



---

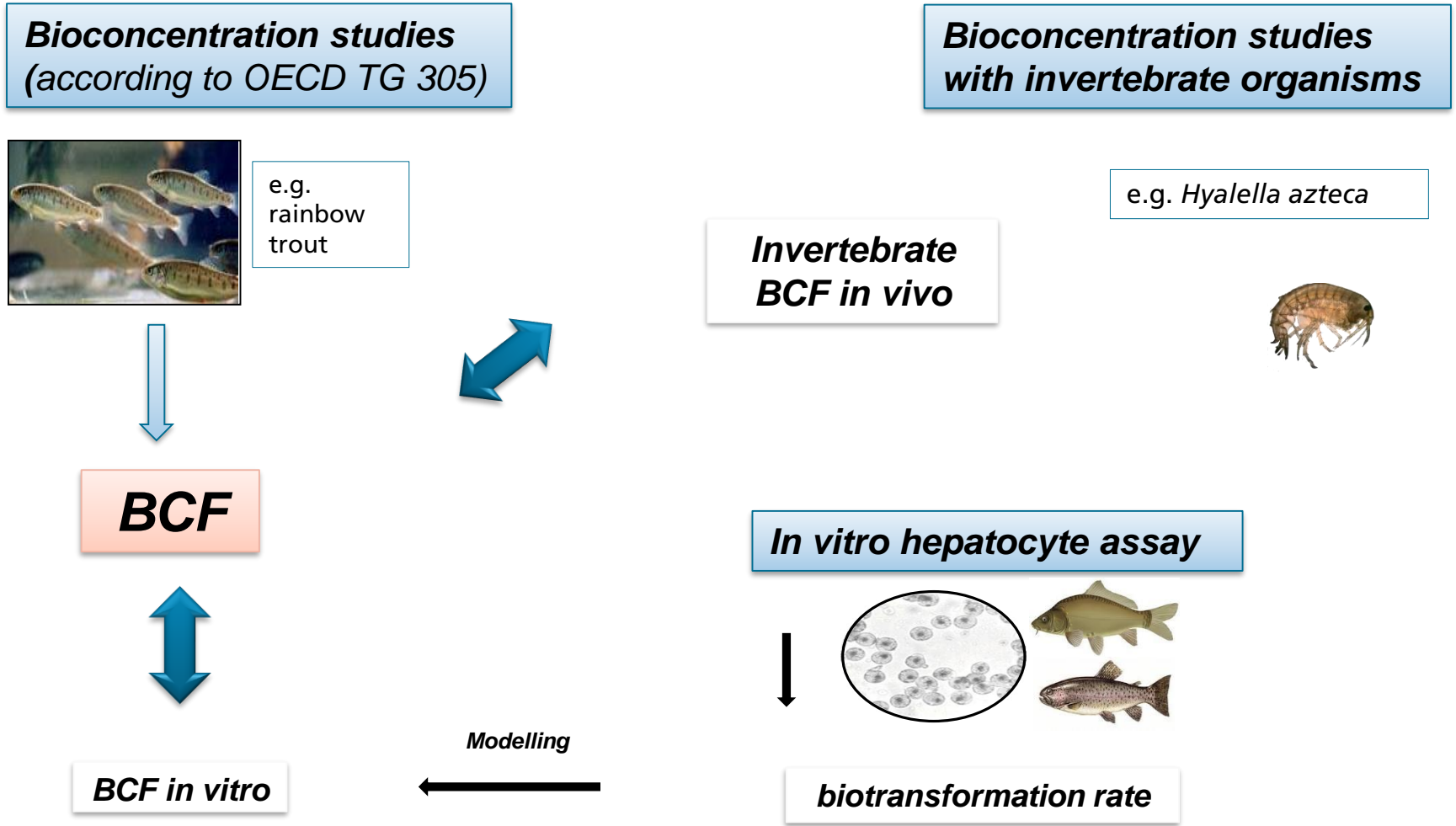
# Validation of the *Hyalella azteca* bioconcentration test (HYBIT)

---

Christian Schlechtriem, Verena Kosfeld, Pascal Pandard, Caren Rauert, Susanne Walter-Rohde

---

# Alternative methods for bioaccumulation testing according to OECD TG 305



# Bioconcentration studies with *Hyalella azteca*

---

- Species: *Hyalella azteca*
- Age: > 3 months
- Weight: 1,5 – 2,5 mg
- Length: 3 - 5 mm
- Sex: male (and female)

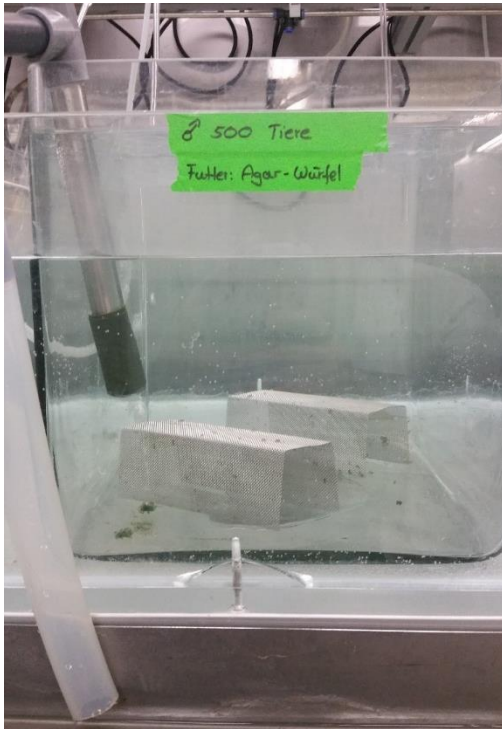


## Advantages as test organism:

- Non vertebrate study
- Simple cultivation
- Laboratory breeding established
- Short generations
- Sufficient sample size



