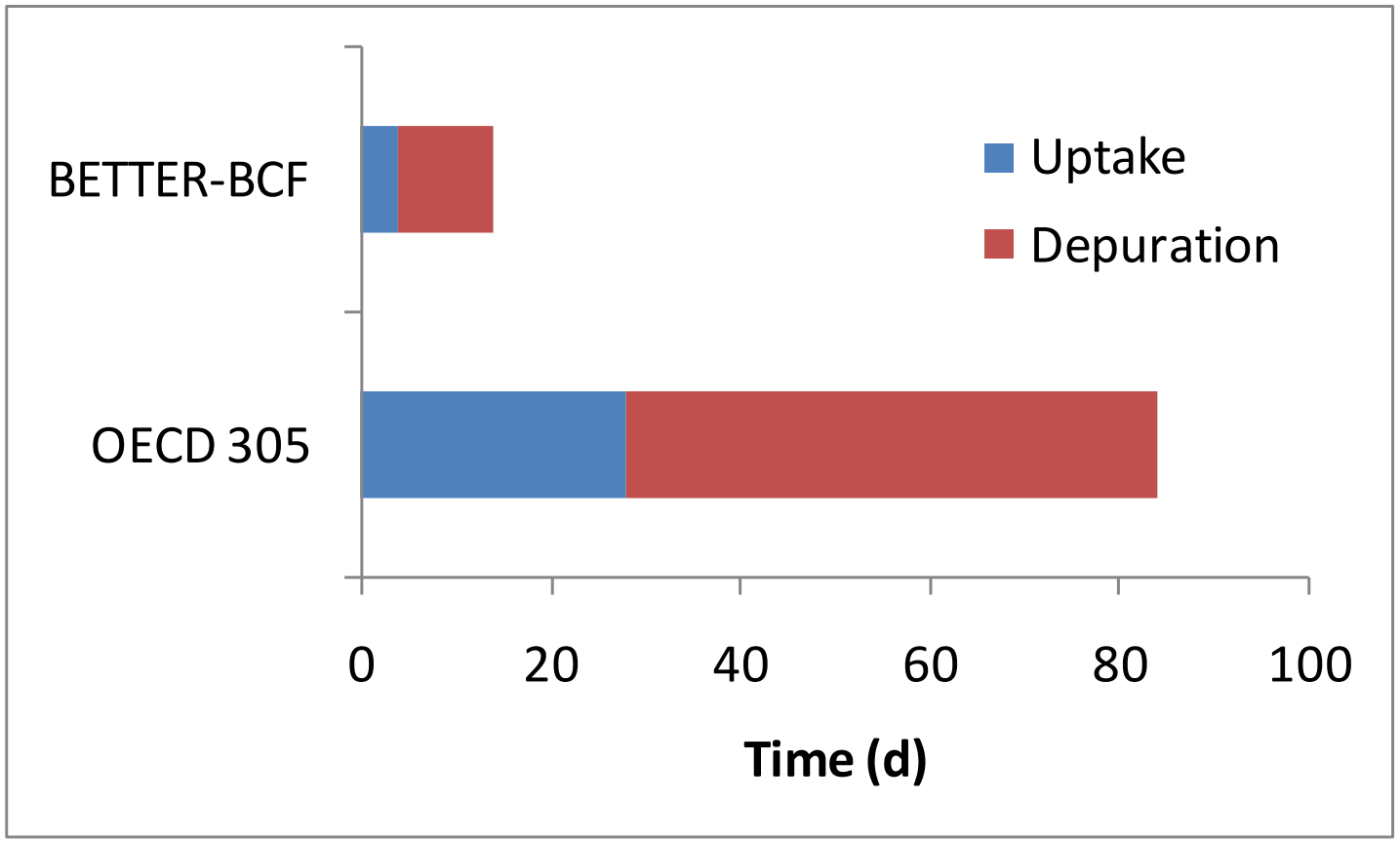
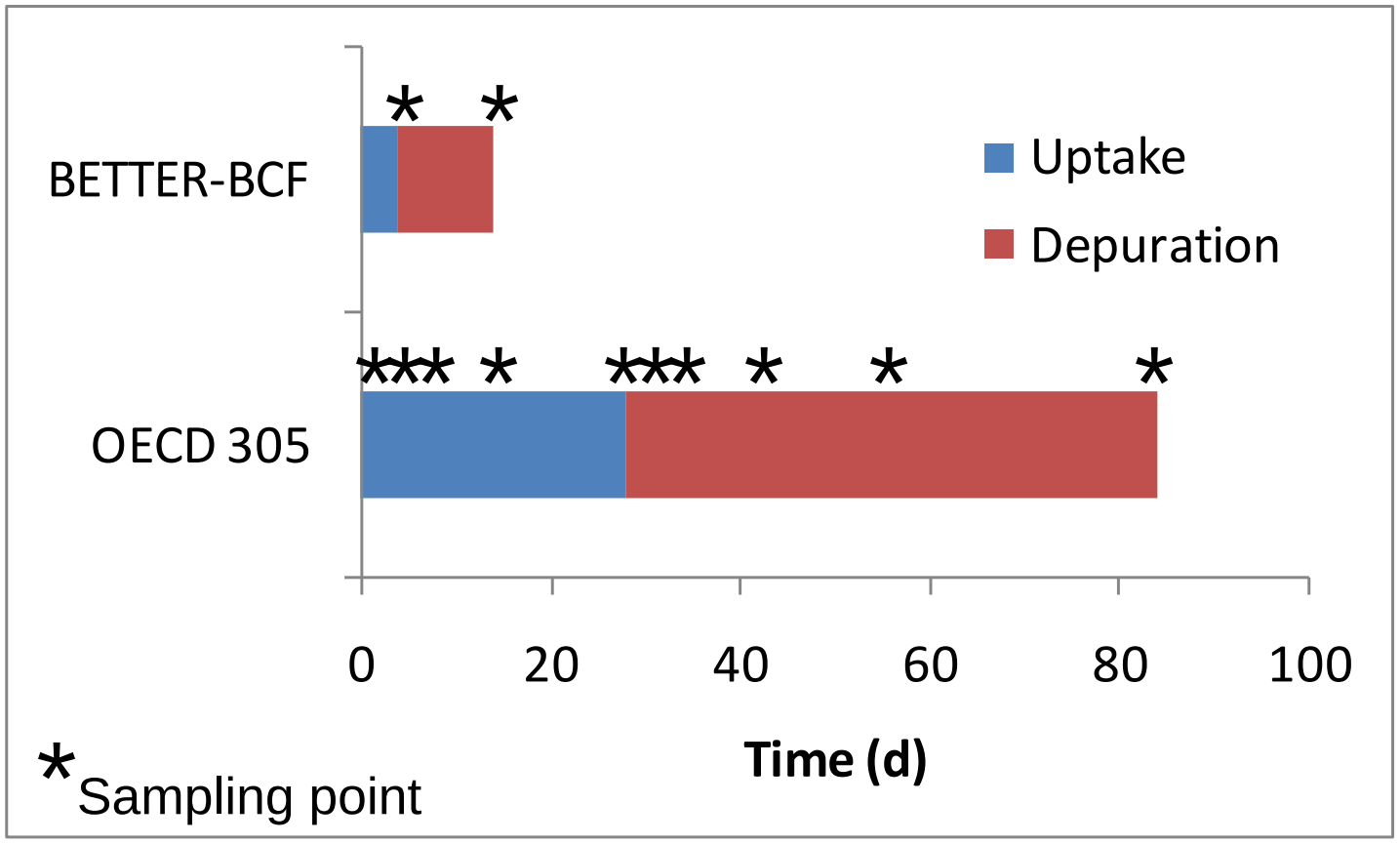
The BETTER-BCF Protocol and Benchmarking

