Bioaccumulation of triclosan in great pond snails from a pond mesocosm study

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Introduction

Triclosan (TCS, 5-chloro-2-(2,4-dichlorophenoxy) phenol) is a frequently used ingredient of everyday articles such as detergents, toothpaste, cosmetics, children's toys, and antibacterial textiles owing to its antibacterial properties. TCS is highly toxic to algae and various microbial species. There is indication of endocrine disruption [1]. Both TCS and its metabolite methyltriclosan (MTCS) are considered bioaccumulative in aquatic organisms [2]. While data for bioaccumulation of TCS in fish [2, 3] are available, empirical findings on bioaccumulation of MTCS and TCS in other taxa are scarce.

A combined fate and effect pond mesocosm study was carried out by the German Federal Environment Agency (UBA). After single dosing to the free water, concentrations of TCS and metabolite MTCS were measured for 120 days in aufwuchs, macrophytes, and snails (*Lymnaea stagnalis*). Even though conditions were not steady-state, the comparison with the measured concentrations in the water allowed for realistic calculations of the bio-accumulation factor (BAF) for both the great pond snail and major part of their diet namely aufwuchs.

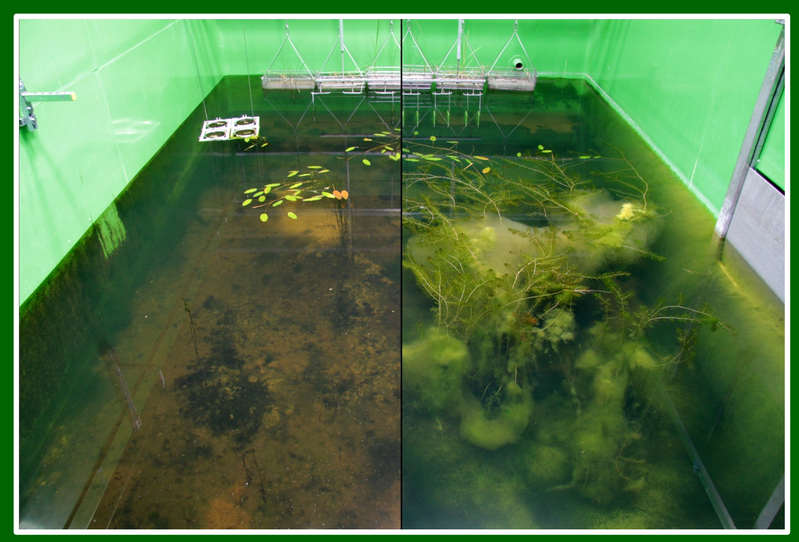

Fig. 1: Pond 778 µg/L 42 d after application of triclosan (left), control (right)

Materials & Methods

Design

Mesocosms:	8 ponds (L6.9 x W3.3 x H2.5m)
Water volume:	21 m^3
Light:	HQI, 13.000 Lux
Ground:	sand covered with natural fine sediment (Lake Schmachter,D)
Macrophytes:	*Myriophyllum spicatum, Potamogeton natans*
Snails:	*Lymnaea stagnalis*
TCS Application:	by spraygun (Fig. 2, 3), solvent: 50 mL ethanol
Concentration:	2 controls, triclosan: 0.1 / 0.6 / 3.6 / 21.6 / 129 / 778 µg/L
Duration:	120 d

Experimental conditions

Water temperature:	16.3 – 22.1°C (T_{avg}: 19.2°C)
pH:	7.5 -9.3 (778 µg/L: 8.0 – 9.9)
Electric conductivity$_{avg}$:	490-506 (778µg/L: 561) µS/cm

Calculations

BAF(non steady state) = $c_{Fresh\ weight\ biota}(t) / c_{Water}(t)$

Analysis of snails and water

0.2 g sample, freeze-dried and homogenized; addition of ^{13}C-TCS and ^{13}C-MTCS as internal standards

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Addtion of 2 g Celite 577® (filter aid)

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Extraction with isopropanol

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Centrifugation, filtration, and evaporation; 6-7 days storage (aging, coagulation of the peptides)

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Extraction with hexane and evaporation

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Derivatisation with acetic anhydride/tertiary butyl methyl ether; redissolution in i-octane

↓

GC-MS with electron ionisation (EI)

Water: 1L water sample; additon of ^{13}C-TCS und ^{13}C-MTCS as internal standards; acidification to pH 2; solid phase extraction (RP-ENV); elution with 5 mL (1 mL and 2x2.5 mL) acetone; addition of squalane; evaporation und redissolution in 100 µL DBOFB (0,2 mg/L in ethylacetate); GC-MS (EI).

Results

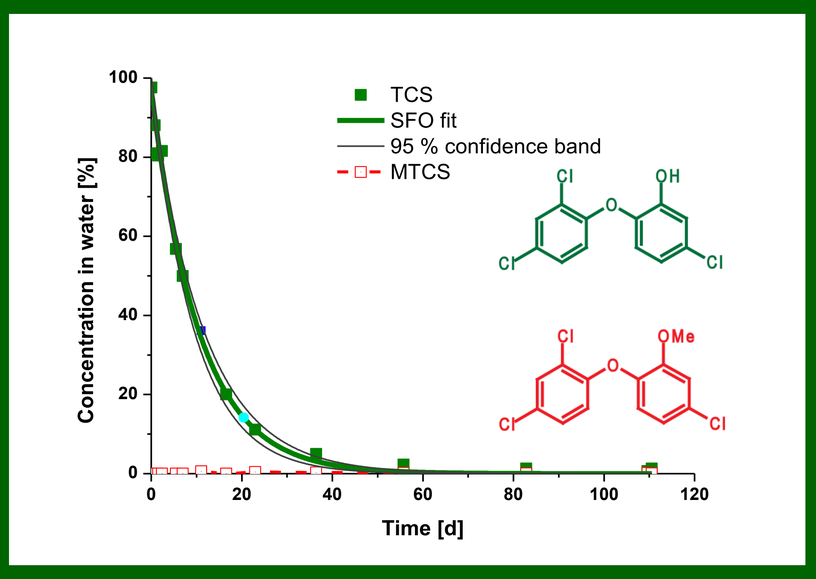

Fig. 2: TCS and its metabolite MTCS in water (Pond 21.6 µg/L)

