Arch Environ Contam Toxicol (2016) 71:60–69
DOI 10.1007/s00244-016-0264-x

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

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Received: 17 July 2015 / Accepted: 19 January 2016 / Published online: 1 February 2016

Abstract Freshwater sediment-dwelling *Lumbriculus variegatus* is known to serve as a vector for the transfer of contaminants from sediments to higher trophic level organisms, but limited data exist on the bioaccumulation of chemicals associated with sediments containing high total organic carbon (TOC). In the current study, sediments from the north shore area of Lake Apopka (Florida, USA), containing very high TOC [39 % (w/w)], were spiked with four chemicals—*p,p′*-dichlorordiphenyldichloroethylene (*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 biosediment 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. The calculated 28-day 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, as a result of the relatively short equilibration time among the various compartments, or due to ingestion of sediment particles by the worms.

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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 (Volner 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 Program 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 phenylpyrazole insecticide that is active against a wide range of insect pests, such as termites, fleas, ticks, and fire ants (United States Environmental Protection Agency (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 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 LC_{50} values for *Procambarus clarkii* crayfish for fipronil, fipronil sulfone, fipronil sulfide, and fipronil desulfinyl are 14.3, 11.2, 15.5, and 68.6 μg/L, respectively (Schlenk et al. 2001). Two antimicrobial compounds used in personal care products, triclosan (TCS) and triclocarban (TCC), are also bioaccumulative, 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 is still being questioned in the United States (Halden 2014).

Lumbriculus variegatus is a class of benthic fauna that plays an essential link in the aquatic food web. *L. variegatus* has extensively been used for bioaccumulation studies because worms can burrow into the sediment and tolerate highly contaminated areas, which makes them good models for screening sediment toxicity and assessing bioaccumulation (USEPA 2000). The 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 approximately 1 at equilibrium. Conversely, when BSAF values are >1, the target contaminants have a greater 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 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) reported that BSAF values for pyrethroids, active ingredients found in many modern insecticides, were relatively low (<1) resulting from the extensive biotransformation of pyrethroids by *L. variegatus*.

To our knowledge, few, if any, studies have addressed the bioaccumulation of organochlorine chemicals in *L. variegatus* exposed to sediments containing very high total organic carbon (TOC), e.g., ≤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, thus making them less bioavailable to worms. Alternatively, the binding characteristics of OC may be influenced by plant-derived material that is apparently less sorptive than other forms of carbon, thus yielding greater concentrations in the porewater and thus greater 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 OC content, but also the characteristics of the OC, 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 (Florida, USA) because 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 them for sediment bioaccumulation studies. Bioaccumulation potential of chemicals was evaluated with BSAF, whereas 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 did 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 overtime 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 no. 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 no. 60-57-1, 98.7 % pure)] were purchased from Aldrich (Milwaukee, Wisconsin, USA). Neat standards of 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4[(trifluoromethyl)-sulfinyl]-1*H*-pyrazole-3-carbonitrile [fipronil (CAS no. 120068-37-3, 98 % pure)] and 2,4,4′-trichloro-2′-hydroxyldiphenyl ether [TCS (CAS no. 3380-34-5, 96 % pure)] were purchased from Enzo Life Sciences (Farmingdale, New York, USA) and TCI America (Montgomeryville, Pennsylvania, USA), respectively. Isotope internal standards (*p,p*′-DDE-$^{13}C_{12}$, dieldrin-$^{13}C_{12}$, and TCS-$^{13}C_{12}$) were obtained from Cambridge Isotope Laboratories (Tewksbury, Massachusetts, USA), whereas 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, New Jersey, USA). 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, which has been historically contaminated with OCPs due to heavily agricultural activities in the past four decades. These sediments are water-saturated soils from marsh areas that were diked up and used for extensive farming from the 1940s to the 1970s. On receipt in the laboratory, the sediments were air-dried for 1 week, sieved (2-mm mesh size) to remove any twigs, leaves, and nonsediment materials, and then stored at 4 °C until use. We reanalyzed the sediment and found that it contained *p,p*′-DDE at a concentration of 0.89 ± 0.04 μg/g dry weight [dw (mean ± SD, $n = 3$)], but concentrations for dieldrin, fipronil, and TCS were lower than the limit of quantitation (LOQ; 20–50 ng/g dw, extraction method described later in the text). Sediment organic matter was determined by the loss-on-ignition method at the Analytical Services Laboratories at the University of Florida (Florida, USA). A conversion factor of 1.72 was then used to convert organic matter to TOC assuming that organic matter contains 58 % OC (Nelson and Sommers 1996). The TOC was thus estimated at 39 % (w/w), which was close to a previous report of 36 % (MACTEC 2005).

