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Bioaccumulation of Legacy and Emerging Organochlorine Contaminants in *Lumbriculus variegatus*

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Abstract

The freshwater sediment-dwelling *Lumbriculus variegatus* is known to serve as a vector for transfer of contaminants from sediments to higher trophic level organisms, but limited data exists on the bioaccumulation of chemicals associated with sediments containing high total organic carbon. In the current study, sediments from the north shore area of Lake Apopka, FL containing very high total organic carbon (39%, w/w) were spiked with four chemicals (*p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), dieldrin, fipronil, and triclosan) individually or in a mixture of the four and then used for bioaccumulation studies. Tissue concentrations of chemicals in *L. variegatus* were measured at 2, 7, 14, 21, and 28 days of exposure and the bioaccumulation potential was evaluated using bio-sediment accumulation factors (BSAF, g_{oc}/g_{lipid}). Increase in total body burdens of all four chemicals in *L. variegatus* was rapid at day 2 and reached a steady state level after 7 days in both single and mixture experiments. Tissue concentrations of fipronil peaked after 2 days and then decreased by 70% in sediment experiments suggesting that in addition to the degradation of fipronil that occurred in the sediment, *L. variegatus* may also be able to metabolize fipronil in. The calculated 28-d BSAF values varied among the chemicals and increased in the order fipronil (1.1) < triclosan (1.4) < dieldrin (21.8) < *p,p'*-DDE (49.8) in correspondence with the increasing degree of their hydrophobicity. The relatively high BSAF values for *p,p'*-DDE and dieldrin probably resulted from lower than expected sorption of chemicals to sediment organic matter either due to the nature of the plant-derived organic matter, or as a result of the relatively short equilibration time among the various compartments, or from ingestion of sediment particles by the worms.

Keywords

L. variegatus; bioaccumulation; organochlorines; high TOC

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INTRODUCTION

Legacy organochlorine pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT) and dieldrin were extensively used from the 1940s to the late 1970s in agriculture for pest control, particularly in the southeastern United States (Voldner and Li 1995). Because OCPs are not metabolized rapidly in sediments and have high bioaccumulation and bioconcentration factors, they were selected by the National Water-Quality Assessment Programs for assessment (Crawford and Luoma 1993). In addition, emerging organochlorine contaminants including modern pesticides and pharmaceuticals and personal care products are of current concern. For example, fipronil, a new phenylpyrazole insecticide which is active against a wide range of insect pests such as termites, fleas, ticks, and fire ants (USEPA 1996), has replaced legacy pesticides in recent years and is now recognized as a potent contaminant. This current-use pesticide is known to be bioaccumulative in fish (Konwick et al. 2006; Baird et al. 2013) and earthworms (Qu et al. 2014). In the sediment-water systems, fipronil can be degraded into fipronil desulfinyl, fipronil sulfide, and fipronil sulfone where the potential formation of each metabolite depends on the environmental conditions (USEPA 1996; Lin et al. 2008; Walse et al. 2004). These metabolites have comparable or greater toxicities for certain aquatic organisms than fipronil itself (Schlenk et al. 2001). For example, the 96-h LC₅₀'s for *Procambarus clarkii* crayfish for fipronil, fipronil sulfone, fipronil sulfide, and fipronil desulfinyl were 14.3, 11.2, 15.5, and 68.6 µg/L, respectively (Schlenk et al. 2001). The two antimicrobial compounds used in personal care products, triclosan (TCS) and triclocarban (TCC), are also bioaccumulative and function as endocrine disruptors and are found in sediments and surface waters worldwide (Chalew and Halden 2009; Miller et al. 2008; Higgins et al. 2009; Delorenzo et al. 2008). Because of their antimicrobial properties, they are believed to potentially inhibit microbial activity and biodegradation of more traditional pollutants, such as *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) and dieldrin (Chalew and Halden 2009; Ela et al. 2011). The European Union has decided to ban the use of TCS in 2010, but the need for regulating TCC and TCS has still been questioned in the United States (Halden 2014).

Lumbriculus variegatus is a class of benthic fauna which plays an essential link in the aquatic food web. *L. variegatus* has extensively been used for bioaccumulation studies as worms can burrow into the sediment and tolerate highly contaminated areas, making them good models for screening sediment toxicity and assessing bioaccumulation (USEPA 2000). The bio-sediment accumulation factor (BSAF) is often used as a screening tool to assess the bioaccumulation potential of hydrophobic contaminants. Its value is calculated as the ratio of lipid-normalized chemical concentration in the organism to the organic carbon-normalized concentration in sediment. If organism lipid and sediment organic matter have the same capacity to bind hydrophobic organic compounds, the BSAF value will be ~ 1 at equilibrium. Conversely, when BSAF values are > 1, the target contaminants have a higher affinity for the organism lipid than for sediment organic carbon, indicating greater bioaccumulation potential. Previous research indicates that the mean values of BSAFs for legacy nonionic OCPs in worms range from 1.2 to 10.4 (You et al. 2006; Mehler et al. 2011) and appear not to be dependent on the octanol-water partitioning coefficient (log K_{ow}) ranging from 2.7 to 7.0 (Tracey and Hansen 1996). Recent studies have also investigated the