- 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!
- Benchmark is an internal standard for whole procedure

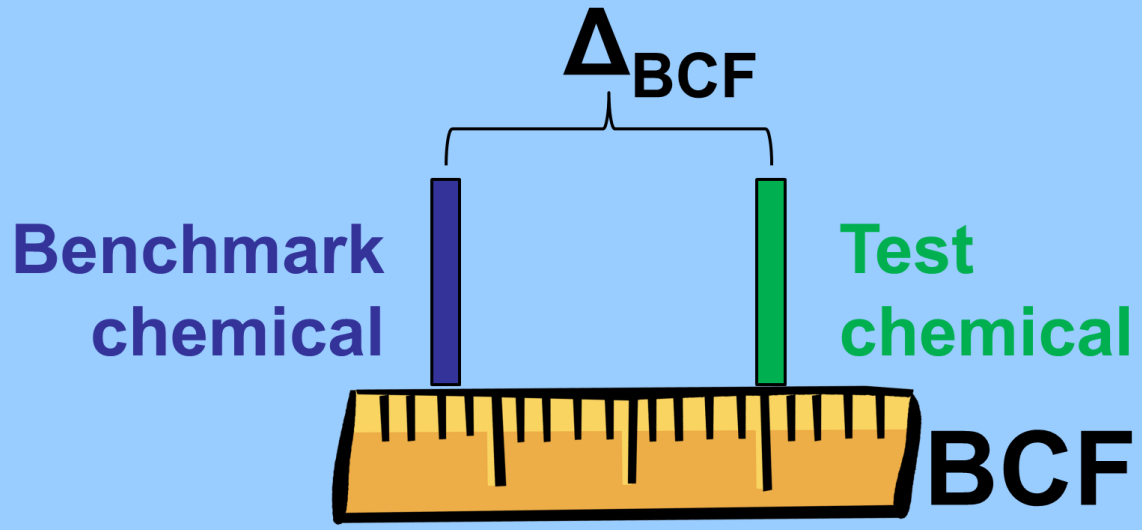
For test chemical x and benchmark chemical bm:

i. Calculate $BCF(x,exp) = k_1(x) / k_T(x)$

ii. Calculate $BCF(bm,exp) = k_1(bm) / k_T(bm)$

iii. $$BCF(x) = \frac{BCF(x, exp)}{BCF(bm, exp)} \times BCF(bm, ref)$$

Benchmarking: The key to simplification



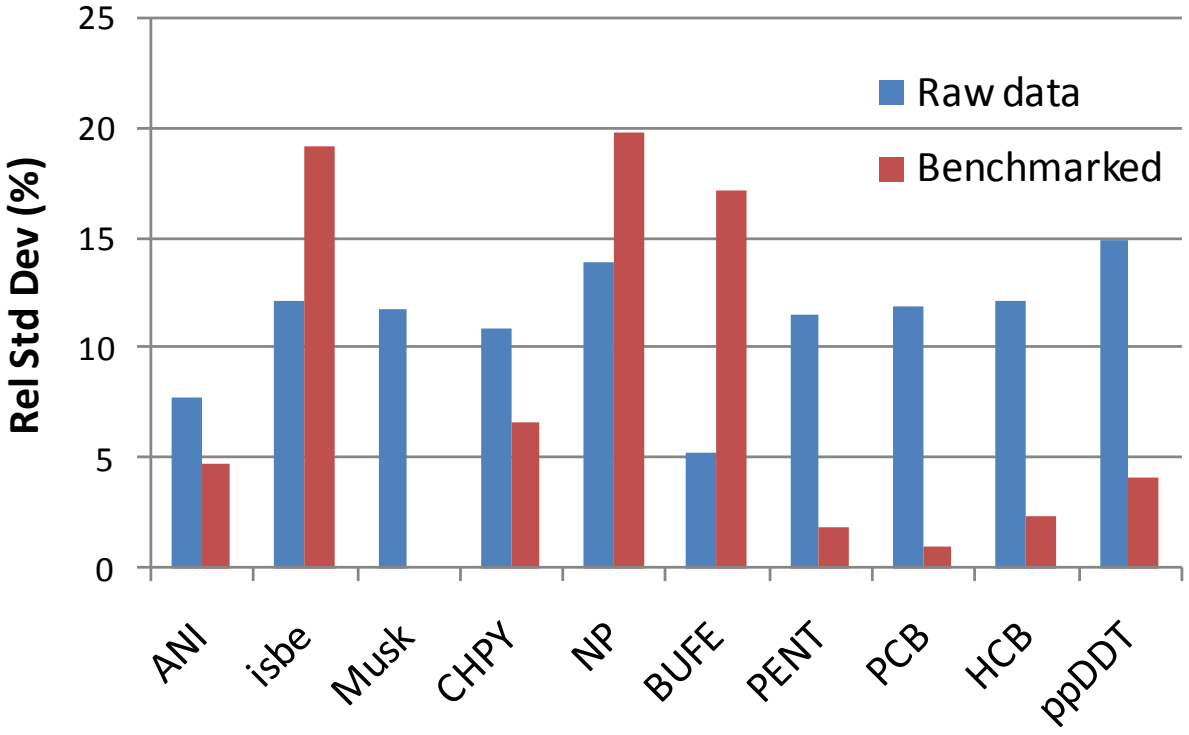
Benchmarking:

