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BIOAVAILABILITY-BASED CHRONIC TOXICITY MEASUREMENTS OF PERMETHRIN TO *CHIRONOMUS DILUTUS*

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Abstract: Compared with acute toxicity, chronic exposures to low levels of contaminants are more environmentally relevant, but fewer data are available. In the present study, sediment toxicity of the pyrethoid permethrin to *Chironomus dilutus* was determined. The whole-life-cycle toxicity testing was conducted with the endpoints covering survival, growth, emergence, and reproduction. Permethrin caused 50% lethality in *C. dilutus* at 1.83 ± 1.13 μg/g organic carbon (OC) and 1.20 ± 0.55 μg/g OC after exposures of 20 d (before pupation) and 58 d (the end of the testing), respectively. The 5% and median effect concentrations (EC5 and EC50) represented the marginal and toxic levels of the sublethal effects, respectively, and effect data were all normalized to the controls before Probit analysis. The EC5s for growth, emergence, and reproduction were 0.034 ± 0.006 μg/g OC, 0.016 ± 0.008 μg/g OC, and 0.009 ± 0.008 μg/g OC, respectively; the respective EC50s were 1.09 ± 0.56 μg/g OC, 0.838 ± 0.077 μg/g OC, and 0.039 ± 0.105 μg/g OC. In addition, a 24-h Tenax extraction was employed to better assess permethrin bioavailability. Ultimately, response spectra with a series of endpoints were developed for permethrin using either OC-normalized bulk sediment concentrations or bioavailability-based Tenax extractable concentrations as the dose metric. The development of bioavailability-based chronic toxicity endpoints for sediment-associated permethrin would provide valuable benchmarks for evaluating ecological risk of this contaminant and contributing to improve sediment management policies. *Environ Toxicol Chem* 2013;32:1403–1411. © 2013 SETAC

Keywords: Bioavailability-based endpoints Chronic toxicity Permethrin Sediment *Chironomus dilutes*

INTRODUCTION

Sediment quality benchmarks are the thresholds above which adverse effects to the benthos occur. The benchmarks have been widely used to characterize sediment risks and define the clean-up goals of contaminated sites [1]. Traditionally, the benchmarks have been derived from toxicity testing data based on bulk sediment concentrations; however, these may overestimate the bioavailable fractions of contaminants [2]. Instead, risk assessments with consideration of bioavailability have taken scientific precedence over the generic evaluations using bulk sediment concentrations because bioavailability of contaminants varies site by site [2,3]. Therefore, bioavailability-based dose metrics are preferred for predicting toxicity. Recently, the US Environmental Protection Agency (USEPA) suggested applying the benchmarks derived from equilibrium partitioning (EqP) estimates to assess sediment risks of hydrophobic organic contaminants (HOCs) such as polycyclic aromatic hydrocarbons [4]. Although organic carbon (OC) normalization significantly improved the accuracy of the assessments, the presence of highly sorptive phases such as black carbon made the EqP prediction less effective [5].

Due to the current limitations of EqP prediction, there is a growing interest in developing bioavailability-based analytical techniques to directly estimate bioavailability of contaminants in sediment; however, applications of these techniques to sediment toxicity evaluation were few [6,7]. As a bioavailability-based measure, 24-h Tenax extractable concentration has previously been shown to be proportional to bioaccumulation of HOCs across sediments [6–9]. In addition, recent studies also showed the potential of using Tenax extractable concentrations to predict acute toxicity of pesticides in sediment [10,11].

Although acute toxicity tests were useful for identifying the highly toxic sites, adverse effects due to long-term exposure to low levels of contaminants are more environmentally realistic under some circumstances. Pyrethroid insecticides have been identified as a principal cause of sediment toxicity to the benthos, and the studies mainly focused on their acute lethality, with less attention paid to their chronic toxicity [12–15]. Pyrethroids were detected in 75% of sediments from agricultural areas in California's central valley, but only 13% of these sediments showed acute toxicity to the midge *Chironomus dilutus* [15]. In cases such as this, evaluating chronic toxicity caused by the presence of trace amounts of pesticide residues in aquatic ecosystems is preferred. Establishing bioavailability-based chronic toxicity thresholds could provide a way for more accurate assessments of site-specific sediment toxicity. Nevertheless, to our knowledge, no study to date has linked bioavailability measurements to chronic sediment toxicity.

The main objective of the present study was to evaluate chronic sediment toxicity of the pyrethroid permethrin to *C. dilutus* using whole-life-cycle bioassays from the newly hatched midge larvae to the hatch of the second generation. The endpoints included lethality at 20 d (before pupation) and 58 d (the end of the whole-life-cycle toxicity testing) as well as the impairments of growth, emergence, and reproduction (sex ratio, egg production, and hatchability). Next, a 24-h Tenax extraction was performed to better assess bioavailability of sediment-associated permethrin. Eventually, response spectra including a variety of endpoints were developed using both OC-normalized sediment concentrations and bioavailability-based Tenax extractable concentrations as the dose metrics to allow better interpretation of site-specific sediment toxicity with the thresholds incorporating bioavailability and chronic toxicity information.

All Supplemental Data may be found in the online version of this article.
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MATERIALS AND METHODS

Chemicals and reagents

The pyrethroid permethrin was chosen as the representative contaminant due to its wide use and frequent detection in the field [13–15]. Permethrin was purchased from ChemService with the purity >97% as certified by the manufacturer. Decachlorobiphenyl was obtained from Supelco and added to all samples before extraction to be used as the surrogate to verify the performance of the analytical processes. The internal standard *trans*-cypermethrin-d6 was purchased from Dr. Ehrenstorfer GmbH and used for gas chromatography–mass spectrometry (GC/MS) quantification.