L. variegatus (California blackworms) ranging from 10 to 15 mg and 2 to 2.5 cm wet weight (ww) and length, respectively, were purchased from Aquatic Foods (Fresno, California, USA) 1–2 weeks before the start of the experiments and cultured at the Center for Environmental and Human Toxicology (CEHT), University of Florida. 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 before 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 1 L filtered freshwater (tap water passed through granular activated carbon followed by filtration using a 0.8-μm filter) to 500 g of air-dried sediment in 2-L glass beakers, and the slurry was conditioned by way of mixing for 28 days using a rotating stainless steel blade powered by a drill. Test compounds, either single chemicals or a mixture of four chemicals, dissolved in methanol were added dropwise into the mixing slurry, for a final mixture of methanol and water of <0.02 %, to obtain a nominal concentration of 2 μg/g dw. Sediment lacking the chemical spike but amended with an equal amount of methanol-and-water mixture served as solvent-control sediments. Approximately 10 ml aliquots of sediment were collected from different locations (top, middle, and 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-day sediment bioaccumulation tests were performed with *L. variegatus* according to a miniaturized version of the USEPA-recommended procedures (USEPA 2000). The exposure was performed in 600-ml glass beakers containing 90 ± 10 g ww (mean ± SD) spiked sediments (approximately 2-cm depth) and approximately 300 ml filtered freshwater. Approximately 0.5 ± 0.1 g ww (mean ± SD) worms were introduced into each beaker after the sediment had settled for 24 h. Exposures were performed with three replicates for each sampling point (days 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-h light-to-dark photoperiod at pH (6.7 ± 0.3) and with an aerated system to maintain adequate dissolved oxygen levels. Overlying water (approximately 15 ml) was added to compensate for any loss by way of 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 h. Worms were then rerinsed, weighed, and frozen at −20 °C before analysis. To mass balance chemicals in the system, approximately 10 g of wet sediment [approximately 4 g dw ($n = 3$)] 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-day exposure study was performed with filtered freshwater. Spiked water was prepared in 230-L fiberglass cylinders by adding the individual chemicals or a mixture of four contaminants, each dissolved in methanol, added 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 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) before chemical analysis. Exposures were performed in triplicate for each chemical and the mixture in 10-L glass tanks and approximately 4 L of spiked water was delivered into each glass tank containing 3 ± 0.2 g ww (mean ± SD) worms. Exposures were performed under semistatic, aerated, constant temperature (20 ± 3 °C), and 16:8-h light-to-dark conditions. Approximately 80 % of the spiked solutions were renewed every 2 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, reweighed, and frozen at −20 °C before analysis.

Sample Analysis

Sediment and tissue samples were thawed and extractions were accomplished by way of sonication, a method optimized before 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 whereas tissues (approximately 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) before extraction. Sediment and tissue samples were solvent-extracted with 10 ml of hexane and acetone [1:1 (v/v)] and 10 ml of hexane and ethyl acetate [1:1 (v/v)], respectively, by shaking in a water bath incubator (32 °C at 130 rpm) for 1 h. The samples were sonicated for an additional 10 min followed by centrifugation in a Beckman centrifuge (for 5 min at approximately 450×*g*). The extractions were performed twice, and the combined supernatants were then transferred to preweighed glass tubes and concentrated to dryness under a gentle stream of nitrogen. For tissue samples, the tubes were reweighed, and the weight difference was considered the lipid mass. The extracts were then redissolved into 1 ml acetonitrile and further filtered using syringe filters (0.45-μm polytetrafluoroethylene); 13-mm diameter).