Measure the difference in BCF, not the absolute value

Experiments

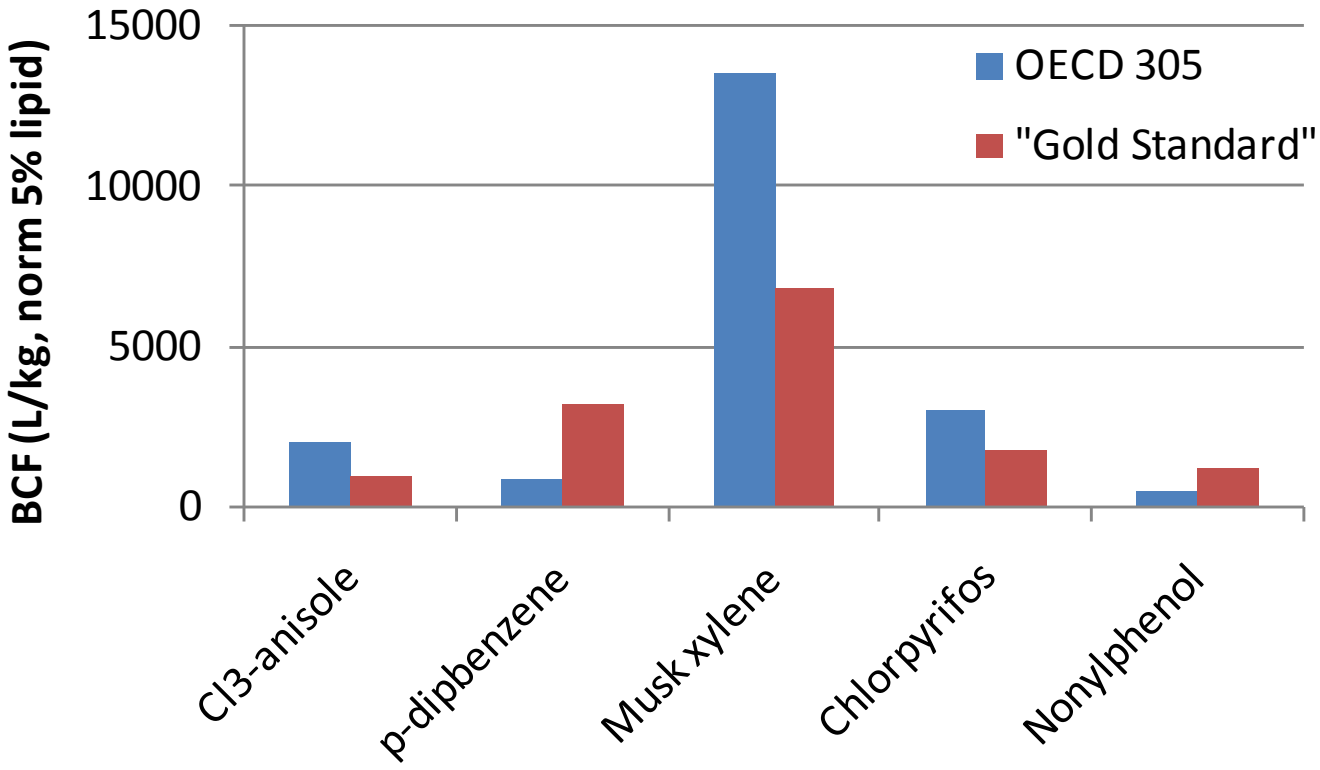
- Running OECD 305, exposing the fish simultaneously to 10 chemicals
- Running the BETTER-BCF method, exposing the fish simultaneously to 10 chemicals
- Comparing the results

