Published in final edited form as:
Environ Toxicol Chem. 2009 December ; 28(12): 2580–2586. doi:10.1897/09-013.1.

Bioaccumulation of Triclocarban in *Lumbriculus variegatus*

Christopher P. Higgins[†,‡], **Zachary J.Paesani**[§], **Talia E. Abbot Chalew**[†], and **Rolf U. Halden**[†,‖]

[†] Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205, USA

[‡] Colorado School of Mines, Golden, Colorado 80401, USA

[§] Ingenuity Project, Baltimore Polytechnic Institute, Baltimore, Maryland 21209, USA

[‖] Biodesign Institute, Arizona State University, Tempe, Arizona 85287, USA

Abstract

The antimicrobial triclocarban (TCC) has been detected in streams and municipal biosolids throughout the United States. In addition, TCC and potential TCC transformation products have been detected at high levels (ppm range) in sediments near major United States cities. Previous work has suggested that TCC is relatively stable in these environments, thereby raising concerns about the potential for bioaccumulation in sediment-dwelling organisms. Bioaccumulation of TCC from sediments was assessed using the freshwater oligochaete, *Lumbriculus variegatus*. Worms were exposed to TCC in sediment spiked to 22.4 ppm to simulate the upper bound of environmental concentrations. Uptake from laboratory-spiked sediment was examined over 56 days for TCC and 4,4′dichlorocarbanilide (DCC), a chemical impurity in and potential transformation product of TCC. The clearance of TCC from worms placed in clean sediment was also examined over 21 d after an initial 35-d exposure to TCC in laboratory-spiked sediment. Concentrations of TCC and DCC were monitored in the worms, sediment, and the overlying water using liquid chromatography tandem mass spectrometry. Experimental data were fitted using a standard biodynamic model to generate uptake and elimination rate constants for TCC in *L. variegatus*. These rate constants were used to estimate steady-state lipid and organic-carbon normalized biota-sediment accumulation factors (BSAFs) for TCC and DCC of 2.2 ± 0.2 and 0.3 ± 0.1 g_{oc}/g_{lip}, respectively. Alternatively, directly-measured BSAFs for TCC and DCC after 56 days of exposure were 1.6 ± 0.6 and 0.5 ± 0.2 g_{oc}/g_{lip}, respectively. Loss of TCC from pre-exposed worms followed first-order kinetics, and the fitted elimination rate-constant was identical to that determined from the uptake portion of the present study. Overall, study observations indicate that TCC bioaccumulates from sediments in a manner that is consistent with the traditional hydrophobic organic contaminant paradigm.

Keywords

Antimicrobials; Sediments; *Lumbriculus variegatus*; Bioaccumulation

Introduction

The antimicrobial chemical triclocarban (TCC) has been frequently detected in various environmental media such as water [1], sediment [2], and sewage sludge [3]. This bacteriostatic chemical is commonly found in many household products including soaps, cosmetics, and

*To whom correspondence may be addressed, Christopher P. Higgins, Environmental Science and Engineering Division, Colorado School of Mines, 1500 Illinois Street, Golden, CO 80401, USA, Tel: 303-384-2002, chiggins@mines.edu.
The current address of C. P. Higgins is Colorado School of Mines, Golden, CO, 80401, USA.

deodorants [4]. When released to domestic waste streams, the vast majority of TCC (65-97%) is removed from the aqueous phase via traditional wastewater treatment and either biodegrades or accumulates in treated municipal sludge (biosolids) [3]. Thus, a small but significant fraction of the TCC entering wastewater treatment plants (WWTPs) is not removed and is subsequently discharged into receiving water bodies. The concentration of TCC in treated effluent is dependent on WWTP-specific loadings and removal efficiencies, resulting in TCC levels in effluent in the 0.1 to 6 μg/L range [5]. Further removal of TCC from the water column in the receiving water body can be expected due to TCC sorption to suspended particles and particle sedimentation. Such processes are likely responsible for the high levels of TCC that have been reported in sediments downstream from WWTPs. Sediment TCC concentrations of up to 25 mg/kg dry weight (ppm) have been reported in a sediment core from a New York, USA estuary, while the sediment core concentration profile of TCC and some of its suspected transformation products at another site suggest that some of the deposited TCC may have undergone reductive dechlorination [2]. Given the likely co-occurrence of TCC and a similar antimicrobial chemical, triclosan (TCS), and the widespread detection of TCS in freshwater streams and rivers [6], it is likely that many freshwater sediments in the receiving water bodies of municipal WWTPs contain significant concentrations of TCC and/or its transformation products. Unfortunately, there are scant data available on the potential bioaccumulation of such chemicals in sediment dwelling organisms. Such data are needed for a complete understanding of the ecological risks associated with TCC releases to aquatic environments.

To date, few studies have examined TCC bioaccumulation in aquatic species. Triclocarban has been shown to bioaccumulate in algae collected downstream from a WWTP in Texas [7]. Wet-weight TCC concentrations in algae ranged from 0.219 to 0.401 μg/g, with wet weight bioaccumulation factors (BAFs) of 1600 to 2700. Similarly, TCC was also found to bioaccumulate in snails exposed to effluent in the same stream, with concentration reaching 0.299 ± 0.09 μg/g on a wet weight basis and a wet weight BAF of 1600 [8]. In both studies, the TCC BAFs and concentrations were higher than those for both TCS and its methylated transformation product (MeTCS), suggesting that TCC may be more problematic with respect to bioaccumulation than TCS. Unfortunately, little is known about the potential adverse effects of TCC on aquatic organisms. Aqueous no-observed-effect concentrations (NOECs) of 1.46 μg/L and 5 μg/L have been reported for *Ceriodaphnia* sp. and the fish species *Pimephales promelas* [9], and a median lethal concentration (LC50) of 30 μg/L has been reported for larvae of the shellfish *Mercenaria mercenaria* [10]. At high doses in rats, TCC was observed to amplify testosterone-induced androgen receptor-mediated transcriptional activity, suggesting TCC may act as an endocrine disrupting compound [11].