bioaccumulation of two ionizable compounds TCS and TCC in biosolid-amended soils and have found BSAF ranges of 0.1 to 15 (Higgins et al. 2009, 2011; Snyder et al. 2011; Macherius et al. 2014). However, bioaccumulation of emerging contaminants from contaminated sediments into worms, especially for current-use insecticides such as fipronil, is not well-understood. You et al. (2009) report that BSAF values for pyrethroids, an active ingredient found in many of the modern insecticides, were relatively low (< 1), resulting from the extensive biotransformation of pyrethroids by *L. variegatus*.

To our knowledge, few, if any, studies address the bioaccumulation of organochlorine chemicals in *L. variegatus* exposed to sediments containing very high total organic carbon (TOC), for instance, up to 40%. Due to the degree of their hydrophobicity, organochlorines may have a strong affinity for sediments with high TOC and therefore may be found in lower concentrations in porewater than expected, making them less bioavailable to worms. Alternatively, binding characteristics of the organic carbon may be influenced by plant-derived material that is apparently less sorptive than other forms of carbon, yielding greater concentration in the porewater and thus higher bioavailability (Kukkonen et al. 2004). It is evident that chemical sorption to sediments comprised of natural organic matter (e.g., vegetative material and decayed remains of animals) can be up to two orders of magnitude lower than the sorption to super sorptive materials (e.g., soot, charcoal, and black carbon) (Ghosh et al. 2003; Cornelissen et al. 2005). Therefore, it is not only sediment organic carbon content but also the characteristics of the organic carbon that determines the partitioning of OCPs in sediments and the further extent of their bioaccumulation into organisms.

The objective of this study was to better understand the bioaccumulation potential of organochlorine contaminants of varying K_{ow} into *L. variegatus* exposed to sediments containing very high TOC. We used sediments from the north shore of Lake Apopka, FL as these sediments have very high TOC, reported previously as 36% (w/w) (MACTEC 2005). Because it is not clear how the tissue concentration for any one chemical is affected by additional chemicals of varying K_{ow} that may be present in a mixture, we spiked sediments with either individual chemicals, *p,p'*-DDE (log K_{ow} 6.5), dieldrin (log K_{ow} 5.4), TCS (log K_{ow} 4.8), and fipronil (log K_{ow} 4.0) or with a mixture of the above and then used for sediment bioaccumulation studies. Bioaccumulation potential of chemicals was evaluated with BSAF, while tissue concentrations were monitored using both time-course sediment and water-only exposures. We found that 1) tissue concentrations of chemicals at each sampling point would not significantly differ regardless of whether the chemicals were present in sediments and water individually or as a mixture; 2) total body burdens of fipronil increased rapidly after a few days of exposure and then decreased over time in sediment exposures; and 3) the bioaccumulation potential of contaminants was highest in *p,p'*-DDE followed by dieldrin, TCS, and fipronil and this was in order of decreasing log K_{ow} .

MATERIALS AND METHODS

Standards and solvents

Neat standards of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p,p'*-DDE, CAS# 72-55-9, 99% pure) and 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,-

endo-5,8-exo-dimethanonaphthalene (dieldrin, CAS# 60-57-1, 98.7% pure) were purchased from Aldrich (Milwaukee, WI). Neat standards of 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4[(trifluoromethyl)-sulfinyl]-1H-pyrazole-3-carbonitrile (fipronil, CAS# 120068-37-3, 98% pure) and 2,4,4'-trichloro-2'-hydroxydiphenyl ether (TCS, CAS# 3380-34-5, 96% pure) were purchased from Enzo Life Sciences (Farmingdale, NY) and TCI America (Montgomeryville, PA), respectively. Isotope internal standards (*p,p'*-DDE-¹³C₁₂, dieldrin-¹³C₁₂, and TCS-¹³C₁₂) were obtained from Cambridge Isotope Laboratories (Tewksbury, MA) while the fipronil surrogate, fipronil des F3, was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Organic solvents and chemical agents were purchased from Fisher Scientific (Fair Lawn, NJ). Standard solutions at a concentration of 1 mg/mL were prepared by dissolving 10 mg neat standards into 10 ml methanol and calibration standards were prepared by a series of dilutions in acetonitrile.