Hexane (high-performance liquid chromatography grade) was purchased from Burdick and Jackson, and dichloromethane and acetone (analytical grade) were obtained from Tianjin Chemical Reagent Factory and redistilled before use. Anhydrous Na_2SO_4 was gained from Tianjin Chemical Reagent Factory and baked at 450 °C for 4 h before being used to remove residual water in the extracts. Silica gel (80–200 mesh; Ocean Chemical Factory) and alumina (80–200 mesh; Wusi Chemical Reagent Company) were baked at 180 °C and 250 °C for 6 h, respectively. Tenax TA sorbents (60–80 mesh) were purchased from Scientific Instrument Service. Reconstituted water was prepared following USEPA protocol [16] and aerated overnight before use.

Organisms and sediment

The midge, *C. dilutus* was selected as the test organism because it is recommended by the USEPA for sediment toxicity testing [16]. The midge life cycle consists of 4 larvae stages, 1 pupa stage, and 1 nonfeeding adult stage. Because midge larvae spend most of their lifespan in sediment, *C. dilutus* is an ideal model organism for chronic sediment toxicity testing. The midges were cultured at Guangzhou Institute of Geochemistry, Chinese Academy of Sciences in accordance with USEPA protocols [16].

Control sediment was collected from a drinking water reservoir in Conghua, China. The sediment was sieved through a 500-μm sieve to remove the debris and native organisms and stored at 4 °C before use. Previous studies detected no permethrin in the sediment, and the sediment exhibited no acute toxicity to the midges [17]. The moisture (water content) of the sediment was 49%. Total OC content of the sediment was analyzed using a Hanau Elementar Vario EL III Analyzer after removing inorganic carbon with 1 mol/L HCl, and the OC content was 1.60 ± 0.14%.

The sediment was spiked with permethrin at 6 concentrations (Table 1), which were predetermined in preliminary range screening tests and acetone was used as the carrier (<6 μL/g sediment). The same amount of acetone was added to control sediment. After spiking, sediments were thoroughly mixed for 4 h using a drill with a stainless-steel blade, and then aged for 17 d at 4 °C in the dark. Before the bioassay initiated, sediments were homogenized again and sediment concentrations were measured in triplicate to ensure the homogeneity.

Whole-life-cycle bioassays

The whole-life-cycle toxicity tests were conducted in 400-mL beakers containing 60 g of wet sediment and 250 mL reconstituted water following the method recommended by the USEPA [16]. The processes of the bioassays and the devices used in the bioassay are shown in Supplemental Data, Figures S1 and S2, respectively. After sediment settled overnight, 20 newly hatched midge larvae (<24 h old) were introduced into each beaker and the testing was performed with a photoperiod of 16:8 light:dark and at 23 ± 1 °C. Temperature, pH, conductivity, and dissolved oxygen were monitored daily; ammonia was measured every 3 d. Overlying water was renewed twice daily with 150 mL each. Considering their physiological difference at different life stages, midge larvae were fed once daily with 1 mL of grounded fish food (Zhongshan Aquatic Products Factory) at various concentrations: no feeding in the first 2 d, 0.6 g/L from days 3 to 7, 3 g/L from days 8 to 12, and then 6 g/L until the termination of bioassays. The major ingredients of fish food were fish meal, shrimp meal, soybean meal, vitamins, minerals, and yeast.

Chronic toxicity tests were performed using 3 groups (A, B, and C) of the midges with 3 replicates in each group (i.e., 9 replicates per concentration), and a wide spectrum of toxic endpoints were assessed (Supplemental Data, Table S1). The treatments in group A were terminated after 20-d exposure and lethality was counted by sieving the midges from the sediments. Moreover, ash-free dry weights of the midges were also measured following the method outlined by Maul et al. [18] to quantify the reduced growth. Briefly, the surviving organisms were placed in preweighed aluminum pans and dried at 60 °C for 3 d to obtain mean weight per replicate. Then the organisms and pans were heated at 550 °C for 3 h and reweighed on a Sartorius AgPro 11 microbalance to calculate ash-free dry weights per replicate.

In addition, the midges in group B were used to evaluate chronic toxicity after exposing them to permethrin for a whole life cycle, which was from the newly hatched larvae to the birth of next generation. Lethality and sublethal endpoints including reduced emergence and impaired reproduction were evaluated (Supplemental Data, Figure S1 and Tables S1 and S2). From the

Table 1. Concentrations of permethrin in the spiked sediments analyzed at 0, 20, and 58 d of the whole-life toxicity testing[a]

	Concentration (μg/g organic carbon)			
Treatment	0 d	20 d	58 d	Average[b]
Level 1	0.80 ± 0.25	0.80 ± 0.07	1.10 ± 0.35	0.92 ± 0.26
Level 2	5.00 ± 0.73	4.70 ± 0.61	4.80 ± 0.99	4.84 ± 0.70
Level 3	6.20 ± 1.40	6.30 ± 3.10	5.70 ± 0.40	6.08 ± 1.76
Level 4	6.60 ± 0.85	7.20 ± 0.50	6.00 ± 0.69	6.60 ± 0.80
Level 5	9.20 ± 0.73	10.4 ± 2.22	10.0 ± 1.19	9.84 ± 1.41
Level 6	15.8 ± 2.04	14.5 ± 1.98	14.0 ± 1.14	14.8 ± 1.72

[a]Data are presented as mean ± standard deviation of three replicates.
[b]Sediment samples were analyzed in triplicate at each timepoint. No significant differences were noted in permethrin concentrations throughout the whole-life-cycle toxicity testing from 0 d (the beginning of test) to 58 d (the end of the test); thus, average concentration was calculated for each level and shown as mean ± standard deviation of 9 replicates.

emergence of the first adult midge at 23 d, male/female emergence and adult mortality were recorded daily until the termination of the bioassays by sieving the remaining midges from sediments at 58 d, which was 7 d past the last recorded emergence in all treatments. Once emerged, the adults were transferred from the exposure beakers to the collection chambers using pipettes for assessing toxicity in reproduction by counting the eggs per female and per replicate. The egg cases were then moved to the incubation jars to measure the hatchability of eggs for an additional 8 d using a ring count method suggested by Benoit et al. [19]. More details on the measurements of the endpoints are presented in Supplemental Data, Table S2.