Though there may be concerns about the impacts of TCC itself on aquatic ecosystems, additional potential concerns are evident if TCC transformation products are also included. A schematic illustrating the various suspected TCC biotransformation pathways is provided in Figure 1. The pathways illustrated reflect metabolic transformations in humans (Path A) [12], transformation during wastewater treatment (Path B) [13] and likely microbial transformation in anaerobic sediments (Path C) [2]. Some of these pathways presumably lead to less toxic chemicals (e.g., Path A), whereas the toxicity of other suspected transformation products such as dichlorocarbanilide (DCC) are unknown (Path C). Yet another pathway (Path B) leads to chemicals for which substantial toxicity data exist (e.g., dichloroaniline). In fact, TCC has been identified as a potential source of dichloroaniline in some aquatic environments [14]. Thus, understanding the bioaccumulation of TCC is important not only with respect to the toxicity of TCC itself, but also the toxicity of its potential transformation products.

The objective of the present study was to determine the extent to which TCC bioaccumulates from sediments into sediment-dwelling organisms. The freshwater oligochaete, *Lumbriculus variegatus*, was chosen as a model organism because of its ease of culture and its direct

ingestion of sediment. In addition, *L. variegatus* is recommended by the U.S. Environmental Protection Agency for assessing bioaccumulation in freshwater sediments [15]. Due to likely reductive dechlorination of the spiked TCC to DCC prior to worm exposure, the bioaccumulation potential of DCC was also assessed. The uptake and elimination kinetics of TCC and DCC in *L. variegatus* were modeled with a standard biokinetic model, and this model was then used to estimate steady-state biota sediment accumulation factors (BSAFs). BSAFs were also calculated directly from the worm and sediment concentrations measured on Day 56 of the study.

Materials and Methods

Chemicals and Sediment

Triclocarban (99%), DCC (99%), $^{13}C_6$-TCC, and d_7-TCC were obtained from Aldrich (Milwaukee, WI, USA), Oakwood Products (West Columbia, SC, USA), and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Standard synthetic freshwater was prepared from MilliQ™ water. All other chemicals and solvents were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Pristine lake sediment obtained from Agvise Laboratories (Northwood, ND, USA) with nondetectable levels of TCC was utilized. Upon receipt in the laboratory, the sediment was wet-sieved (2 mm) and stored at 4°C until spiking. The fraction of organic carbon (f_{oc}) of the sediment, as determined by a commercial laboratory using the Walkley-Black method, was 3.3%. For the TCC-spiked sediment, a small aliquot of a stock standard solution of TCC in methanol/acetone (50:50) was added to sediment-water mixture to obtain a nominal concentration of 50 ppm TCC (dry wt basis). Preliminary 10-d acute toxicity experiments indicated no mortality of *L. variegatus* due to TCC exposure at sediment concentrations of up to 100 ppm (dry wt basis; data not shown). Sediment-water mixtures were equilibrated on an orbital shaker for two weeks. The solvent control and depuration sediment (no TCC added) was equilibrated in an identical fashion.

Organisms

A culture of *L. variegatus* was purchased from a local aquatic pet supply store approximately two weeks prior to the start of the experiment and was maintained (unfed) in an aerated aquarium with 1 to 2 cm of solvent-washed sand. Immediately prior to the start of the experiment, 200 to 250 mg (wet wt) aliquots of worms were removed via disposable polypropylene pipet and placed into polystyrene weigh boats. Intact adult worms 2 to 3 cm in length were chosen to minimize effects of growth and reproduction.

Experimental Setup

Semi-static bioaccumulation experiments were conducted in 150 ml glass beakers. After spiking, TCC-spiked or solvent-control sediment was added to each glass beaker (~ 40 g dry wt sediment per beaker) and allowed to settle for 4 d prior to worm addition. This mass of TCC-spiked sediment corresponded to a target mass of TCC per beaker of approximately 2 mg. At the start of the uptake experiment, two large-volume (3 L) glass beakers were also prepared in a similar manner and a correspondingly larger mass of worms added. On day 35, worms added to these beakers were removed from the TCC-spiked sediment and placed in 150 ml glass beakers containing settled clean sediment for the elimination experiment. As per U.S. Environmental Protection Agency recommendations, the ratio of sediment organic carbon to worm mass (dry wt) was at least 50:1 for all experiments [15]. The beakers were maintained in aquaria in a temperature-monitored water bath with a 16:8 photoperiod. Over the course of the experiments, the water bath temperature was 23 ± 2 °C. The depth of the overlying water (OLW) was maintained at approximately 2 cm to ensure adequate dissolved oxygen levels. Starting on the day prior to worm addition, the OLW was removed from each beaker and replaced with clean synthetic freshwater at a rate of approximately 20 ml every other day. The

OLW that was removed was pooled for each week of exposure and frozen for TCC analysis. The OLW pH and total ammonia were monitored weekly with commercially available kits and were found to be relatively constant over the course of the experiment. Triplicate beakers were prepared and terminated at each time point, which were days 0, 1, 3, 7, 10. 14, 21, 28, 35, 42, 49, and 56 for the uptake experiment and days 35 (Down day 0), 36, 38, 40, 42, 45, 49, and 56 for the elimination experiment. Triplicate beakers of control sediment were prepared anal terminated on days 28 and 56.

Worm, sediment, and water analysis

At each time point, worms were removed from the sediment by sieving, transferred to polystyrene weigh boats, and allowed to depurate in clean synthetic freshwater for 6 h. After 6 h, the worms were transferred to clean weigh boats, thoroughly rinsed, and the rinse water removed. The worms were then transferred by dental pick to pre-weighed glass vials, and a wet weight was obtained. One worm from each beaker was also transferred to a second pre-weighed glass vial and frozen (-20 °C) for lipid analysis using a method previously described [16]. The remaining worms were frozen and stored at -20 °C until extraction. After worm removal, the sediment from each time point was pooled, allowed to settle, and then a subsample was removed and frozen (-20°C) until extraction.