Sediment collection and organisms

Sediment (top 40 cm) was collected from the least contaminated area on the north shore of Lake Apopka, FL, which has been historically contaminated with OCPs due to heavily agricultural activities in the past 4 decades. These sediments are water-saturated soils from marsh areas that were diked up and used for extensive farming from the 1940's to the 1970's. Upon receipt in the laboratory, the sediments were air-dried for a week, sieved (2 mm mesh size) to remove any twigs, leaves, and non-sediment materials, and then stored at 4 °C until use. We reanalyzed the sediment and found it contained *p,p'*-DDE at a concentration of 0.89 ± 0.04 µg/g dry wt (mean $\pm$ SD, n=3) but concentrations for dieldrin, fipronil, and TCS were below the limit of quantitation (20–50 ng/g dry wt, extraction method described below). Sediment organic matter was determined by the loss-on-ignition method at the Analytical Services Laboratories (IFAS), University of Florida, FL. A conversion factor of 1.72 was then used to convert organic matter to TOC assuming that organic matter contains 58% organic C (Nelson and Sommers 1996). The TOC was thus estimated at 39% (w/w) which was close to a previous report of 36% (MACTEC 2005).

Lumbriculus variegatus (California blackworms) ranging from 10–15 mg and 2–2.5 cm in wet weight and length, respectively were purchased from Aquatic Foods (Fresno, CA) one to two weeks prior to the start of the experiments and cultured at the Center for Environmental and Human Toxicology (CEHT), University of Florida, FL. Worms were maintained unfed in an aerated, continuous-flow tank containing a 1.5 cm thick layer of quartz sand. A subsample of *L. variegatus* was collected for background levels of chemicals prior to exposures.

Sediment spiking

Spiked sediment was prepared using a slurry technique (Foster et al., 1987; Landrum and Faust, 1991; Landrum et al., 1992) by adding a slurry of 1 L filtered freshwater (tap water passed through granular activated carbon followed by filtration using 0.8 µm filter) and 500 g of air-dried sediment in 2-L glass beakers and the slurry was conditioned via mixing with a rotating stainless steel blade powered by a drill for over 28 days. Test compounds, either single chemicals or a mixture of 4 chemicals, dissolved in methanol were added dropwise into the mixing slurry for a final methanol:water <0.02%, to obtain a nominal concentration

of 2 µg/g dry wt. Sediment lacking the chemical spike but amended with an equal amount of methanol:water served as solvent-control sediments. About 10-ml aliquots of sediment were collected from different locations (top-middle-bottom) within each beaker at 7, 14, 21, and 28 days of mixing for measurement of total sediment chemical concentrations. Background levels of *p,p'*-DDE were determined for the unspiked sediments and subtracted from all samples. Sediments were then rinsed with freshwater to remove unbound chemicals and the spiked and unspiked sediments were then stored at room temperature for an additional 30 days before use to allow equilibration.

Sediment bioaccumulation test

Spiked sediments prepared as described in the spiking test were used for the sediment bioaccumulation study and 28-d sediment bioaccumulation tests were performed with *L. variegatus* following a miniaturized version of the U.S. EPA recommended procedures (USEPA 2000). The exposure was conducted in 600 ml glass beakers containing 90 ± 10 g wet wt (mean $\pm$ SD) spiked sediments (~ 2 cm depth) and approximately 300 ml filtered freshwater. Approximately 0.5 ± 0.1 g wet wt (mean $\pm$ SD) worms were introduced into each beaker after the sediment had settled for 24 hr. Exposures were performed with three replicates for each sampling point (day 2, 7, 14, 21, and 28) and treatments included: 1) worms exposed to sediments spiked with individual chemicals; 2) worms exposed to sediments spiked with a mixture of 4 chemicals; and 3) worms exposed to unspiked sediments (solvent-control treatment). Worms were not fed during the course of the exposure. Exposure was performed in a constant temperature (20 ± 3 °C) with a 16:8 light:dark photoperiod, pH (6.7 ± 0.3), and with an aerated system to maintain adequate dissolved oxygen levels. Overlying water (~ 15 ml) was added to compensate for any loss via evaporation throughout the experiment. Worms were separated from sediments by sieving (500 µm mesh size) and rinsed 3 times, and allowed to depurate in filtered freshwater for 24 hr. Worms were then re-rinsed, weighed, and frozen at -20 °C prior to analysis. To mass balance chemicals in the system, about 10 g of wet sediment (~ 4 g dry wt, $n=3$) was also collected from each of the treatments at the beginning (day 0) and at the end of the exposures (day 28) for measuring total sediment chemical concentrations.