# Bioconcentration studies with *Hyalella azteca* under flow-through conditions



## BCF-Studies:

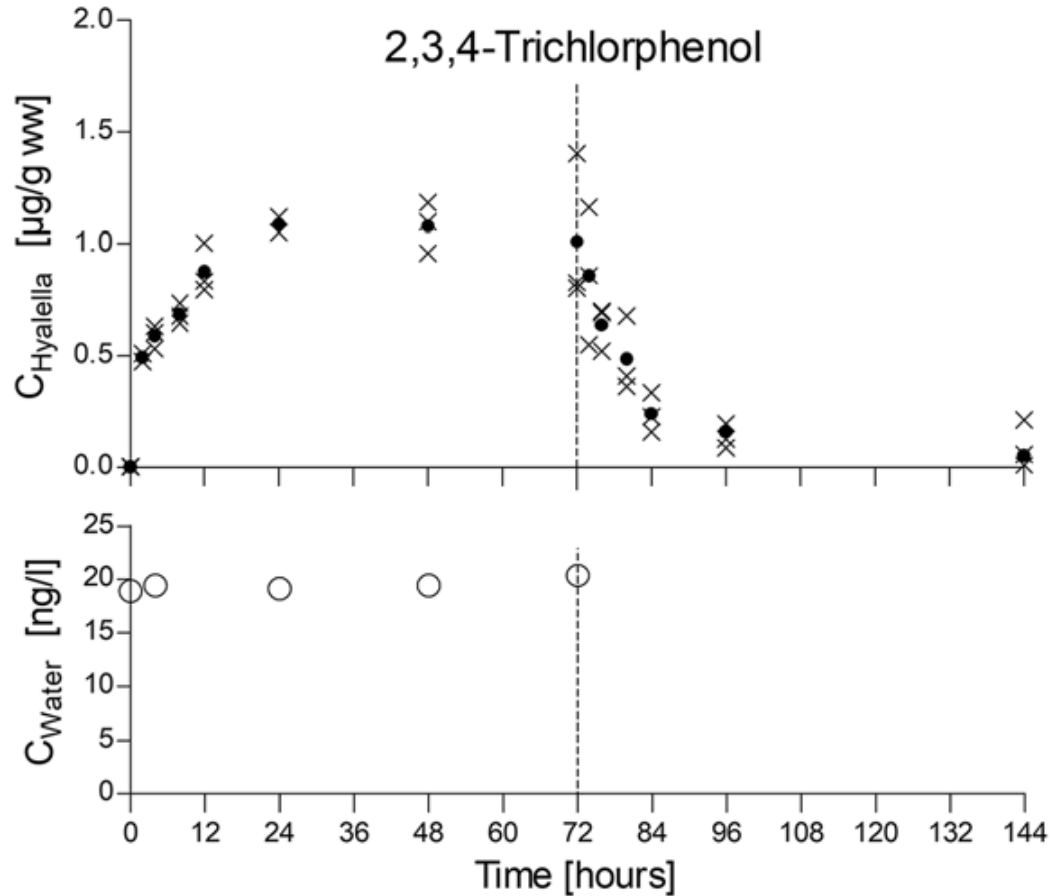
- Continuous supply of dissolved test substance



## Application of Decotabs

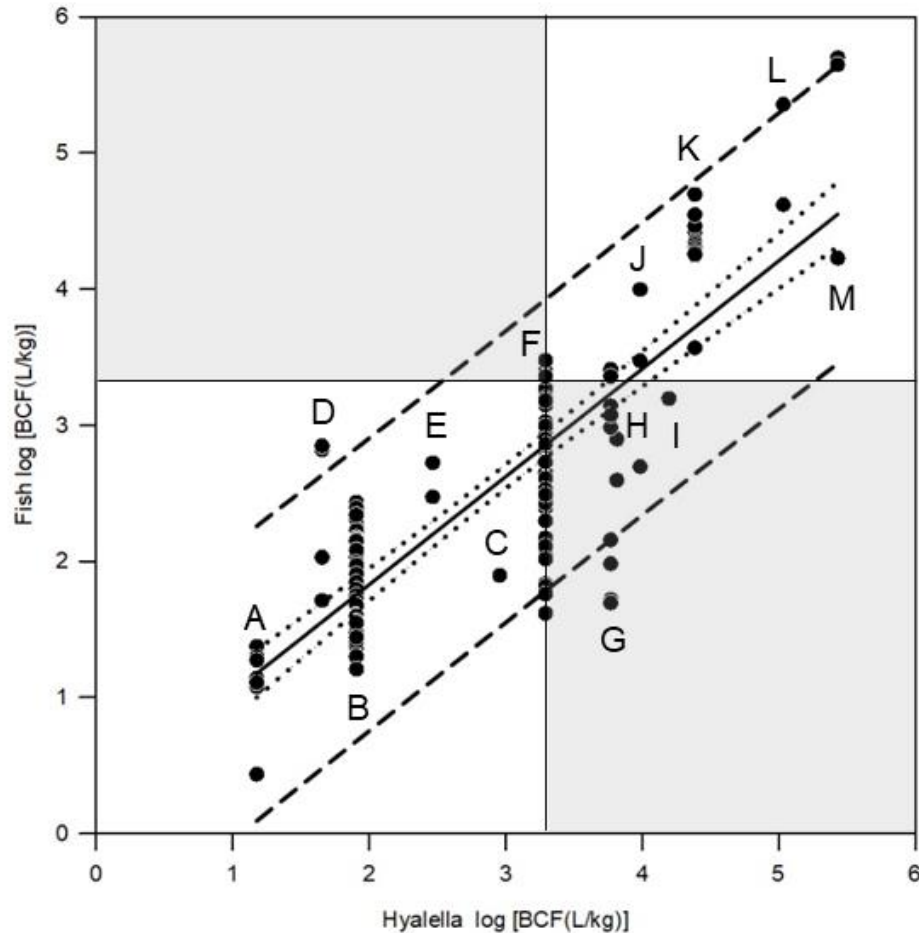
Test chamber (20 L volume)  
~ 1200 – 1300 amphipods / tank

# *Hyalella azteca* flow-through bioconcentration study with 2,3,4-Trichlorophenol



Schlechtriem et al. 2019

# Experimental fish BCFs from different studies vs. individual experimental kinetic BCFs estimated for male *Hyalella azteca*



- A: <sup>14</sup>C-simazine
- B: diazinon
- C: <sup>14</sup>C-Low hydrophobic compound
- D: 1,2,3-trichlorobenzene
- E: 2,4,5-trichlorophenol
- F: chlorpyrifos
- G: <sup>14</sup>C-pyrene
- H: benzo(a)pyrene
- I: methoxychlor
- J: o-terphenyl
- K: hexachlorobenzene
- L: PCB77
- M: PCB 153