Immediately prior to extraction, 100 to 500 ng of $^{13}C_6$-TCC was added to each sediment and worm sample to determine TCC recoveries. For extraction of TCC from worm tissues, 5 ml of acetonitrile was added to each glass vial and the vial placed on an orbital shaker for 1 h. After settling, the supernatant was removed via glass pipet, transferred to a 20 ml glass vial, and the procedure repeated twice. For each sample, all three extracts were combined and evaporated to dryness under low heat and gentle nitrogen. The extract was reconstituted in 1 ml of a 70:30 methanol:water mixture and transferred to a 2 ml microcentrifuge tube. The tube was spun at 18,000 RCF for 30 min before an aliquot was removed and diluted as appropriate for TCC analysis via LC-MS/MS. Sediments were extracted in a similar manner with a mixture of 50:50 methanol/acetone. All sediment extracts were reconstituted in methanol, centrifuged, and diluted with MilliQ™ water to obtain a final extract in 70% methanol. Water samples were extracted via solid phase extraction (SPE) as described previously [1] with all methanol extracts diluted with MilliQ™ water to obtain a final extract in 70:30 methanol:water. For all samples, 500 μl of extract was transferred to a 2 ml autosampler vial, 50 μl of the internal standard d_7-TCC was added, and the extract analyzed by LC-MS/MS. The limits of quantitation (LOQs) were matrix and run-dependent, but were approximately 0.03 $\mu g/g_{lip}$ for worms, 2 $\mu g/g_{oc}$ for sediment, and 10 ng/L for water for both TCC and DCC.

Analysis via LC-MS/MS was conducted under conditions similar to those previously reported [3]. Chromatography was performed using an aqueous ammonium acetate (2 mM) and methanol gradient delivered at a flow rate of 200 μl/min by a Waters 2795 LC system (Milford, MA, USA). Samples and standards were injected (50 μl) onto a Ultra IBD C18 column (5 μm particle size, 150mm × 2.1mm; Restek Corporation, Bellefonte, PA, USA). Initial eluent conditions were 70% methanol. The percent methanol was increased to 100% at 5 min, held 100% for 3 min, decreased to 70% over 2 min, and held at 70% for the remaining 5 min. A Waters Quattro Micro triple quadrupole mass spectrometer operating in negative electrospray ionization multiple reaction monitoring (MRM) mode was employed for sample analysis. For all analytes, the cone voltage was 30 V and the collision energy was 13 eV. The MRM transitions monitored for each analyte, surrogate, and internal standard are listed in Table 1. A dwell time of 100 ms was used for each transition. Nitrogen was provided by a Parker-Balston N2-14 generator (Haverhill, MA, USA). Quantitation was performed using Waters QuanLynx™, with all calibration curves having r^2 values greater than 0.99 and the accuracy of each calibration point within 30% of its expected value. All values reported are corrected

for recovery of the $^{13}C_6$-TCC surrogate standard, which was generally greater than 75% for all samples in all matrices.

Data Analysis

Modeling of worm accumulation was performed using Origin 8 (Northampton, MA, USA). Bioaccumulation data were fitted using non-linear regression to a first-order kinetic model [17]:

$$C_{org}=\frac{(k_s C_{sed,0})}{k_e-\lambda}\left(e^{-\lambda t}-e^{-k_e t}\right)$$

where C_{org} is the lipid-normalized concentration of TCC in the worm (μg/g_{lip}), $C_{sed,0}$ is the initial organic-carbon normalized concentration of TCC in the sediment (μg/g_{oc}), k_s is the uptake rate constant (g_{oc}/g_{lip}h), k_e is first-order elimination rate constant (h^{-1}), and λ is a rate constant (h^{-1}) that allows for decreasing levels of contaminant and/or decreasing contaminant bioavailability over the course of the experiment [17]. For the depuration phase, data were fitted using a simple first-order decay:

$$C_{org}=C_{org}^{t=0}e^{-\alpha t}$$

where $C_{org}^{t=0}$ is the lipid-normalized concentration of TCC in the worm at the start of the depuration phase of the experiment (μg/g_{lip}) and α is first-order elimination rate constant (h^{-1}). Estimation of the steady-state biota-sediment accumulation factor ($BSAF_{ss}$; g_{oc}/g_{lip}) was performed by relating the uptake (k_s) and elimination (k_e) rate constants according to the following:

$$BSAF_{ss}=\frac{k_s}{k_e}$$

In addition, $BSAF_{56}$ values were calculated directly from the lipid-normalized worm concentrations and organic-carbon normalized sediment concentrations on day 56 of the study using the equation:

$$BSAF_{56}=\frac{C_{org}}{C_{sed}}$$

Results and Discussion

Levels of TCC and DCC in Sediment and Water

As determined by analytical measurements, the concentration of TCC in the laboratory-spiked sediment was 22.4 ± 7.6 μg/$g_{dry\ wt}$ or 652 ± 230 μg/g_{oc} (mean ± standard deviation), and was constant over the course of the experiment. A production impurity and transformation product of TCC also was detected in the laboratory-spiked sediment: the concentration of DCC averaged 0.7 ± 0.2 μg/$g_{dry\ wt}$ or 21 ± 7 μg/g_{oc} and also remained constant over the course of the experiment. This level corresponds to approximately 3% (by mass) of the TCC present in

the sediment. Since the DCC mass exceeded the fraction of impurities (1%) contained in the analytical-grade TCC used to spike the sediment, at least a fraction of the observed DCC was likely generated in situ during the sediment equilibration procedure. The biological generation of DCC from TCC presumably is an anaerobic reductive dechlorination process that is likely mediated by sediment-dwelling anaerobic bacteria. While the redox conditions during sediment equilibration were not directly monitored, the production of volatile sulfides was observed at the end of the equilibration phase, suggesting that highly anaerobic conditions were evident (data not shown). Observation of DCC formation in the present study is consistent with prior reports of evidence for reductive dechlorination of TCC in field sediments [2]. Currently available data on potential transformation pathways for TCC are summarized in Figure 1. The analytical methodology chosen here did not lend itself to the analysis of the mono- and dichlorinated anilines shown in Path B of Figure 1.

