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EFFECT OF SEDIMENT-ASSOCIATED PYRETHROIDS, FIPRONIL, AND METABOLITES ON *CHIRONOMUS TENTANS* GROWTH RATE, BODY MASS, CONDITION INDEX, IMMOBILIZATION, AND SURVIVAL

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Abstract—Pyrethroids and fipronil insecticides partition to sediment and organic matter in aquatic systems and may pose a risk to organisms that use these matrices. It has been suggested that bioavailability of sediment-sorbed pesticides is reduced, but data on toxicity of sediment-associated pesticides for pyrethroids and fipronil are limited. In the current study, 10-d sediment exposures were conducted with larval *Chironomus tentans* for bifenthrin, lambda-cyhalothrin, permethrin, fipronil, fipronil-sulfide, and fipronil-sulfone, the last two being common fipronil metabolites. Sublethal endpoints included immobilization, instantaneous growth rate (IGR), body condition index, and growth estimated by ash-free dry mass (AFDM). Pyrethroid lethal concentrations to 50% of the population (LC50s) were 6.2, 2.8, and 24.5 μg/g of organic carbon (OC) for bifenthrin, lambda-cyhalothrin, and permethrin, respectively; with the former two lower than previously published estimates. Fipronil, fipronil-sulfide, and fipronil-sulfone LC50 values were 0.13, 0.16, and 0.12 μg/g of OC, respectively. Ratios of LC50s to sublethal endpoints (immobilization, IGR, and AFDM) ranged from 0.90 to 9.03. The effects on growth observed in the present study are important because of the unique dipteran life cycle involving pupation and emergence events. Growth inhibition would likely lead to ecological impacts similar to mortality (no emergence and thus not reproductively viable) but at concentrations up to 4.3 times lower than the LC50 for some compounds. In addition, *C. tentans* was highly sensitive to fipronil and metabolites, suggesting that dipterans may be important for estimating risk and understanding effects of phenylpyrazole-class insecticides on benthic macroinvertebrate communities.

Keywords—*Chironomus tentans* Fipronil Pyrethroids Sediment toxicity Sublethal responses

INTRODUCTION

Use of synthetic pyrethroids and the phenylpyrazole insecticide fipronil to control insect pests has steadily increased in both agricultural and urban environments. Within aquatic systems, many of these compounds readily partition to sediment and organic matter [1,2], potentially reducing toxicity and risk to aquatic species such as fish and some aquatic invertebrates [3,4]. However, benthic organisms that use sediment and organic matter as a food resource and habitat may potentially be at increased risk via adsorption of pyrethroids and fipronil to these solid matrices [5].

Research on toxicity of these compounds to aquatic invertebrates has addressed zooplankton and macroinvertebrate species that use the water column, but fewer studies have addressed sediment-associated toxicity [3–7]. Because of this data limitation, sediment-associated toxicity to benthic invertebrates often is estimated based on the equilibrium partitioning theory using aqueous concentrations lethal to 50% of the population (LC50s), partition coefficients (K_d), and organic carbon (OC) content of the sediment. In fact, acute pyrethroid toxicity endpoints for some species have been estimated from other pyrethroids and ecologically distinct species, rather than being measured directly [8]. Comparisons made between sediment LC50s estimated via these approaches and measured sediment LC50s can vary dramatically [8]. The relationship may be further confounded by the observed variation in bioavailability of pyrethroids among sediments with similar OC content or after normalizing for OC [5,9]. Generation of point estimates for sediment LC50 and effective concentration to 50% of the population (EC50) is important for understanding risk to benthic organisms because pyrethroids and other contaminants often are detected in sediments [8,10] and LC50s are commonly used in a toxic unit approach to identify sources of toxicity [8].

In several recent studies, numerous sediments from creeks receiving agricultural and urban runoff had concentrations of bifenthrin, lambda-cyhalothrin, and permethrin that were at levels likely to contribute to more than 0.5 toxic units for *Chironomus tentans* [8,11]. These estimates for toxic units were based on mortality endpoints (LC50s). Given that *C. tentans* endpoints such as growth no-observed-effect concentrations (NOECs) can range from 2.7- to 4.4-fold lower than lethal responses for cypermethrin [5], reported environmental concentrations of other pyrethroids in many agricultural and urban sediments may affect sublethal responses of benthic invertebrates that have sensitivities similar to *C. tentans*. However, sublethal benthic invertebrate responses to sediment-associated pyrethroid and fipronil insecticides have received limited research attention. Such endpoints are necessary for fully understanding the risk of these compounds to populations of benthic invertebrates. In the present study, we examined daily instantaneous growth rates (IGRs), body mass, body condition index (BCI), immobilization, and survival of *C. tentans* in response to exposure to the commonly used pyrethroids bifenthrin, permethrin, and lambda-cyhalothrin, as well as fi-

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pronil and its fipronil-sulfide and fipronil-sulfone degradation products.