Because the peak emergence of female midges generally occurred approximately 1 week later than male emergence, auxiliary male midges were needed to mate with female adults for assessing reproduction impairments during the latter stage of the emergence period. Hence, additional midges (group C) were also raised at the same condition as group B, but 10 d later to ensure a sufficient supply of reproductively viable males (Supplemental Data, Figure S1 and Table S2).

Sediment extraction

Concentrations of permethrin were analyzed at 0 d (after 17-d aging period), 20 d (before pupation), and 58 d (end of the bioassays) of the toxicity testing (3 replicates for each analysis) to identify the possible degradation of permethrin in sediment. Sediment samples were extracted using a Xintuo CW-2000 ultrasound-assisted microwave extractor following a previously developed method [20]. In brief, 2 g to10 g of freeze-dried sediment was extracted with 100 mL of a mixture of hexane and acetone (1:1, v/v) for 6 min using an ultrasound-assisted microwave extractor. Activated copper powder was added to the sediment to eliminate sulfur interference, and 50 ng of decachlorobiphenyl was added as the surrogate. The ultrasound and microwave power was set at 50 W and 100 W, respectively. After decanting the extract, the sediment was extracted with additional 50 mL of extraction solution, and the extracts were combined, filtered, and evaporated, and the solvent exchanged to approximately 1 mL of hexane using a Xintuo XT-NS-1 Turbovap. A self-packed column with 12 cm of silica gel, 6 cm of alumina, and 1 cm of anhydrous Na_2SO_4 from the bottom to the top was used to clean the extracts, with 70 mL of 30% dichloromethane in hexane as the eluting solvents. The cleaned extracts were concentrated and solvent exchanged to hexane, and 50 ng/mL of trans-cypermethrin-d6 was added as the internal standard for GC/MS quantification.

Tenax extraction

The 24-h Tenax extractable concentrations have been shown to be proportional to bioaccumulation of sediment-associated HOCs [8,9]. To better estimate bioavailability of permethrin, 24-h Tenax extraction was performed in triplicate following a previously developed method [9]. After the spiked sediments were aged for 17 d, approximately 5 g wet sediment, 0.5 g Tenax, 5 mg NaN_3 (which was used to prevent microbial growth), and 45 mL reconstituted water were added into a 50-mL tube. The tubes were rotated at 20 revolutions per min for 24 h on a QB-228 rolling incubator (Kylin-Bell Lab Instruments). Upon completion, Tenax beads were separated from the sediment slurry and sonicated sequentially with 5 mL of acetone and 5 mL of a mixture of acetone:hexane (1:1, v/v) twice. The sonication time was 5 min for each extraction. After adding 50 ng of decachlorobiphenyl, the extracts were combined, concentrated and solvent exchanged to 1 mL of hexane, cleaned with the alumina/silica gel columns mentioned above, and then concentrated and solvent extracted to hexane. Prior to GC/MS analysis, 50 ng/mL of *trans*-cypermethrin-d6 was added to the extracts.

Instrumental analysis

The cleaned extracts were analyzed on a Shimadzu QP-2010 plus series GC/MS in negative chemical ionization mode, with methane being the reaction gas. A DB-5HT column (15 m × 0.25 mm, 0.10-μm film thickness) was used to separate the analytes, and helium was used as the carrier gas at a flow rate of 1.2 mL/min. Temperature of ion source and transfer line was set at 250 °C and 280 °C, respectively. The extract (1 μL) was injected with a programmable temperature vaporizing injector, and the initial injector temperature was 50 °C, heated to 300 °C at 230 °C/min after holding at 50 °C for 0.1 min, and held at 300 °C for 5 min. The oven temperature was set at 60 °C for 1 min, heated to 200 °C at 30 °C/min, heated to 220 °C at 10 °C/min, held at 220 °C for 5 min, heated to 300 °C at 50 °C/min, and then held at 300 °C for 10 min.

Identification of analytes was based on the detection of target and qualifier ions within 1% retention time window, and internal standard calibration was used for chemical quantification. The range of the calibration standards was 5 ng/mL to 200 ng/mL for permethrin and decachlorobiphenyl, whereas the concentration of *trans*-cypermethrin-d6 remained constant at 50 ng/mL. A calibration standard was analyzed after every 10 samples on GC/MS, and the relative differences between the calibration curve and the daily calibrations were within 20% for all analytes. A method blank (solvent), matrix blank (control sediment), matrix spike, and matrix spike duplicate were included in the analyses for every 20 samples. No permethrin was detected in the blanks. In addition, recoveries of the surrogate decachlorobiphenyl were $105 \pm 38.3\%$ and $96.0 \pm 20.6\%$ for sediment and Tenax samples, respectively.

Data analysis

The median lethal concentration (LC50) and the 5% and median effect concentrations (EC5 and EC50) were all determined using 2 types of dose metrics including OC-normalized bulk sediment concentrations and Tenax extractable sediment concentrations. These values were estimated from the bioassays using Probit analyses with SPSS 13.0 software (International Business Machines). The effect data of growth, emergence, and reproduction were all normalized to the controls before estimation of the EC values using Probit analysis and control mortality was included for LC50 estimation. Toxicity among the treatments was compared using analysis of variance (ANOVA) and Dunnett's multiple comparisons using SPSS 13.0 software. Significant difference was set at $p < 0.05$.