Correlation: Black Regression Line [fish log BCF = 0.251 + (0.792 \* Hyalella log BCF)]; R<sup>2</sup>=0.687) with 95% Confidence Interval (dotted lines) and Prediction Interval (short dash)

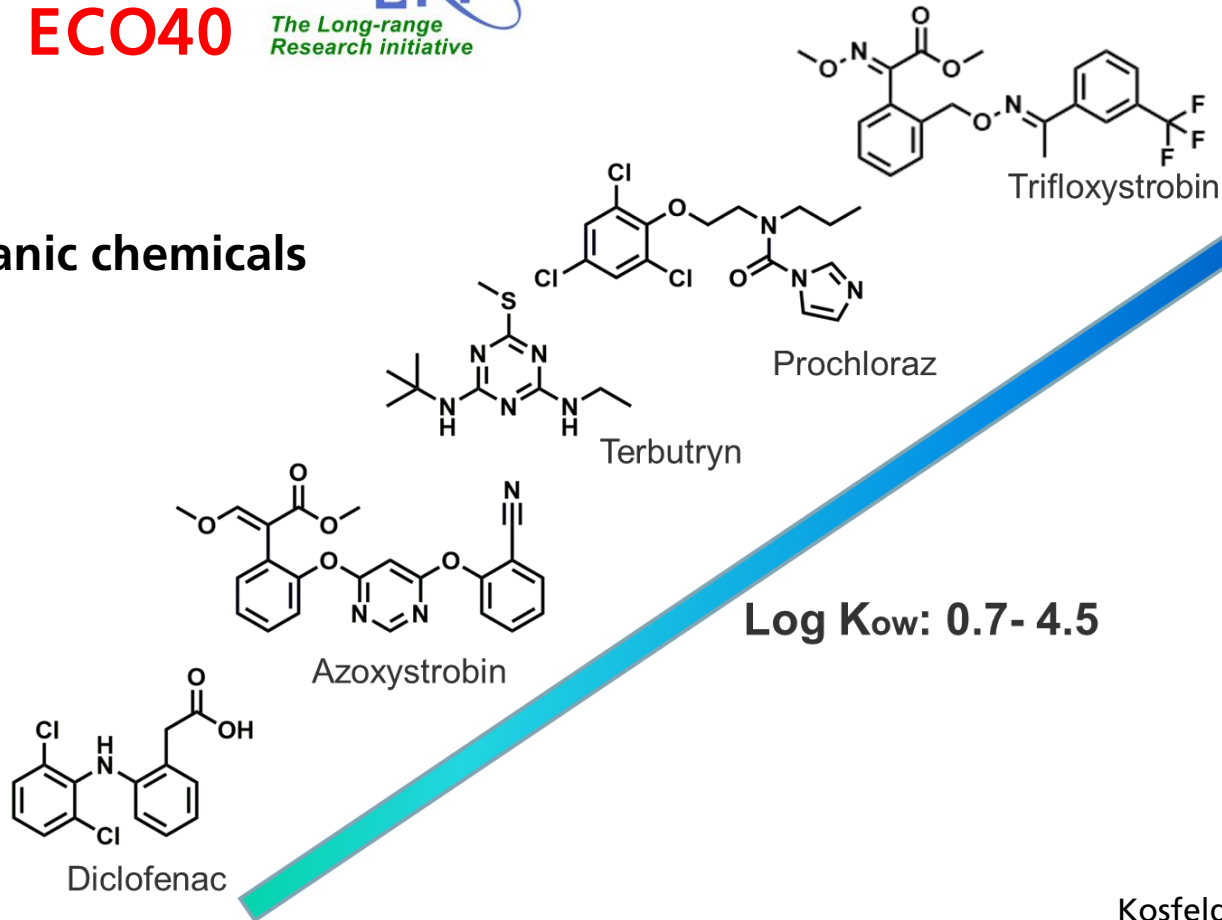
Schlechtriem et al. 2019

# Investigations on the bioconcentration of xenobiotics in the freshwater amphipod *Hyalella azteca*

CEFIC LRI ECO40

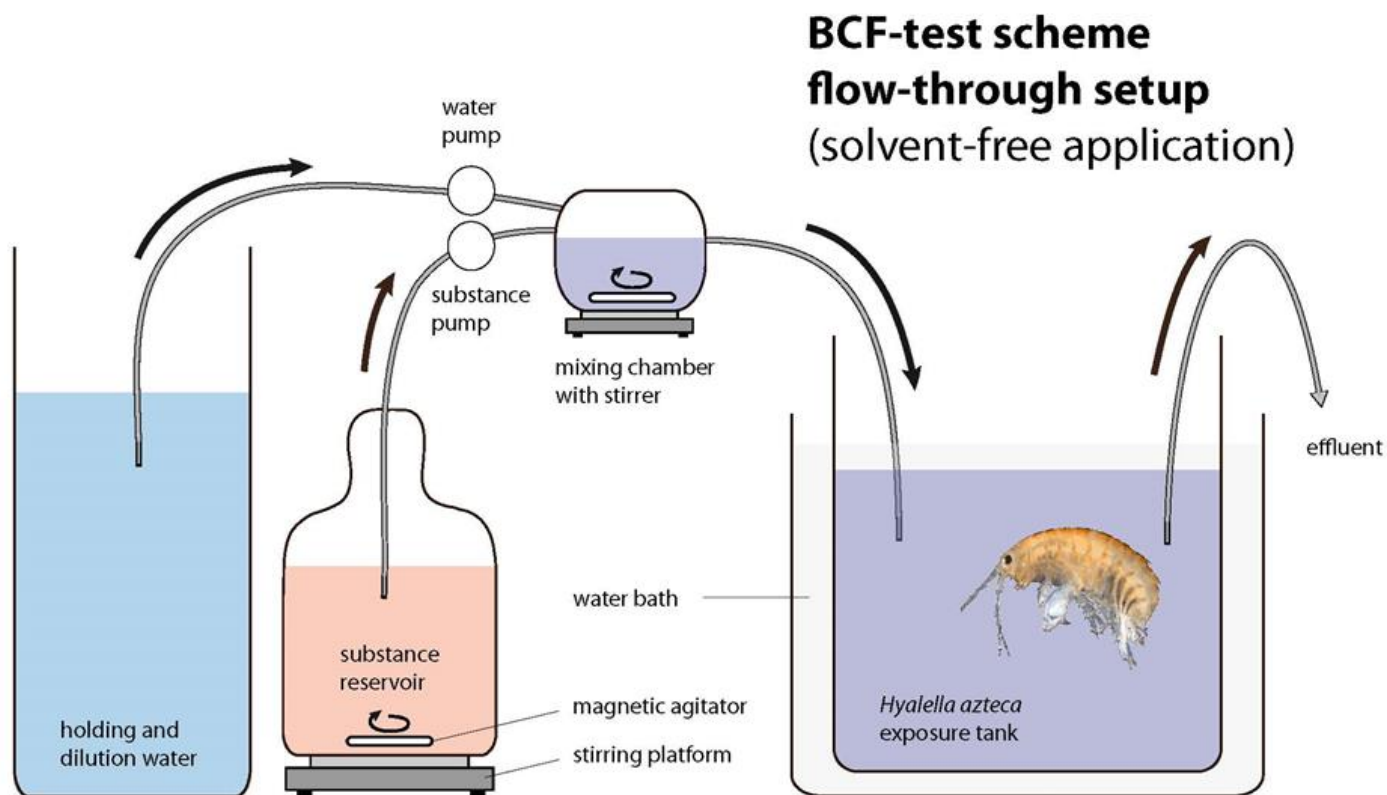
**LRI**  
The Long-range  
Research initiative