The levels of TCC and DCC in the overlying water (OLW) also suggested TCC transformation. Contaminant concentrations in the composite OLW samples were determined beginning with week 2, when 430 ng/L of DCC was measured. Throughout the remainder of the experiment (week 3-8), the observed average OLW concentration of DCC was 22 ± 4 ng/L. In contrast, the concentration of TCC in the OLW was constant at 820 ± 220 ng/L throughout the course of the experiment.

These data suggest an initial rapid flux of DCC from the sediment to the OLW during the first two weeks of the experiment, followed by a steady-state release of both TCC and DCC into the OLW (which was renewed every other day) throughout the remainder of the experiment. However, as noted above, no temporal trends were apparent in sediment concentrations of either DCC or TCC. Indeed, using the average sediment and OLW levels of TCC and DCC over the course of the experiment, flux losses from the sediment of < 0.1% for TCC and < 0.5% for DCC were calculated, even after accounting for the higher initial levels of DCC in the OLW. As will be discussed, these data suggest that the decreased bioaccumulation of TCC over time was likely due to a decrease in TCC bioavailability in the sediment as opposed to a flux of TCC out of the sediment.

Measures of Oligochaete Health

No acute mortality of worms due to TCC exposure was apparent. The wet weight of worms collected from both TCC-exposed and control worms decreased over time until reaching approximately 60% of the initial worm mass on day 35 and then remained constant until the end of the experiment. A similar weight loss was previously observed for worms exposed to sediment from the same site [18]. To account for this weight loss for the purposes of the biodynamic modeling, all worm wet weights were normalized to the initial mass of worms added to each beaker. Alternatively, $BSAF_{56}$ values were calculated using the actual worm masses measured on day 56 of the study (i.e., not corrected for weight loss). Though the worms did lose weight over the course of the experiment, at 3.2 ± 1.0%, worm lipid content (as a percentage of measured wet weight) did not vary over the course of the experiment. Thus, all tissue concentrations were normalized to the average lipid content for all worms over the course of the experiment.

Accumulation of TCC and DCC in *L. variegatus*

As is clear from Figure 2, both TCC and DCC rapidly accumulated in *L. variegatus* over the course of the experiment. However, substantial differences were apparent in the biokinetic profiles of TCC and DCC. As shown in Figure 2A, the maximum body burden of TCC was observed on day 5, with an observed concentration of 1310 ± 60 $\mu g/g_{lip}$ (42 ± 2 $\mu g/g_{wet\ wt}$; mean ± standard deviation). After day 5, body burdens of TCC in *L. variegatus* declined over the course of the experiment. In contrast, the maximum DCC body burden of 7.85 ± 0.29 μg/

g_{lip} (0.250 ± 0.009 μg/$g_{wet\ wt}$) was observed on day 56. These clear differences in bioaccumulation kinetics between TCC and DCC may be due to differences in bioavailability and/or the potential transformation of TCC to DCC within *L. variegatus*. While the latter is certainly a possibility (particularly if the transformation is due to gut microflora), the metabolic capability of *L. variegatus* has been characterized as fairly limited [19,[20]].

The generic biokinetic model provided an excellent fit to the data, though the fit was much better for TCC than DCC. The fitted model parameters are provided in Table 2. In particular, the TCC data were best fit if λ, the fitting parameter meant to account for changes in bioavailability, was 6.7 ± 0.3 (x 10^{-4}; h^{-1}), while the best fit for DCC was obtained with λ set to zero. This implies that the bioavailability of TCC decreased over the course of the experiment while the bioavailability of DCC did not decrease. As the measured TCC sediment concentration did not decrease over time, this suggests an actual decrease in the bioavailable fraction of TCC in the sediment over time as opposed to a declining total sediment concentration. Similar decreases in bioavailability over time in laboratory-spiked systems have been observed [17]. As noted above, no significant flux of TCC out of the sediment was observed, and thus the decrease in TCC bioavailability may have been due to the slow diffusion of TCC into the sediment particles where it is rendered less bioavailable to the worms.

An alternative interpretation of the declining TCC body burden over time would be that TCC was being biotransformed by *L. variegatus* after an initial lag period. This lag period could be attributed to the time needed to induce the enzymes necessary for TCC biotransformation. While this explanation has been used to explain decreasing body burdens of polycyclic musks in midge larvae over time [21], the same study did not observe any evidence of enzyme induction in *L. variegatus*. Coupled with the reported low metabolic capabilities of *L. variegatus*, these data suggest that the decreasing body burden of TCC over time was likely due to a decrease in TCC bioavailability. The lack of a decrease of DCC bioavailability may simply be due to the fact that the DCC, which was presumably produced by microbial transformation processes, was produced at or near the surface of the sediment particles where it was readily taken up by the worms. Clearly, further research with field-contaminated sediments are needed to verify that the decrease in body burden observed in *L. variegatus* in these laboratory spiked systems is due to a change in bioavailability as opposed to a non-linear metabolic response to TCC exposure.