Water-only exposure

To further examine time-course tissue concentrations of contaminants in *L. variegatus*, a 28-d exposure study was conducted with filtered freshwater. Spiked water was prepared in 230-L fiberglass cylinders by adding the individual chemicals or a mixture of 4 contaminants, each dissolved in methanol, dropwise into 226 L of filtered freshwater (<0.001% methanol v/v, final conditions) to obtain a nominal concentration of 0.1 µg/L for *p,p'*-DDE and TCS and of 1 µg/L for dieldrin and fipronil. Solvent control treatments were also prepared by adding only an equal amount of methanol into the water. The spiked water in the cylinders was thoroughly mixed by aeration throughout the study. Three replicates of one liter of the spiked water from the prepared cylinders in the mixture experiment were collected at day 0 and another at day 28 in amber glass bottles, and stored in a cold room (4 °C) prior to chemical analysis. Exposures were performed in triplicate for each chemical and the mixture in 10-L glass tanks and about 4 L of spiked water was delivered into each glass tank containing 3 ± 0.2 g wet wt (mean $\pm$ SD) worms. Exposures were conducted under semi-

static, aerated, constant temperature (20 ± 3 °C), and 16:8 hr light:dark conditions. Approximately 80% of the spiked solutions were renewed every two days to ensure that chemicals were not limiting in the water. Sampling was terminated at 2, 7, 14, 21, and 28 days of the exposures. At each sampling point, worms from three random tanks were removed, rinsed with filtered freshwater, re-weighed, and frozen at -20 °C prior to analysis.

Sample analysis

Sediment and tissue samples were thawed and extractions were accomplished via sonication, a method optimized prior to this study for these chemicals. Sediments were air-dried at room temperature for a week and approximately 0.5 g of air-dried sediments was used for extraction while tissues (~ 0.2 – 0.3 g wet) were homogenized with 10 times the amount of anhydrous sodium sulfate (10:1 chemical to tissue, v/v). Sediments and tissues were then spiked with 100 ng internal standards or fipronil surrogate (fipronil des F3) prior to extraction. Sediment and tissue samples were solvent-extracted with 10 ml of hexane:acetone (1:1 v/v) and 10 ml of hexane:ethyl acetate (1:1 v/v), respectively, by shaking in a water bath incubator (32 °C at 130 rpm) for 1 hr. The samples were sonicated for an additional 10 min followed by centrifugation in a Beckman centrifuge (5 min at $\sim 450 \times g$). The extractions were performed twice and the combined supernatants were then transferred to pre-weighed glass tubes and concentrated to dryness under a gentle stream of nitrogen. For tissue samples, the tubes were reweighed and the weight difference was the lipid mass. The extracts were then redissolved into 1 ml acetonitrile and further filtered using syringe filters (0.45 μ m PTFE; 13 mm in diameter).

Water extractions were performed within a week of collection using a solid-phase extraction method. About 1 L water samples were also spiked with 100 ng internal standards or fipronil surrogate followed by vigorous shaking for 2 min. Water was then passed through a solid-phase extraction (SPE) cartridge (Oasis HLB, 6 cc, 500 mg sorbent; Waters Corp., Milford, MA) at a rate of 5 ml/min under vacuum. The elution was performed with 2 $\times$ 4 mL dichloromethane:ethyl acetate (1:1 v/v). Eluates were dried with 2–3 g of anhydrous sodium sulfate, concentrated to near dryness under a gentle stream of nitrogen, reconstituted into 1 mL acetonitrile, and filtered.

Analysis of *p,p'*-DDE and dieldrin was performed on a 6890 GC-MS (HP, Santa Clara, CA) using a ZB-5MS column (Zebron, Torrance, CA; 30 m length, 0.25 mm diameter, and 0.25 μ m film thickness). Helium in ultra high purity (99.999%) was used as a carrier gas. The GC temperature program started at 100 °C, increased in increments of 15 °C/min to a final temperature of 280 °C, and then was held for 2 min. The injector and MS source temperatures were set at 280 °C and 230 °C, respectively. Analysis of fipronil and TCS was accomplished using isotope dilution liquid chromatography negative electrospray ionization tandem mass spectrometry (LC-ESIMS/MS). Mass spectrometric analyses were carried out on an API-4000 MS/MS (Applied Biosystems, Framingham, MA) coupled to a Shimadzu Prominence HPLC (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA), controlled by Analyst 1.5 software (Applied Biosystems, Framingham, MA, USA). Reversed phase separation was carried out using either an Ultra IBD column (5 μ m particle size, 2.1×150 mm; Restek Corporation, Bellefonte, PA) or X-Bridge® C-18 column (3.5 μ m particle size,

2.1 × 150 mm; Waters Corporation, Milford, MA). The mobile phase consisted of 40% acetonitrile and 60% water flowing at a rate of 400 µL/min with a total runtime of 11 min, and a gradient profile of 10% acetonitrile/min starting at t = 1.00 min. Acetonitrile concentration was then held at 90% for 2.00 min before ramping back to 40%. Analytes were introduced into the mass spectrometer using an electrospray ionization probe operating in negative mode, and multiple reaction monitoring (MRM) was used for quantitative analysis. All analytes were identified using the two most abundant ion transitions, with the most abundant transition being used for quantitation, and the second transition for qualification.

