Bioconcentration and metabolism of laurate in the freshwater amphipod *Hyalella azteca*

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Introduction

Regulatory assessment of bioaccumulation from water is commonly based on bioconcentration factors (BCF) derived from fish flow-through tests (OECD 305). Such experiments require a lot of laboratory animals and are time-consuming and costly. An alternative test set-up for organic, neutral compounds using the freshwater amphipod Hyalella azteca was suggested (Schlechtriem et al., 2018). However, there have been no studies yet elucidating the bioconcentration of ionic compounds in *H. azteca*. As the test item laurate, the anion of lauric acid (dodecanoic acid), a saturated fatty acid with a chain length of 12 carbon atoms, was chosen. Lauric acid is applied in high tonnages (EU: 10 000 – 100 000 tonnes per year) as surfactant in hygienic and cosmetic products. In contrast to most industrial chemicals, laurate is no xenobiotic. It is a natural part of the physiology and lipid metabolism of any organism used for testing.

This study was the first approach to assess the fate of an ionic compound in the alternative test system *H. azteca*. The aim was to elucidate the bioaccumulation potential and metabolite pattern of ^{14}C-labelled laurate as well as the spatial distribution of radioactivity in the test organism. The results were compared to the results of a previously conducted study by Van Egmond et al. (1999) using the zebrafish *Danio rerio*.

Methods

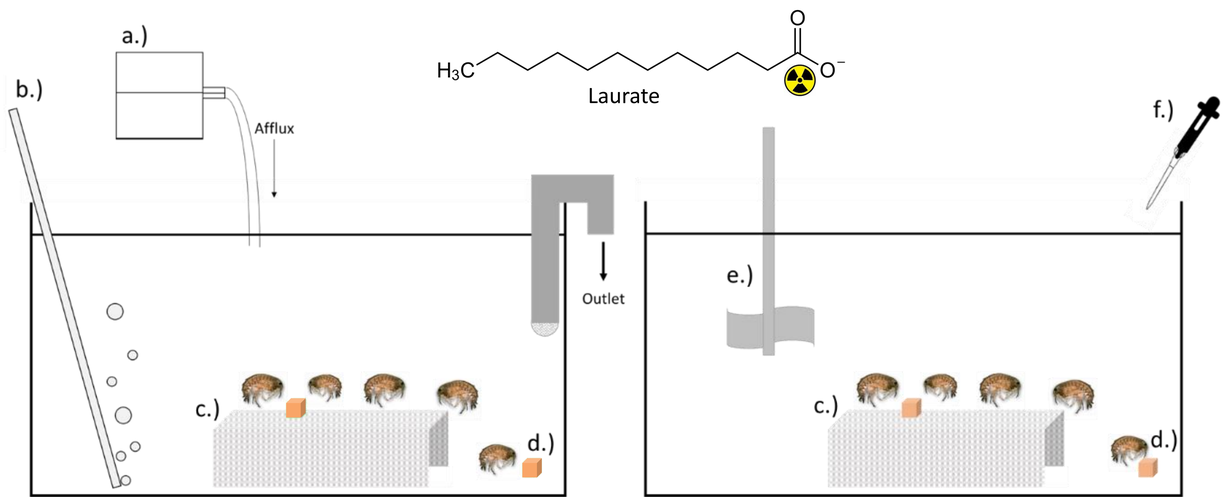


Figure 1: Experimental set-up of the flow-through system (left) and the semi-static system (right). a.) mixing chamber; b.) aeration; c.) steel grid; d.) food; e.) stirrer; f.) spiked medium.