Elimination of TCC in *L. variegatus*

The elimination of TCC from *L. variegatus* was rapid and followed simple first-order decay kinetics (Fig. 3). After 21 d in clean sediment, the TCC body burden in *L. variegatus* declined to 9.6 ± 0.3 μg/g_{lip} (0.31 ± 0.01 μg/$g_{wet\ wt}$). These levels were still above the trace levels of TCC measured in the control (nonexposed) worms on day 56 (0.09 ± 0.12 μg/g_{lip} (0.003 ± 0.004 μg/$g_{wet\ wt}$). As is apparent in Table 2, there are no statistical differences between the first-order elimination rate constant determined from the elimination experiment (α) when compared to the elimination rate constant determined from the uptake experiment (k_e). This value of approximately160 (x 10^{-4} h^{-1}) is on the lower end of the elimination rate constants in *L. variegatus* observed for polybrominated diphenyl ethers (150-660) [22], and is within the range of values observed for polychlorinated biphenyls[23].

Biota sediment accumulation factors

Using the fitted uptake and elimination rate constants, $BSAF_{ss}$ values (g_{oc}/g_{lip}) were calculated for both TCC and DCC. These values were 2.2 ± 0.2 and 0.3 ± 0.1 for TCC and DCC, respectively. In addition, $BSAF_{56}$ values were calculated to be 1.6 ± 0.6 and 0.5 ± 0.2 for TCC and DCC, respectively. The partitioning theory for traditional hydrophobic organic contaminants (HOCs) predicts a theoretical organic carbon and lipid-normalized steady-state

BSAF value of 1.6 for nonmetabolized HOCs if the log of the octanol water partition coefficient (log K_{OW}) is less than 6 [24]. Thus, TCC appears to bioaccumulate from sediments in a manner that is consistent with traditional HOC partitioning theory. The slightly lower $BSAF_{56}$ value for TCC, as compared to the $BSAF_{ss}$ value, reflects the fact that the bioavailability of TCC appeared to decrease over time. Conversely, the slightly higher $BSAF_{56}$ value for DCC, as compared to the $BSAF_{ss}$ value, reflects the fact that the calculation of the $BSAF_{56}$ value used the actual worm weights recorded on day 56, and thus did not account for the weight loss observed. However, for both TCC and DCC, both approaches for calculating BSAFs yielded values that are approximately equal (i.e., within experimental error). Clearly, field-based data on TCC bioaccumulation from sediments are needed to verify that TCC does, in fact, follow HOC partitioning theory under field conditions. However, field-based measurements of BSAFs for HOCs are highly variable, and field-measured BSAF values appear to be slightly higher than theory would predict [25]. The significantly lower BSAF for DCC is somewhat surprising. Further research is needed to aid in determining the cause of this lower bioaccumulation.

Comparison with other studies

To the best of our knowledge, this is the first study to demonstrate TCC bioaccumulation from sediments. However, as noted above, a few studies have examined the bioaccumulation of TCC from the aqueous phase under field conditions [7,[8]]. These studies have yielded wet-weight bioaccumulation factors (BAFs) for TCC of 1600 to 2700. The $BSAF_{ss}$ value estimated for TCC in the present study has been normalized to the lipid content of the organism and sediment organic carbon. If the fraction of lipids (f_{lip}) and the log K_{OC} values are known or estimated and an assumption of equilibrium conditions (with respect to sediment porewater) is made, the corresponding wet weight BAF value can be estimated using the following relationship:

$$BAF_{\text{wet wt}} = \frac{C_{\text{org,wet wt}}}{C_w} = \frac{C_{\text{org,lip}} \times f_{\text{lip}}}{C_{\text{sed,oc}}/K_{\text{OC}}} = BSAF_{\text{oc,lip}} \times f_{\text{lip}} \times K_{\text{OC}}$$

In the present study, the steady-state $BSAF_{oc,lip}$ was 2.2 ± 0.2, the f_{lip} was 3.2 ± 1.0% and a log K_{OC} of 4.5 has been reported for TCC [26], yielding a $BAF_{wet\ wt}$ of approximately 2200, which is well within the range of $BAF_{wet\ wt}$ values reported previously [7,[8]] and very close to the average algal $BAF_{wet\ wt}$ of 2300 reported from three separate sites [7]. In fact, if the slighltly lower day 56 $BSAF_{oc,lip}$ value of 1.6 ± 0.6 is used, the estimated $BAF_{wet\ wt}$ is ~1600, which is at the lower end of the range of values reported previously. Given the uncertainty with respect to both the presence of steady-state conditions in this study and the reported log K_{OC} value for TCC, these estimates should be viewed with caution. However, these values do suggest that the results of this study are consistent with the few studies reporting the bioaccumulation of TCC under field conditions.

Conclusions

The commonly-used antimicrobial chemical TCC was observed to bioaccumulate in the sediment-dwelling organism *L. variegatus*. This bioaccumulation was consistent with traditional HOC partitioning theory, and the elimination kinetics of TCC from L. variegatus was similar to the elimination kinetics of other persistent halogenated organic compounds. Thus, this study demonstrated that TCC can accumulate in sediment-dwelling organisms at the base of the food-web. Further research is needed to determine whether TCC will undergo trophic transfer and biomagnification in (aquatic) food chains. The present study also provides evidence suggesting that TCC may undergo dechlorination by microorganisms within sediments to yield DCC. In turn, DCC also appears to bioaccumulate from sediments, though

to a lesser extent than TCC. Further research is needed to verify that the BSAF value measured for TCC in this study is representative of field conditions.

Acknowledgments

Support for this project was provided by the National Institute of Environmental Health Sciences (NIEHS) Training Grant ES 07141for CPH, NIEHS research grant 1R01ES015445, and the JHU Center for a Livable Future. The authors would like to thank Renee Gardner and Erica Hartmann for assistance with worm care, and Lynn Roberts and Joseph Bressler for access to instrumentation.

