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Acute Toxicity of Sediment-Sorbed Endrin, Methoxychlor, and Endosulfan to *Hyalella azteca* and *Chironomus tentans*

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Although once widely used, most organochlorine pesticides (OCs) have been de-registered in the United States for 20-30 years. Due to their hydrophobic nature, OCs tend to accumulate and persist in sediments. Currently, sediment residues of OCs are still routinely found in aquatic environments and depending on their availability to aquatic organisms, they may pose a significant threat (You et al. in press).

In our previous study (Weston et al. 2004), 70 sediments samples were collected and toxicity evaluated from a 10-county area in the agriculturally dominated Central Valley of California. In this study, pyrethroids were determined to be the main contributor to the observed toxicity of the collected sediments; however, residues of OCs including endrin, methoxychlor and endosulfan were detected routinely and may be responsible for the toxicity found at several sites. Endrin has been banned for agricultural use for more than two decades, while endosulfan and methoxychlor are currently being applied for a variety of agricultural activities. To assess the toxicity data in our previous study (Weston et al. 2004), an extensive literature review was performed of 10-d sediment LC50 values of OCs for *H. azteca* and *C. tentans*. However, the information on the acute lethality of endrin, endosulfan and methoxychlor in sediments to these two aquatic organisms was lacking. Therefore, the objectives of this study were to fill data gaps by conducting 10-d sediment toxicity tests with endosulfan (the α- isomer, β-isomer and endosulfan sulfate compounds individually), endrin and methoxychlor to *H. azteca* and *C. tentans*. In addition, the relative sensitivity of the selected OCs also was compared between the two species.

MATERIALS AND METHODS

Quail-grade endrin was purchased from PolyScience Corp (Nile, IL, USA). Methoxychlor (99%), α-endosulfan (99%), β-endosulfan (99%) and endosulfan sulfate (98%) were purchased from ChemService (West Chester, PA, USA). The reagents (anhydrous magnesium sulfate and anhydrous sodium sulfate) and

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various solvents were all purchased from Fisher Scientific (Pittsburgh, PA, USA). The solvents used in this study were all pesticide grade.

The amphipod, *H. azteca,* and the midge, *C. tentans,* were chosen as test organisms for toxicity testing in this study due to their wide distribution throughout North and Central America, relative sensitivity to contaminants within the sediment, ease of culture and usefulness in interlaboratory comparisons of toxicity data (Ingersoll et al. 1995; U.S. EPA 2000). Juvenile *H. azteca* which passed through a 1-mm sieve, but were retained on a 0.5-mm sieve (2 to 3 week old) and 3rd instar *C. tentans* were used throughout our experiments. These invertebrates were obtained from the stock cultures maintained in the Fisheries and Illinois Aquaculture Center, Southern Illinois University at Carbondale from cultures originally obtained from the U.S. EPA Environmental Research Laboratory, Duluth, Minnesota.

Dosed sediments were prepared from a soil collected in Florissant, Missouri. This soil was composed of 26% sand, 56% silt and 18% clay, its total organic carbon (TOC) content was approximately 1%, and it has been used as a reference material in previous studies (Schuler et al. 2002). The soil was sieved with a 0.5-mm screen and mixed with water to produce sediment with a dry:wet ratio between 60 and 70%. This sediment was dosed with 100 μL of five different concentrations of each pesticide using acetone as a carrier. A solvent control was prepared by adding 100 μL of acetone carrier alone to the reference sediment. After dosing, the sediments were thoroughly mixed using a stainless steel paddle driven by an overhead motor for 1 h to ensure homogeneity of the pesticides within the sediment. Sediments were then stored at 10°C for 7 d and homogenized again prior to use in the toxicity tests.

