Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment Microcosm

Peng, F-J., Ying, G. G., Pan, C. G., Selck, H., Salvito, D., & van den Brink, P. J.

Bioaccumulation and biotransformation of triclosan and galaxolide in the freshwater oligochaete *Limnodrilus hoffmeisteri* in a water/sediment microcosm

Feng-Jiao Peng [†], Guang-Guo Ying [‡, *], Chang-Gui Pan [§], Henriette Selck [Φ], Daniel Salvito [δ], Paul J. Van den Brink [†, ξ, *]

[†]Aquatic Ecology and Water Quality Management group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands

[‡]The Environmental Research Institute, MOE Key Laboratory of Environmental Theoretical Chemistry, South China Normal University, Guangzhou 510006, China

[§]School of Marine Sciences, Guangxi University, Nanning 530004, China

[Φ]Department of Science and Environment, Roskilde University, Universitetsvej 1, Denmark

[δ]Research Institute for Fragrance Materials, 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

[ξ]Wageningen Environmental Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands

* Corresponding author.

Email address: guangguo.ying@gmail.com; guangguo.ying@m.scnu.edu.cn

Email address: paul.vandenbrink@wur.nl

ABSTRACT: Personal care products are widely used in our daily life in considerable quantities and discharged through the down-the-drain route to the aquatic environments, resulting in potential risks to aquatic organisms. We investigated bioaccumulation and biotransformation of two widely used personal care products, triclosan (TCS) and galaxolide (HHCB) spiked to sediment, in the oligochaete worm *Limnodrilus hoffmeisteri* in water/sediment microcosms. After 7 days of sediment exposure to 3.1 μg TCS or HHCB /g dry weight (dw) sediment, the accumulation of TCS and HHCB in *L. hoffmeisteri* reached equilibrium, at which point the biota-sediment accumulation factors (BSAFs) were 2.07 and 2.50 for TCS and HHCB, respectively. The presence of *L. hoffmeisteri* significantly accelerated the dissipation of TCS and HHCB in the microcosms, with approximately 9.03% and 2.90% of TCS and HHCB eliminated from the water-sediment systems after 14 d exposure in presence of worms, respectively. Two biotransformation products, methyl triclosan and triclosan-O-sulfate, were identified for TCS in the worm tissue, whereas only methyl triclosan was identified in the sediment. Unlike TCS, no evidence of biotransformation products was found for HHCB in either worm tissue or sediment. These experiments demonstrate that *L. hoffmeisteri* biotransformed TCS through methylation and sulfation, whereas HHCB biotransformation was undetectable.

INTRODUCTION

Personal care products (PCPs) are widely used in our daily life and can be a potential risk to the aquatic environment due to their incomplete removal in wastewater treatment plants (WWTPs) and negative effects on aquatic ecosystems.[1] Sediments may act as ‘sinks’ and long-term reservoirs for hydrophobic PCPs released into the aquatic environment.[2] Those hydrophobic PCPs can accumulate in aquatic organisms and may cause bio-magnification through dietary transfer in the food web,[3] or may potentially be biotransformed as observed in

annelid worm *Capitella teleta*, important for sediment biogeochemistry and sediment-associated contaminant turnover,[4] exposed to sediment-associated acetyl cedrene[5], thereby reducing the body burden. Oligochaete worms prevail in aquatic environments worldwide and are exposed to sediment-associated hydrophobic PCPs. However, little is known about their potential to biotransform these chemicals.

Triclosan (TCS) and galaxolide (HHCB) are two ingredients widely used in personal care products and are ubiquitous in a variety of aquatic environments.[6] For example, our chemical monitoring results show that TCS and HHCB were the most frequently detected hydrophobic chemicals used in personal care products in the subtropical urban rivers, with concentrations up to 1 μg/g dw.[7] With their hydrophobic nature, these two chemicals may sorb to settling particles and bio-accumulate in deposit-feeding macroinvertebrates.[8, 9] To date, laboratory degradation studies of TCS and HHCB have been limited to soil bacterial cultures,[10] wastewater microorganisms,[11] fungi,[12, 13] diatom,[14] algae,[15] activated sludge[16-19] and iron and manganese oxides.[20] For example, TCS can be transformed into methyl triclosan (Me-TCS) in activated sludge under aerobic conditions[16] and in biosolid-amended agricultural soil by microorganisms[21] or earthworms.[22] Similarly, the biological oxidation of HHCB into HHCB-lactone has been reported in wastewater treatment processes[23] and fish samples.[24] However, little to no research has been performed to investigate their degradation under more ecologically realistic conditions, such as water/sediment systems with the presence of oligochaete worms that may efficiently biotransform organic contaminants. For example, *Lumbriculus variegatus* (Oligochaeta) was reported to biotransform pyrene into 1-hydroxypyrene.[25]

Oligochaete worms are an important group of freshwater benthic macroinvertebrates, ubiquitous and abundant in sediments of freshwater ecosystems, such as rivers, ponds and lakes.[26] They are thus widely used to evaluate the toxicity and accumulation of sediment-

associated hydrophobic organic contaminants.[27-29] *Limnodrilus hoffmeisteri* (Naididae, Oligochaeta) is the dominant taxon within oligochaete worms in the Pearl River (South China), and it can achieve a density of up to 50.000 ind./m^2.[30] Our recent biological monitoring also demonstrated that *L. hoffmeisteri* was the predominant benthic macroinvertebrates identified in six urban rivers of Guangzhou City, South China. As conveyor-belt feeder, *L. hoffmeisteri* ingests small particles in sediments and egests them as faecal pellets on the sediment surface.[31, 32] Sediment-associated hydrophobic organic contaminants may go through bioaccumulation and biotransformation in the body of *L. hoffmeisteri*,[33] thereby influencing the fate of chemicals in environment. During the biotransformation process, both more water-soluble and more hydrophobic products can be produced. For example, 2,4-dichlorophenol, a metabolite of TCS, is more water-soluble but less toxic than its parent compound.[11, 34] However, Macherius et al.[22] reported that *Eisenia fetida* can biotransform TCS into Me-TCS that is more environmentally persistent, lipophilic[35, 36] and toxic to *Vibrio fischeri* than TCS.[37] Although both TCS and HHCB are hydrophobic chemicals, they have different physicochemical properties. TCS is an ionizable compound with water solubility of 10 mg/L and a log octanol-water partition coefficient (Kow) of 4.8, whereas HHCB is a non-ionizable compound with water solubility of 1.75 mg/L and a log Kow of 5.9. [38] Besides, they have different steric configuration and molecular size. As such, TCS and HHCB are likely to show different bioaccumulation and biotransformation in oligochaete *L. hoffmeisteri*.[39, 40] This, however, has not been studied thus far, despite the importance of understanding the metabolic pathway of TCS and HHCB for evaluation of their persistence and risk in the environment.

This study aims to evaluate the importance of *L. hoffmeisteri* in the dissipation of sediment-associated TCS and HHCB in microcosms simulating static water systems. The microcosms were divided into two treatment groups: with and without addition of *L.*

hoffmeisteri. The exposures lasted for 14 d, and worms were sampled on day 1, 3, 7, 10 and 14 to investigate the bioaccumulation kinetics of TCS and HHCB. Our results will improve the understanding of the dissipation kinetics of TCS and HHCB in a water/sediment system, and the accumulation and biotransformation of sediment-associated TCS and HHCB in *L. hoffmeisteri*.

MATERIALS AND METHODS

Standards and Reagents. Standards of triclosan (TCS), methyl triclosan, 2,4-dichlorophenol, 4-chlorocatechol, and d_3-Tonalide (d_3-AHTN) were purchased from Dr. Ehrensorfer (Germany), while triclosan-O-β-D-glucuronide sodium salt (TCSG), triclosan-O-sulfate sodium salt and galaxolidone (HHCB-lactone) were obtained from TRC (Canada). The standard galaxolide (HHCB; 1,3,4,6,7,8-hexahydro-4,6,6, 7, 8, 8-hexamethyl cyclopenta (g)-2-benzopyran) was kindly provided by International Flavors & Fragrances (USA), containing about 10% HHCB-lactone, a technical product.[23] The internal standards $^{13}C_{12}$-triclosan and $^{13}C_{12}$-methyl triclosan were obtained from Cambridge Isotope Laboratories (Andover, USA). Sylon BTZ containing trimethylchlorosilane, N,O-bis(trimethylsilyl) acetamide, and N-trimethylsilylimidazole was obtained from Supelco. Further details are provided in the Supporting Information (Text S1).

Test sediment and spiking. The experimental sediment was collected from an uncontaminated reservoir (113˚47'42"N, 23˚46'01"E) [7, 41], a drinking water source of Guangzhou city (South China). The natural sediment was wet-sieved (300 μm) with deionized water, and then allowed to settle overnight. After removing the overlying water, the resultant sediment was kept frozen at -20 °C until use. The sediments used in the microcosms consisted of 0.49% sand, 40.82% silt, and 58.69% clay, and they had a water content of 57% (24 h at 105 °C; n = 4), an organic matter (OM) content of 20.6‰, a total nitrogen (TN) content of

1.45‰, a total phosphorus (TP) content of 0.45‰ and an ammonia (NH_4^+) content of 0.11‰.[42] The background TCS and HHCB concentrations in the sediment were around 0.002 µg/g dry weight (dw), and considered negligible for the purposes of this study. Before chemical application, sediment was thawed at 27 ± 1 °C in the dark and rinsed with Milli-Q water.