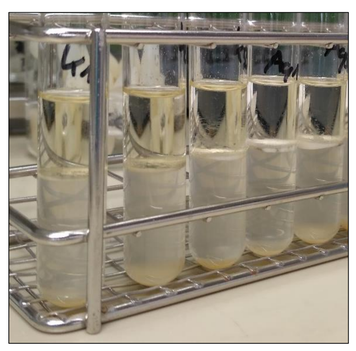


Figure 2: *Hyalella* extraction

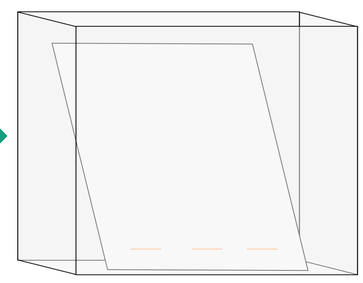


Figure 3: Radio-TLC analysis

Figure 4: Exemplary *Hyalella* exoskeleton after combustion (unextracted). E.g. CaO residues.

Metabolites

$BCF_{ss} = \frac{C_{Hyalella}}{C_{Water}}$

$BCF_{kin} = \frac{uptake\ rate}{elimination\ rate}$

Autoradiography

Male individuals of *H. azteca* with an age of 2-3 months were exposed for 48 h to radiolabelled laurate in a flow-through (150 µg/L) and a semi-static (1.5 mg/L) bioconcentration test system. For the following 48 h animals were transferred into an uncontaminated water tank for depuration. The flow-through system was run with a medium exchange rate of 4 h and 2 d of preconditioning prior to the *Hyalella* insertion. The medium of the semi-static study was renewed every 12 h to avoid microbial growth and degradation.

During the studies animal and media samples were collected in regular periods.

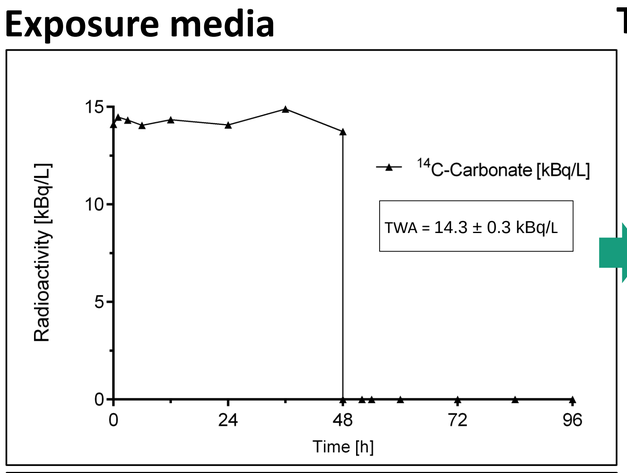
Media samples were analysed by liquid scintillation counting (LSC) to determine the total radioactivity and by radio-thin layer chromatography (TLC) to identify the proportion of untransformed test item.

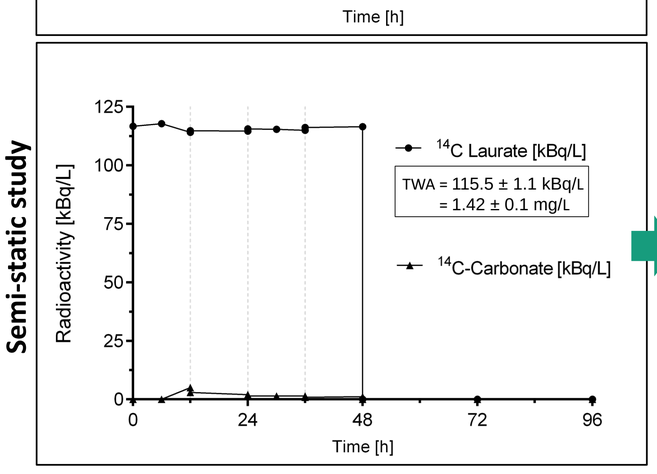
Hyalella samples were extracted and non-extractable residues (NERs) were determined by LSC after combustion. Extracts were analysed by LSC and radio-TLC.

Bioconcentration factors (BCFs) were determined under steady state (BCF_{ss}) conditions and using the modelled kinetic (BCF_{kin}) approach. BCFs were lipid normalised for the comparison with fish.