RESULTS AND DISCUSSION

Quality control

Throughout the bioassays, dissolved oxygen (5.8 ± 1.4 mg/L), pH (7.51 ± 0.16), temperature (23.8 ± 0.4 °C), conductivity (302 ± 17 μs/cm), and ammonia concentrations (0.46 ± 0.21 mg/L) of the overlying water were all within the acceptable ranges [16]. After exposing them to control sediment for 20 d and 58 d, $78 \pm 5.8\%$ and $68 \pm 5.8\%$ *C. dilutus* survived, respectively. The survivorship fulfilled the USEPA requirements of ≥70% at 20 d and ≥65% at the end of the whole-life-cycle toxicity testing [16], but were significantly lower than the survivorship in the 10-d bioassays ($94 \pm 6.5\%$) in our previous

study where 3rd instar *C. dilutus* were exposed to the same sediment [21]. Both the younger stage of the midges being used and the longer exposure time contributed to less control survival noted in the present study compared with Du et al. [21]. In addition, ash-free dry weights of the survived midges after the 20-d exposure to control sediment was 0.75 ± 0.19 mg per midge, which exceeded the minimum requirement of 0.48 mg per midge in the USEPA protocol [16].

Whole life-cycle bioassays

Concentrations of permethrin in sediment were measured at 0 d, 20 d, and 58 d of the bioassays (Table 1). No significant difference was noted among permethrin concentrations measured at different timepoints, indicating degradation of permethrin was minimal through the whole-life-cycle bioassays. Hence, the average concentrations of permethrin measured at the 3 timepoints (9 replicates) were used for further toxicity assessments. The responses of *C. dilutus* for the measured toxicity endpoints are presented in Table 2.

Lethality. Survivorship of *C. dilutus* after 20 and 58 d exposure are shown in Figure 1. When permethrin concentrations increased, midge survival decreased from $78.3 \pm 5.8\%$ and $68.3 \pm 5.8\%$ in the control to $3.3 \pm 5.8\%$ and $3.3 \pm 2.9\%$ in the sediment at the highest permethrin concentration of 14.8 ± 1.72 μg/g OC at 20 d and 58 d, respectively.

Although not significantly different, lethality of permethrin to *C. dilutus* was slightly increased when exposure time was prolonged, with the LC50 values being 1.83 ± 1.13 μg/g OC at 20 d and 1.20 ± 0.55 μg/g OC at 58 d. Sibley et al. [22,23] also observed a decrease in midge survival with extended exposure duration, and the elevated mortality may be a result from the losses of larvae between 20 d and the onset of pupation [19]. Although no information on chronic toxicity of permethrin to the midges was available, the acute 10-d LC50 value of 24.5 μg/g OC has been reported [18]. The acute–chronic factor was the ratio of acute and chronic toxicity values, and 12.4 was suggested to be the acute–chronic factor of pyrethroids to the benthos [12]. By dividing the 10-d LC50 and acute–chronic factor values from the literature [12,18], a chronic LC50 value was calculated (1.98 μg/g OC). The value was similar to the chronic LC50s (1.83 μg/g OC at 20 and 1.20 μg/g OC at 58 d) in the present study, indicating the reliability of the chronic lethality data.

Reduced growth and emergence. Reduced growth was estimated by determining ash-free dry weights of the midge larvae after 20 d exposure (Figure 1). With permethrin concentrations increased, *C. dilutus* ash-free dry weights declined from 15.1 ± 3.72 mg/replicate in the control to 1.91 ± 0.00 mg/replicate in the sediment with the highest permethrin concentration (14.8 ± 1.72 μg/g OC). The reduced growth of the midges in contaminated sediment may contribute to a shift in energy allocation from devoting to growth to recovering from the toxic effects [24]. Compared with 20-d lethality, permethrin affected the growth of *C. dilutus* at a lower level, with a 20-d EC50 of 1.09 ± 0.56 μg/g OC, and it was approximatelt 25 times less than the 10-d ash-free dry weight EC50 value of 27.4 μg/g OC reported by Maul et al. [18]. The lower ash-free dry weight EC50 value in the present study was reasonable for the longer exposure duration and younger stage of organisms being used.

In addition, changes in emergence were also used to assess the chronic toxicity to Chironomidae [25]. Figure 2 plots the cumulative emergence of the midges that were exposed to permethrin at differing concentrations versus exposure time. As

Table 2. The responses for the lethal and sublethal endpoints of *Chironomus dilutus* exposed to different levels of sediment-associated permethrin[a]

	Lethal endpoint		Sublethal endpoint						
Treatment[b]	20-d survival (%)	58-d survival (%)	Growth (AFDW, mg/replicate)	Emergence (%)	No. of female per replicate	No. of eggs per female	No. of eggs per replicate	Hatchability of eggs (%)	Sex ratio (female/male)
Control	78.3 ± 5.80	68.3 ± 5.80	15.1 ± 3.72	68.3 ± 2.89	6.30 ± 1.50	815 ± 328	5363 ± 3245	99.4 ± 0.40	1.84 ± 3.04
Level 1	55.0 ± 18.0	45.8 ± 13.8	16.6 ± 3.77	60.0 ± 5.00	3.00 ± 1.00	867 ± 723	2122 ± 1258	99.1 ± 0.60	2.06 ± 3.42
Level 2	31.7 ± 20.8	23.3 ± 8.80	14.1 ± 2.18	33.3 ± 2.89	1.50 ± 0.70	1066 ± 718	1853 ± 1832	97.9 ± 1.30	1.15 ± 1.32
Level 3	26.7 ± 23.6	22.5 ± 4.30	13.1 ± 0.50	16.7 ± 5.77	NA	NA	NA	NA	0.23 ± 0.26
Level 4	26.7 ± 10.4	23.3 ± 3.80	13.4 ± 7.33	23.3 ± 2.89	1.00 ± 1.00	907 ± 0	1815 ± 0	98.7 ± 0	0.50 ± 0.36
Level 5	10.0 ± 8.70	8.30 ± 2.90	9.86 ± 4.90	18.3 ± 2.08	NA	NA	NA	NA	0.96 ± 0.73
Level 6	3.30 ± 5.80	3.30 ± 2.90	1.91 ± 0	11.7 ± 1.53	NA	NA	NA	NA	0.67 ± 0.58

[a]Data are presented as mean ± standard deviation of three replicates.
[b]Permethrin concentration in sediment in each level is presented in Table 1.
AFDW = ash-free dry weight; NA = Not available due to no emergence of female midge.