Selected organic chemicals



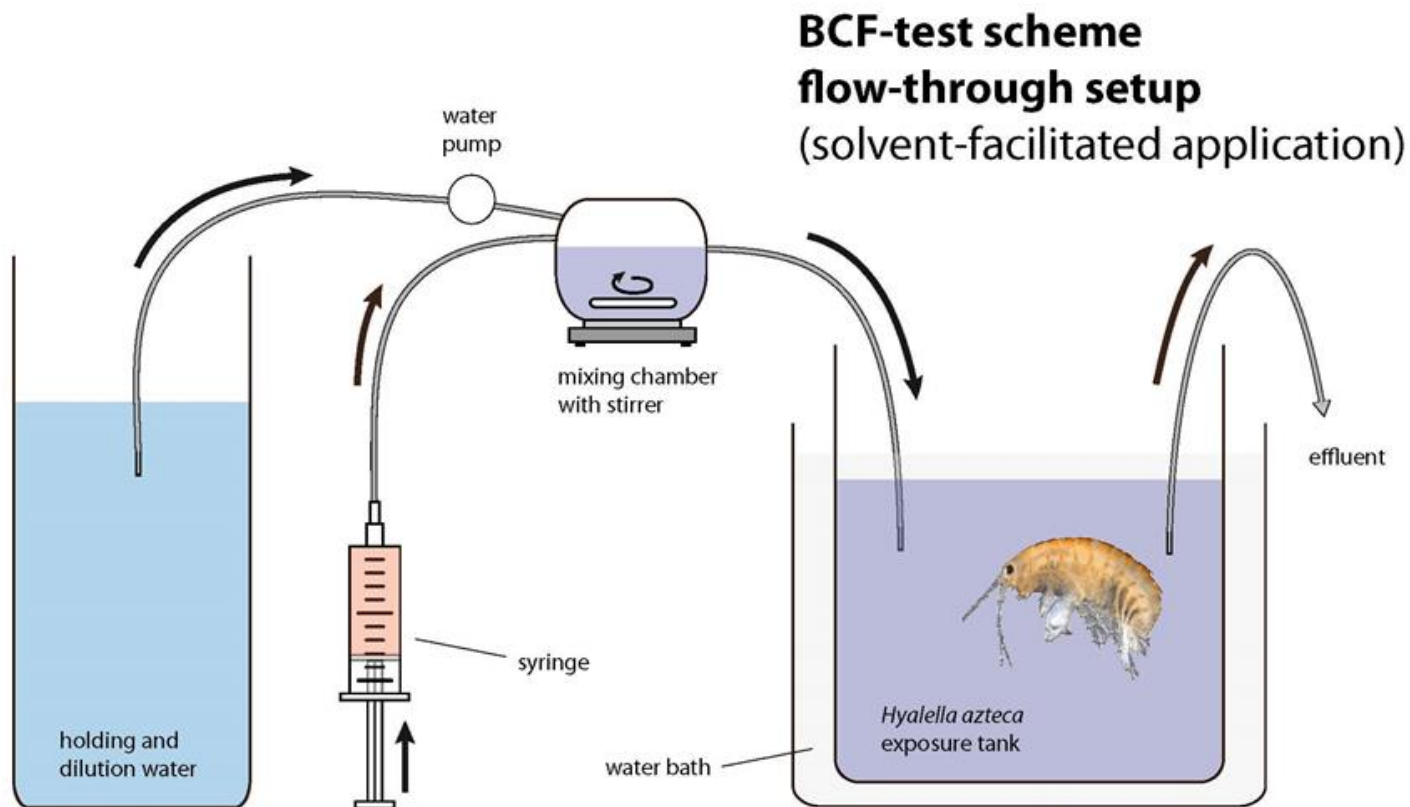
Kosfeld et al. 2020

# Schematic overview of the flow-through test setup (solvent-free application)



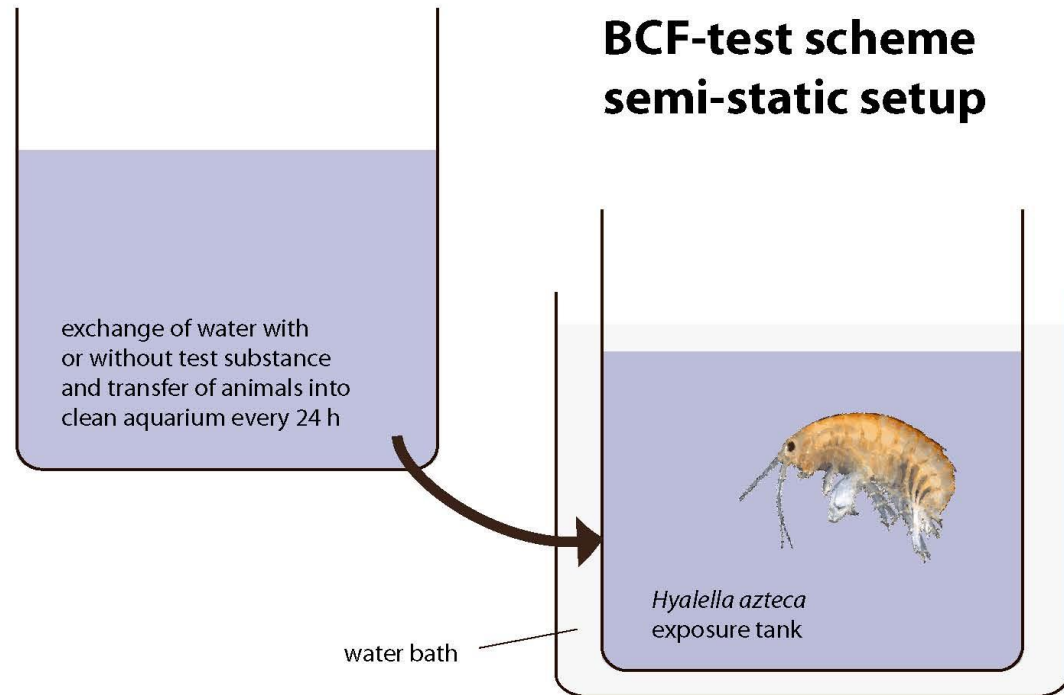


# Schematic overview of the flow-through test setup (solvent-facilitated application)



# Schematic overview of the experimental setup of the semi-static test

---



---

# HYBIT – RING TEST

---

## Institution/Company

- L'Oreal, France
- IES, Switzerland
- Biotechnologie BT, Italy
- BASF, Germany
- INERIS, France
- Eurofins, Germany
- ECT, Germany
- UBA, Germany
- Noack-Lab, Germany
- IBACON, Germany