Calibration standards for target analytes were constructed using a 6-point standard curve with analyte concentrations from 2–1000 µg/L and internal standard/fipronil surrogate concentrations of 100 µg/L. Blank spikes and duplicate matrix spikes were included. The limits of quantitation (LOQ) were approximately 2–10 ng/L in water, 0.2–1.1 µg/g lipid in worms, and 20–50 ng/g dry wt in sediment. The recoveries for the internal standards for each chemical and for fipronil surrogate ranged from 80–97%, 70–96%, and 52–75% in sediment, tissue, and water, respectively.

Data and statistical analysis

Bio-sediment accumulation factors (BSAFs) for chemicals were calculated for day 28 of the study using the following equation:

$$\text{BSAF}_{28} = \frac{C_{\text{org}}}{C_{\text{sed}}} \quad (1)$$

where C_{org} is the lipid-normalized concentration (µg/g lipid) of contaminant in the worm and C_{sed} is the organic-carbon normalized concentration (µg/g OC) of contaminant in the sediment. The average sediment and tissue concentrations of parent compounds measured at day 28 were used for calculation. The TOC of the sediments used in BSAF estimation was 39% (w/w).

Concentrations of chemicals in worms were reported as averages of three replicates measured after 2, 7, 14, 21, and 28 days and data were expressed as microgram per gram lipid. SigmaPlot version 11 was employed for statistical analysis. Concentrations in worms among sampling periods in treatments were compared by using one-way analysis of variance (ANOVA) with Tukey honestly-significant-difference tests ($p < 0.05$).

RESULTS

Sorption of chemicals to spiked sediments

Spiked sediments were prepared by adding chemicals to the sediments for a final nominal concentration of 2 µg/g dry sediment. Three aliquots were collected randomly from different locations (top-middle-bottom) within each beaker at 7, 14, 21, and 28 days of mixing and mean concentrations in the single and mixture spiking tests are presented in Table 1. Data reported in Table 1 for sediment sorption of *p,p'*-DDE included only the amount of freshly added chemical which was calculated as the difference between the amount in spiked and

unspiked sediments. Chemical stability in sediment, as shown by low standard deviations among replicates, was achieved after 7 days of mixing with either single chemicals or a mixture of 4 chemicals. Concentrations of *p,p'*-DDE and dieldrin in the mixture experiments were higher than in the single experiments, but the mean concentrations between the individual and mixture experiments were not statistically different ($p < 0.05$). Measured sediment concentration of TCS was about 2 times higher than a nominal concentration at day 7 and then remained at $\sim 2 \mu\text{g/g}$ dry wt in both single and mixture experiments. An exception was that the sediment concentration of TCS significantly decreased by day 14 in both the single chemical experiment and the mixture experiments and then further decreased in the mixture experiment by day 28 (Table 1). This relatively low concentration on day 28 could be attributed to analytical errors in the measurement of TCS in the mixture experiment. Concentrations of fipronil on day 28 were almost 50% lower than the nominal concentration in both the single and mixture experiments, which is likely due to losses via degradation, volatilization, or to a combination of these processes as fipronil can undergo oxidation to fipronil sulfone (Bobé et al. 1998), reduction to fipronil sulfide (Ramesh et al. 1998), and photolysis to fipronil desulfinyl (Hainzl et al. 1996).

Sediment bioaccumulation test

For the sediment bioaccumulation experiment, we used the sediments from the spiking test with their actual chemical concentrations measured, as indicated above. Acute mortality of worms was not observed during any of the exposures. No significant differences in lipid content were determined for *L. variegatus* exposed to control and spiked sediments over the 28-d exposure with a mean of $2.4 \pm 0.4\%$. Unexposed (negative control) worms showed background levels of target compounds (below LOQ). Concentrations of *p,p'*-DDE, dieldrin, and TCS in the sediment at the beginning (day 0) and at the end (day 28) of the exposures changed $< 5\%$, while fipronil concentration in the sediment decreased by 40 to 50% after 28 days of the exposures suggesting degradation of fipronil in the sediment. Tissue concentrations of chemicals at different times in the single experiment (Fig. 1a) were not statistically different from that in the experiment with a mixture of 4 chemicals (Fig. 1b) ($p < 0.05$). Concentrations of *p,p'*-DDE in worms plotted in the figure is only the amount of accumulation of freshly spiked *p,p'*-DDE. Concentrations of aged *p,p'*-DDE in worms exposed to unspiked sediment followed the same accumulation pattern, but to a lower (4–6 fold) extent.