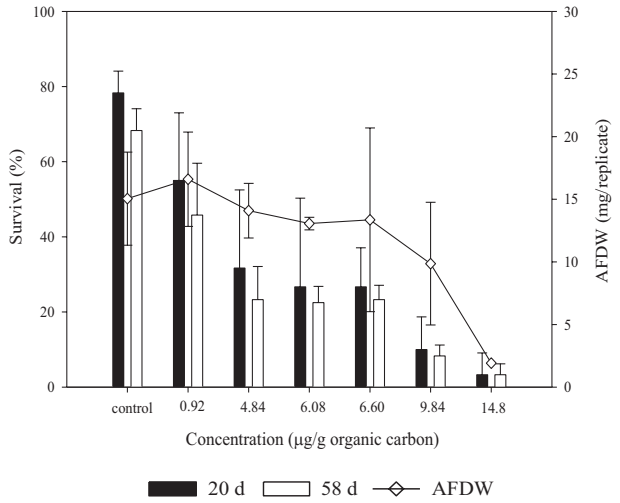

Figure 1. Survival percentage and ash-free dry weight (AFDW; mg/replicate) of *Chironomus dilutus* after chronic exposure to various concentrations of permethrin in sediment. Survival was measured at 20 d (before pupation) and 58 d (the end of whole-life-cycle toxicity testing). Growth as presented as AFDW was assessed only at 20 d.

shown in Figure 2, the cumulative emergence of the midges exposed to all permethrin-contaminated sediments was significantly different from the control ($p < 0.001$), except that from the sediment spiked at the lowest level (0.92 ± 0.26 μg/g OC; $p = 0.056$). The EC50 values for cumulative emergence were calculated after normalizing effect data to the control (0.838 ± 0.077 μg/g OC).

Rate of emergence (the time required to reach a certain emergence percentage) was a more sensitive emergence endpoint than the cumulative emergence [23]. A delay in emergence was evident for the midges being exposed to permethrin (Figure 2). Although the time to reach 30% emergence in the control and the sediment with the lowest permethrin concentration (0.92 ± 0.26 μg/g OC) was about 34 d, it took 43 d for the midges in the second lowest concentration (4.84 ± 0.70 μg/g OC). The midges in the remaining treatments never reached 30% emergence. It generally takes 5 wk to 6 wk for *C. dilutus* to complete a life cycle under normal conditions [26]. For the midges that lived in contaminated sediment, however, this time extended to 60 d to 75 d [19,22,23], and the last emergence of the midge was recorded at 51 d in the present study.

Reduced growth was usually associated with declined emergence, and the failure of larvae to complete pupation in the presence of contaminants may be one of the reasons for the developmental delays [27]. Previous studies implied that emergence began to defer when a midge had an ash-free dry weight below 0.8 mg and that emergence may cease with an ash-free dry weight < 0.5 mg/midge [23,28]. Compared with the control, 87% reduction in growth occurred when the midges were exposed to 14.8 ± 1.72 μg/g OC of permethrin (the highest level); this was associated with an 83% reduction in emergence (Figures 1 and 2). This was similar to the results reported by Liber et al. [28], who noted an 86% to 100% drop in emergence along with a 64% to 73% drop in growth for the affected midges

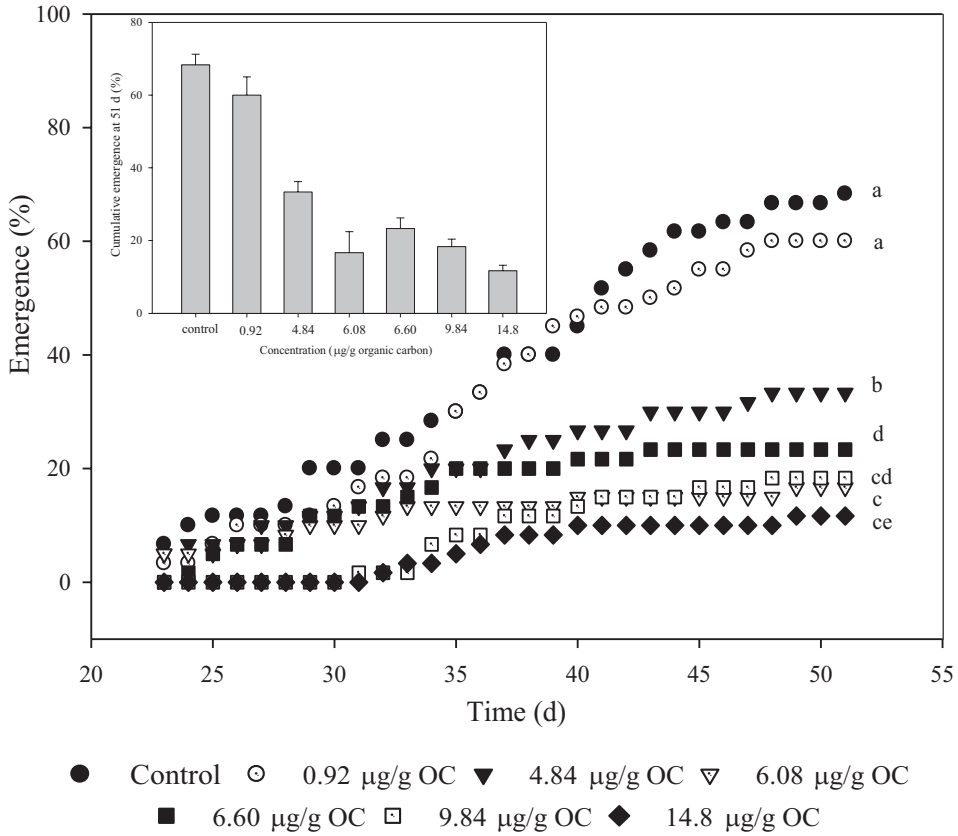

Figure 2. Cumulative emergence of *Chironomus dilutus* after exposure to various concentrations of permethrin versus exposure time. Cumulative emergence at 51 d when the last emergence was recorded is presented with standard deviation in the small graph. Significant differences ($p < 0.05$) among the treatments are indicated by different letters. OC = organic carbon.

compared with the controls. The sublethal effects on growth and emergence may be attributed to the decreases in feeding activity [29], assimilation efficiency [30], protein synthesis, and/or rates of biotransformation and damage repair [31]; but further identifying the mechanisms by which permethrin interfered with developmental processes of the midges was beyond the scope of the present study.