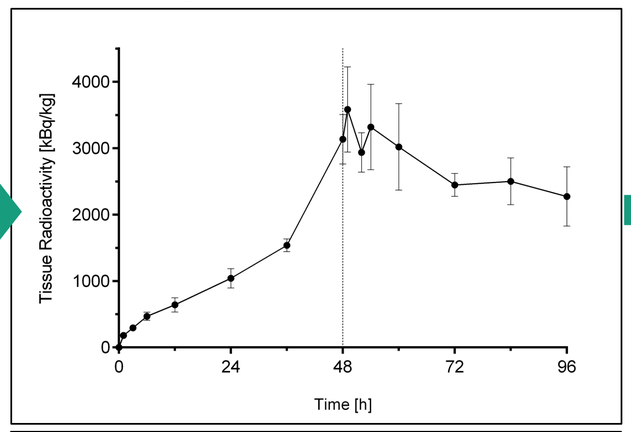
Results

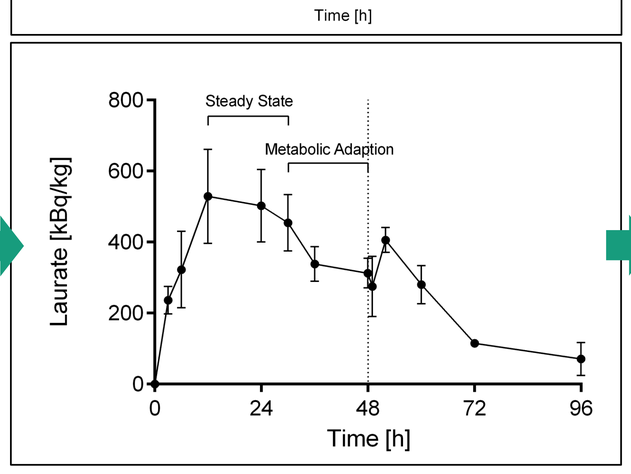
Exposure media





Tissue analysis





No stable exposure

- Stable radioactivity, but completely mineralised Test item
- No extractable radioactivity
- Intense uptake and low elimination of the ^{14}C-carbonate
- No BCF could be determined

Autoradiography

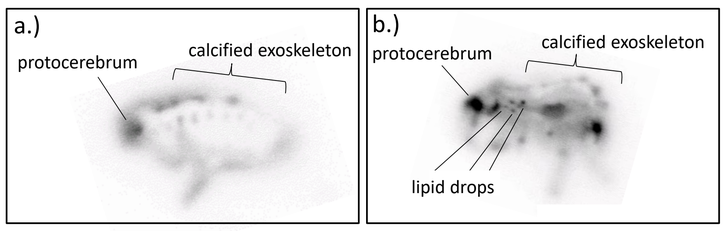
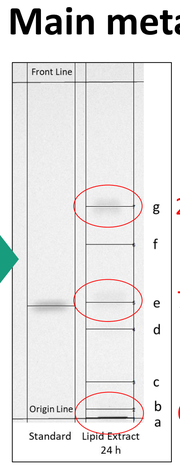


Figure 5: Autoradiograms of *H. azteca* taken after 48h exposure from (a.) the flow-through study and (b.) the semi static study. Autoradiograms of a pure ^{14}C- carbonate exposure showed the same pattern than (a.).

Main metabolites



23% Triacylglycerides (TAG)

7% Fatty acids (laurate)

66% Polar lipids

Comparison to fish

BCFs

D. rerio:

- 255 ± 22

H. azteca:

- BCFss: 14.8 ± 2.5
- BCFkin: approx. 30

Identified metabolites

D. rerio:

- TAG

H. azteca:

- TAG, Polar lipids
- 8 more (including cholesterol and cholesterol esters)

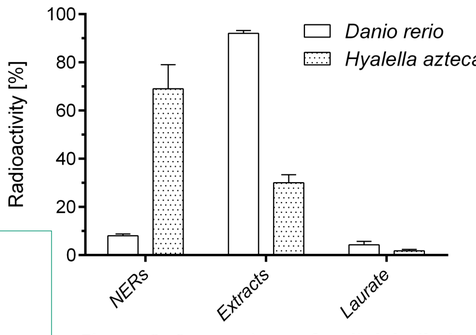


Figure 6: Comparison of radiolabelled fractions in *D. rerio* and *H. azteca*.