References

1. Halden RU, Paull DH. Analysis of triclocarban in aquatic samples by liquid chromatography electrospray ionization mass spectrometry. Env Sci Technol 2004;38:4849–4855. [PubMed: 15487795]
2. Miller TR, Heidler J, Chillrud SN, Delaquil A, Ritchie JC, Mihalic JN, Bopp R, Halden RU. Fate of triclosan and evidence for reductive dechlorination of triclocarban in estuarine sediments. Env Sci Technol 2008;42:4570–4576. [PubMed: 18605588]
3. Heidler J, Sapkota A, Halden RU. Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment. Env Sci Technol 2006;40:3634–3639. [PubMed: 16786704]
4. (SCCP) SCoCP. Opinion on Triclocarban for other uses than as a preservative. European Commission: Health and Consumer Protection Directorate-General; 2005.
5. Chalew TEA, Halden RU. Environmental Exposure of Aquatic and Terrestrial Biota to Triclosan and Triclocarban. Journal of the American Water Resources Association 2009;45:4–13.
6. Halden RU, Paull DH. Co-occurrence of triclocarban and triclosan in U.S. water resources. Env Sci Technol 2005;39:1420–1426. [PubMed: 15819193]
7. Coogan MA, Edziyie RE, La Point TW, Venables BJ. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater treatment plant receiving stream. Chemosphere 2007;67:1911–1918. [PubMed: 17275881]
8. Coogan MA, La Point TW. Snail bioaccumulation of triclocarban, triclosan, and methyltriclosan in a North Texas, USA, stream affected by wastewater treatment plant runoff. Environ Toxicol Chem 2008;27:1788–1793. [PubMed: 18380516]
9. Consortium, T. High Production Volume (HPV) Chemical Challenge Program Data Availability and Screening Level Assessment for Triclocarban CAS# 101-20-2. U.S. Environmental Protection Agency; Washington, DC: 2002.
10. Davis HC, Hidu H. Effects of pesticides on embryonic development of clams and oysters and on survival and growth of the larvae. Fisheries Bulletin 1969;67:393–404.
11. Chen J, Ki CA, Gee NA, Ahmed MI, Duleba AJ, Zhao L, Gee SJ, Hammock BD, Lasley BL. Triclocarban enhances testosterone action: A new type of endocrine disruptor? Endocrinology 2008;149:1173–1179. [PubMed: 18048496]
12. Gruenke LD, Craig JC, Wester RC, Maibach HI, Northroot H, Corbin NC. A Selected Ion Monitoring Gc/Ms Assay for 3,4,4′-Trichlorocarbanilide and Its Metabolites in Biological-Fluids. Journal of Analytical Toxicology 1987;11:75–80. [PubMed: 3573729]
13. Gledhill WE. Biodegradation of 3,4,4′-Trichlorocarbanilide, TCC, in Sewage and Activated-Sludge. Water Res 1975;9:649–654.
14. Halden RU. Comment on “accumulation of contaminants in fish from wastewater treatment wetlands”. Env Sci Technol 2006;40:3437. [1]. [PubMed: 16749718]
15. USEPA. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. EPA; 2000. 600/R-99/064
16. Van handel E. Rapid-Determination of Total Lipids in Mosquitos. Journal of the American Mosquito Control Association 1985;1:302–304. [PubMed: 2906672]
17. Landrum PF. Bioavailability and Toxicokinetics of Polycyclic Aromatic-Hydrocarbons Sorbed to Sediments for the Amphipod Pontoporeia-Hoyi. Env Sci Technol 1989;23:588–595.

18. Higgins CP, McLeod PB, Macmanus-Spencer LA, Luthy RG. Bioaccumulation of perfluorochemicals in sediments by the aquatic oligochaete Lumbriculus variegatus. Env Sci Technol 2007;41:4600–4606. [PubMed: 17695903]

19. Harkey GA, Landrum PF, Klaine SJ. Comparison of whole-sediment, elutriate and pore-water exposures for use in asssessing sediment-associated organic contaminants in bioassays. Environ Toxicol Chem 1994;13:1315–1329.

20. Schuler LJ, Wheeler M, Bailer AJ, Lydy MJ. Toxicokinetics of sediment-sorbed benzo[a]pyrene and hexachlorobiphenyl using the freshwater invertebrates Hyalella azteca, Chironomus tentans, and Lumbriculus variegatus. Environ Toxicol Chem 2003;22:439–449. [PubMed: 12558178]

21. Artola-Garicano E, Sinnige TL, van Holsteijn I, Vaes WHJ, Hermens JLM. Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environ Toxicol Chem 2003;22:1086–1092. [PubMed: 12729218]

22. Leppanen MT, Kukkonen JVK. Toxicokinetics of sediment-associated polybrominated diphenylethers (flame retardants) in benthic invertebrates (Lumbriculus variegatus, oligochaeta). Environ Toxicol Chem 2004;23:166–172. [PubMed: 14768881]

23. Sun XL, Werner D, Ghosh U. Modeling PCB Mass Transfer and Bioaccumulation in a Freshwater Oligochaete Before and After Amendment of Sediment with Activated Carbon. Env Sci Technol 2009;43:1115–1121. [PubMed: 19320167]

24. Morrison HA, Gobas FAPC, Lazar R, Haffner GD. Development and verification of a bioaccumulation model for organic contaminants in benthic invertebrates. Env Sci Technol 1996;30:3377–3384.