Impaired reproduction. The reproductive endpoints included sex ratio of the midges, egg productivity (the numbers of eggs per female and the numbers of eggs per replicate), as well as hatchability (i.e., embryo viability). Benoit et al. [19] suggested that the reproductive endpoints were important in the life-cycle toxicity testing because they were not only sensitive to natural or anthropogenic stressors but also reflective to the effects on larval growth. Maintaining a relatively even sex ratio was necessary to viable reproduction [19]. Compared with the control, the ratio of female to male midges dropped after being exposed to permethrin (Table 2). Although the difference in the ratios was not significant because of high variability among replicates, the observation suggested that female midges may be more vulnerable to permethrin contamination than males.

The numbers of eggs per female and the numbers of females per replicate are shown in Figure 3A. There were fewer female adults in permethrin-exposed treatments compared with the control, and no adult female was found in 3 treatments with permethrin concentrations at 6.08 ± 1.76 μg/g OC, 9.84 ± 1.41 μg/g OC, and 14.8 ± 1.72 μg/g OC. Hatchability has been proposed as another reproductive endpoint for the midges [25]. As shown in Figure 3B, no significant inhibition of egg hatchability occurred for all treatments, with an average of 98.9% eggs being hatched. Sibley et al. [22] also denoted the ineffectiveness of hatchability as the reproductive endpoint in sediment toxicity testing, and the unequal distribution of contaminants in different parts of eggs may be the reason for its high variability and the subsequent ineffectiveness [32].

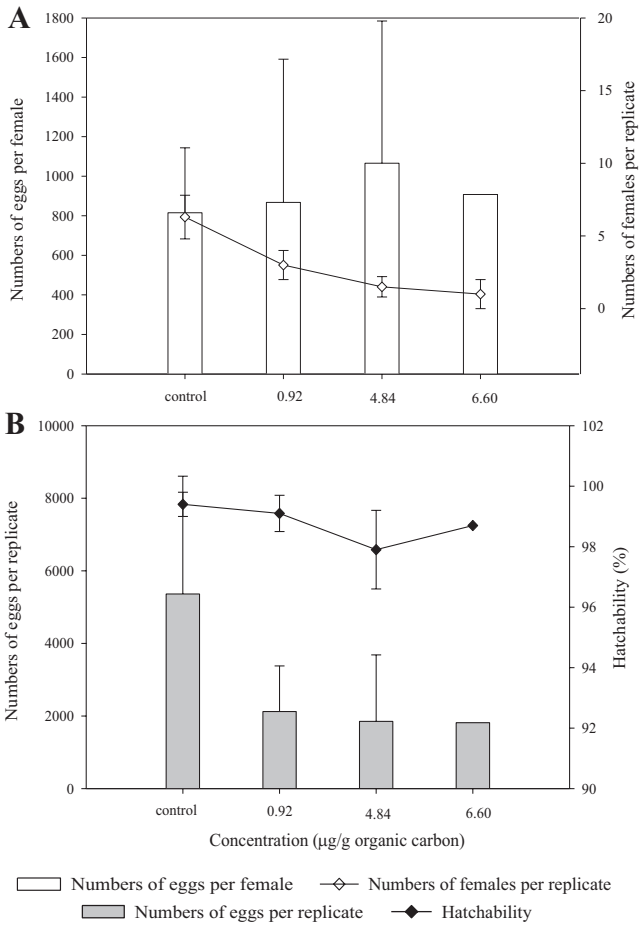

Figure 3. Adverse effects of permethrin on reproduction of *Chironomus dilutus* with endpoints including the numbers of females per replicate and the numbers of eggs per female (**A**), as well as the number of eggs per replicate and the hatchability of eggs (**B**).

Consequently, the number of eggs per replicate was used as the reproductive endpoint because it took into consideration both the number of females and the number of eggs [19]. As shown in Figure 3B, the number of eggs per replicate dropped from 5363 ± 3245 in the control to 1815 ± 0 in the treatment at permethrin concentration of 6.60 ± 0.80 μg/g OC, and the EC50 value of reproduction was 0.039 ± 0.105 μg/g OC.

Bioavailability measured by Tenax extraction

Bulk sediment concentrations were conventionally applied to predict sediment toxicity with normalizing to OC content in a specific sediment to minimize the variation among sites as suggested by EqP theory [4]. Nevertheless, the presence of highly sorptive carbonaceous materials in sediment may diminish the accuracy in predicting bioavailability using EqP [5]. The freely dissolved chemical concentrations measured by solid-phase microextraction have demonstrated a great potential to estimate bioavailability and toxicity of pesticides in sediment [33,34], but the sensitivity of detection limited its use for the highly toxic chemicals in the field [11].

Rather, Tenax extraction has the advantages of greater sensitivity of detection and less operation time, which made it a good choice for measuring bioavailability of pyrethroids that exhibited toxicity to aquatic invertebrates at extremely low concentrations [11,35]. You et al. [10] used Tenax extractable concentrations to better interpret the unexpected low toxicity in field-collected pyrethroid-contaminated sediments compared with the traditional OC-normalized concentrations. The applicability of a literature-based Tenax model (24-h Tenax extraction) was validated for assessing bioavailability of HOCs to the oligochaetes across various sediments [8]. Recent studies have also shown that Tenax extractable concentration was directly related to the bioaccumulation of sediment-associated HOCs to the midges [11,21,35,36]. Therefore, 24-h Tenax extractable concentration was selected as the bioavailability-based dose metric for permethrin chronic toxicity evaluation.

