The freshwater amphipod *Hyalella azteca* as alternative test organism for bioaccumulation studies

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1. Introduction

The ultimate decisive bioaccumulation-criterion as part of the REACH regulation (Annex XIII) is the bioconcentration factor (BCF) reflecting the uptake of a test substance from the contaminated surrounding medium. Bioconcentration factors (BCF) for regulatory purposes are usually determined by fish flow-through tests according to TGD OECD 305. Alternatively, biomagnification factors (BMF) can be determined in dietary exposure tests for such groups of substances where this is considered more suitable than an aqueous exposure test. Fish bioaccumulation studies are time consuming, expensive, and use many animals. Alternative methods that replace the use of fish for BCF (BMF) testing would therefore be of value. The bioaccumulation of sediment-associated contaminants by benthic invertebrates can be assessed by using the bioaccumulation test TGD OECD 315. However, endobenthic aquatic oligochaetes burrow in the sediment and are therefore exposed to contaminants via multiple uptake routes including direct dermal contact, ingestion of contaminated sediment particles, porewater, and overlying water. Therefore, the test endpoint is defined as a bioaccumulation factor (BAF). A suitable method to deduce BCFs from BAFs derived from OECD 315 studies is still missing leading to a limited value of this test from the regulatory point of view. The aim of this study was to investigate whether the freshwater amphipod *Hyalella azteca* can be used as alternative test organism for bioaccumulation studies, providing the opportunity to explain bioaccumulation from water (bioconcentration) and food (biomagnification) separately.

2. Materials and method

In a first approach, a diet suitable to grow the freshwater amphipod was identified using a simple filter feeding system. The selected diet was then enriched with the highly lipophilic test items hexachlorobenzene (HCB) and ortho-terphenyl (o-TP) as well as the perfluorinated acid perfluoroctane sulfonate (PFOS). The enriched diets were applied in dietary exposure tests with *H. azteca*. In a further approach the uptake and accumulation from water of the same substances as well as of a low lipophilic pesticide (logKow =3) was investigated. Column generated concentrations were applied where required to avoid the use of solvents. The bioaccumulation of test items in male and female animals was compared. Animals collected during the bioaccumulation studies were analysed for their tissue concentrations. Based on the kinetic study design the depuration and uptake rates for the test items were determined which were further used to calculate species specific BCF or BMF estimates. The results were compared with BCF and BMF values obtained from fish

bioaccumulation studies in rainbow trout (*Oncorhynchus mykiss*) which were previously carried out according to the revised TGD OECD 305. Additional animals were collected during the studies for lipid determination. Tissue concentrations were normalized to a lipid content of 5% to make the results obtained from bioaccumulation tests using fish or Hyalella comparable.

3. Results and discussion

Bioconcentration and biomagnification factors determined in Hyalella bioaccumulation studies are similar to those obtained from fish tests (Table 1). Steady state tissue concentrations were reached in all studies within significantly shorter uptake periods compared to fish studies on the same test items. The collection of only ten adult amphipods resulted in pooled biomass sufficient to quantify tissue concentrations. Tissue concentrations estimated for male animals were less variable compared to the concentrations measured in pooled samples of female animals. Uptake and elimination rates could be determined for Hyalella used in bioconcentration testing. Compared to studies on rainbow trout significantly shorter depuration periods were required to reach 90% elimination of accumulated test items.

	HCB	o-TP
Rainbow trout (juvenile)	25000	10000
***Hyalella azteca* (male)**	23000	9000
***Hyalella azteca* (female)**	18000	14000

Table 1. Steady state bioconcentration factors (BCFs) estimated for hexachlorobenzene (HCB) and ortho-terphenyl (o-TP) in flow-through tests with rainbow trout and adult amphipods (Hyalella azteca).

4. Conclusions

H. azteca provides the opportunity to investigate bioaccumulation from water (bioconcentration) and food (biomagnification) separately and thus has a high potential to be used as alternative test organism for bioaccumulation studies.

BCF and BMF estimates from bioaccumulation studies with *H. azteca* are similar to those obtained from fish tests.

Bioaccumulation studies with *H. azteca* support animal welfare considerations using a non-vertebrate species, improve efficiency and reduce costs for BCF/BMF-testing.

Investigations on further substances are required to confirm the suitability of BCF/BMF-tests with *H. azteca*.

5. References

[1] OECD Test No- 315: Bioaccumulation in Sediment-dwelling Benthic Oligochaetes.
[2] OECD Test No. 305: Bioaccumulation in fish: Aqueous and dietary exposure.