25. Wong CS, Capel PD, Nowell LH. National-scale, field-based evaluation of the biota-sediment accumulation factor model. Env Sci Technol 2001;35:1709–1715. [PubMed: 11355183]

26. Young TA, Heidler J, Matos-Perez CR, Sapkota A, Toler T, Gibson KE, Schwab KJ, Halden RU. Ab initio and in situ comparison of caffeine, triclosan, and triclocarban as indicators of sewage-derived microbes in surface waters. Env Sci Technol 2008;42:3335–3340. [PubMed: 18522115]

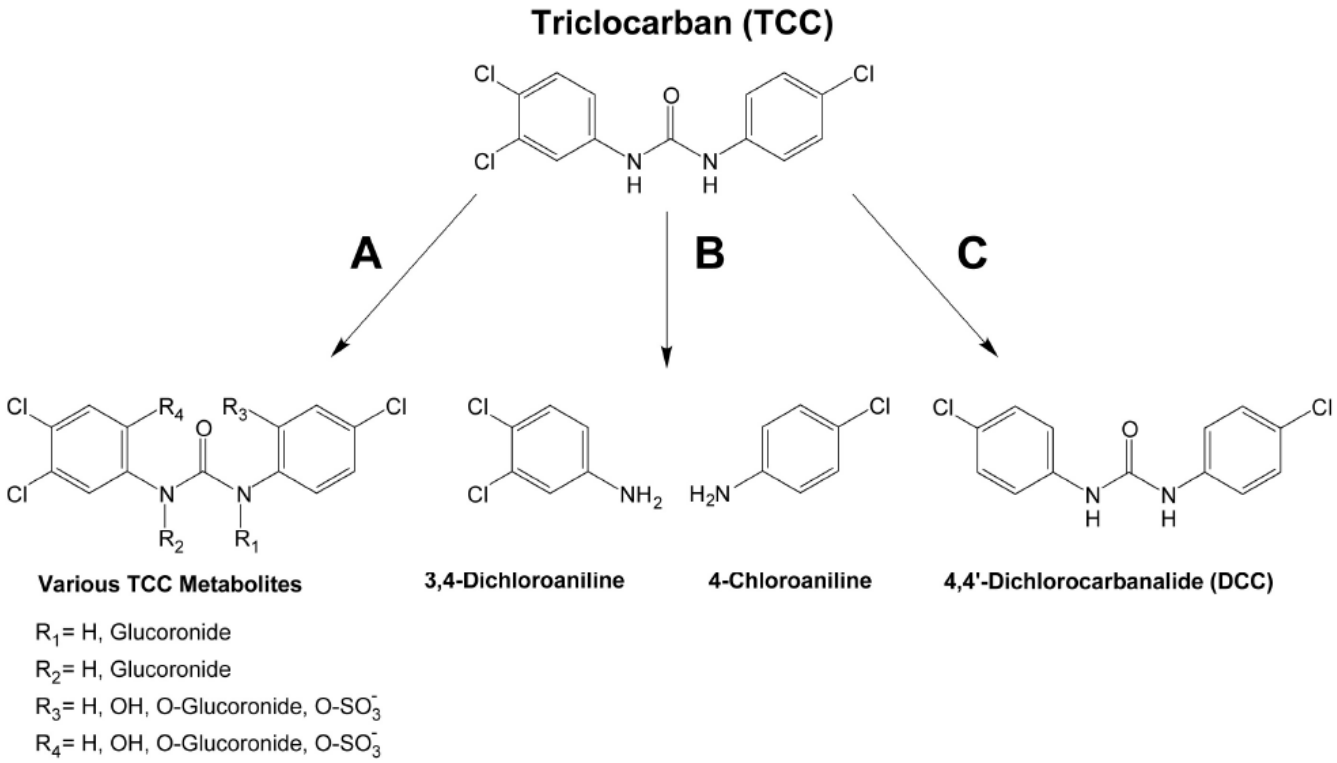

Figure 1.
Potential transformation pathways of triclocarban (TCC). Path A: Metabolic oxidation and/or conjugation in humans [12]. Path B: microbial transformation and hydrolysis to chloroanilines [13]. Path C: reductive dechlorination to dichlorocarbanilide (DCC) [2].

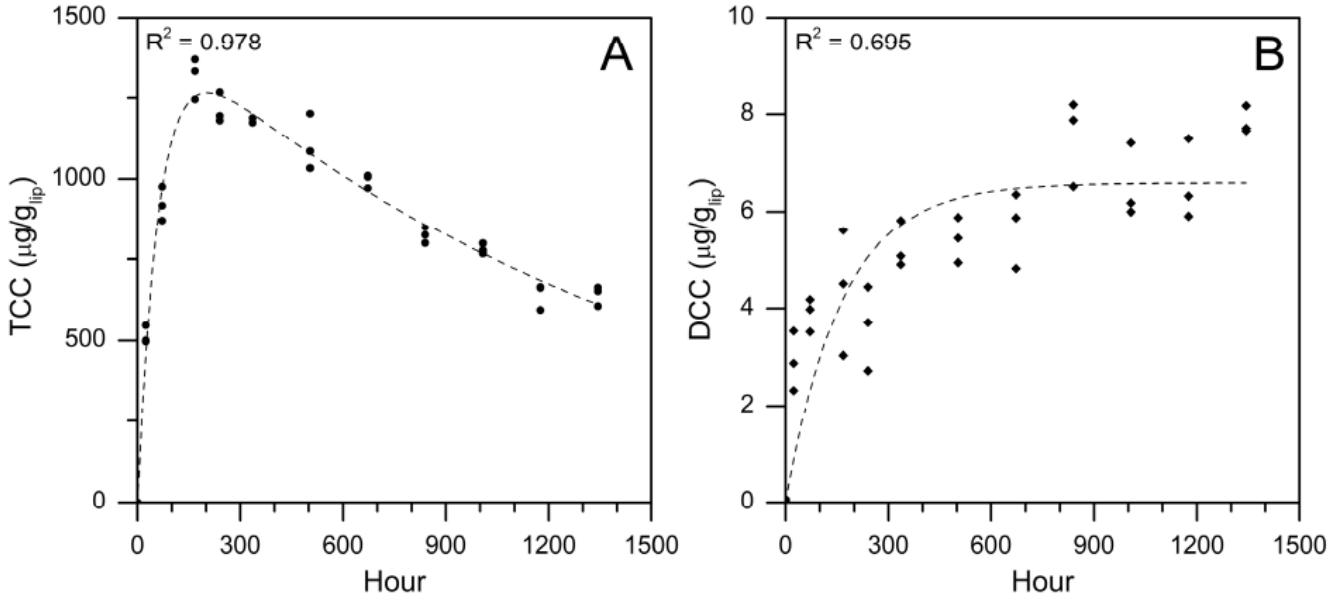

Figure 2.
Bioaccumulation of triclocarban (TCC; **A**) and dichlorocarbanilide (DCC; **B**) in lake sediment spiked with TCC by *Lumbriculus variegatus*. Symbols denote individual jars (n = 3 for each time point), and the dotted line denotes the model fit using parameters listed in Table 2.