Ten-day toxicity experiments were conducted following U.S. EPA recommended procedures (U.S. EPA 2000). Three replicates were used in all exposures including the solvent and negative controls. Experiments were conducted in 300 ml tall-form beakers containing 100 g (wet weight) of thoroughly homogenized sediment and 175 ml of overlying water. The overlying water was renewed twice daily by the addition of 100 mL of moderately hard water (MHW) using a water delivery system. The MHW used in this study was prepared following U.S. EPA methodologies, where the appropriate salts were dissolved into distilled water and then aerated for 24 h to ensure water quality. Ten organisms were randomly selected and assigned to each beaker after the sediment settled overnight. Tests were performed at 23-27°C with a 16:8 L:D photoperiod under fluorescent lights. Temperature and dissolved oxygen were measured daily, while pH, conductivity and ammonia of overlying water were measured at the beginning and end of the tests. *H. azteca* were fed 1.0 mL of yeast-cerophyl-trout chow (YCT) and *C. tentans* were fed 1.5 mL of a 4 g/L suspension of ground Tetrafin® goldfish food daily. Following the 10-d exposure, organisms were removed from the sediment by sieving with a 0.5-mm screen and surviving organisms counted.

Chemical analyses of the pesticides were performed using an Agilent 6890 series GC equipped with an Agilent 7683 autosampler, electron capture detector (Agilent Technologies, Palo Alto, CA, USA) and a DB-608 capillary column (30m×0.25mm×0.25μm film thickness, Agilent Technologies, Palo Alto, CA, USA). Standard solutions were made by dissolving 10, 50 or 100 μg/mL of each pesticide in hexane and the calibration curves were linear within this concentration range.

In preliminary experiments, pesticide concentrations in the sediments decreased with holding time; therefore, sediment samples were analyzed following the 7-d holding period just prior to the start of each toxicity test. Pesticides were extracted from the sediments by washing approximately 2 $g_{(ww)}$ of sediment with a series of 5 mL solvent washes using the following solvent systems: acetone, 4:1 acetone: hexane (v/v), 5:5 acetone: hexane (v/v) and 1:4 acetone: hexane (v/v). All solvent washes were decanted, combined and dried with anhydrous magnesium sulfate. The washed sediment was then sonicated with 30 mL of hexane for 3 min in pulse mode using a high intensity ultrasonic processor (Model VCX 400, Sonics and Materials Inc., Newtown, CT, USA). Extracts from the washing and sonicating steps were combined and evaporated under a stream of nitrogen using a TurboVap II evaporator (Zymark, Hopkinton, MA, USA) to approximately 10 mL. The evaporated solution was then either diluted with hexane or evaporated under a gentle stream of nitrogen using a Pierce Model 1878 Reactivap™ (Rockford, IL, USA) to a concentration in the range of the calibration curves prior to GC analysis. This extraction method was validated using 2 $g_{(ww)}$ of control sediment spiked with 1 mg of each pesticide and extracted after 30 min of holding time. Validation of the sediment extraction method yielded extraction efficiencies of 94, 110, 95, 92 and 114% for endrin, methoxychlor, α-endosulfan, β-endosulfan and endosulfan sulfate, respectively.

LC50 values were calculated using the standard probit analysis using SAS Version 8.02 (SAS Institute Inc., Cary, NC, USA). The LC50 values were estimated from three distribution models, namely normal, logistic and Weibull.

RESULTS AND DISCUSSION

Following the 7-d holding period, the measured concentrations of the test pesticides decreased to approximately 50% of the nominal concentrations (Table 1). The 'loss of compound' agrees with results from Navarro et al. (2000), which showed a 49.6% disappearance of endosulfan from unsterilized sediments following a 7-d holding period. The differences between the nominal and measured concentrations at the beginning of toxicity test (after 7-d aging) may be attributed to either the degradation of the test pesticide or the tight binding of the pesticide to the matrix which makes the test pesticide less extractable (Peterson and Batley 1993). No target pesticides were detected in the control sediment. The measured concentrations of the pesticides following the 7-d holding period were used in all toxicity calculations.

Table 1. Measured pesticide concentrations in sediments after the 7-d holding period and prior to the start of the toxicity tests.