MATERIALS AND METHODS

Chemicals

Bifenthrin, lambda-cyhalothrin, and permethrin were obtained from Chem Service (West Chester, PA, USA), and fipronil, fipronil sulfone, and fipronil sulfide were obtained from Accustandard (New Haven, CT, USA). The surrogate standards 4,4′-dibromooctafluorobiphenyl and decachlorobiphenyl were obtained from Supelco (Bellefonte, PA, USA). Anhydrous sodium sulfate (Na_2SO_4), chromatographic silica gel, and all pesticide grade solvents were obtained from Fisher Scientific (Pittsburgh, PA, USA); diatomaceous earth was obtained from Dionex (Sunnyvale, CA, USA).

Sediment spiking

Reference soil was collected at a location 15 km south of Carbondale, Illinois, USA. Soil was sieved through a 500-μm sieve and hydrated with moderately hard water [12] to form a reference sediment slurry. The OC content was 0.69% [3] for the sediment slurry for permethrin, fipronil, fipronil-sulfide, and fipronil-sulfone experiments. A second collection made from the same location had an OC content of 0.97% and was used for the bifenthrin and lambda-cyhalothrin experiments.

Stock solutions were prepared from neat chemical in an acetone carrier. Dosing stocks were prepared from a stock solution for each concentration, and concentrations used in the definitive tests were based on results from preliminary experiments. Stock solution and dosing stock concentrations were confirmed using a gas chromatograph and an electron capture detector (as described later).

Sediment was prepared in bulk in 1-L glass jars for each concentration. Dosing stock solutions were added dropwise to the sediment slurry, and the slurry was stirred for 1 h using a stainless-steel paddle stirrer powered by an overhead motor. Bulk sediment for each concentration received 150 μl of acetone carrier. Likewise, bulk solvent control sediment was spiked with 150 μl of acetone. Sediment was covered with aluminum foil and aged for 14 d in darkness at 4°C. The 14-d aging period is intended to simulate pesticide-sorbed sediment in an aqueous environment such as a stream or lake. After the aging period, approximately 50 g (dry weight) of spiked bulk sediment was distributed to experimental chambers. A subsample of sediment from each concentration was collected after spiking (time 0), before exposure (time 14), and at the end of the 10-d toxicity test (time 24) and was stored at −20°C before analysis.

Toxicity tests

Standard 10-d sediment toxicity bioassays were used to evaluate effects of these insecticides on BCIs, IGRs, immobilization, and survival of the benthic invertebrate, *C. tentans* [12]. Although the species name has been changed to *C. dilutus*, *C. tentans* is used throughout this text to be consistent with referenced methodology for culturing and experimentation [12]. Third and fourth instar *C. tentans* were obtained from mass cultures at Southern Illinois University (Carbondale, IL, USA) that were cultured following standard protocols [12]. Mid- to late-third instar organisms were used for the pyrethroid experiments, while early- to mid-fourth instar organisms were used for fipronil and fipronil metabolite experiments. Initial organism selection for all toxicity experiments was guided by the recommended range for total body length typical for third instar *C. tentans* [12]. Ultimately, instar determinations (as described earlier) were based on measured head capsule widths; thus, organisms used for the fipronil experiments had a slightly larger initial head capsule width than expected for the corresponding body length.

Experimental units consisted of an 800-ml jar containing approximately 50 g of sediment (dry weight) with approximately 700 ml of overlying reconstituted moderately hard water [12]. Five replicate experimental units were used at each of five or six concentrations, negative control, and solvent control. Ten organisms were randomly distributed to each experimental unit, and an additional group was preserved in 10% formalin. This group was used for determinations of initial mass and size of *C. tentans* used for each experiment. To minimize selection bias, individuals were selected singly after distributing organisms to each experimental chamber. After addition of organisms, the experimental chambers were covered with aluminum foil and maintained at 23°C, with a 16:8-h light:dark photoperiod in a Precision Scientific 818 environmental chamber (Chicago, IL, USA). Each experimental chamber had 75% of overlying water renewed and received daily 1 ml of a 6 g/L of Tetrafin™ solution (Tetra Werke, Melle, Germany). Experimental chambers were arranged in a randomized block design. Water quality parameters including dissolved oxygen, pH, and conductivity were monitored daily, before water changes, with a YSI 55 water quality meter (Yellow Springs Instrument, Yellow Springs, OH, USA) and an Oakton conductivity meter (Oakton Instruments, Vernon Hills, IL, USA).