To spike each test compound into sediment, 15 g wet sediment was weighed into a centrifuge tube (50 mL), producing a sediment height of approximately 2.5 cm, and amended with 10 µL of TCS or HHCB stock solution to achieve a final concentration of 3.1 µg/g dw sed. It should be noted that the presence of HHCB-lactone in the HHCB stock solution resulted in a spiked HHCB-lactone concentration of 0.34 µg/g dw in the spiked sediment. Two controls were used in the experiments: a water control and an acetone control, which were created by replacing the chemical solution with the same volume of Milli-Q water and acetone, respectively. Tubes were wrapped with aluminium foil to minimize photolysis of TCS and HHCB. After 15 min of solvent evaporation in the fume hood under darkness, each tube was vortexed for 5 min and then shaken on a horizontal shaker for 12 h in the dark at 16 °C to achieve homogeneity.

Test Organisms. The *L. hoffmeisteri* was obtained from an aquarium market (Guangzhou, South China). It was acclimatized in a 18-L glass tank containing aerated deionized water and thawed sediment (27 ± 1 °C, dark). The acclimatization phase lasted three weeks before the start of exposure. The culturing water and sediment were renewed once during the acclimatization. The total lipids were extracted with acetone/hexane (1/1, v/v) and quantified gravimetrically.[43] TCS, Me-TCS, HHCB and HHCB-lactone concentrations were below the method quantification limits (MQLs) in the unexposed worm tissue. Therefore, worms used here were suitable for the purposes of this study.

Experimental Design. TCS and HHCB biotransformation experiments were performed separately in water/sediment microcosms. After sediment spiking, 30 mL of aerated Milli-Q water was gently pipetted into each glass vial along the wall, and stored at 4 °C in the dark for 2 days to enable potential suspended particles to settle down. Then 30 *L. hoffmeisteri* (length: 20.48±3.17 mm; width: 0.375±0.032 mm; wet weight: 0.0021±0.0006 g) at larval stage were introduced into each tube belonging to system with worms. A parallel set of vials without worms were also included to assess microbial degradation. Constant gentle aeration was provided through a glass Pasteur pipette in each tube of both systems at the water surface. Microcosms were incubated statically at 27±1 °C in the dark. The experiment ran for 14 d. During the exposure period, no food was added into the microcosms as worms live on the organic matter associated with the sediment particles. As such, the exposure used here cannot last for a long period. Nevertheless, 14-d exposure is enough for the purpose of studying the bioaccumulation and biotransformation of chemicals in the worm tissue, as demonstrated by the degradation of acetyl cedrene by *C. teleta*.[5] Water evaporation was minimized by covering the tubes with parafilm during the exposure period. According to our previous experience, the evaporation was negligible after 14 d culturing. To measure the abiotic loss of TCS and HHCB during the exposure period, blanks were prepared by adding 30 mL of aerated Milli-Q water containing TCS or HHCB at concentration of 2 µg/L into microcosms. All experiments were performed in four replicates, thus there were 72 tubes in total for each experiment. To analyse bioaccumulation and dissipation kinetics of TCS or HHCB, 8 tubes were sacrificed on days 0, 1, 3, 7, 10 and 14, respectively. Blank and control vessels were sacrificed only at the start and end of exposure. The TCS and HHCB concentrations in the water phase were determined only at the start of exposure. As Me-TCS and HHCB-lactone have been reported as the main product of TCS[22] and HHCB[12], their concentrations were also measured on each sampling date in the worms and water-sediment phases. The pH value in the overlying water

was measured at the start and end of exposure. The biotransformation products were determined on days 0, 7 and 14 in worm tissue and in water-sediment phases.

Sample Pre-treatment. Tubes from system without worms were directly frozen (-20 °C) until lyophilization. Tubes from system with worms were gently vortexed, the resultant water-sediment mixture was then sieved (300 µm). Worms were transferred to glass beakers with 400 mL of aerated tap water, left to depurate overnight, weighted into a 50-mL polypropylene centrifuge tube for chemical extraction. Water and sediment were separated by centrifugation at 4000 rpm. The resultant water phase was immediately filtered through 0.7-µm glass fibre filters, combined with the above tap water, diluted to 1000 mL and extracted using solid-phase extraction (SPE) as previously described.[44] The collected sediment and filters were frozen (-20 °C), lyophilized and stored at 4 °C in the dark until extraction. The detailed explanation for SPE is given in the supporting information (Text S2).

Sediment samples were extracted by ultrasonic extraction combined with purification by SPE cartridges. Briefly, 15 mL of methanol (for TCS extraction) or acetone/dichloromethane (1:1, v/v) (for HHCB extraction) was added into each tube with dry sediment, vortexed for 5 min and further shaken on a horizontal shaker for 2.5 h at 16 °C to thoroughly mix the sediment and solvent. Samples were then extracted in an ultrasonic bath for 0.5 h, and centrifuged at 3000 rpm for 10 min. The clear supernatant was transferred to a 300-mL flat-bottomed flask using a glass pipette. The extraction procedure was repeated three times. For the fourth extraction of TCS, 15 mL of methanol containing 0.1 % (v/v) formic acid was used as extraction solvent. Extraction procedures for particles on the filters were the same as the sediment samples. The supernatants of the sediment and filter from the same microcosm were combined, allowed to evaporate at 37 °C to about 20 mL for TCS whereas to almost dry and reconstituted in 20-mL methanol for HHCB, and diluted with Milli-Q water to a volume of

300 mL. Each diluted extract was then purified and enriched on an Oasis HLB cartridge (200 mg, 6 mL) using the same procedures for the extraction of water samples.

Worms in the tubes were first spiked with 100 ng of d_3-AHTN in case of HHCB samples and 100 ng of $^{13}C_{12}$-triclosan and $^{13}C_{12}$-methyl triclosan in case of TCS samples, vortexed for 30 s, and equilibrated at 4 °C for 30 min. Worm tissue was then homogenized in 4 mL of acetonitrile with two ceramic homogenizers. The homogenates were ultra-sonicated (30 min, 20 °C) and centrifuged (10 min, 4000 rpm). The clear supernatants were transferred to 15 mL d-SPE tubes containing 900 mg anhydrous $MgSO_4$, 150 mg PSA, and 150 mg C_{18} to remove lipids. The extraction procedure was repeated twice for each sample. In the third extraction, acetonitrile was replaced by acetonitrile containing 0.2 % acetic acid. The d-SPE tubes containing supernatants were shaken for 2 min and centrifuged (15 min, 4000 rpm). The final supernatants were transferred to 15-mL glass tubes, dried under gentle nitrogen stream, re-dissolved in 1 mL of methanol, filtered through 0.22-μm membrane filters into 2-mL amber glass vials and stored at -20 °C until instrumental analysis.

To identify biotransformation products of HHCB, the extracts were derivatized following the procedure described by Martin et al.[12] The details of derivatization are given in the supporting information (Text S3).

Instrumental Analysis. TCS in the extracts was quantified using an Agilent 1200 high performance liquid chromatograph (Agilent, USA) coupled to an Agilent 6460 triple quadrupole mass spectrometer with electrospray ionization under negative ionization modes (HPLC-MS/MS, ESI-). Me-TCS, HHCB and HHCB-lactone in the extracts were determined by an Agilent 6890N gas chromatograph (Agilent, USA) connected to an Agilent 5975B MSD mass spectrometer (GC-MS), equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific Co., USA), in the selected-ion-monitoring (SIM) mode under electron-impact ionization (EI). Qualification of TCS biotransformation products

in worm tissue and sediment was performed using Waters ACQUITY UPLC-I Class with Xevo G2-XS QTOF, whereas Agilent 7250 GC/Q-TOF was used to analyse biotransformation products of HHCB. The detailed procedures used for the quantitative and qualitative analysis are provided in the supporting information (Text S4).

Quality Assurance, Quality Control, and Data Analysis. Solvent blanks and procedural blanks were determined successively for each batch of samples to check background contamination and ensure the performance of the analytical procedure. The MQLs were defined as 10 times the ratio of the signal to instrument noise (Table S1). The recoveries of TCS, Me-TCS, HHCB and HHCB-lactone in each compartment were separately assessed by spiking a standard solution at three levels (0.1, 0.5, and 2) in clean Milli-Q water (µg/L), sediment (µg/g dw), sediment particles on the filters (µg/g dw) and worm tissue (µg/g ww), respectively. All recoveries were in the range of 60% to 110% (Table S2). Concentration data below MQLs were treated as not detected (ND). TCS, Me-TCS, HHCB and HHCB-lactone concentrations were below the MQLs in the clean Milli-Q water and worm tissue in the controls at the end of experiment.

The dissipation kinetics of TCS and HHCB in the water/sediment systems were described using both zero-order and first-order kinetic models. For zero-order kinetic model, $C_{(t)} = C_{(t=0)} - kt$ and half-life $t_{1/2} = C_{(t=0)} / 2k$; for first-order kinetic model, $C_{(t)} = C_{(t=0)} \times \exp^{(-kt)}$ and half-life $t_{1/2} = \ln(2) / k$, where $C_{(t)}$ (µg/g dw) is the TCS or HHCB concentration in the sediment at sampling time t (days) and k is the elimination rate constant.

The biota-sediment-accumulation-factor (BSAF) was calculated at each sampling point using the following equation:[45] $BSAF = (C_o / f_l) / (C_s / f_{OM})$, where C_o is the chemical concentration in the organism (µg/g wet weight (ww)) at each sampling point, f_l is the lipid fraction of the organism (g lipid/g ww) at the start of exposure, C_s is the chemical

concentration in the sediment (μg/g dw) at the corresponding sampling point, and f_{OM} is the organic matter fraction of the sediment (g organic matter/g dw) at the start of exposure.

