

Fragrances not only scent

Pre-experimental toxicity testing for the freshwater amphipod *Hyalella azteca* bioconcentration test (HYBIT) with difficult to test chemicals

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Medium preparation

WAF like approach

- 2 h stirring
- Brown WAF glass bottle
- Cu²⁺ reduced H₂O
- Range finder:
 - Below max. water solubility
 - Rangefinder A: broad range
 - Rangefinder B: refined range
 - Ten fold dilution steps

Alternative approaches

- Passive dosing
- Solvent spiking

Hyalella azteca

- Amphipod
- Benthic
- Freshwater
- Origin: America
- Omnivor
- Grazing and deposit feeding
- Low generation time
- No seasonal dependence



Implementation

- 6 beakers per – concentration – control
- ¼ Decotab
- 20 *H. azteca*
- 1 Refuge, stainless steel
- 200 mL medium



Daily

- Mortality evaluation
- Medium change
- Feeding Decotabs ad libitum

Measurement

pH

O₂

Temperature



Duration 3 – 12 d

Parameter _c

23 ± 2°C

500 – 1000 lx 16 : 8

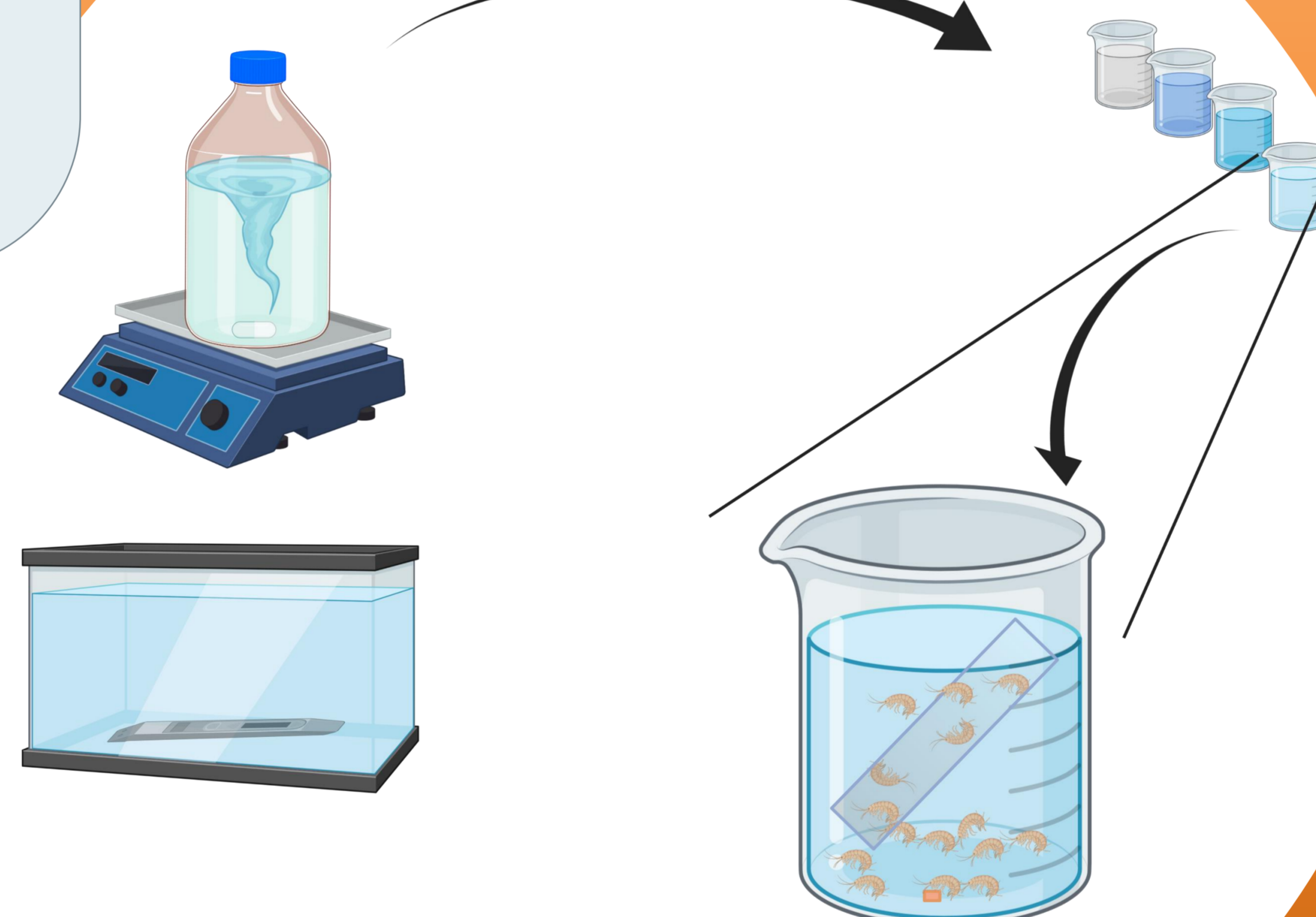
Validity:

- Control mortality < 20 %
- Temperature: 23 °C ± 2 °C
- Oxygen saturation > 60 %



BCF-Test

Suitable concentration
54.22 – 89.39 µg/L



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Analytcs

LC/MS (or GC/MS)

Stability check

At each medium change

fresh

used, pooled

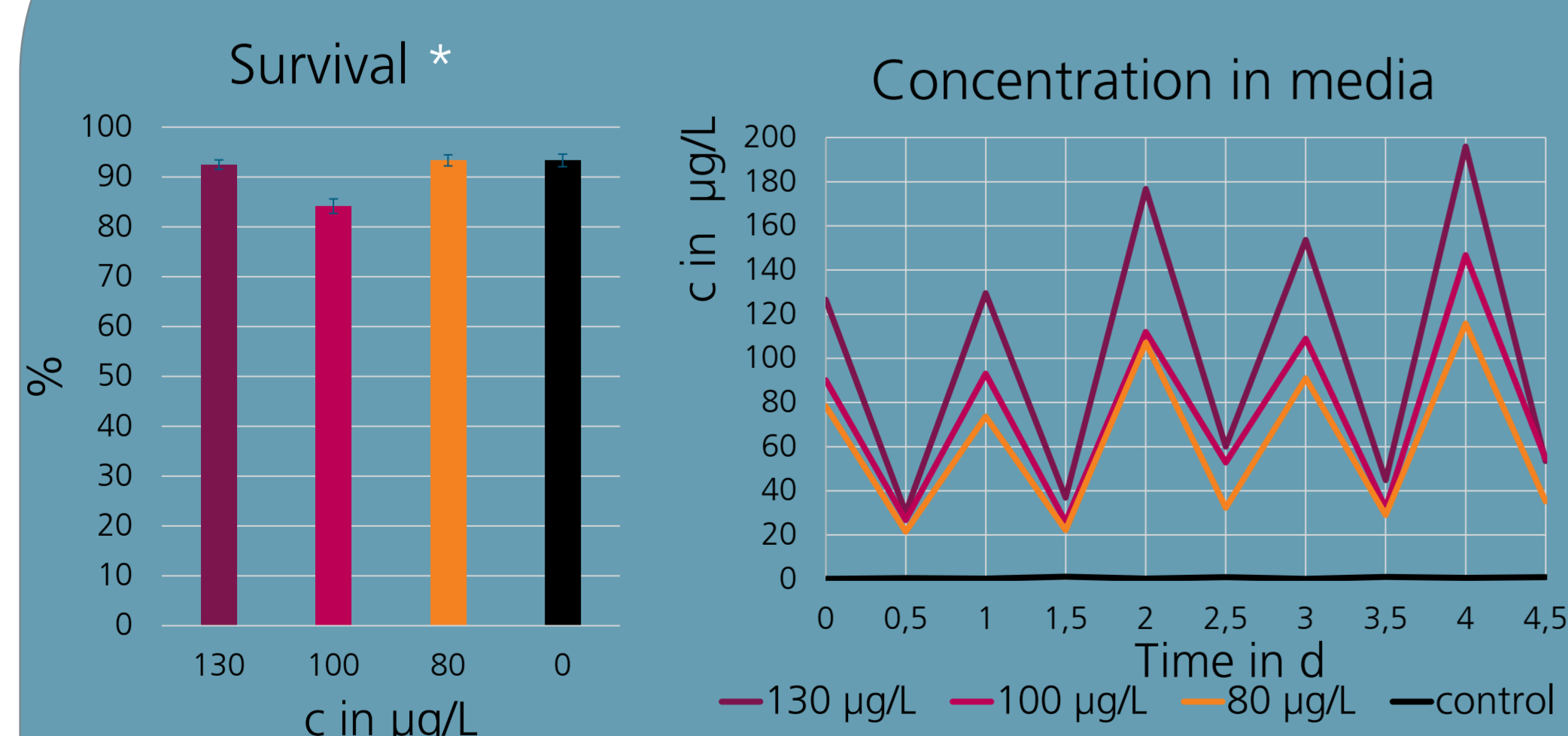


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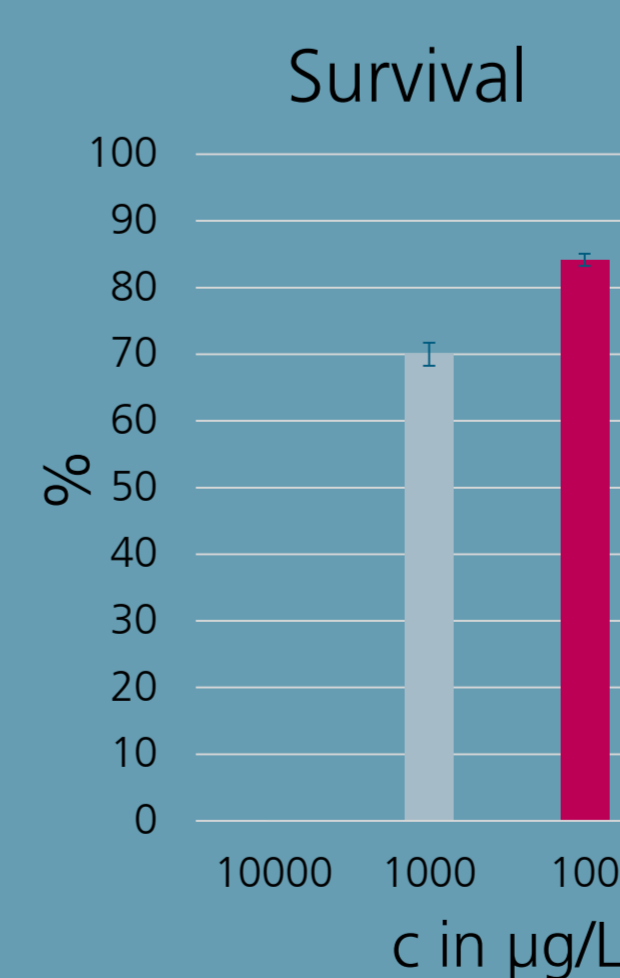
Rangefinder B

Results

Rangefinder A



c nominal µg/L	mean µg/L
10000	6946
1000	684
100	68.8
0	4.4



* ANOVA On Ranks (equal variance test failed) $p = 0.244$

No significant difference between treatments and control

c nominal µg/L	TWA µg/L
130	89.39
100	67.83
80	54.22
0	0.47

Refined Approach

Exemplary work

- Moderate lipophilic Substance $\log K_{ow}$: 3.68
- 6 d exposure period
- LC/MS for analytics
- Fragrance substance

^A Strong Jr, D. R. (1972). "Life history variation among populations of an amphipod (*Hyalella azteca*).^A *Ecology* 53(6): 1103-1111.

^B Schlechtriem, C., Kampe, S., Bruckert, H. J., Bischof, I., Ebersbach, I., Kosfeld, V., ... & L'Haridon, J. (2019). Bioconcentration studies with the freshwater amphipod *Hyalella azteca*: are the results predictive of bioconcentration in fish?. *Environmental Science and Pollution Research*, 26(2), 1628-1641.

^C US Environmental Protection Agency. (2000). Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. *Testing manual (Inland testing manual)*.

Abbreviations

ANOVA Analysis Of Variance
BCF Bioconcentration Factor
c concentration
LC/MS Liquid Chromatography–Mass Spectrometry
TWA Time Weighted Average
WAF Water Accommodated Fraction