Concentration data: Raw versus benchmarked to MUSK



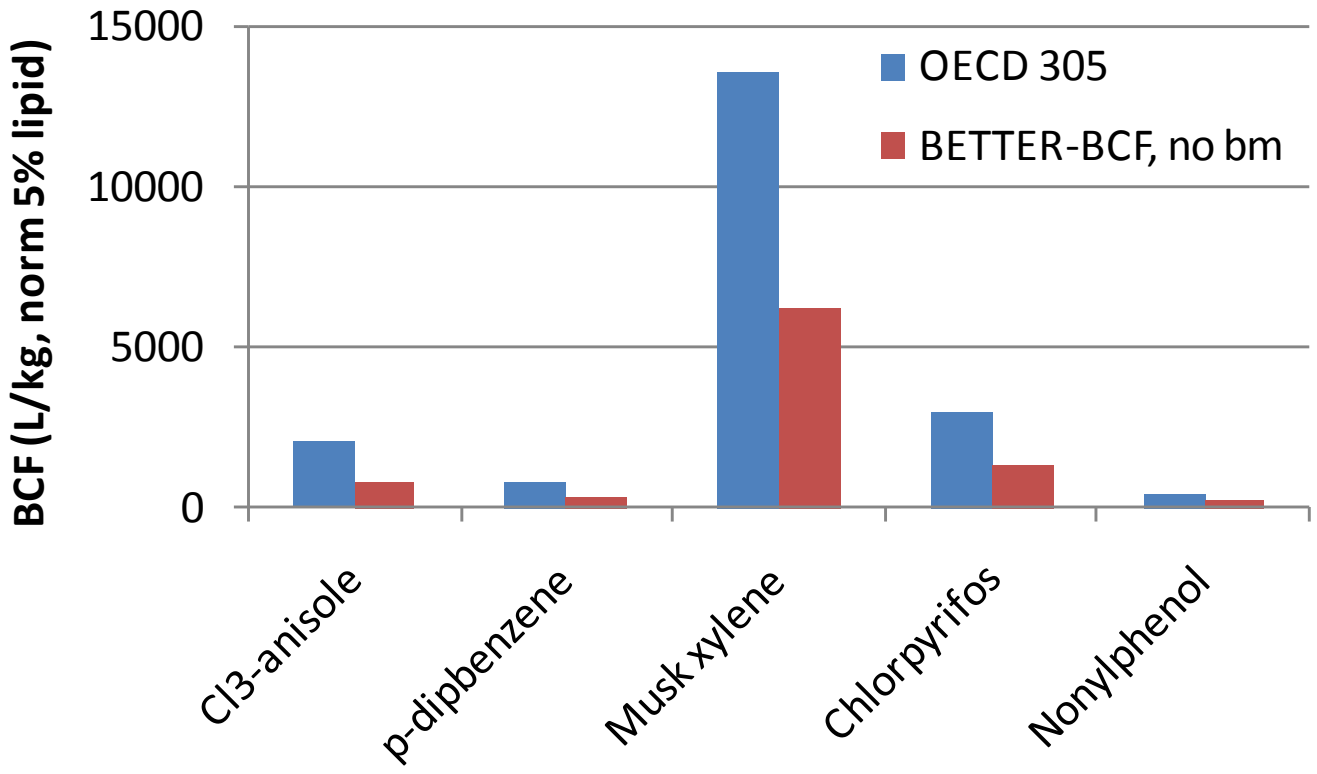
- choose benchmark wisely to capture dominant source of variability

OECD 305 versus “Gold Standard”