Statistical analyses were performed with the software SPSS Statistics (Ver 23.0.0). Two-way ANOVA (factors: presence of *L. hoffmeisteri* and sampling time) with Tukey's multiple comparison tests was used to determine the statistical differences in the chemicals concentrations between systems with and without worms or among sampling dates. Data were checked for normality and variance homogeneity with Shapiro-Wilk test and Levene's test, respectively. Statistical significance was accepted at $p < 0.05$ level.

RESULTS

Concentrations and Dissipation Kinetics of TCS and HHCB in the Microcosms. The concentrations of TCS and HHCB in the sediment were measured on days 0, 1, 3, 7, 10 and 14 and are shown in Table S3 and Figure 1. TCS and HHCB concentrations in the blank samples remained at 2 μg/L during the 14 d incubation period. At the start of exposure, TCS and HHCB concentrations in the water phase were 0.59 μg/L and 0.48 μg/L, respectively. Over the course of experiment, both TCS and HHCB gradually disappeared from the microcosms. However, TCS dissipated faster than HHCB, as demonstrated by greater negative slopes of TCS relative to HHCB in the zero-order model (Figure 1A and B). After 14 d exposure, the TCS concentrations decreased from 3.1 μg/g dw to 2.8 μg/g dw (9.03%) and 3.0 μg/g dw (3.23%) in systems with and without worms, respectively (Table S3). The HHCB concentrations declined slightly from 3.10 μg/g dw to 3.02 μg/g dw (2.90%) and 3.08 μg/g dw (0.65%) in systems with and without worms, respectively (Table S3). Furthermore, there was a significant difference in TCS and HHCB concentrations between systems with and without worms (two-way ANOVA, $p < 0.05$). Compared to the original spiked concentration,

TCS and HHCB concentrations significantly decreased from day 3 and 10 onwards, respectively (two-way ANOVA, $p < 0.05$).

Dissipation data of TCS and HHCB fitted well to both zero-order and first-order reaction kinetic models in both systems with and without worms (Figure 1). Under zero-order model (Figure 1A and B), estimated $t_{1/2}$ values for TCS were 79 d and 218 d, and for HHCB were 320 d and 1105 d in systems with and without worms, respectively. However, under first-order model (Figure 1C and D), estimated $t_{1/2}$ values for TCS were 103 d and 301 d, and for HHCB were 433 d and 1386 d in systems with and without worms, respectively.

Identification of Biotransformation Products in the Sediment. The concentrations of Me-TCS increased in both systems during the exposure period, with significantly higher concentrations in systems with than without worm presence (two-way ANOVA, $p < 0.05$) (Table S3 and Figure 2). HHCB-lactone concentration remained at similar levels throughout the exposure period in both systems (two-way ANOVA, $p > 0.05$), with values around the initial spiked concentration, i.e. 0.34 µg/g dw (Figure 2). However, after 14 d exposure, the final HHCB-lactone concentration was slightly lower in the system with (~0.33 µg/g dw) than without (~0.34 µg/g dw) worms (Table S3). Me-TCS concentrations significantly increased from day 7 onwards (two-way ANOVA, $p < 0.05$), whereas there was no significant difference in HHCB-lactone between sampling dates (two-way ANOVA, $p > 0.05$). No other products were found for TCS or HHCB in the sediment by UPLC- QTOF and GC-QTOF, respectively.

Bioaccumulation and Biotransformation Products of TCS and HHCB in the Worm Tissue. The lipid content of *L. hoffmeisteri* was 2.26% ww. During the 14 d exposure period, there was no mortality of *L. hoffmeisteri* in any treatments. The pH was around 6.6 in the overlying water at the start and end of exposure. TCS and HHCB concentrations showed similar change trends in the worm tissue, i.e. increasing from day 0 to day 3 and remaining

stable from day 7 onwards (Figure 3). After 1 d exposure, the TCS and HHCB concentrations were 2.4 µg/g ww and 6.5 µg/g ww, respectively. After 3 d exposure, the TCS and HHCB concentrations reached 6.5 and 8.4 µg/g ww, respectively (Table S3 and Figure 3). The BSAF values of TCS and HHCB were in the range of 0.70 to 2.07 and 1.84 to 2.50 during the exposure period, respectively (Table S3).

As was observed in the sediment, Me-TCS and HHCB-lactone were also detected in the worm tissue, with concentrations in the range of 0.06-0.15 µg/g ww and 0.70-0.81 µg/g ww, respectively (Table S3 and Figure 3). Moreover, the results of mass balance show that HHCB-lactone accumulation in worms was responsible for the loss of HHCB-lactone in systems with worm presence. Me-TCS and HHCB-lactone concentrations reached the steady state in *L. hoffmeisteri* on day 7 and day 10, respectively.

In addition to Me-TCS, triclosan-O-sulfate (TCS-O-sulfate) was detected by LC-Q-TOF (Figure 4 and Figure S1). The identification of TCS-O-sulfate was further confirmed by its authentic standard. However, no biotransformation products were identified for HHCB in the worm tissue by GC-QTOF, except for HHCB-lactone.

DISCUSSION

This study showed that the TCS and HHCB dissipation in the microcosms fitted well to both zero-order and first-order reaction kinetics models. Likewise, fitting to both models has been reported for TCS[16, 19, 46] and HHCB[12] dissipation by biosolids-amended soil microorganisms and fungi, respectively. TCS dissipated slowly in systems without worm presence with a $t_{1/2}$ value of 218 d (zero-order model) or 301 d (first-order model). While these values are larger than the $t_{1/2}$ value of 58 d detected in the pond water-silty clay loam sediment system under aerobic conditions[47], they are comparable to the $t_{1/2}$ value of 239 d in the lake water-silty clay sediment system with dissolved oxygen levels above 3 mg/L.[48] These

differences are likely related to different microbial communities and sediment properties including organic matter and clay content between studies.[21, 49, 50] In addition, although in this study the oxygen was supplied in the overlying water during the incubation period, the sediment in systems without worms was likely under reducing condition due to the microbial respiration[51] and lack of bioturbation, which might hamper the dissipation of TCS because TCS dissipated faster under aerobic than anaerobic conditions.[9, 10] The estimated $t_{1/2}$ for sediment-associated HHCB were > 300 d in both systems under both kinetics models, suggesting that HHCB was persistent in the water/sediment system under the conditions in the present study. However, in the EU Risk Assessment Report (EU RAR) for HHCB, $t_{1/2}$ of 79 d in the sediment was deemed most relevant for modelling the fate of HHCB in sediment using the European Union System for the Evaluation of Substances (EUSES) model.[52] These differences could be attributed to differences in sediment properties, microbial communities and exposure scenarios. Under both kinetics models, the estimated $t_{1/2}$ values of HHCB were ~3 and ~4 times longer than those of TCS in systems with and without the presence of worms, respectively, indicating that HHCB was more persistent than TCS in the water-sediment system. Likewise, a longer $t_{1/2}$ value of HHCB (900 d[53]) relative to TCS (258 d[53] and 107 d[21]) has been reported in biosolid-amended soils in field. However, a faster dissipation of HHCB in biosolid-amended soils has been described by DiFrancesco et al.[54] The corresponding $t_{1/2}$ values were 141 and 144 d in the spiked and unspiked biosolids-amended soils, respectively.[52] The dissipation of TCS and HHCB were faster in systems with than without worms, suggesting that *L. hoffmeisteri* stimulated the dissipation of the two hydrophobic compounds in the water/sediment systems in this study. This is likely to be associated with the bioaccumulation and biotransformation in *L. hoffmeisteri* and enhanced microbial degradation due to the sediment reworking by worms.[55, 56]

TCS, Me-TCS, HHCB and HHCB-lactone were detected in the worm tissue, with concentrations increasing from exposure day 1 to 7 and reaching the steady state from then onwards (Table S3), which indicates that *L. hoffmeisteri* can accumulate these hydrophobic compounds. Similar time to reach steady state has previously been observed for sediment-associated polybrominated diphenyl ether (PBDE) accumulation in the oligochaete *Lumbriculus variegatus* (a similar species to *L. hoffmeisteri*).[57, 58] The stabilized BSAF values of TCS (~2.07) in *L. hoffmeisteri* were larger than the 28-day BSAF value (1.4) reported by Dang et al,[59] who studied the bioaccumulation of TCS in *L. variegatus*. However, another study has reported a greater BSAF (9.04) of TCS in *L. variegatus* than the present study.[60] These differences are most likely related to differences in sediment characteristics and species traits between the studies.[60, 61] The stabilized BSAFs of HHCB were around 2.50 in *L. hoffmeisteri*, similar to the values (1.5-2.5) reported in carps from the Haihe River (China).[62] HHCB showed higher BSAF values than TCS in *L. hoffmeisteri*, which is likely associated with the lower metabolism and water solubility but higher log K_{ow} value of HHCB than TCS.[39, 40]

Me-TCS was detected in both the sediment and worm tissue whereas TCS-O-sulfate was only detected in the worm tissue. These two metabolites were products from phase II reaction, i.e., methylation and sulfation. However, no phase I (e.g., oxidation, reduction and hydrolysis reactions) products were observed in this study. This may be related to the fast transformation of phase I to phase II products, as described by Malmquist et al.[63] who investigated the biotransformation of pyrene by the benthic invertebrate *Nereis diversicolor*. Also, analyses of the overlying water would have provided more information on the fate of phase I products. In the future work, we therefore recommend to analyse metabolites in the overlying water. Yet, the formation of Me-TCS via biological methylation has been reported for different stages of wastewater treatment plants.[16, 64] Besides, Macherius et al.[22] found that TCS was transformed

into Me-TCS by earthworms in biosolid-amended agricultural field. However, compared to TCS, Me-TCS is more persistent and also more prone to bio-accumulate in aquatic organisms.[36] The formation of TCS-O-sulfate has been reported in activated sludge,[17] plants[65], rats[66] and human urine[67].