Water extractions were performed within 1 week of collection using a solid-phase extraction (SPE) method. Approximately 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 an SPE cartridge (Oasis HLB, 6 cc, 500 mg sorbent; Waters, Milford, Massachusetts, USA) at a rate of 5 ml/min under vacuum. The elution was performed with 2 × 4 mL dichloromethane and 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 gas chromatography-mass spectrometer (HP, Santa Clara, California USA) using a ZB-5MS column (Zebron, Torrance, California, USA; 30-m length, 0.25-mm diameter, and 0.25-μm film thickness). Helium in ultra high-purity (99.999 %) was used as a carrier gas. The gas chromatography temperature program was started at 100 °C, increased in increments of 15 °C/min to a final temperature of 280 °C, and then held for 2 min. The injector and mass spectrometry (MS) source temperatures were set at 280 and 230 °C, respectively. Analysis of fipronil and TCS was accomplished using isotope dilution liquid chromatography negative electrospray ionization tandem MS (LC–ESI–MS/MS). Mass spectrometric analyses were performed on an API-4000 MS/MS (Applied Biosystems, Framingham, Massachusetts, USA) coupled to a Shimadzu Prominence high-performance liquid chromatograph (Shimadzu, Columbia, Maryland, USA) controlled by Analyst 1.5 software (Applied Biosystems). Reversed-phase separation was performed using either an Ultra IBD column (5-μm particle size, 2.1 × 150 mm; Restek, Bellefonte, Pennsylvania, USA) or X-Bridge C-18 column (3.5-μm particle size, 2.1 × 150 mm; Waters,

Milford, Massachusetts, USA). 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 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 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 six-point standard curve with analyte concentrations from 2 to 1000 µg/L and internal standard/fipronil surrogate concentrations of 100 µg/L. Blank spikes and duplicate matrix spikes were included. The LOQs were approximately 2–10 ng/L in water, 0.2 to 1.1 µg/g lipid in worms, and 20 to 50 ng/g dw in sediment. Recoveries for the internal standards for each chemical and for the fipronil surrogate ranged from 80 to 97, 70 to 96, and 52 to 75 % in sediment, tissue, and water, respectively.

Data and Statistical Analysis

(BSAFs for chemicals were calculated for day 28 of the study using the following equation:

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

where C_{org} is the lipid-normalized concentration (µg/g lipid) of contaminant in worms, and C_{sed} is the organic-carbon normalized concentration (µg/g OC) of contaminant in 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 software was employed for statistical analysis. Concentrations in worms among sampling periods in treatments were compared using one-way analysis of variance with Tukey's honestly significant difference test ($p < 0.05$).

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, and bottom) within each beaker at 7, 14, 21, and 28 days of mixing, and mean concentrations in the single and mixture spiking tests are listed in Table 1. Data listed in Table 1 for sediment sorption of *p,p′*-DDE include 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 SDs among replicates, was achieved after 7 days of mixing with either single chemicals or a mixture of the four chemicals. Concentrations of *p,p′*-DDE and dieldrin in the mixture experiments were greater than those 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 approximately 2 times greater than a nominal concentration at day 7 and then remained at approximately 2 µg/g dw 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-chemical and mixture experiments, which is likely due to losses by way of degradation, volatilization, or to a combination of these processes because fipronil can undergo oxidation to fipronil sulfone (Bobé et al. 1998), reduction to fipronil sulfide (Ramesh and Balasubramanian 1999), and photolysis to fipronil desulfinyl (Hainzl and Casida 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 previously. 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-day exposure with a mean of 2.4 ± 0.4 %. Unexposed (negative control) worms showed background levels of target compounds (lower than 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 %, whereas fipronil concentration in the sediment decreased by 40–50 % after 28 days of the exposures suggesting the degradation of fipronil in sediment. Tissue concentrations of chemicals at different times in the single-chemical experiment (Fig. 1a) were not

Table 1 Sediment concentrations (μg/g dry sediment, $n = 3$) for chemicals measured in 28-day spiking tests

	Single-chemical experiment				Mixture experiment			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
p,p′-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

Values represent the mean of three replicates ± SD

[a] Analytical error

statistically different from that in the experiment with a mixture of the four chemicals (Fig. 1b) ($p < 0.05$). Concentrations of *p,p′*-DDE in worms plotted in the figure represent 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 (i.e., four to sixfold) extent.

For both single-chemical 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 to 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 (Fig. 1a, b) because 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 listed 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 (*i.e.*, 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 >1 suggesting that these chemicals have a greater affinity for organism lipid compared with sediment OC, thus indicating greater bioaccumulation potential.