Test pesticide	*H. azteca* Tests		*C. tentans* Tests	
	NC (ng/g_{dw})	MC (ng/g_{dw})	NC (ng/g_{dw})	MC (ng/g_{dw})
Methoxychlor	312	160	150	130
	625	265	300	253
	1,250	620	600	301
	2,500	1,385	1,200	838
	5,000	2,495	2,400	1,562
α-Endosulfan	625	423	38	8
	1,250	792	75	31
	2,500	1,327	150	52
	5,000	2,963	300	131
	10,000	4,313	600	314
β-Endosulfan	1,250	908	75	40
	2,500	1,720	150	72
	5,000	3,034	300	118
	10,000	6,416	600	356
	20,000	10,099	1200	679
Endosulfan Sulfate	250	188	75	58
	500	391	150	73
	1,000	760	300	201
	1,500	899	600	245
	2,000	1,797	1,200	428
Endrin	nd	nd	120	80
	nd	nd	240	199
	nd	nd	480	339
	nd	nd	960	596
	nd	nd	1,920	1,336

NC: Nominal Concentrations;
MC: Measured Concentrations
nd: not determined

The 10-d sediment LC50s were estimated using probit analysis with three different distribution models including normal, logistic and Weibull. The χ^2 values of the three models were used to compare their relative goodness-of-fit (Newman 2000). The χ^2 values using the Weibull distribution were the smallest of the three models tested, therefore the Weibull model was chosen for the determination of LC50s. The pesticide LC50 values, normalized to TOC and corrected for control mortality, are presented in Table 2. Control survival averaged 98% for *H. azteca* exposures and 90% for *C. tentans* exposures.

The 10-d sediment LC50 values for methoxychlor were estimated as 36.7 and 85.8 $\mu g/g_{oc}$ for *C. tentans* and *H. azteca*, respectively. Literature values for sediment exposed aquatic invertebrates to methoxychlor were not found. However, 10-d LC50 values for DDT-spiked sediments (similar chemical structure as methoxychlor) using *H. azteca* were 101 to 140 $\mu g/g_{oc}$ and 272 to 473 $\mu g/g_{oc}$ (Nebeker et al. 1989; Schuytema et al. 1989, respectively).

Because endosulfan exists as two stereoisomers, α- and β-endosulfan, the toxicity of individual isomers was evaluated. In addition, the toxicity of endosulfan sulfate, one of the major degradation products of endosulfan, also was examined. The 10-d sediment LC50 values for *C. tentans* were 0.96, 3.24 and 5.22 $\mu g/g_{oc}$, while the LC50 values for *H. azteca* were 51.7, >1000 and 873$\mu g/g_{oc}$ for α-endosulfan, β-endosulfan and endosulfan sulfate, respectively. The toxicity values determined in this study compare well with values obtained by McLeese et al. (1982), which reported a 12-d sediment LC50 value of 17 $\mu g/g_{oc}$ for endosulfan to *Nereis virens*. Also, a 10-d sediment LC50 value for technical endosulfan (α/β:7/3) was 0.16 $\mu g/g_{ww}$ to the mayfly nymph, *Jappa kutera* (Leonard et al. 2001).

The difference in the toxicity between the α- and β- isomers may have been caused by their different chemical properties. β -endosulfan has a greater binding affinity to sediment than α- endosulfan (World Health Organization 1984), which may have resulted in the compound being less bioavailable and subsequently less toxic to the test organisms. In addition, Leonard et al. (2001) compared the toxicity of endosulfan isomers to *J. kutera* using water-only toxicity tests and determined that the α-isomer was more toxic than the β-isomer suggesting that the α-isomer is more potent.

Differences in sensitivity between the two organisms in this study was statistically significant for each of the endosulfan compounds where the toxicity of each of the endosulfan compounds was greater to *C. tentans* compared to *H. azteca*. For example, the LC50 for β– endosulfan to *C. tentans* was 5.22 $\mu g/g_{oc}$, whereas no toxicity was observed at the highest test concentration for *H. azteca*. Endosulfan sulfate, the primary oxidative metabolite of endosulfan, has also been shown to be toxic (NRCC 1975). The World Health Organization (1984) suggested that toxicity of the sulfate was equally as toxic as the parent compound and this was supported in this study, where the LC50 values varied by less than a factor of two for both test species.

Table 2. Ten-day sediment LC50 values for *C. tentans* and *H. azteca* with test pesticides calculated using a Weibull model.