Unlike TCS, no products were identified for HHCB. Although HHCB-lactone was detected in both sediment and worm tissue, the results of mass balance show that the presence of HHCB-lactone was due to the spiking rather than HHCB degradation by microorganisms or worms. HHCB-lactone has been reported as a HHCB degradation metabolite for activated sludge[8] and cultures of fungi such as *Myrioconium* sp.[12] and *Phanerochaete chrysosporium*.[8] However, our results demonstrate that *L. hoffmeisteri* and microorganisms in the sediment did not degrade HHCB or HHCB-lactone to a measurable degree. Unlike HHCB, Dai et al.[5] found that after 14 days of exposure sediment-associated acetyl cedrene (another fragrance material) was reduced by 88-99% and 13-31% in the sediment with and without *C. teleta*, respectively. However, another study reported that acetyl cedrene in the sediment decreased 72% in both treatments with and without *C. teleta* after 16 days.[68] One explanation for these findings is that the microbial activity was very low initially in the present study due to the freezing of the sediment, which would potentially decrease microbial degradation compared to a full-active microbial community in previous studies. In addition, it seems that macrofaunal biotransformation is both species- and chemical specific (e.g., Malmquist et al.[63]).

In conclusion, our results demonstrate that oligochaete worm presence significantly accelerated HHCB and TCS dissipation in water-sediment systems. *L. hoffmeisteri* either cannot or has a very low ability to biodegrade HHCB but can biotransform TCS through methylation and sulfation. However, currently little information is available for TCS-O-sulfate. Further work is therefore needed to evaluate the (eco)toxicity and persistence of TCS-O-sulfate.

ASSOCIATED CONTENT

Supporting Information

Additional information about sample preparation, instrumental analysis, measured chemical concentrations and predicted biotransformation pathways.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors would like to acknowledge the financial support from the Research Institute for Fragrance Materials and the National Natural Science Foundation of China (NSFC 41473105). We also thank application staff of Waters and Agilent in Guangzhou for their help and support with the identification of biotransformation products.

REFERENCES

1. Díaz-Cruz, M. S.; Barceló, D., Personal care products in the aquatic environment. Springer: **2015**; Vol. 36.
2. Burton, J. A. G., Sediment quality criteria in use around the world. *Limnology* **2002,** *3*, (2), 65-76.
3. Janssen, E. M. L.; Beckingham, B. A., Biological responses to activated carbon amendments in sediment remediation. *Environmental Science & Technology* **2013,** *47*, (14), 7595-7607.
4. Mendez, N.; Linke-Gamenick, I.; Forbes, V. E.; Baird, D. J., Sediment processing in Capitella spp.(Polychaeta: Capitellidae): strain-specific differences and effects of the organic toxicant fluoranthene. *Marine Biology* **2001,** *138*, (2), 311-319.
5. Dai, L.; Selck, H.; Salvito, D.; Forbes, V. E., Fate and effects of acetyl cedrene in sediments inhabited by different densities of the deposit feeder, Capitella teleta. *Environmental toxicology and chemistry* **2012,** *31*, (11), 2639-2646.
6. Ying, G.-G., Personal care products. In *Analysis of Endocrine Disrupting Compounds in Food*, Wiley-Blackwell: **2010**; pp 413-428.
7. Peng, F.-J.; Pan, C.-G.; Zhang, M.; Zhang, N.-S.; Windfeld, R.; Salvito, D.; Selck, H.; Van den Brink, P. J.; Ying, G.-G., Occurrence and ecological risk assessment of emerging organic chemicals in urban rivers: Guangzhou as a case study in China. *Science of the Total Environment* **2017,** *589*, 46-55.
8. Balk, F.; Ford, R. A., Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU: I. Fate and exposure assessment. *Toxicology Letters* **1999,** *111*, (1–2), 57-79.
9. Bedoux, G.; Roig, B.; Thomas, O.; Dupont, V.; Le Bot, B., Occurrence and toxicity of antimicrobial triclosan and by-products in the environment. *Environmental Science and Pollution Research* **2012,** *19*, (4), 1044-1065.
10. Ying, G.-G.; Yu, X.-Y.; Kookana, R. S., Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling. *Environmental Pollution* **2007,** *150*, (3), 300-305.
11. Lee, D. G.; Zhao, F.; Rezenom, Y. H.; Russell, D. H.; Chu, K.-H., Biodegradation of triclosan by a wastewater microorganism. *Water Research* **2012,** *46*, (13), 4226-4234.
12. Martin, C.; Moeder, M.; Daniel, X.; Krauss, G.; Schlosser, D., Biotransformation of the polycyclic musks HHCB and AHTN and metabolite formation by fungi occurring in freshwater environments. *Environmental Science & Technology* **2007,** *41*, (15), 5395-5402.
13. Vallecillos, L.; Sadef, Y.; Borrull, F.; Pocurull, E.; Bester, K., Degradation of synthetic fragrances by laccase-mediated system. *Journal of Hazardous Materials* **2017,** *334*, 233-243.
14. Ding, T.; Lin, K.; Yang, M.; Bao, L.; Li, J.; Yang, B.; Gan, J., Biodegradation of triclosan in diatom Navicula sp.: Kinetics, transformation products, toxicity evaluation and the effects of pH and potassium permanganate. *Journal of hazardous materials* **2018,** *344*, 200-209.
15. Wang, S.; Poon, K.; Cai, Z., Removal and metabolism of triclosan by three different microalgal species in aquatic environment. *Journal of hazardous materials* **2018,** *342*, 643-650.
16. Chen, X.; Nielsen, J. L.; Furgal, K.; Liu, Y.; Lolas, I. B.; Bester, K., Biodegradation of triclosan and formation of methyl-triclosan in activated sludge under aerobic conditions. *Chemosphere* **2011,** *84*, (4), 452-456.
17. Chen, X.; Casas, M. E.; Nielsen, J. L.; Wimmer, R.; Bester, K., Identification of triclosan-O-sulfate and other transformation products of triclosan formed by activated sludge. *Science of The Total Environment* **2015,** *505*, 39-46.

18. Armstrong, D. L.; Rice, C. P.; Ramirez, M.; Torrents, A., Influence of thermal hydrolysis-anaerobic digestion treatment of wastewater solids on concentrations of triclosan, triclocarban, and their transformation products in biosolids. *Chemosphere* **2017,** *171*, 609-616.
19. Armstrong, D. L.; Lozano, N.; Rice, C. P.; Ramirez, M.; Torrents, A., Degradation of triclosan and triclocarban and formation of transformation products in activated sludge using benchtop bioreactors. *Environmental research* **2018,** 161, 17-25.
20. Ding, J.; Su, M.; Wu, C.; Lin, K., Transformation of triclosan to 2,8-dichlorodibenzo-p-dioxin by iron and manganese oxides under near dry conditions. *Chemosphere* **2015,** *133*, 41-46.
21. Lozano, N.; Rice, C. P.; Ramirez, M.; Torrents, A., Fate of triclosan in agricultural soils after biosolid applications. *Chemosphere* **2010,** *78*, (6), 760-766.
22. Macherius, A.; Lapen, D. R.; Reemtsma, T.; Römbke, J.; Topp, E.; Coors, A., Triclocarban, triclosan and its transformation product methyl triclosan in native earthworm species four years after a commercial-scale biosolids application. *Science of The Total Environment* **2014,** *472*, 235-238.
23. Bester, K., Polycyclic musks in the Ruhr catchment area-transport, discharges of waste water, and transformations of HHCB, AHTN and HHCB-lactone. *Journal of Environmental Monitoring* **2005,** *7*, (1), 43-51.
24. Hühnerfuss, H.; Biselli, S.; Gatermann, R., Enantioselective analysis of polycyclic musks as a versatile tool for the understanding of environmental processes. *Series anthropogenic compounds* **2004**, 213-231.
25. Navarro, V. C.; Brozinski, J. M.; Leppänen, M. T.; Honkanen, J. O.; Kronberg, L.; Kukkonen, J. V., Inhibition of pyrene biotransformation by piperonyl butoxide and identification of two pyrene derivatives in *Lumbriculus variegatus* (Oligochaeta). *Environmental toxicology and chemistry* **2011,** *30*, (5), 1069-1078.
26. Vivien, R.; Tixier, G.; Lafont, M., Use of oligochaete communities for assessing the quality of sediments in watercourses of the Geneva area (Switzerland) and Artois-Picardie basin (France): proposition of heavy metal toxicity thresholds. *Ecohydrology & Hydrobiology* **2014,** *14*, (2), 142-151.
27. Lotufo, G. R.; Fleeger, J. W., Toxicity of sediment-associated pyrene and phenanthrene to *Limnodrilus hoffmeisteri* (oligochaeta: Tubificidae). *Environmental toxicology and chemistry* **1996,** *15*, (9), 1508-1516.
28. Di, S.; Huang, L.; Diao, J.; Zhou, Z., Selective bioaccumulation and elimination of hexachlorocyclohexane isomers in *Tubifex tubifex* (Oligochaeta, Tubificidae). *Environmental Science and Pollution Research* **2016,** *23*, (7), 6990-6998.
29. Yang, X.; Yu, L.; Chen, Z.; Xu, M., Bioavailability of polycyclic aromatic hydrocarbons and their potential application in eco-risk assessment and source apportionment in urban river sediment. *Scientific reports* **2016,** *6*, 23134.
30. Jang, W.-X.; Lai, Z.-N.; Peng, S.-Y.; Gao, Y.; Yang, W.-N.; Pang, S.-X., Primary Study of Macroinvertebrate Community Structure in the Pearl River Guangzhou Portion. *Environmental Monitoring in China* **2011**, *5*, 020.
31. Kaster, J. L.; Klump, J. V.; Meyer, J.; Krezoski, J.; Smith, M. E., Comparison of defecation rates of *Limnodrilus hoffmeisteri* Claparede (Tubificidae) using two different methods. *Hydrobiologia* **1984,** *111*, (3), 181-184.
32. Dafoe, L. T.; Rygh, A. L.; Yang, B.; Gingras, M. K.; Pemberton, S. G., A new technique for assessing tubificid burrowing activities, and recognition of biogenic grading formed by these oligochaetes. *Palaios* **2011,** *26*, (1), 66-80.