- Fraunhofer IME, Germany



**HYBIT: Bioconcentration in the amphipod *Hyaella azteca***

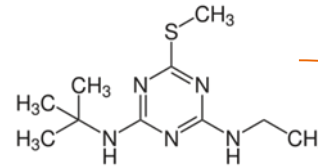
DRAFT PROTOCOL Version 1.0 (funded by project CEFIC LRI ECO40)

# HYBIT: Selected organic chemicals

## Terbutryn

$\log K_{ow} = 3.6$

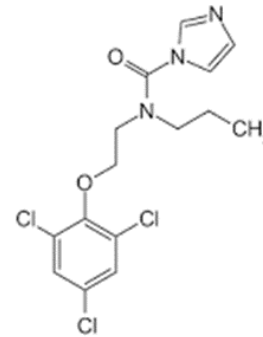
Moderately hydrophobic test item



## Prochloraz

$\log K_{ow} = 4.4$

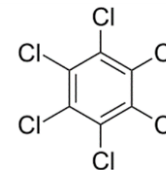
Hydrophobic test item metabolizable in *H. azteca*



## Hexachlorobenzene (HCB)

$\log K_{ow} = 5.86$

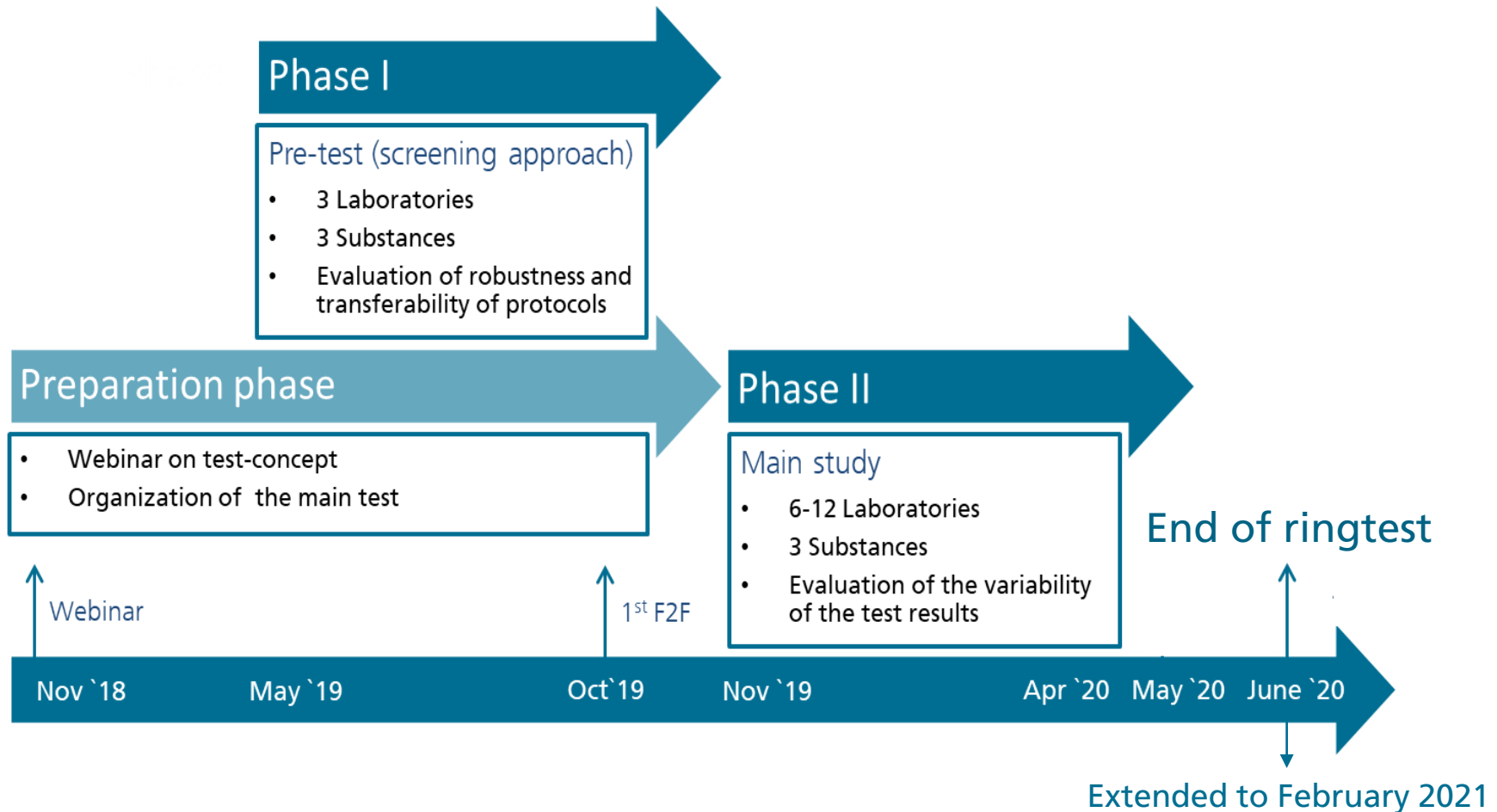
Highly hydrophobic test chemical non-metabolizable in *H. azteca* (and fish)