For both single and mixture exposure scenarios for all of the chemicals, the tissue concentrations increased rapidly in the first 2 days and reached a plateau within 7 days, albeit to different body burdens. The highest body burdens were observed for *p,p'*-DDE and dieldrin in correspondence with their high $\log K_{ow}$ of 6.5 and 5.4, respectively. The initial increase in tissue concentration at 2 days for TCS in either the single chemical experiment or the mixture experiment appeared to be slower than what was observed at 7 days or for the other chemicals, suggesting that the worms were either able to metabolize TCS rapidly or excrete it. By 7 days, total body burdens of TCS reached a steady state level for both experiments. The pattern for fipronil differed from the other chemicals in both experiments (Figs. 1a, 1b) as it peaked at day 2 and then decreased by 70% suggesting that in addition to

the degradation of fipronil that occurred in the sediment, the worms may also be able to metabolize fipronil.

The bioaccumulation potential at day 28 was expressed as BSAFs for the four chemicals from sediments to *L. variegatus* and are presented in Table 2. BSAF values for *p,p'*-DDE were estimated using total chemical concentrations (freshly spiked and aged chemicals) measured in sediment and tissues. BSAF values varied among the four compounds and the order was fipronil (1.1) < TCS (1.4) < dieldrin (21.8) < *p,p'*-DDE (49.8) in correspondence with the increasing degree of their hydrophobicity (log K_{ow} 4.0, 4.8, 5.4, and 6.5 for fipronil, TCS, dieldrin, and *p,p'*-DDE, respectively). Overall, BSAF values were all greater than 1 suggesting that these chemicals have a higher affinity for organism lipid compared with sediment organic carbon indicating greater bioaccumulation potential.

Water-only exposure

A 28-d water-only exposure was conducted to further investigate the time course for body burdens of contaminants in worms. Spiked water from the mixture experiment was only collected at day 0 and at day 28 of the exposures for chemical analysis. The concentrations in the spiked water were fairly stable over 28 days with changes <10% for all chemicals, except for TCS where the concentration decreased by 40% from day 0 to day 28. Decrease in concentrations of TCS might be due to sorption to the exposure cylinders and biodegradation. In addition, TCS is known to be subject to photodegradation (Singer et al. 2002; Aranami and Readman 2007; Buth et al. 2010), with half-lives reported to be as low as 4 days. The exposures in the current study were performed in a 16:8 light:dark cycle and therefore; the reduced concentrations of TCS from day 0 to day 28 in the spiked water could be due to photodegradation.

L. variegatus did not die in any of the exposures, however, they experienced about 20% weight loss, as they were not fed. The percentage of parent compounds in *L. variegatus* exposed to water spiked with 4 chemicals individually or in a mixture peaked at 2 days and then remained stable over time (Figs. 2a, 2b). TCS was below the limit of quantification for the single chemical exposure (Fig. 2a) but was measurable in the mixture exposure (Fig. 2b). Again, the initial increase in body burden of TCS into worms in the mixture experiment appeared to be slower at 2 days than at 7 days and slower for the increases at this time point for the other chemicals, showing very low accumulation in the first 2 days, suggesting that the worms were either able to metabolize TCS rapidly or excrete it. By 7 days, tissue concentration of TCS reached a steady state level.

DISCUSSION

Sediment bioaccumulation of chemicals into *L. variegatus*

L. variegatus can serve as a potential vector for the transfer of contaminants from sediments to higher trophic level organisms and it is thus important to understand bioaccumulation of contaminants in worms. In the sediment spiking test (Table 1), measured concentrations of *p,p'*-DDE, dieldrin, and TCS in sediment were similar to nominal amounts of chemicals added to the sediment (2 µg/g dry wt), suggesting that these hydrophobic contaminants