Toxicity thresholds and their application

The main goal of the present study was to develop response spectra to describe whole-life-cycle toxicity of sediment-associated permethrin to the midges. Response spectra summarized a variety of toxic effects of a contaminant at different exposure levels, and different dose metrics were utilized, including water concentrations [37] and internal body residues [32,38]. In addition to providing a means to directly assess toxicity of a contaminant through its exposure, response spectra also elucidated the relative sensitivity of various toxic endpoints. Using body residue as a measure of exposure takes bioavailability into consideration and improves the accuracy in toxicity assessments [32,38]. Unfortunately, body residue measurements can be expensive and labor -intensive. In some situations, it may be difficult to collect enough organisms for analyzing body

residues. Thus, it is desirable to develop response spectra with bioavailability-based dose metrics that could be measured with sensitive, simple, reliable, and cost-effective techniques. Tenax extraction is one of the techniques.

The reliability of the traditional indices used in chronic toxicity testing including the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC), were under debate recently [39]. Therefore, the 5% and median lethality and effect (reduced growth, emergence, and reproduction) values derived from the dose-response curves (Figure 4 and Supplemental Data, Figure S3) were used to represent the marginal and toxic levels of chronic toxicity, respectively. Although Tenax extractable concentration is a bioavailability-based measure, OC-normalized concentration has been widely used for toxicity evaluation; thus it is useful to include OC-normalized data in the present study for comparison with other studies. Accordingly, 2 dose metrics including OC-normalized sediment concentrations and bioavailability-based Tenax extractable concentrations were used to construct the response spectra (Figure 5).

Reproduction was the most sensitive endpoint, with EC5 and EC50 values of 0.009 ± 0.008 μg/g OC and 0.039 ± 0.105 μg/g OC, respectively. Conversely, mortality at 20 d was the least toxic endpoint, with LC5 and LC50 values of 0.143 ± 0.022 μg/g OC and 1.83 ± 1.13 μg/g OC, respectively. Toxic sensitivity of growth and emergence fell between mortality and reproduction, with the EC5 and EC50 values being 0.034 ± 0.006 μg/g OC and 1.090 ± 0.564 μg/g OC for growth and 0.016 ± 0.008 μg/g OC and 0.838 ± 0.077 μg/g OC for emergence, respectively (Figure 5A). Moreover, larger derivations were noted for the endpoints that were more sensitive. As shown in Supplemental Data, Figure S3, data in the dose-response curves of the reproduction endpoint were the most scattered among all the curves, although the r^2 values were not significantly different and all dose-response relationships were significant ($p < 0.0001$). In their study of the chronic toxicity of fluoranthene and pentachlorobenzene to the midges, Schuler et al. [32] made a similar conclusion—the more biologically complex endpoints were, the more sensitive and variable.

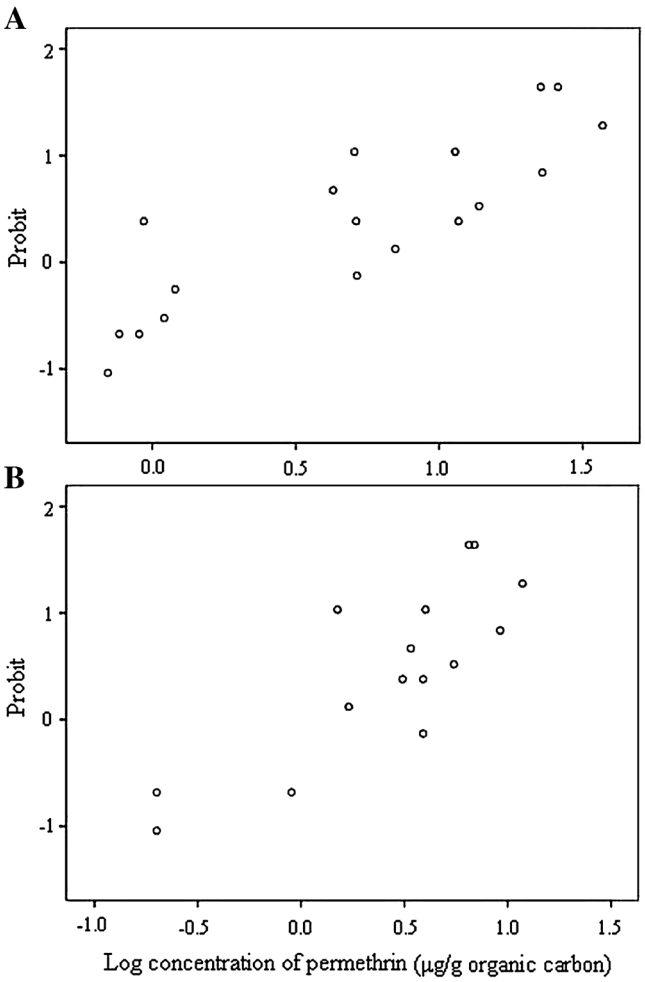

Figure 4. Probit-transformed dose-response curves of the lethality of *Chironomus dilutus* after 20-d exposure to permethrin using the dose metric of bulk sediment concentration (**A**) or Tenax extractable concentration (**B**). The equations of the relationships were Probit = 0.328 (±0.009) log concentration + 0.390 (±0.010) ($r^2 = 0.988$, $p < 0.0001$) (**A**); and Probit = 0.367 (±0.010) log concentration + 0.481 (±0.010) ($r^2 = 0.976$, $p < 0.0001$) (**B**).