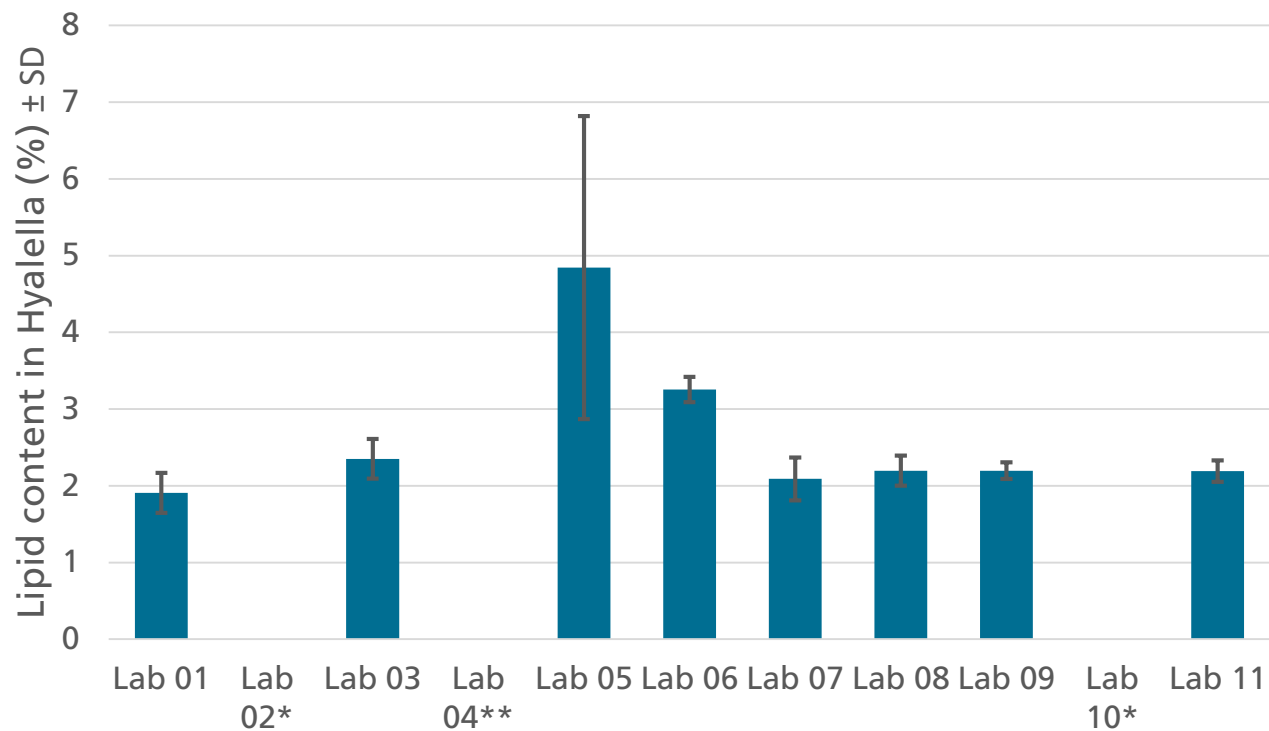
Semi-static approach

Flow-through approach

# Two-step validation process of the HYBIT protocols



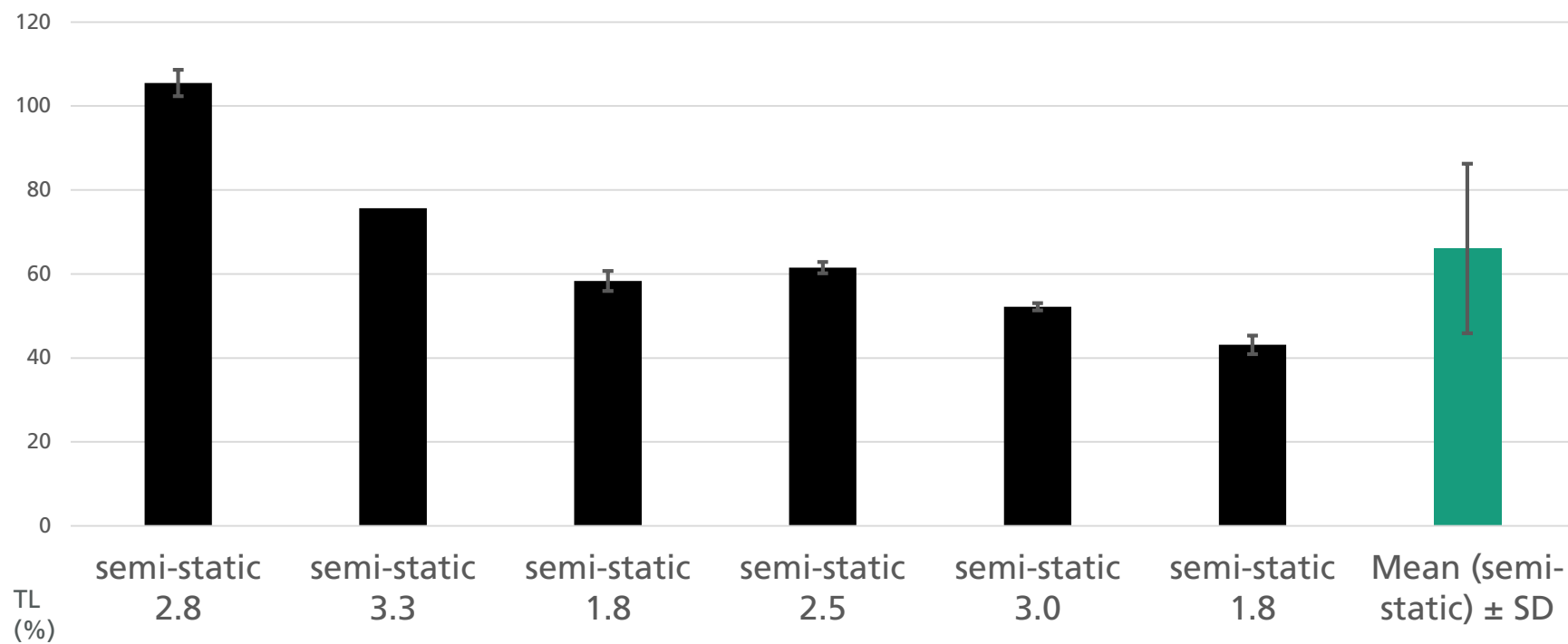
# Lipid pre-ringtest



- \* lipid analysis following analysis of main test samples
- \*\* No equipment available. Analysis of main test samples performed by Fraunhofer IME