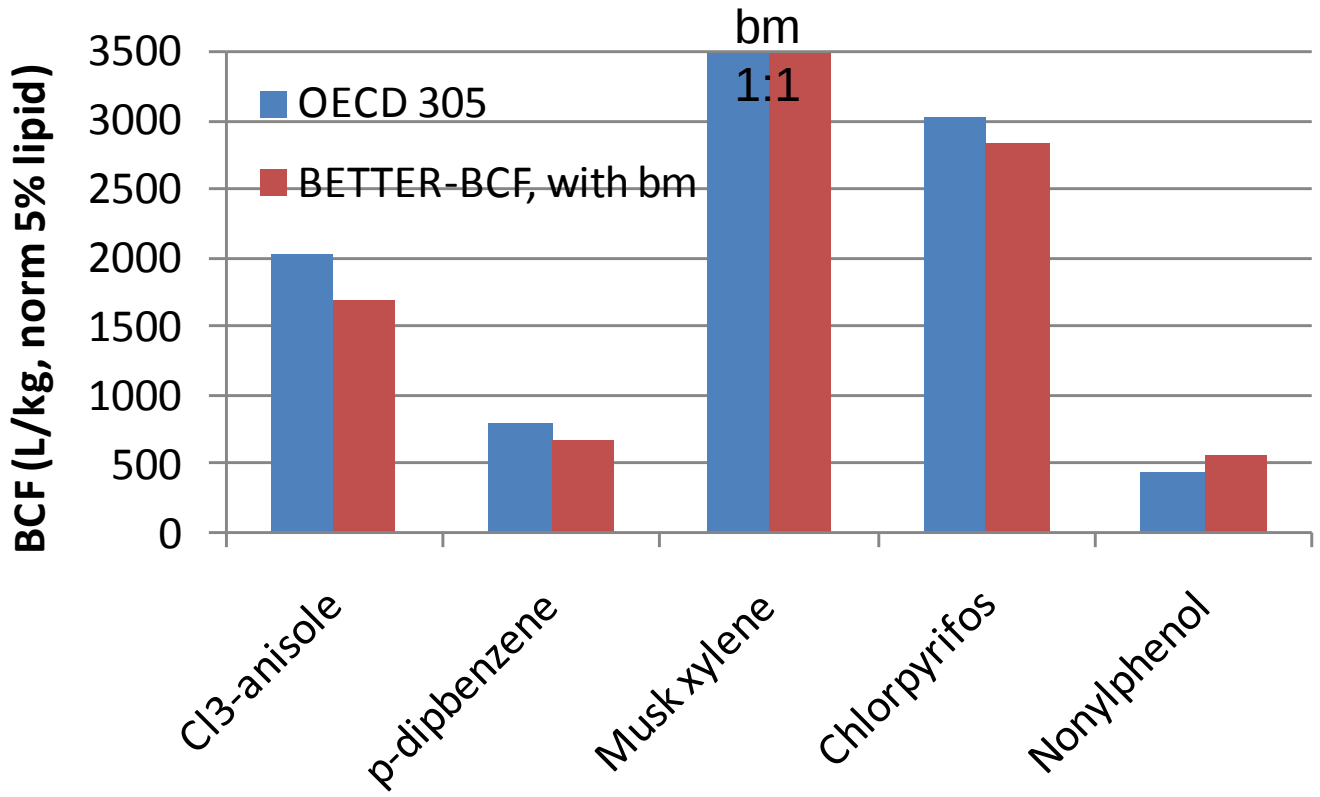
- precision of OECD 305 poor

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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A better BCF test is possible!

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)

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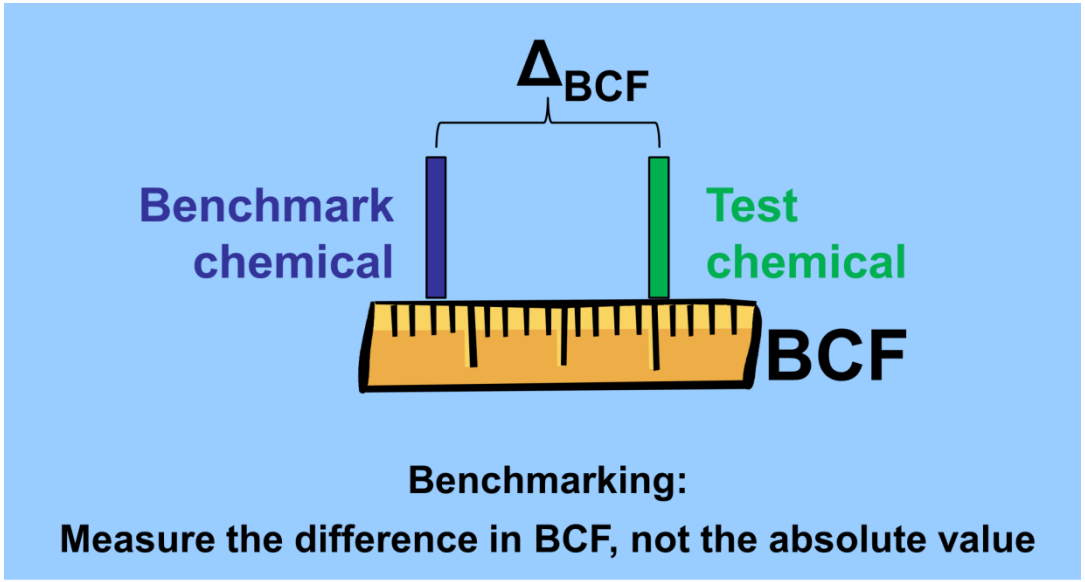
Evolution or Revolution?