Although acute toxicity of permethrin to *C. dilutus* has been reported [18], little information on its chronic toxicity is available. Fleming et al. [40] evaluated emergence of *C. riparius* using a natural sediment with 1.23% OC, and 63% reduction in emergence compared with the control was noted at a nominal permethrin concentration of 65 μg/g OC. This value was an order greater than that in the present study, and the use of nominal concentrations in the study by Fleming et al. [40] and the different sensitivity of the 2 midge species may be the reasons. Although having the same total OC contents, Fleming et al. noted that 2 artificial sediments prepared from α-cellulose and peat showed significantly greater degrees of reduction in emergence than the natural sediment [40]. The difference in toxicity among the sediments with the same OC contents indicated that factors other than OC content were affecting sediment bioavailability [40] and that a bioavailability-based dose metric would better predict chronic toxicity.

To our knowledge, no study has established dose-response relationships to directly link Tenax extractable concentrations and sediment toxicity. Figure 5B plots the chronic toxicity endpoints using Tenax extractable concentration as the dose metric. Due to sequestration, only a portion of permethrin was extractable by Tenax; thus, the values of bioavailability-based dose metric were smaller than the bulk sediment concentrations, ranging from reproduction EC50 value of 0.187 ± 0.071 μg/g OC to 20-d LC50 value of 1.13 ± 0.898 μg/g OC (Figure 5B). As shown in Figure 5, variations in the spectrum of Tenax-extractable concentrations slightly decreased compared with response spectrum using bulk sediment concentrations. Similarly, the dose-response curves in Figure 4 and in Supplemental Data, Figure S3, show better correlations between the biological effects to Tenax extractable concentrations than to bulk sediment concentrations; the differences in r^2 values, however, are not significant.

The development of bioavailability-based response spectrum including a variety of chronic toxicity endpoints for sediment-associated permethrin provided valuable thresholds for evaluating ecological risk of this contaminant. Subsequently, it helps to better understand the site-specific relationship between the contamination and toxicity of pyrethroids. It is particularly useful in aquatic ecosystems where organisms are exposed to a low level of pesticide residues that may not cause acute lethality. Additionally, the application of a bioavailability-based dose metric for predicting long-term sublethal effects may also provide a better understanding of population-level impacts of a certain contaminant.

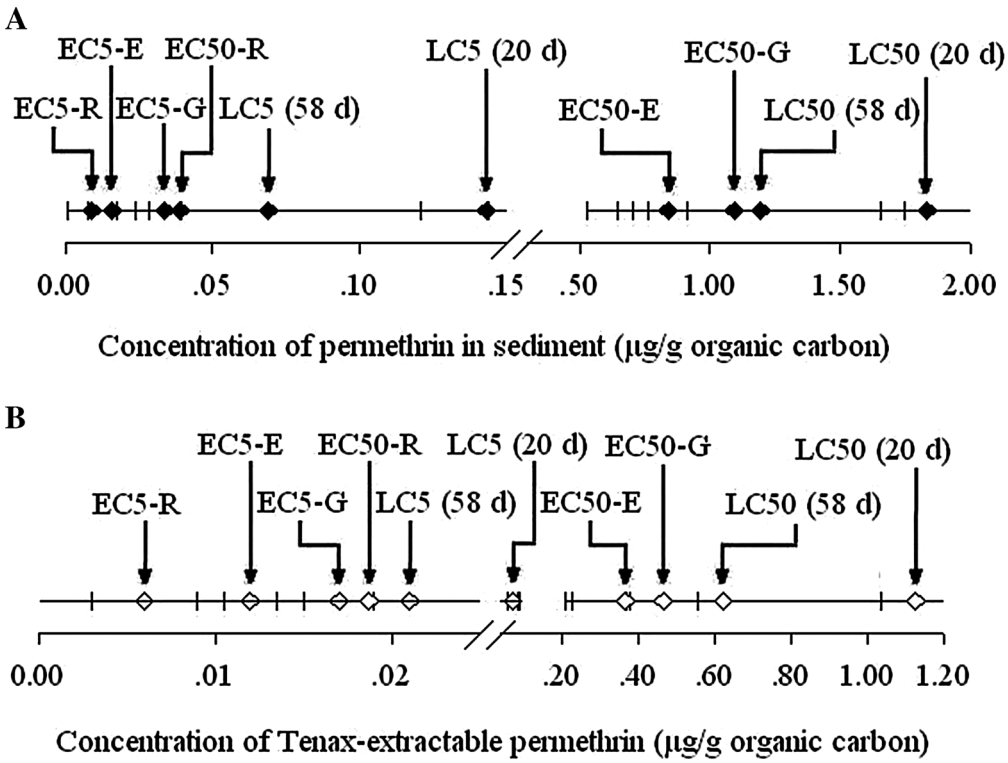

Figure 5. Response spectra of toxic endpoints ranging from lethality to sublethal effects on growth, emergence, and reproduction for *Chironomus dilutus* exposed to sediment-associated permethrin. Organic carbon (OC)-normalized sediment concentration (**A**) and 24-h Tenax extractable concentration (**B**) were used as the dose metrics. The solid and hollow diamonds represent OC-normalized and Tenax extractable concentrations, respectively. LC5 and LC50 represent 5% and median lethal concentrations, and EC5 and EC50 represent 5% and median effect concentrations, respectively. The lethality was recorded at both 20 d and 58 d. LC5 = 5% lethal concentration; LC50 = median lethal concentration; EC5 = 5% effective concentration; EC50 = median effective concentration; R = reproduction; E = emergence; G = growth.

CONCLUSIONS

The dose-response spectra generated from the present study attempts to improve the accuracy in toxicity evaluation by linking a bioavailability-based dose metric to the whole-life-cycle toxicological response in *C. dilutus*. By incorporating bioavailability and chronic sublethal effects into risk assessments, the development of bioavailability-based chronic toxic thresholds for the pyrethroid permethrin provides valuable benchmarks for evaluating ecological risk of this contaminant and contributes to the improvement of sediment management policies.

SUPPLEMENTAL DATA

Tables S1 and S2.
Figures S1 to S3. (537 KB PDF).

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