partitioned as expected to the sediment. In the case of fipronil, concentrations decreased by 40 to 50% after 28 days in both the single chemical experiment and mixture experiment, suggesting that microbial activity in the sediments or photodegradation occurred. The addition of TCS, an antimicrobial chemical, to the mixture experiment did not decrease the degradation of fipronil, suggesting it might be more from abiotic sources (USEPA 1996; Lin et al. 2008; Walse et al. 2004). We used the sediments from this spiking test for bioaccumulation tests. Bioaccumulation of four chemicals was measured in worms after 28-d of exposure at chemical concentrations ranging from 0.8 to 1.7 $\mu\text{g/g}$ dry sediment (2.2 to 4.2 $\mu\text{g/g}$ OC, Table 2). BSAFs values varied among the compounds and were > 1 with the highest BSAF value for *p,p'*-DDE (49.8) followed by dieldrin (21.8) and TCS (1.7) and fipronil (1.1). Tracey and Hansen (1995) indicated that BSAF values were uniform (mean of medians = 1.36) for nonionic pesticides with $\log K_{ow}$ ranging from 2.7 to 6.9. Although fipronil, *p,p'*-DDE, and dieldrin used in the current study are nonionic pesticides with their $\log K_{ow}$ of 4.0, 5.4, and 6.5, respectively, only the BSAF value for fipronil appeared to follow the previous estimation. In addition, the BSAF values for *p,p'*-DDE and dieldrin in the current study were much higher than those in previous studies that reported BSAF values for *p,p'*-DDE and dieldrin ranging from 2.0 to 10.4 (You et al. 2006; Mehler et al. 2011) and from 0.5 to 2.5 (Standley, 1997), respectively. Relatively high BSAF values for *p,p'*-DDE and dieldrin in the current work may have resulted from three distinct processes. First, characteristics of Lake Apopka sediment may be dominated by vegetative debris and decayed remains of animals. Literature reports that sorptive behavior of hydrophobic compounds is less strong for sediment organic carbon associated with these natural sources than for sediments containing organic carbon derived from particles of coal, charcoal, and soot (Ghosh et al. 2003; Cornelissen et al. 2005; Gustafsson et al. 1996). Perhaps pesticides are less sorptive to Lake Apopka sediments yielding greater bioavailability than expected. The second reason for the higher BSAF values might be due to the relatively short time the contaminants were associated with the sediments prior to the addition of the worms. In our experiments, the sediments were mixed with the contaminants for 28 days and then further allowed to mature for another 30 days before the introduction of worms. However, this may still not have allowed full partitioning of contaminants to sediments. You et al. (2006) indicated that the distribution of some organochlorine chemicals to the slow compartments would require more than 1 yr for equilibrium. The estimations of very high BSAF values for *p,p'*-DDE and dieldrin in the current study might partly result from the relatively short equilibrium time among the various compartments. The third reason may be from ingestion of the sediments, which is a route of chemical exposure in oligochaetes (USEPA 2000). The worms were not fed during the experiment and we noted sediments within their gastrointestinal (GI) tracts after 28 days. While the worms were rinsed extensively and allowed to depurate in clean water for 24 hr with no sediments still visible in the GI tracts, it is possible that because of the low sorption of the TOC in the sediments, that the contaminants more easily partitioned into the lipid content of the gut.

Uptake of emerging organochlorine chemicals (e.g., TCS and fipronil) from sediment into worms has been slightly studied. Karlsson et al. (2015) measured concentrations of TCS in sediment and in *L. variegatus* and found that 48-h BSAF values ranged from 6.6 to 9.0. The BSAF value for TCS in the current study was about 5 to 6 fold lower than the BSAFs

measured in Karlsson's study. Possible explanations for the differences between the results of the current study and the previous study could be differences in sediment characteristics, differences in species traits, or possible biotransformation of TCS in the test system or in the worms themselves. However, Higgins et al (2009) estimated a 56-d BSAF for triclocarban, a similar compound, for *L. variegatus* to be 1.6 ± 0.6 , which is similar to the 28-d BSAF for TCS (1.4 ± 0.3) in the current study. It has been suggested that the capacity of lipids of organisms to bind non-metabolized hydrophobic organic contaminants (HOCs) was approximately twice as large as the binding capacity of organic carbon in the sediment, and the equilibrium partitioning theory predicted a steady-state BSAF value of 1.7 for HOCs if their log K_{ow} is less than 6 (Di Toro et al. 1991). TCS (log K_{ow} 4.8) is thus able to bioaccumulate into worms from sediments in a manner that is consistent with the equilibrium partitioning of traditional hydrophobic organic contaminants. Conversely, bioaccumulation of current-use insecticide fipronil in organisms was previously not well-understood. You et al. (2009) report that BSAF values for pyrethroids, an active ingredient found in many of the modern insecticides, were relatively low (< 1), which was lower than the BSAF values for fipronil in the current study (> 1). In addition, biotransformation of fipronil was observed in earthworms (*Eisenia feotida*) exposed to fipronil-spiked soils (Qu et al. 2014), which could partially explain the severe body burden reduction we observed in our study. To our knowledge, this is the first study that investigated the bioaccumulation potential of fipronil in *L. variegatus*.

Water-only exposures.