33. Liu, J.; Qu, R.; Yan, L.; Wang, L.; Wang, Z., Evaluation of single and joint toxicity of perfluorooctane sulfonate and zinc to Limnodrilus hoffmeisteri: Acute toxicity, bioaccumulation and oxidative stress. *Journal of hazardous materials* **2016,** *301*, 342-349.
34. Yin, D.; Jin, H.; Yu, L.; Hu, S., Deriving freshwater quality criteria for 2, 4-dichlorophenol for protection of aquatic life in China. Environmental Pollution 2003, 122, (2), 217-222.
35. James, M. O.; Marth, C. J.; Rowland-Faux, L., Slow O-demethylation of methyl triclosan to triclosan, which is rapidly glucuronidated and sulfonated in channel catfish liver and intestine. *Aquatic toxicology* **2012**, 124-125, 72-82.
36. Pycke, B. F. G.; Roll, I. B.; Brownawell, B. J.; Kinney, C. A.; Furlong, E. T.; Kolpin, D. W.; Halden, R. U., Transformation products and human metabolites of triclocarban and triclosan in sewage sludge across the United States. *Environmental Science & Technology* **2014,** *48*, (14), 7881-7890.
37. Farré, M.; Asperger, D.; Kantiani, L.; González, S.; Petrovic, M.; Barceló, D., Assessment of the acute toxicity of triclosan and methyl triclosan in wastewater based on the bioluminescence inhibition of Vibrio fischeri. *Analytical and Bioanalytical Chemistry* **2008,** *390*, (8), 1999-2007.
38. EPA, U., Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. *United States Environmental Protection Agency, Washington, DC, USA* **2012**.
39. Tulp, M. T. M.; Hutzinger, O., Some thoughts on aqueous solubilities and partition coefficients of PCB, and the mathematical correlation between bioaccumulation and physico-chemical properties. *Chemosphere* **1978,** *7*, (10), 849-860.
40. Geyer, H.; Sheehan, P.; Kotzias, D.; Freitag, D.; Korte, F., Prediction of ecotoxicological behaviour of chemicals: relationship between physico-chemical properties and bioaccumulation of organic chemicals in the mussel Mytilus edulis. *Chemosphere* **1982,** *11*, (11), 1121-1134.
41. Zhao, J.-L.; Ying, G.-G.; Liu, Y.-S.; Chen, F.; Yang, J.-F.; Wang, L., Occurrence and risks of triclosan and triclocarban in the Pearl River system, South China: from source to the receiving environment. *Journal of hazardous materials* **2010,** *179*, (1), 215-222.
42. Clesceri, A.; Greenberg, A., Eaton. Standard methods for the examination of water and wastewater, 20th ed, *American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC* **1998**.
43. Bligh, E. G.; Dyer, W. J., A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology* **1959,** *37*, (8), 911-917.
44. Chen, Z.-F.; Ying, G.-G.; Lai, H.-J.; Chen, F.; Su, H.-C.; Liu, Y.-S.; Peng, F.-Q.; Zhao, J.-L., Determination of biocides in different environmental matrices by use of ultra-high-performance liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* **2012,** *404*, (10), 3175-3188.
45. Ankley, G. T.; Cook, P. M.; Carlson, A. R.; Call, D. J.; Swenson, J. A.; Corcoran, H. F.; Hoke, R. A., Bioaccumulation of PCBs from sediments by oligochaetes and fishes: comparison of laboratory and field studies. *Canadian Journal of Fisheries and Aquatic Sciences* **1992,** *49*, (10), 2080-2085.
46. Waria, M.; O'Connor, G. A.; Toor, G. S., Biodegradation of triclosan in biosolids-amended soils. *Environmental toxicology and chemistry* **2011,** *30*, (11), 2488-2496.
47. Union, E., Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. *Off J Eur Union L* **2012,** *167*, 1-116.
48. Huang, X.; Wu, C.; Hu, H.; Yu, Y.; Liu, J., Sorption and degradation of triclosan in sediments and its effect on microbes. *Ecotoxicology and Environmental Safety* **2015**, *116*, 76-83.

549 49. Marshall, K., Clay mineralogy in relation to survival of soil bacteria. *Annual Review of*
550 *Phytopathology* **1975,** *13*, (1), 357-373.
551 50. Mashtare, M. L.; Lee, L. S.; Nies, L. F.; Turco, R. F., Transformation of 17 alpha-
552 Estradiol, 17 beta-Estradiol, and Estrone in Sediments Under Nitrate- and Sulfate-Reducing
553 Conditions. *Environmental Science & Technology* **2013,** *47*, (13), 7178-7185.
554 51. Boros, G.; Søndergaard, M.; Takács, P.; Vári, Á.; Tátrai, I., Influence of submerged
555 macrophytes, temperature, and nutrient loading on the development of redox potential around
556 the sediment-water interface in lakes. *Hydrobiologia* **2011,** *665*, (1), 117-127.
557 52. EC (European Commission). 2008. European Union Risk Assessment Report for
558 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-a-2-benzopyran (1,3,4,6,7,8-
559 hexahydro-4,6,6,7,8,8-hexamethylin-deno[5,6-C]pyran-HHCB), CAS No. 1222-05-5,
560 EINECS No. 214-916-9, Risk Assessment, Final Approved Version. Office for Official
561 Publications of the European Communities, Luxembourg, The Netherlands.
562 http://esis.jrc.ec.europa.eu/doc/risk_assessment/REPORT/hhcbreport414.pdf. Accessed
563 September 27, 2012.
564 53. Chen, F.; Ying, G.-G.; Ma, Y.-B.; Chen, Z.-F.; Lai, H.-J.; Peng, F.-J., Field dissipation
565 and risk assessment of typical personal care products TCC, TCS, AHTN and HHCB in
566 biosolid-amended soils. *Science of the Total Environment* **2014,** *470*, 1078-1086.
567 54. DiFrancesco, A. M.; Chiu, P. C.; Standley, L. J.; Allen, H. E.; Salvito, D. T.,
568 Dissipation of fragrance materials in sludge-amended soils. *Environmental science &*
569 *technology* **2004,** *38*, (1), 194-201.
570 55. Kristensen, E.; Holmer, M., Decomposition of plant materials in marine sediment
571 exposed to different electron acceptors (O_2, NO_3^-, and SO_4^{2-}), with emphasis on substrate
572 origin, degradation kinetics, and the role of bioturbation. *Geochimica et Cosmochimica Acta*
573 **2001**, 65, 419-433.
574 56. Madsen, S. D.; Forbes, T. L.; Forbes, V. E., Particle mixing by the polychaete
575 Capitells species 1: coupling fate and effect of a particle-bound organic contaminant
576 (fluoranthene) in a marine sediment. *Marine Ecology Progress Series* **1997**, 129-142.
577 57. Leppänen, M. T.; Kukkonen, J. V., Toxicokinetics of sediment-associated
578 polybrominated diphenylethers (flame retardants) in benthic invertebrates (Lumbriculus
579 variegatus, oligochaeta). *Environmental toxicology and chemistry* **2004,** *23*, (1), 166-172.
580 58. Ciparis, S.; Hale, R. C., Bioavailability of polybrominated diphenyl ether flame
581 retardants in biosolids and spiked sediment to the aquatic oligochaete, Lumbriculus
582 variegatus. *Environmental Toxicology and Chemistry* **2005,** *24*, (4), 916-925.
583 59. Dang, V. D.; Kroll, K. J.; Supowit, S. D.; Halden, R. U.; Denslow, N. D.,
584 Bioaccumulation of legacy and emerging organochlorine contaminants in Lumbriculus
585 variegatus. *Archives of environmental contamination and toxicology* **2016,** *71*, (1), 60-69.
586 60. Karlsson, M. V.; Marshall, S.; Gouin, T.; Boxall, A. B. A., Routes of uptake of
587 diclofenac, fluoxetine, and triclosan into sediment-dwelling worms. *Environmental*
588 *Toxicology and Chemistry* **2016,** *35*, (4), 836-842.
589 61. Diepens, N. J.; Van den Heuvel-Greve, M. J.; Koelmans, A. A., Modeling of
590 bioaccumulation in marine benthic invertebrates using a multispecies experimental approach.
591 *Environmental Science & Technology* **2015,** *49*, (22), 13575-13585.
592 62. Hu, Z.; Shi, Y.; Cai, Y., Concentrations, distribution, and bioaccumulation of synthetic
593 musks in the Haihe River of China. *Chemosphere* **2011,** *84*, (11), 1630-1635.
594 63. Malmquist, L. M. V.; Christensen, J. H.; Selck, H., Effects of Nereis diversicolor on
595 the Transformation of 1-Methylpyrene and Pyrene: Transformation Efficiency and
596 Identification of Phase I and II Products. *Environmental Science & Technology* **2013,** *47*,
597 (10), 5383-5392.