# HYBIT BCFs - Terbutryn

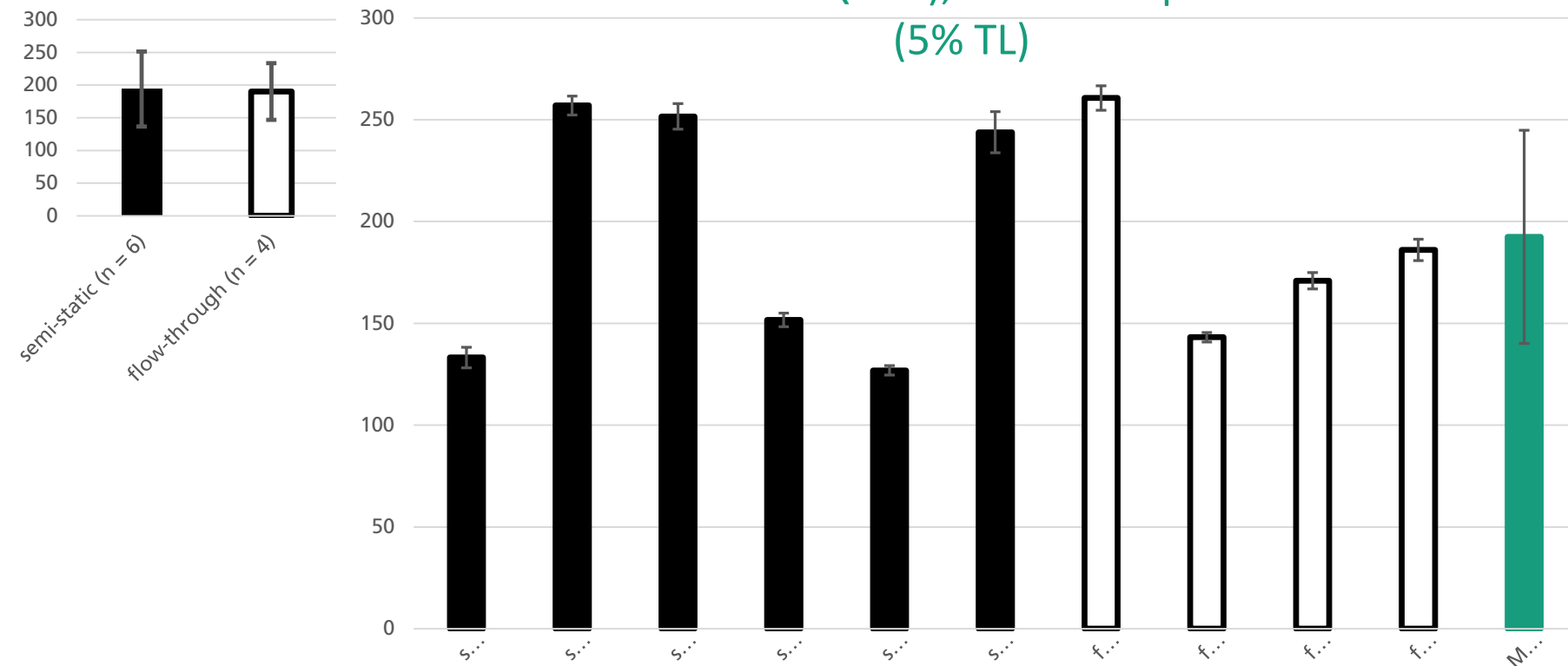
## Terbutryn BCFs lipid normalized ( $\pm$ SE)