Conclusions

- The flow-through approach did not guarantee a stable exposure concentration.
- High amounts of radioactivity were taken up from the test-medium as ^{14}C-carbonate and stored in calcified structures in the exoskeleton of *H. azteca*. This ^{14}C was not extractable. We suggest a similar fate of ^{14}C-carbonate released from metabolism.
- Extractable radioactivity was stored in lipid-rich compartments (lipid drops and nervous tissue) as expected.
- Metabolite pattern differed between *D. rerio* and *H. azteca.*
- In contrast to neutral compounds bioaccumulation potential of laurate was lower in *H. azteca* than in fish.
- Mechanisms of bioaccumulation of laurate and the uptake of radioactivity vary between *H. azteca* and zebrafish.
- In a regulatory perspective both species BCFs were below the threshold value of 3000, suggesting *H. azteca* as a suitable alternative for testing ionic compounds.
- However, more ionic compounds need to be tested.

References

OECD. (2012). Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure. OECD.

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Van Egmond, R., Hambling, S., & Marshall, S. (1999). Bioconcentration, biotransformation, and chronic toxicity of sodium laurate to zebrafish (*Danio rerio*). Environmental Toxicology and Chemistry, 18(3), 466–473.

Session: New Developments in the Science of vPvB and PBT Assessment (P)
Poster, Exhibition hall, ID TH141
Thursday May 30th, 2019, 8:30 AM

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Keywords: bioconcentration, *Hyalella azteca*, ionic compounds

Regulatory assessment of bioaccumulation from water is commonly based on bioconcentration factors (BCF) derived from fish flow-through tests (OECD 305). Such experiments require a lot of laboratory animals and are time-consuming and costly. Further, the 3R principle postulates the replacement, reduction and refinement of vertebrate tests. An alternative test set-up for organic, neutral compounds using the freshwater amphipod *Hyalella azteca* was suggested. However, there is a lack of information on the bioaccumulation of ionic compounds in *H. azteca*. In this study the bioconcentration of the lauric acid anion laurate in *H. azteca* was investigated to elucidate the bioaccumulation potential of the test item in comparison to published data on zebrafish.

In a first study, radiolabeled 1-^{14}C sodium laurate was applied to *H. azteca* in a flow through system. Media samples were analysed via LSC and radio TLC. Tissue concentrations were determined via LSC after combustion. Laurate showed to be rapidly biodegradable. Therefore, a second BCF-test was carried out under semi-static conditions to ensure stable exposure concentrations. Methods for tissue analysis were adjusted to allow an accurate quantification of the test item.

Laurate was incorporated into various lipid fractions of *H. azteca*. However, more than 60 % of the total radioactivity found in the amphipod tissue was not extractable but could partially be driven out by acidification and captured as CO_2. The results suggest that also carbonate (CO_3^-) resulting from the mineralisation of laurate was bioaccumulated in the calcified exoskeleton of *Hyalella azteca* consequently leading to an overestimation of tissue concentrations, if accumulated compound was simply measured as total radioactivity and not further fractionated. The calculated BCF estimates differ from the one described in the literature for zebrafish.

The results show that bioaccumulation of laurate varies between *H. azteca* and zebrafish. The mechanisms involved in the bioconcentration of the anionic compound seem to be more complex and the results cannot be extrapolated to other species without further research. Special care is required for *Hyalella* bioaccumulation studies with ^{14}C-labelled compounds which are rapidly mineralised.