64. Lozano, N.; Rice, C. P.; Ramirez, M.; Torrents, A., Fate of triclocarban, triclosan and methyltriclosan during wastewater and biosolids treatment processes. *Water research* **2013,** *47*, (13), 4519-4527.
65. Macherius, A.; Eggen, T.; Lorenz, W.; Moeder, M.; Ondruschka, J.; Reemtsma, T., Metabolization of the bacteriostatic agent triclosan in edible plants and its consequences for plant uptake assessment. *Environmental science & technology* **2012,** *46*, (19), 10797-10804.
66. Wu, J. l.; Liu, J.; Cai, Z., Determination of triclosan metabolites by using in-source fragmentation from high-performance liquid chromatography/negative atmospheric pressure chemical ionization ion trap mass spectrometry. *Rapid Communications in Mass Spectrometry* **2010,** *24*, (13), 1828-1834.
67. Ranganathan, A.; Gee, S. J.; Hammock, B. D., An immunoassay for the detection of triclosan-O-glucuronide, a primary human urinary metabolite of triclosan. *Analytical and bioanalytical chemistry* **2015,** *407*, (24), 7263-7273.
68. Ellegaard-Petersen, L.; Selck, H.; Priemé, A.; Salvito, D.; Forbes, V., Investigation of the fate and effects of acetyl cedrene on *Capitella teleta* and sediment bacterial community. *Ecotoxicology* **2010,** *19*, (6), 1046-1058.

List of Figure Captions

Figure 1. Time courses of TCS (A and C) and HHCB (B and D) concentrations in the sediment from microcosms with and without *Limnodrilus hoffmeisteri*.

Figure 2. Time courses of Me-TCS (A) and HHCB-lactone (B) concentrations in the sediment from microcosms with and without *Limnodrilus hoffmeisteri*.

Figure 3. Time courses of TCS, HHCB (A), Me-TCS and HHCB-lactone (B) concentrations (μg/g ww) in the tissue of *Limnodrilus hoffmeisteri*.

Figure 4. UPLC-Q-TOF product ion spectra and chromatogram of sulfonated metabolite of TCS in worm tissue.

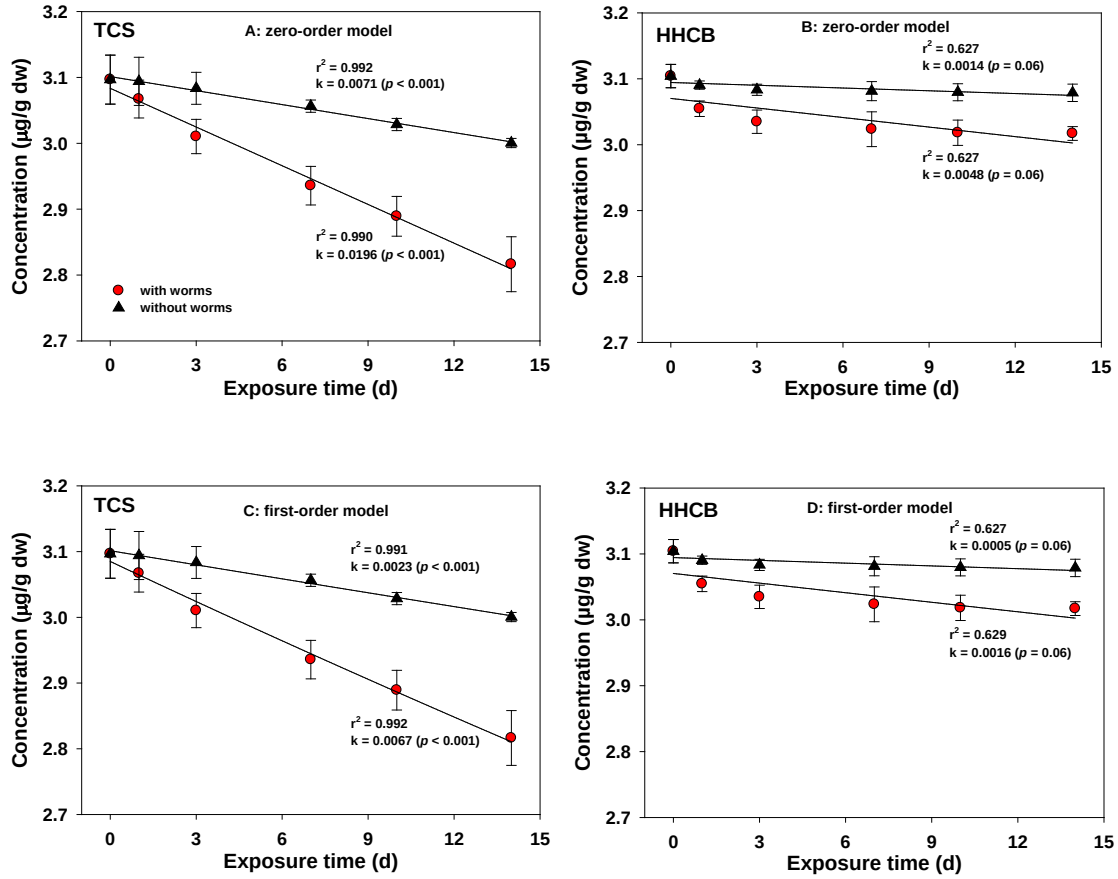
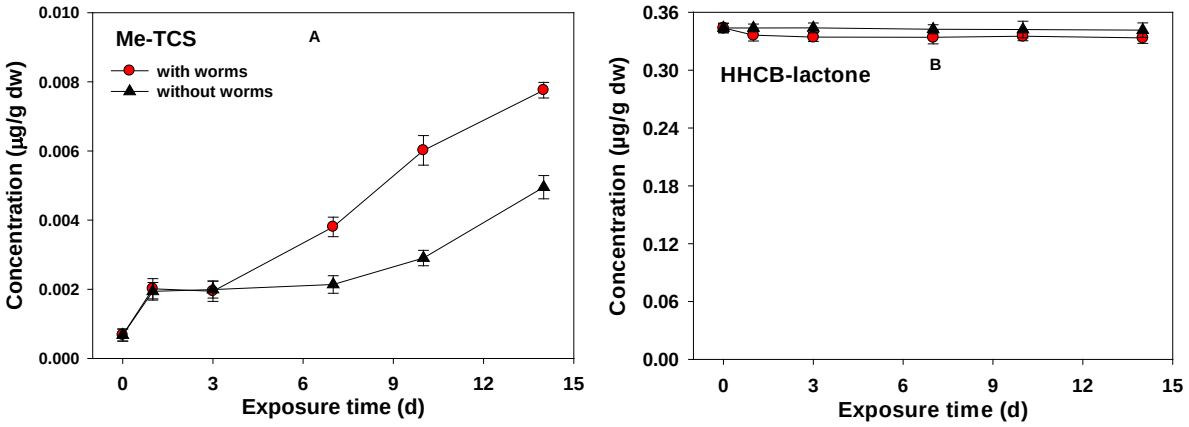


Figure 1. Time courses of TCS (A and C) and HHCB (B and D) concentrations in the sediment from microcosms with and without *Limnodrilus hoffmeisteri*. Red circle symbols and black up triangle symbols represent averages of chemical concentrations in systems with and without worms, respectively.



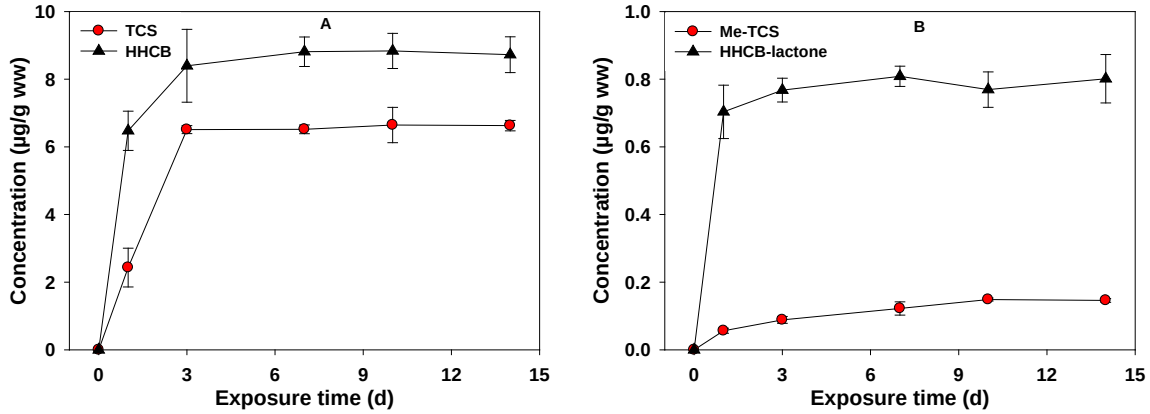
632

633 Figure 2. Time courses of Me-TCS (A) and HHCB-lactone (B) concentrations in the sediment

634 from microcosms with and without *Limnodrilus hoffmeisteri*. Red circle symbols and black up

635 triangle symbols represent averages of chemicals concentration in systems with and without

636 worms, respectively.



637

638 Figure 3. Time courses of TCS, HHCB (A), Me-TCS and HHCB-lactone (B) concentrations

639 (µg/g ww) in the tissue of *Limnodrilus hoffmeisteri*.