	BC(A)F	Species
TCS	≤ 4157	*Danio rerio* [2]
	≤ 7900	*Danio rerio* [4]
	(BAF) 500	*Helisoma trivolis* [5]
MTCS	≤ 5200	Diff. species of fish [3]
	(BAF) 1200	*Helisoma trivolis* [5]
	(BAF) 1600	*Cladophora* spp [5]

Tab. 1: BCF and BAF for different trophic levels

BAF

Fig. 3: TCS , MTCS in biota (Pond 21.6 µg/L)

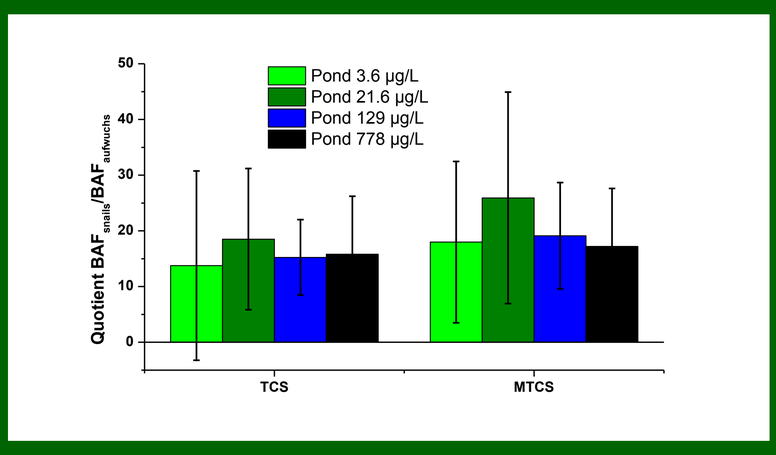

Fig. 4: Enrichment factor in relation to food, calculated as BAF ∅ $_{Snails}$/BAF∅ $_{Aufwuchs}$

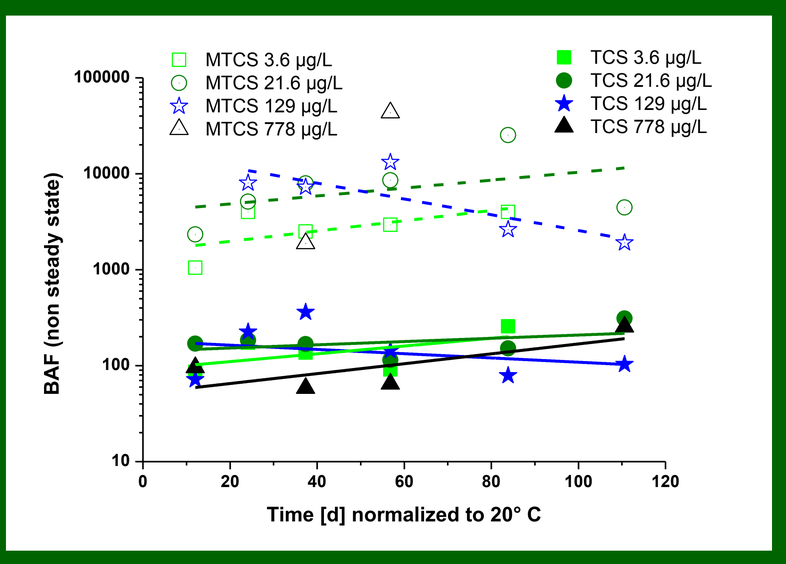

Fig. 5: BAF of TCS und MTCS in great pond snails over time; lines are adjusted linearly (MTCS dashed lines)

Nom. Konz. µg/L	TCS BAF ∅	TCS BAF max.	MTCS BAF ∅	MTCS BAF max.
778	126	254		(43,382)*
129	182	361	6,592	13,173
21.6	186	311	10,288	25,347
3.6	164	256	3,362	4,003

*Tab. 2: BAF (average day 20-120) and BAF$_{max}$ of TCS and MTCS in great pond snails; * max. of 2 values*

Discussion

- Concentration of TCS in biota decreased rapidly and followed the concentration in water (Fig. 2, 3).
- Only low concentrations of MTCS were detectable in water (Fig. 2); in pond 778 µg/L mainly < LoQ.
- BAF after day 20 was surprisingly constant despite decreasing TCS concentrations in water (Fig. 5).
- Cascading enrichment of MTCS from water to aufwuchs (BAF max. 880) and finally to snails.
- Increase from aufwuchs to snails by factors of 16 (TCS) and 20 (MTCS) (Fig. 4) likely due to biomagnification.
- Moderate accumulation of TCS in biota (BAF max. 361; Tab. 2); for comparison to literature data see Tab. 1.
- The transformation product MTCS was strongly bioaccumulated in snails with BAF up to 25,000 (Fig. 5, Tab. 2) also in comparison to literature data (Tab.1).

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Literature:

[1] Veldhoen N, Skirrow RC, Osachoff H, *et al.* (2006): *Aquat. Toxicol.* **80** (3): 217–27.
[2] Revised Environmental Fate Science Chapter for the Triclosan Reregistration Eligibility Decision (RED) Document; US EPA 2008.
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[4] Schettgen et al. (2000); Organohalogen Compounds 43, 49-52.
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