Water-Only Exposure

A 28-day water-only exposure was performed to further investigate the time course for body burdens of contaminants in worms. Spiked water from the mixture experiment

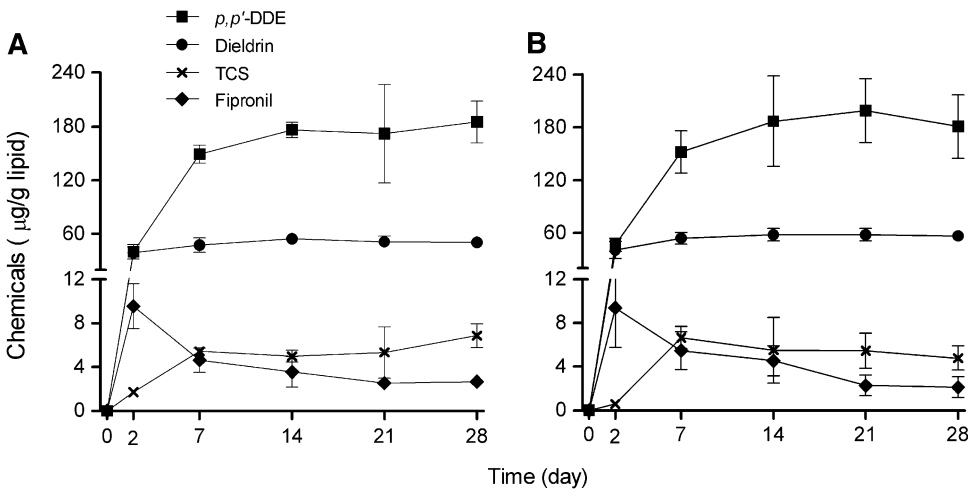

Fig. 1 Tissue concentrations of chemicals (μg/g lipid, $n = 3$) measured in *L. variegatus* exposed to spiked sediments at different sampling times. Values represent the mean of three replicates ±SD. **a** Single-chemical experiment. **b** Mixture experiment

Table 2 Sediment concentrations (C_{sed}), concentration in *L. variegatus* (C_{org}), and BSAFs for four chemicals measured at day 28 of the exposures

Chemical	C_{sed} (μg/g dw)	C_{sed} (μg/g OC)	C_{org} (μg/g lipid)	BSAF (g OC/g lipid)
p,p′-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

Values represent the mean of three replicates ± SD for sediment and worm data

OC organic carbon

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 TCS concentrations 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-h light-to-dark cycle, and therefore the decreased 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 approximately 20 % weight loss because they were not fed. The percentage of parent compounds in *L. variegatus* exposed to water spiked with four chemicals individually or in a mixture peaked at 2 days and then remained stable overtime (Fig. 2a, b). TCS was lower than the LOQ for the single-chemical exposure (Fig. 2a), but it 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, thus suggesting that the worms were either able to metabolize TCS rapidly or excrete it. By 7 days, the 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 greater trophic level organisms, and thus it is important to understand the 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 dw) suggesting that these hydrophobic contaminants partitioned to the sediment as expected. In the case of fipronil, concentrations decreased by 40–50 % after 28 days in both the single-chemical and mixture experiments 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 that its degradation 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 days of exposure at chemical concentrations ranging from 0.8 to 1.7 μg/g dry sediment (2.2–4.2 μg/g OC [Table 2]). BSAF 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 (1996) 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 log K_{ow} values 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 greater 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 OC associated with these natural sources than for sediments containing OC 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 greater BSAF values might be due to the relatively short time the contaminants were associated with the sediments before the addition of the worms. In our