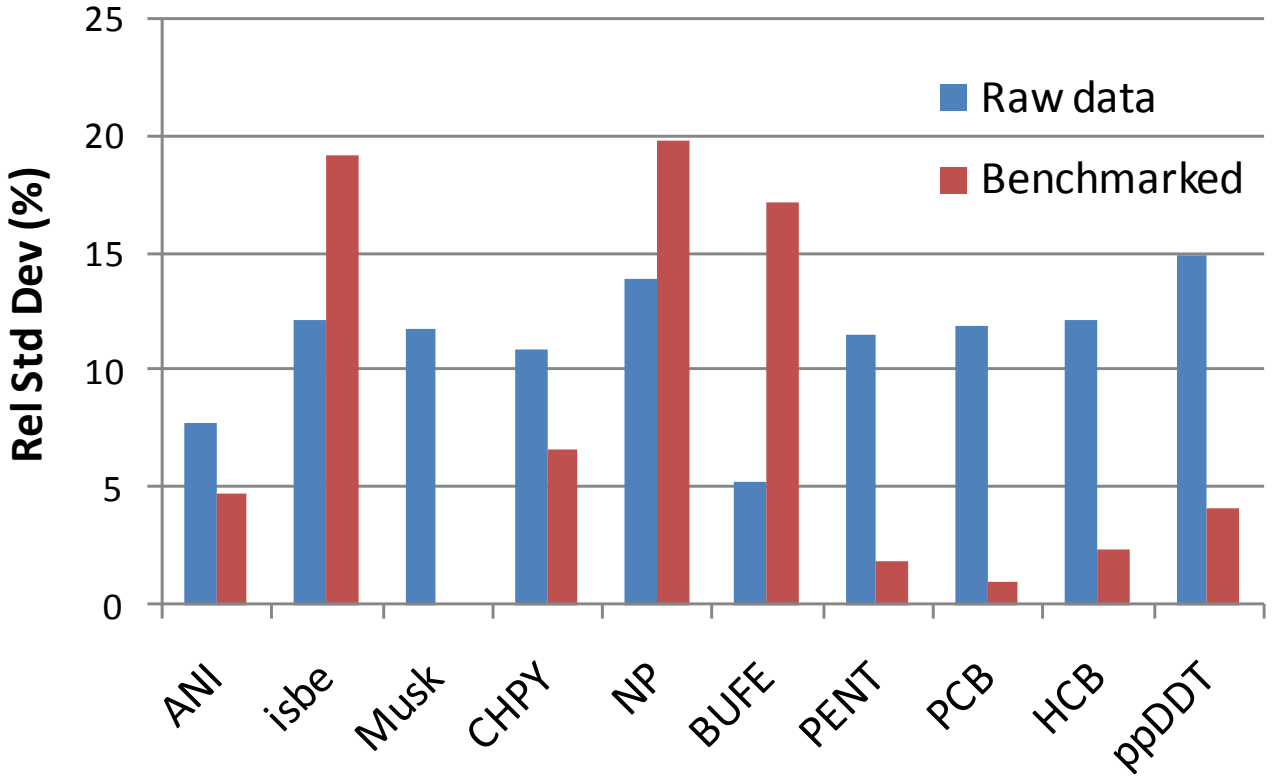
Evolution towards Revolution?



Acknowledgement

Thanks to the Cefic-LRI for funding and to the ECETOC research liaison team for their support.

RSD of C_{fish} (n=4) fish from the uptake phase: Raw data vs. benchmarked against Musk



- benchmarking markedly reduces variability for most chemicals
- does not necessarily reduce variability when metabolism important (here isbe, NP)