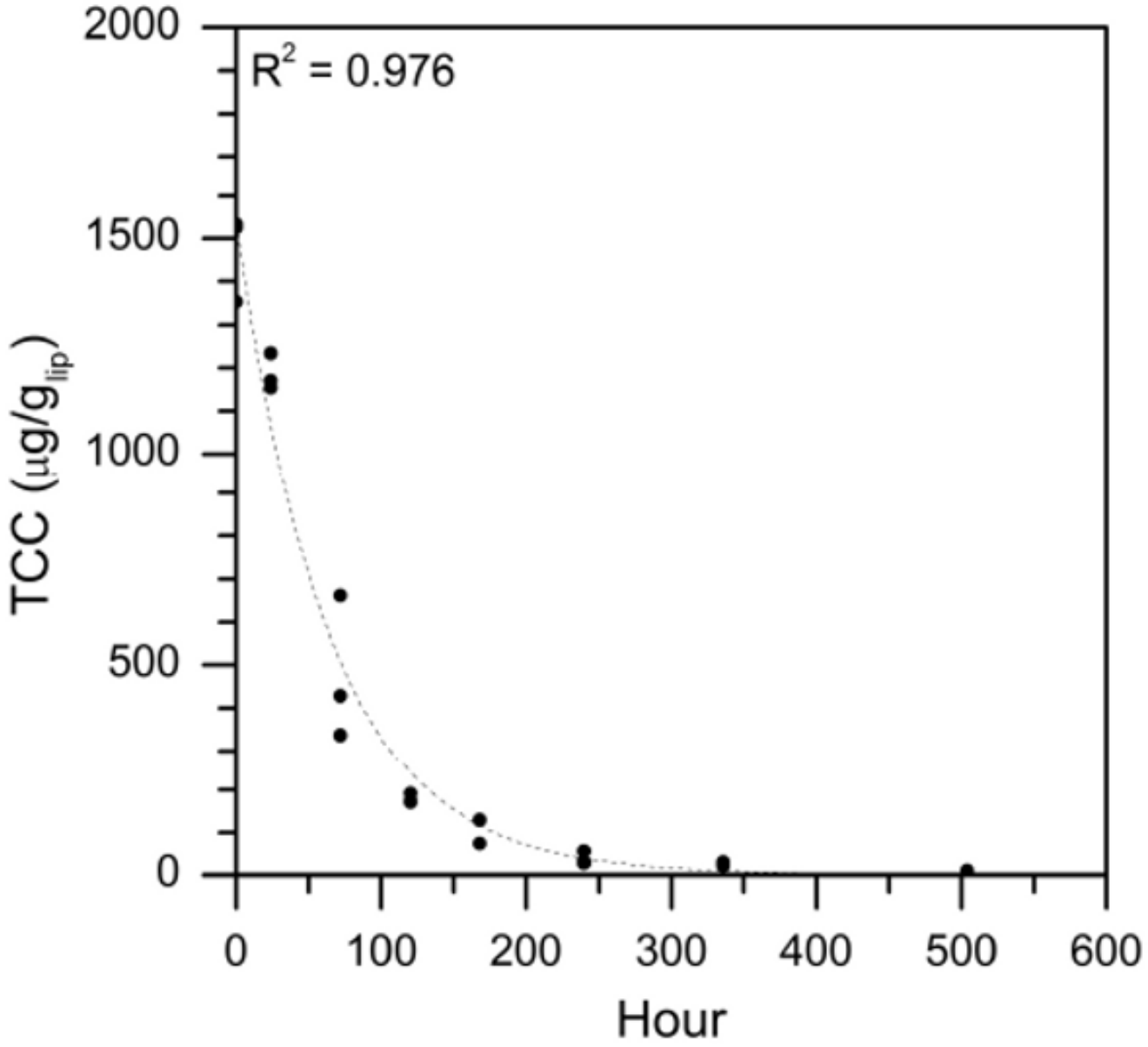

Figure 3.
Elimination of triclocarban (TCC) from *Lumbriculus variegatus* after 35-day exposure and transfer to non-spiked lake sediment. Symbols denote individual jars (n = 3 for each time point), and the dotted line denotes the fit of a first-order exponential decay model using the parameters listed in Table 2.

Table 1

MRM Transitions Monitored by LC-MS/MS

Chemical	**MRM Transitions (*m/z* → *m/z*)**
TCC	313 → 160
$^{13}C_6$-TCC	319 → 160
d_7-TCC	322 → 165[a]
DCC	279 → 126

[a] Due to overlap from $^{13}C_6$-TCC transition, the ^{37}Cl isotope transition was used for the internal standard d_7-TCC

Table 2

Biokinetic Model Parameters TCC and DCC Bioaccumulation in *L. variegatus*

Parameter (units)	Description	TCC	DCC (λ = 0)
k_s (g_{oc}/g_{lip}hr) × 10^4	Uptake Rate Constant [a]	364 ± 18	19 ± 3
λ (hr^{-1}) × 10^4	Rate Constant for Change in Bioavailability [a]	6.7 ± 0.3	--
k_e (hr^{-1}) × 10^4	Elimination Rate Constant [a]	164 ± 10	61± 12
α (hr^{-1}) × 10^4	Elimination Rate Constant [b]	154 ± 10	--

[a] Determined from the 56 day uptake experiment.

[b] Determined from the 21 day elimination experiment (after 35 days of exposure).