640

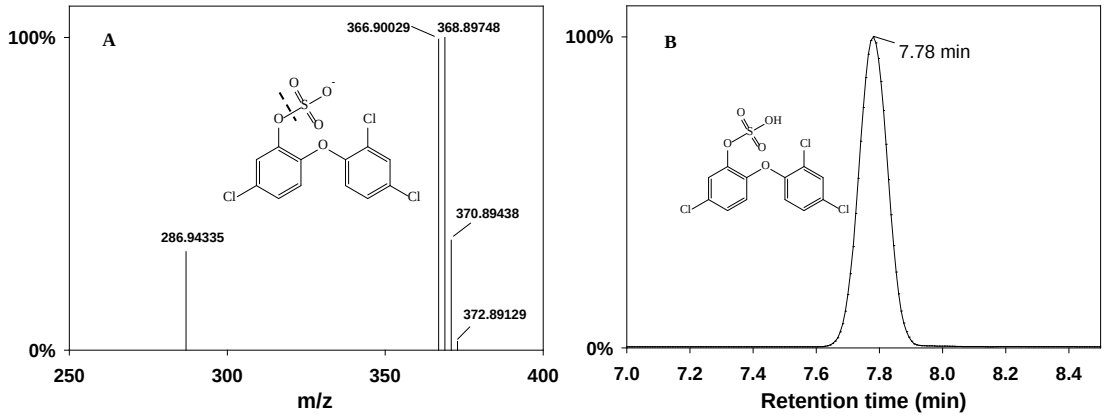


Figure 4. UPLC-Q-TOF product ion spectra and chromatogram of sulfonated metabolite of TCS in worm tissue. (A) Product ion spectra of the m/z 368.89748 peak (7.78 min), the product was identified as TCS-O-sulfate. (B) Extracted ion chromatogram of TCS-O-sulfate in the worm tissue.

Supporting Information

Bioaccumulation and biotransformation of triclosan and galaxolide in the freshwater oligochaete *Limnodrilus hoffmeisteri* in a water/sediment microcosm

Feng-Jiao Peng [†], Guang-Guo Ying [‡, *], Chang-Gui Pan [§], Henriette Selck [Φ], Daniel Salvito [δ], Paul J. Van den Brink [†, ξ, *]

[†]Aquatic Ecology and Water Quality Management group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands

[‡]The Environmental Research Institute, MOE Key Laboratory of Environmental Theoretical Chemistry, South China Normal University, Guangzhou 510006, China

[§] School of Marine Sciences, Guangxi University, Nanning 530004, China

[Φ]Department of Science and Environment, Roskilde University, Universitetsvej 1, Denmark

[δ]Research Institute for Fragrance Materials, 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

[ξ]Wageningen Environmental Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands

* Corresponding author.

Email address: guangguo.ying@gmail.com; guangguo.ying@m.scnu.edu.cn

Email address: paul.vandenbrink@wur.nl

Number of pages: 14

Number of texts: 4

Number of tables: 3

Number of figures: 1

Contents:

Text S1 Standards and Reagents

Text S2 Details of solid phase extraction

Text S3 Derivatization of HHCB and its biotransformation products

Text S4 Details of instrumental analysis

Table S1 Method quantification limits of target compounds in surface water, sediment, particles and worms by HPLC-MS/MS or GC-MS.

Table S2 Recoveries of target compounds in surface water (µg/L), sediment (µg/g dw), particles (µg/g dw) and worm (µg/g ww).

Table S3 TCS, Me-TCS, HHCB and HHCB-lactone concentrations in sediment (µg/g dw) and worm tissue (µg/g ww) during the exposure period.

Figure S1 Predicted biotransformation pathways of TCS in *Limnodrilus hoffmeisteri*.

References

Text S1 Standards and Reagents

All solvents used for chemical analysis, including methanol, ethyl acetate, n-hexane, acetone, dichloromethane, and acetonitrile were of high-performance liquid chromatography (HPLC) grade and purchased from CNW Technologies (Shanghai, China) or Merck (Germany). Acetic acid and ammonium acetic were bought from Sigma-aldrich (St.Louis, USA), while formic acid was obtained from Tedia (USA). Oasis HLB cartridges (60 mg, 3 mL) and Oasis HLB cartridges (200 mg, 6 mL) were supplied by Waters Corporation (Milford, MA, USA). Glass fiber filters (GF/F, pore size 0.7 μm) were obtained from Whatman (Maidstone, UK). Ceramic homogenizer, Z-Sep tube, anhydrous magnesium sulfate ($MgSO_4$), primary-secondary amine (PSA) and C_{18} bulk sorbent were purchased from Agilent (Santa Clara, USA). Ultrapure water was provided by a Milli-Q system from Millipore (Watford, UK). TCS and HHCB were dissolved in acetone to make a stock solution of 2 g/L. The resultant stock solutions with concentration of 100 mg/L in methanol were individually prepared, and stored at -18 ˚C until use. It should be noted that HHCB stock solution contains about 10% HHCB-lactone.

Text S2 Details of solid phase extraction

Oasis HLB SPE cartridges were preconditioned with 10 mL of methanol and 10 mL of Milli-Q water. Samples were passed through cartridges at a flow rate of 5-10 mL/min. Each sample bottle was rinsed twice with 50 mL of Milli-Q water containing 5 % methanol (v/v) and passed through the SPE cartridge. The cartridges were then dried under vacuum for 3 h. The cartridges were eluted with 3×3 mL of methanol followed by 3×3 mL of ethyl acetate, 3×3 mL of dichloromethane and 3×3 n-hexane. The eluates were dried under a gentle nitrogen gas, re-dissolved in 1 mL of methanol, transferred to a 2 mL amber glass vial with filtering through a 0.22 μm nylon membrane filter, and finally stored at -18 $^{\circ}$C until analysis.

Text S3 Derivatization of HHCB and its biotransformation products

The derivatization method for HHCB and its biotransformation products reported by Martin et al.[1] was used in this study. Specifically, 100 μL of an extract in methanol was transferred to a 2 mL amber glass vial with polytetrafluoroethylene (PTFE) screw cap and dried under a gentle nitrogen stream, added with 100 μL of Sylon BTZ and derivatized at 60 ˚C for 1 hour. Then, 200 μL of Milli-Q water at pH 3.0 was added to remove the excess derivatization reagent. The derivatization products were extracted with 500 μL of n-hexane, dried over anhydrous sodium sulfate, concentrated to a final volume of 100 μL, and analysed by GC-Q-TOF.

Text S4 Details of instrumental analysis

LC-MS/MS for TCS quantification: TCS was analysed by an Agilent 1200 rapid resolution liquid chromatograph coupled to Agilent G6460A triple quadrupole mass spectrometer under electrospray negative ionization (ESI) mode[2]. A 10 μL aliquot of each extract was injected into an Agilent SB-C18 column (3.0 mm × 100 mm ID, 1.8 μm particle size) at temperature of 40 ˚C with an RRLC in-line pre-column filter (4.6 mm, 0.2 μm filter), with Milli-Q water containing 0.01% acetic acid (v/v) (solvent A) and acetonitrile : methanol (1:1, v/v) (solvent B) as the mobile phase at a flow rate of 0.3 mL/min. The gradient program was given as follows: 60% B at 0 min, then increased to 90% B at 3 min and kept at 90% B for 4 min, then returned to the initial 60% B at 9 min and let column re-equilibrate for 6 min. The capillary was maintained at 3500 V. Dry and sheath gas flows were kept at 8 and 12 mL/min, respectively. Both dry and sheath temperatures were kept at 350 ˚C.

GC-MS for Me-TCS quantification: Me-TCS was analysed using an Agilent 6890N GC interfaced to a 5975B MSD (GC-MS), equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific Co., USA), under electron-impact ionization (EI) mode. Helium (purity > 99.999%) was used as the carrier gas at a constant flowrate of 1.0 mL/min. Splitless mode was applied for injection, with injection volume of 1 μL for each samples. The temperatures for the GC-MS interface, ion source, quadrupole and injector were kept at 300 ˚C, 230 ˚C, 150 ˚C and 250 ˚C, respectively. The column temperature was programmed as follows: from 100 ˚C (2 min) to 180 ˚C at 5 ˚C/min (2 min), from 180 ˚C to 300 ˚C at 10 ˚C/min (2 min), and then to the temperature 310 ˚C at 10 ˚C/min (10 min). The characteristic ions were 314, 264 and 243.9 for $^{13}C_{12}$-Me-TCS, 301.9, 251.9 and 232 for Me-TCS.

ACQUITY UPLC-I Class with Xevo G2-XS QTOF: the qualification of TCS biotransformation products were analysed by an Waters ACQUITY UPLC-I Class with Xevo G2-XS QTOF under a negative ion mode. For chromatographic conditions, a 2 μL aliquot of each extract was injected into a HSS T3 column (2.1 × 100 mm ID, 1.8 μm particle size) at temperature of 40 ˚C, with Milli-Q water containing 10 mM ammonium acetate (solvent A) and methanol (solvent B) as the mobile phase at a flow rate of 0.4 mL/min. The gradient program was given as follows: kept 2% B from 0 to 0.25 min, increased to 98% B at 12 min and kept at 98% B for 3 min, then returned to the initial 2% B at 18 min and let column re-equilibrate for 6 min. For mass spectrometry conditions, the capillary and cone voltage were maintained at 2500 V and 20 V, respectively. The cone gas flow was kept at 50 L/h, and the source temperature was 120 ˚C. The desolvation temperature and desolvation gas flow were set as 500 ˚C and 800 L/h. The samples were scanned using MSE scan mode at a range of 50-1000 m/z with scan time of 0.2 s. Leucine-enkephalin was used as reference for mass correction. The data were processed using the UNIFI Scientific Information System to identify the putative compounds present in the extracts.

