Introduction
Triclosan (TCS, 5-chloro-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.

Materials & Methods

Design
Mesocosms: 8 ponds (L.6.9 x W.3.3 x H.2.5m)
Water volume: 21 m³
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 (Tavg: 19.2°C)
pH: 7.5 – 9.3 (778 µg/L; 8.0 – 9.9)
Electric conductivity: 490-506 (778µg/L: 561) µS/cm

Calculations
BAF(non steady state) = Cfresh weight (total) / Cwater (f)

Analysis of snails and water
0.2 g sample, freeze-dried and homogenized; addition of 13C-TCS and 13C-MTCS as internal standards
Addition of 2 g Cellite 577® (filter aid)
Extraction with isopropanol
Centrifugation, filtration, and evaporation; 6-7 days storage (aging, coagulation of the peptides)

Extraction with hexane and evaporation
Derivatisation with acetic anhydride/tertiary butyl methyl ether; redissolution in i-octane

GC-MS with electron ionisation (EI)

Water: 1L water sample; addition of 13C-TCS and 13C-MTCS as internal standards; acidification to pH 2; solid phase extraction (RP-ENV); elution with 5 mL (1 mL and 2x2.5 mL) aceton; addition of squalane; evaporation and redissolution in 100 µL DSOFB (0.2 mg/L in ethylacetate); GC-MS (EI).

Results

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

Table 1: BAF and BAF for different trophic levels

| Species | BAF | Data
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>TCS</td>
<td>0.1</td>
<td>Pond 778 µg/L</td>
</tr>
<tr>
<td>TCS</td>
<td>0.6</td>
<td>Pond 21.6 µg/L</td>
</tr>
<tr>
<td>TCS</td>
<td>3.6</td>
<td>Pond 129 µg/L</td>
</tr>
<tr>
<td>TCS</td>
<td>21.6</td>
<td>Pond 778 µg/L</td>
</tr>
<tr>
<td>MTCS</td>
<td>0.15</td>
<td>Pond 778 µg/L</td>
</tr>
<tr>
<td>MTCS</td>
<td>0.3</td>
<td>Pond 129 µg/L</td>
</tr>
<tr>
<td>MTCS</td>
<td>0.6</td>
<td>Pond 21.6 µg/L</td>
</tr>
<tr>
<td>MTCS</td>
<td>0.7</td>
<td>Pond 778 µg/L</td>
</tr>
</tbody>
</table>

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

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 TCS from water to aufwuchs (BAF max. 880) and finally to snails.

BAF after day 20 was surprisingly constant despite decreasing TCS concentrations in water (Fig. 5).

Fig. 4: Enrichment factor in relation to food, calculated as BAF_TCS/BAF_MCTS

Tab. 2: BAF in great pond snails over time; lines are adjusted linearly (MTCS dashed lines)

<table>
<thead>
<tr>
<th>Nom, µg/L</th>
<th>TCS BAFl 0</th>
<th>TCS BAFl max.</th>
<th>MTCS BAFl</th>
<th>MTCS BAFl max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>778</td>
<td>300</td>
<td>126</td>
<td>254</td>
<td>(43,382)*</td>
</tr>
<tr>
<td>129</td>
<td>380</td>
<td>182</td>
<td>361</td>
<td>6,592</td>
</tr>
<tr>
<td>21.6</td>
<td>77.5</td>
<td>186</td>
<td>311</td>
<td>10,286</td>
</tr>
<tr>
<td>3.6</td>
<td>15.0</td>
<td>184</td>
<td>256</td>
<td>3,762</td>
</tr>
</tbody>
</table>

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

• 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).

Literature:

Thanks to:

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