Time-course tissue concentrations of four contaminants were also assessed using water-only exposures. In the water-only exposures, spiked water was exchanged every two days to make sure that concentrations of contaminants were not limiting in the exposure tanks. Total body residue concentrations of all four contaminants in the water exposures increased rapidly by day 2 and remained a steady state level after 7 days of exposure. Uptake of TCS into worms in the mixture experiment (Fig. 2b) was slow in the first two days and then peaked at 7 days (Fig. 2b), similar to uptake patterns observed in the sediment exposures (Fig. 1), suggesting that the worms were either able to metabolize TCS rapidly or excrete it. Tissue concentrations of fipronil, however, did not decrease in the water exposures after 7 days, as seen in the sediment exposures. A possible explanation for this difference was that fipronil was constantly replenished from the originally mixed solution in cylinders into the exposure tanks (every 2 days) making it highly bioavailable for worms, while fipronil in the sediments could have been diminished either by abiotic or biotic means. Literature has shown that *L. variegatus* is able to biotransform PAHs (Lyytikäinen et al. 2007; Leppänen and Kukkonen 2000), trinitrotoluene (TNT) (Belden et al. 2005), and pyrethroid insecticides (You et al. 2009), but no data exists for induction of biotransformation of fipronil in this species. Our results from the 28-d water exposures do not strongly support increased biotransformation of fipronil in worms and further research is needed to verify whether or not this biological process occurs.

In conclusion, our study suggests that pesticides with relatively high K_{ow} found in sediments with high TOC derived from vegetative sources may be more bioavailable to organisms than predicted by equilibrium partitioning theory (Di Toro et al. 1991) or even from the non-

equilibrium, steady state model proposed by Morrison et al. (1996). This may be the mechanism by which high bioaccumulation of OCPs was observed in fish (Martyniuk et al. submitted) and birds (SJRWMD, 2006) in the north shore area of Lake Apopka, FL.

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Figure 1.

Tissue concentrations of chemicals (µg/g lipid, n=3) measured in *L. variegatus* exposed to spiked sediments at different sampling times. Values represent the mean of three replicates $\pm$ standard deviation. (A) Single chemical experiment. (B) Mixture experiment.

Figure 2.

Tissue concentrations of chemicals (µg/g lipid, n=3) measured in *L. variegatus* exposed to spiked water at sampling different times. Values represent the mean of three replicates $\pm$ standard deviation. (A) p,p' -DDE and TCS in single experiments. TCS concentrations were below limit of quantitation. (B) p,p' -DDE and TCS in the mixture experiment. (C) Dieldrin and fipronil in single experiments. (D) Dieldrin and fipronil in the mixture experiment.

Table 1

Sediment concentrations ($\mu\text{g/g}$ dry sediment, $n=3$) for chemicals measured in 28-d spiking tests. Values represent the mean of three replicates $\pm$ standard deviation.

	Individual experiment				Mixture experiment			
	d7	d14	d21	d28	d7	d14	d21	d28
<i>p,p'</i> -DDE	2.4 ± 0.7	2.6 ± 0.7	2.4 ± 0.6	1.9 ± 0.5	4.6 ± 1.0	3.9 ± 0.8	3.7 ± 0.3	3.3 ± 0.8
dieldrin	2.2 ± 0.4	1.8 ± 0.2	1.6 ± 0.2	1.5 ± 0.1	3.1 ± 0.2	2.5 ± 0.6	2.3 ± 0.7	2.2 ± 0.3
TCS	4.0 ± 1.4	2.0 ± 0.7	2.3 ± 0.7	2.2 ± 0.3	4.5 ± 0.3	2.5 ± 0.5	2.2 ± 0.3	1.3 ± 0.3^a
fipronil	1.9 ± 0.6	1.6 ± 0.4	1.5 ± 0.3	1.2 ± 0.2	3.2 ± 0.3	1.7 ± 0.3	1.4 ± 0.4	1.1 ± 0.2

^a Analytical error

Table 2

Sediment concentrations (C_{sed}), concentration in *L. variegatus* (C_{org}), and bio-sediment accumulation factors (BSAF) for 4 chemicals measured at day 28 of the exposures. Values represent the mean of three replicates $\pm$ standard deviation for sediment and worm data. OC: organic carbon.

	C_{sed} ($\mu\text{g/g dry wt}$)	C_{sed} ($\mu\text{g/g OC}$)	C_{org} ($\mu\text{g/g lipid}$)	BSAF (g OC/g lipid)
<i>p,p'</i> -DDE	1.7 ± 0.2	4.2 ± 0.6	209.7 ± 43.0	49.8 ± 10.2
dieldrin	0.9 ± 0.1	2.3 ± 0.4	50.1 ± 5.5	21.8 ± 2.4
TCS	1.4 ± 0.2	3.5 ± 0.5	5.0 ± 1.2	1.4 ± 0.3
fipronil	0.8 ± 0.1	2.2 ± 0.3	2.6 ± 1.2	1.1 ± 0.5