Test pesticide	*C. tentans*		*H. azteca*	
	LC50 ($\mu g/g_{oc}$)	95% CI ($\mu g/g_{oc}$)	LC50 ($\mu g/g_{oc}$)	95% CI ($\mu g/g_{oc}$)
Methoxychlor	36.7	27.2 - 46.8	85.8	72 - 103
α-Endosulfan	0.96	0.41 - 1.46	51.7	39 - 62
ß-Endosulfan	3.24	1.46 - 4.27	>1000 *	------
Endosulfan Sulphate	5.22	3.23 - 5.82	873	660 - 1139
Endrin	4.22	0.7 - 8.11	nd	nd

* No mortality was found at the highest test concentration
nd: not determined

In the current experiments, the 10-d LC50 value for endrin with *C. tentans* was 4.22 (0.7-8.11) $\mu g/g_{oc}$. This value is lower than estimates for other species, where the 96-h LC50 values for the oligochaetes *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri* exposed to endrin-contaminated sediment were 2,588 µg/g and 2,725 $\mu g/g_{(dw)}$ sediment, respectively (Keilty et al. 1988). McLeese et al. (1982) reported 2 of 5 *Nereis virens* died at an endrin concentration of 1400 $\mu g/g_{oc}$ in a 288-h sediment toxicity test, while Nebeker et al. (1989) reported 10-d LC50 values for *H. azteca* ranging from 53.6 to 146.7 $\mu g/g_{oc}$ for three sediments with varying TOC content (3.0 to 11.2). Similar toxicity values for *H. azteca* were reported by Schuytema et al. (1989) and ranged from 94 to 170 $\mu g/g_{oc}$. The toxicity of endrin in the current study was not determined for *H. azteca*.

Comparisons of the 10-d sediment LC50 values for *C. tentans* and *H. azteca* with methoxychlor and endosulfan indicated *C. tentans* was the more susceptible species. These results agree with the study of Phipps et al. (1995), where the relative sensitivity of the *C. tentans* was greater to the pesticides than *H. azteca*. Using a phthalate ester, Call et al. (2001) showed the sediment toxicity to *C. tentans* was an order of magnitude greater compared to *H. azteca*. These results may be due to differences in habitat and feeding preferences between these two species, where *H. azteca* is epibenthic and *C. tentans* is a tube-dwelling organism (Ingersoll 1995; Schuler et al. 2002). These differences may affect their contact with the sediment and thus their contact with the test pesticide. Whiteman et al. (1996) examined the sediment toxicity of ammonia for *Lumbriculus variegates*, *C. tentans* and *H. azteca* and suggested that the reduced toxicity observed for *H.*

azteca was due to their ability to avoid contaminated sediments by moving into the upper-water column thereby reducing their exposure.

As part of an earlier study evaluating the toxicity of field-collected sediments from Central Valley of California, this work was conducted due to the limited availability of laboratory-derived toxicity values for the selected test pesticides. In the previous study, the 10-d toxicity of 70 field-collected sediments was evaluated using *H. azteca* and *C. tentans*. Of the 70 sediments, 23 and 10 were found to be toxic to *H. azteca* and *C. tentans*, respectively. Following an extensive chemical analysis of the field sediments (see Weston et al. 2004 for specific analytes), a toxic unit (TU) approach was employed to examine the contribution of each class of pesticide (i.e. pyrethroids and OCs) to the overall toxicity found for each sediment. The result of these analyses suggested that the observed toxicity was primarily due to pyrethroids in the sediments; however, there were significant concentrations of some OC pesticides, namely methoxychlor, endosulfan and endrin, in the sediments as well. At the time of that analysis, literature toxicity values were unavailable for those compounds using *H. azteca* and *C. tentans*. Using the values determined in this study, the contribution of the OCs to the toxicity of *H. azteca* was minimal with only 2 of the 23 sediments having any significant mortality as a result of the OCs. For *C. tentans*, seven of the 10 sediments had concentrations of OCs that would be expected to cause significant mortality; however, four of these sediments, each exhibiting nearly 100% mortality, also had concentrations of pyrethroids that would be expected to cause complete mortality (i.e. TU values 2.0 – 4.7). Therefore, from the results of this study, we can conclude that the observed toxicity from the field-collected sediments from the Central Valley of California was primarily a result of pyrethroids not OCs.

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