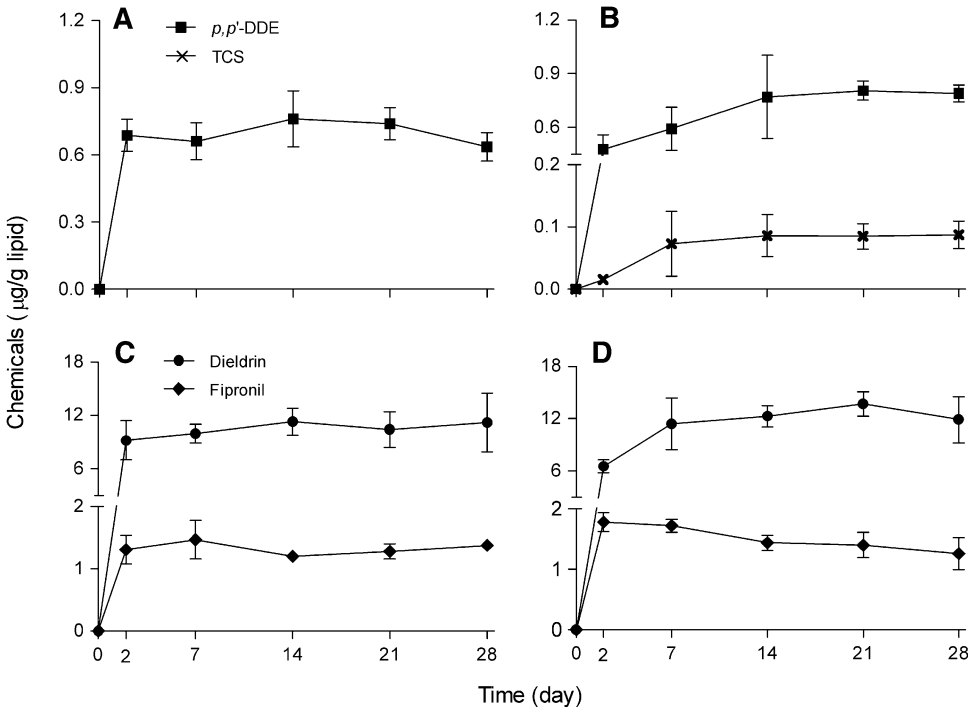

Fig. 2 Tissue concentrations of chemicals (μg/g lipid, $n = 3$) measured in *L. variegatus* exposed to spiked water at different sampling times. Values represent the mean of three replicates ±SD. **a** *p,p′*-DDE and TCS in single-chemical experiments. TCS concentrations were lower than LOQ. **b** *p,p′*-DDE and TCS in the mixture experiment. **c** Dieldrin and fipronil in the single-chemical experiments. **d** Dieldrin and fipronil in the mixture experiment

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 >1 year 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 the 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. Although the worms were rinsed extensively and allowed to depurate in clean water for 24 h, with no sediments still visible in the GI tracts, it is possible that because of the low sorption of the TOC in the sediments, 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 only 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 approximately five to sixfold 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 the 56-day BSAF for triclocarban, a similar compound, for *L. variegatus* to be 1.6 ± 0.6, which is similar to the 28-day BSAF for TCS (1.4 ± 0.3) in the current study. It has been suggested that the capacity of lipids of organisms to bind nonmetabolized hydrophobic organic contaminants (HOCs) was approximately twice as large as the binding capacity of OC 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 <6 (Di Toro et al. 1991). Thus, TCS (log K_{ow} 4.8) is able to bioaccumulate into worms from sediments in a manner consistent with the equilibrium partitioning of traditional hydrophobic organic contaminants. Conversely, bioaccumulation of the current-use insecticide fipronil in organisms was previously not well understood. You et al. (2009) reported that BSAF values for pyrethroids, an active ingredient found in many modern insecticides, were relatively low (<1), which was lower than the BSAF values for fipronil in the current study (>1). In addition, the 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 2 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 2 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 to 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), thus making it highly bioavailable for worms, whereas fipronil in the sediments could have been diminished either by abiotic or biotic means. Literature has shown that *L. variegatus* is able to biotransform polycyclic aromatic hydrocarbons (Lyytikäinen et al. 2007; Leppänen and Kukkonen 2000), trinitrotoluene (Belden et al. 2005), and pyrethroid insecticides (You et al. 2009), but no data exist for the induction of biotransformation of fipronil in this species. Our results from the 28-day 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 nonequilibrium, steady-state model proposed by Morrison et al. (1996). This may be the mechanism by which the high bioaccumulation of OCPs was observed in fish and birds (SJRWMD 2006) in the north shore area of Lake Apopka, FL.

Acknowledgments We thank Emily R. Pszczola for help with sample collection. This project was supported by the National Institute of Environmental Health Sciences (Award No. R01-ES020899).

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