GC-MS for HHCB and HHCB-lactone quantification: HHCB and HHCB-lactone in the extracts were measured using an Agilent 6890 N GC interfaced to a 5975B MSD (GC-MS), equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific Co., USA), in selected ion monitoring (SIM) mode under electron-impact ionization (EI). Helium (purity > 99.999%) was used as carrier gas at a constant flow of 1.0 mL/min. Splitless mode was used for injection, with injection volume of 2 μL for each samples. The temperatures for the GC-MS interface, ion source, quadrupole and injector were 280 ˚C, 250 ˚C, 150 ˚C and 280 ˚C, respectively. The GC oven temperature was programmed as follows:

80 ˚C for 0 min, increased to 170 ˚C at 15 ˚C/min, from 170 ˚C to 185 ˚C at 1 ˚C /min, then to 300 ˚C at a rate of 20 ˚C /min for 5 min.

GC/Q-TOF for HHCB biotransformation qualification: HHCB biotransformation products qualification was analysed using an Agilent 7890B GC interfaced to a 7250 QTOF, equipped with a HP-5MS UI column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific Co., USA) under electron-impact ionization (EI). Helium (purity > 99.999%) was used as carrier gas at a constant flow of 1.0 mL/min. Splitless mode was applied for injection, with injection volume of 2 μL for each samples. The temperatures for the GC/Q-TOF interface, ion source, quadrupole and injector were 300 ˚C, 200 ˚C, 150 ˚C and 280 ˚C respectively. The GC oven temperature was programmed as follows: 80 ˚C for 0 min, increased to 170 ˚C at 15 ˚C/min, from 170 ˚C to 185 ˚C at 1 ˚C /min, then to 300 ˚C at a rate of 20 ˚C /min for 5 min. The samples were scanned using full scan TOF mode at a range of 50-550 m/z with scan time of 0.20 s.

Table S1 Method quantification limits of target compounds in surface water, sediment, particles and worms by HPLC-MS/MS or GC-MS.

Compound	Surface water (ng/L)	Sediment (ng/g)	Particles (ng/g)	Worm (ng/g)
Triclosan	0.08	0.10	0.14	0.16
Methyl triclosan	0.94	1.17	1.28	1.55
Galaxolide	1.01	1.36	1.41	1.78
Galaxolidone	1.40	1.72	2.05	2.36

Table S2 Recoveries of target compounds in surface water (µg/L), sediment (µg/g dw), particles (µg/g dw) and worm (µg/g ww).

Compounds	Spiked concentrations in water			Spiked concentrations in sediment			Spiked concentrations in particles			Spiked concentrations in worm		
	0.1	0.5	2	0.1	0.5	2	0.1	0.5	2	0.1	0.5	2
Triclosan	105 ± 5.31	98.1 ± 4.84	94.1 ± 4.66	102 ± 4.15	95.3 ± 5.16	91.9 ± 2.28	102 ± 4.17	97.5 ± 4.82	92.5 ± 3.94	102 ± 5.17	105 ± 4.36	95.7 ± 3.09
Methyl triclosan	97.0 ± 4.19	93.9 ± 4.07	85.9 ± 5.65	101 ± 5.14	90.6± 5.38	82.5 ± 3.16	103 ± 5.43	89.4 ± 5.39	80.7 ± 4.62	105± 4.31	100 ± 4.75	97.9 ± 4.82
Galaxolide	90.4 ± 4.97	82.4 ± 4.11	75.8 ± 3.96	79.6 ± 4.36	70.6 ± 4.47	62.7 ± 2.43	81.3 ± 4.37	74.2 ± 4.19	64.9 ± 4.42	99.3 ± 4.97	103 ± 3.76	106 ± 4.24
Galaxolidone	92.3 ± 5.12	85.7 ± 4.65	77.6 ± 3.77	82.3 ± 4.18	75.4 ± 3.53	68.1 ± 3.16	83.0 ± 5.13	77.9 ± 5.77	70.5 ± 3.46	105 ± 4.83	98.5 ± 3.77	94.7 ± 4.98

Three replicates were used to determine recovery.

Table S3 TCS, Me-TCS, HHCB and HHCB-lactone concentrations in sediment (μg/g dw) and worm tissue (μg/g ww) during the exposure period.

Exposure time (d)	Sediment								Worm				BSAFs	
	TCS		Me-TCS		HHCB		HHCB-lactone		TCS	Me-TCS	HHCB	HHCB-lactone	TCS	HHCB
	with worms	without worms	with worms	without worms	with worms	without worms	with worms	without worms						
0	3.10±0.04	3.10±0.04	< MQL	< MQL	3.10±0.02	3.10±0.02	0.34±0.01	0.34±0.01	< MQL	< MQL	< MQL	< MQL	-	-
1	3.07±0.03	3.09±0.04	0.0020±0.0003	0.0019±0.0003	3.05±0.01	3.09±0.01	0.34±0.01	0.34±0.01	2.43±0.57	0.06±0.01	6.47±0.58	0.70±0.08	0.70±0.17	1.84±0.16
3	3.01±0.03	3.08±0.02	0.0019±0.0003	0.0020±0.0002	3.03±0.02	3.08±0.01	0.33±0.01	0.34±0.01	6.51±0.12	0.09±0.01	8.40±1.08	0.76±0.04	1.90±0.04	2.38±0.31
7	2.94±0.03	3.06±0.01	0.0038±0.0003	0.0021±0.0003	3.02±0.03	3.08±0.01	0.33±0.01	0.34±0.01	6.52±0.13	0.12±0.02	8.81±0.44	0.81±0.03	1.95±0.04	2.50±0.12
10	2.89±0.03	3.03±0.01	0.006±0.0004	0.0029±0.0002	3.02±0.02	3.08±0.01	0.33±0.01	0.34±0.01	6.65±0.52	0.15±0.02	8.84±0.52	0.77±0.05	2.02±0.16	2.50±0.15
14	2.82±0.04	3.00±0.01	0.0078±0.0002	0.0050±0.0003	3.02±0.01	3.08±0.01	0.33±0.01	0.34±0.01	6.63±0.15	0.15±0.01	8.72±0.53	0.80±0.07	2.07±0.04	2.47±0.15

MQL means method limit of quantitation.

with and without worms represent microcosms with and without worm, respectively.

BSAFs: biota-sediment accumulation factor.

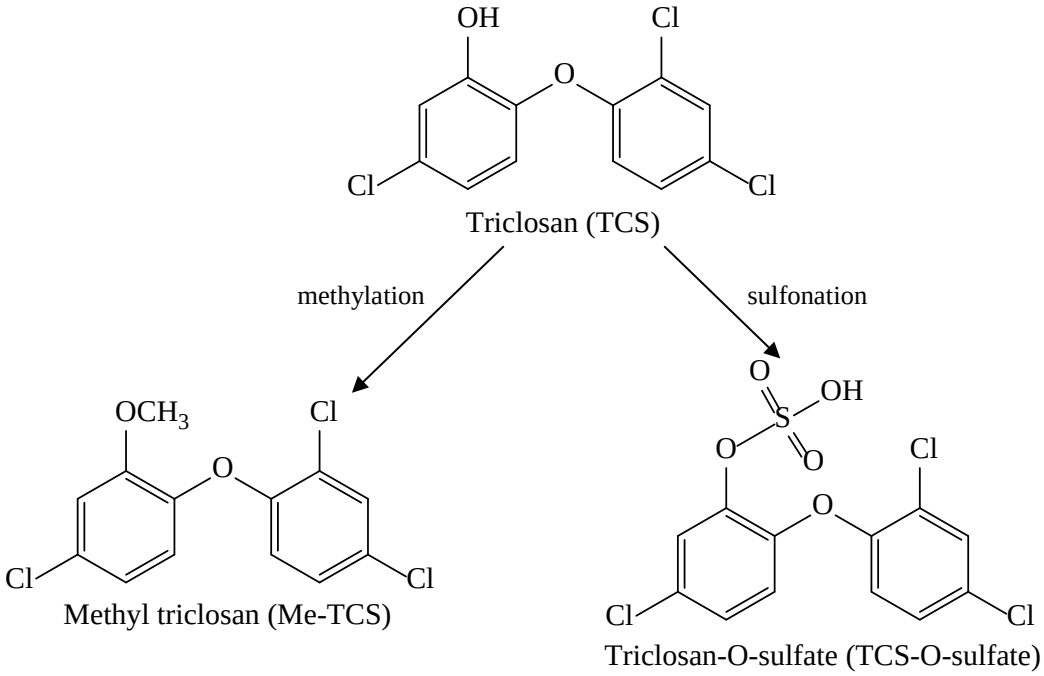


Figure S1 Predicted biotransformation pathways of TCS in *Limnodrilus hoffmeisteri*.

References

1. Martin, C.; Moeder, M.; Daniel, X.; Krauss, G.; Schlosser, D., Biotransformation of the polycyclic musks HHCB and AHTN and metabolite formation by fungi occurring in freshwater environments. *Environmental Science & Technology* **2007,** *41*, (15), 5395-5402.

2. Chen, Z.-F.; Ying, G.-G.; Lai, H.-J.; Chen, F.; Su, H.-C.; Liu, Y.-S.; Peng, F.-Q.; Zhao, J.-L., Determination of biocides in different environmental matrices by use of ultra-high-performance liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* **2012,** *404*, (10), 3175-3188.