# HYBIT BCFs - Prochloraz

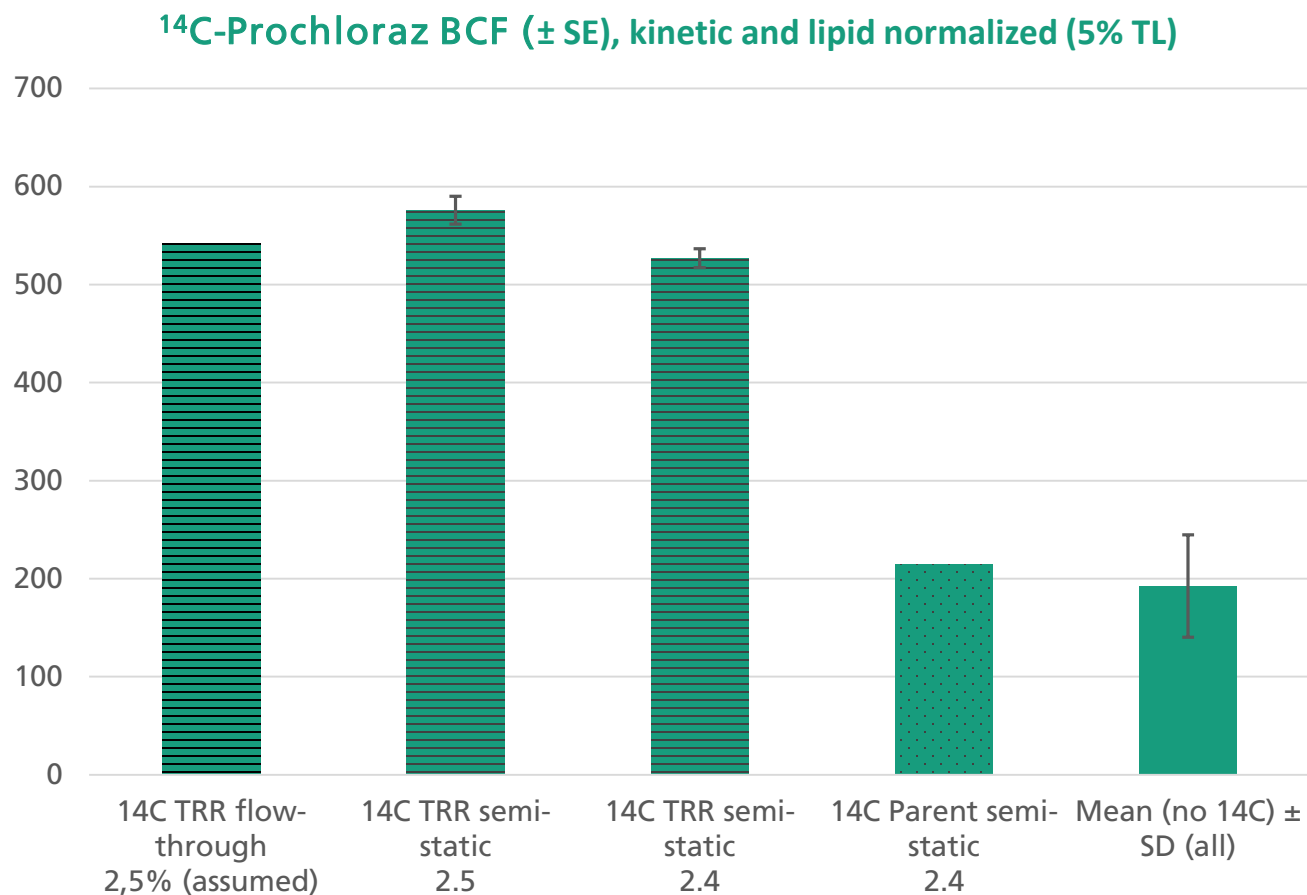
Prochloraz

Prochloraz BCFs ( $\pm$  SE), kinetic & lipid normalized  
(5% TL)



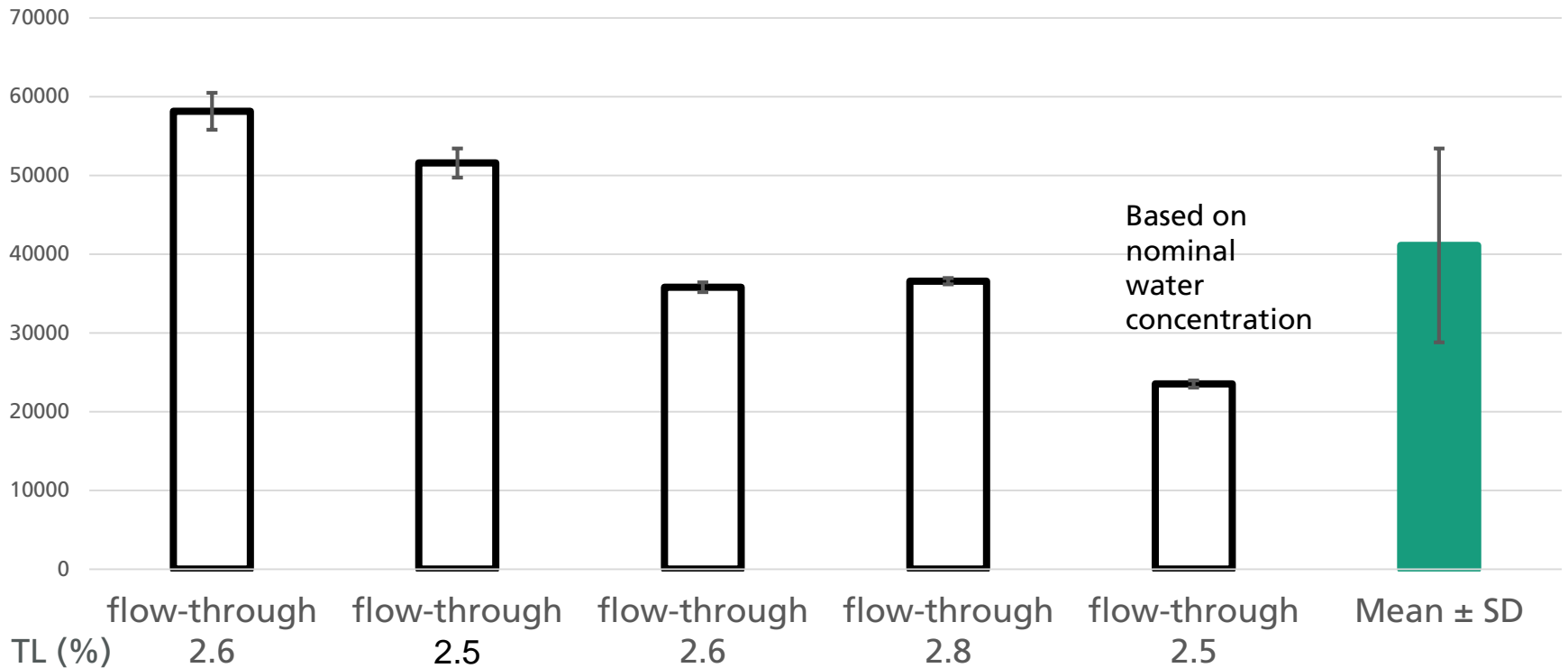


# HYBIT BCFs – Prochloraz $^{14}\text{C}$ experiments



# HYBIT BCFs - HCB

## HCB BCFs ( $\pm$ SE), kinetic & lipid normalized (5% TL)



# New TG on *Hyalella azteca* Bioconcentration Test (HYBIT)

---



## HYBIT: Bioconcentration in the amphipod *Hyalella azteca*

DRAFT PROTOCOL Version 1.0 (funded by project CEFIC LRI ECO40)

- Development of draft TG: Q2 2021
- Expert group and WNT commenting rounds, TG finalization (Q2 2021 - Q1 2022)
- Earliest adoption of TG by OECD WNT (2022)

# Acknowledgements

---

Studies presented were funded by

CEFIC

Project: LRI-ECO40



Umweltbundesamt

Project: FKZ 3718 67 401 0



Fraunhofer



Thanks to OECD (Leon Vanderwal and colleagues) for supporting the ring test process



# Contact

---