Organism responses

Test organisms were retrieved after 10 d of exposure by gently passing the organisms and sediment through a 500-μm sieve. Organisms were transferred to a clean pan containing moderately hard water, and the number of *C. tentans* surviving was recorded. Of those organisms surviving, the sublethal responses of BCI, IGR, and immobilization were evaluated. Immobilization was defined as the inability to perform the typical S-shape response after gentle probing.

Size of organisms collected at the end of the experiment and from the initial group was evaluated by taking digital pictures of each individual using a Leica MZ9.5 stereomicroscope (Leica Microsystems, Bannockburn, IL, USA). As an indicator of size, head capsule width and length were measured to the nearest 0.001 mm from digital pictures using ImageJ software (Ver 1.32j; National Institutes of Health; rsb.info.nih.gov/ij/). Head capsule size is a reliable indicator of larval *C. tentans* growth and commonly used for age and instar determinations [12]. Head capsule length and width data were analyzed using principal component analyses, and scores from the first principal component were used as a composite score of body size, an approach that may provide more accurate estimates of structural size than individual morphological measurements [13].

All *C. tentans* were dried at 60°C for 3 d to constant mass, and mass was estimated to the nearest 0.001 mg with a Cahn C-33 microbalance (Cahn Instruments, Cerritos, CA, USA). Organisms were ashed at 550°C for 3 h to determine ash-free dry mass (AFDM). A BCI was calculated for *C. tentans* by regressing AFDM of control organisms against head capsule size score (from the first principal component) of exposed organisms using the model II regression method, standard ma-

jor axis regression [13,14]. The residuals ($x + 10$) of these data from the predicted relationship (i.e., derived from control data) were used as a BCI [13]. A BCI value of 10 indicated an AFDM that was expected for the corresponding structural size of the organism based on the linear regression model. Sublethal toxicity or suboptimal resource conditions might not be reflected in head capsule size measurements alone; thus, the use of a BCI was examined to predict a mass expected for a particular head capsule size under the experimental condition.

Daily IGRs also were evaluated as a sublethal response to these insecticides. This endpoint differs from the BCI in that initial mass estimates are incorporated and morphology is not evaluated. Previously, IGRs have been used to assess environmental [15] and contaminant [16] stress on aquatic invertebrates. Daily IGRs were estimated using the following equation [17]:

$$IGR = [\ln(m_f/m_i)]/d$$

where m_f is the mean final organisms AFDM for an experimental unit, m_i is the mean AFDM from the initial group of organisms before the start of the experiment, and $d = 10$ (length of growth period in days).

Sediment extractions and chemical analyses

Frozen sediment from each time point was thawed, and excess water was removed by centrifuging at 3,300 *g*. After centrifugation, the sample was thoroughly homogenized and 10 g of sediment (for pyrethroids) or 40 g of sediment (for fipronil and metabolites) was prepared for pressurized liquid extraction with the Accelerated Solvent Extractor 200 (Dionex). The sample preparation method was modified from the U.S. Environmental Protection Agency method 3545A and the *ASE 200 Accelerated Solvent Extractor Operator's Manual* [18,19]. For the pyrethroid insecticides, each sample was mixed with 5 g of diatomaceous earth and 1 g of silica until dryness. Silica was added to aid the removal of polar compounds. A cellulose filter and 1 g of silica were added to the 33-ml extraction cell before addition of the dried sample. Each sample was spiked with 50 ng of the surrogates, 4,4′-dibromooctafluorobiphenyl and decachlorobiphenyl. Fipronil, fipronil sulfone, and fipronil sulfide samples were prepared similarly with the exception of the reagents and surrogate used. Silica was not added since it may remove a portion of the analytes of interest. For the fipronil, fipronil-sulfone, and fipronil-sulfide experiments, each sample was spiked with 25 ng of a bifenthrin surrogate. Since only 10 g of sediment can be placed in each extraction cell, four cells were used to extract one sample for fipronil and its metabolites. After the extraction cells were packed, the samples were extracted with 1:1, dichloromethane:acetone (v/v) at 100°C and 1,500 pounds per square inch during two 5-min static cycles. The cells were flushed with 60% solvent for 60 s. After extraction, 12 g of anhydrous Na_2SO_4 was added to each extract and shaken to ensure complete removal of water. The Na_2SO_4 was washed twice with 10 ml of hexane. The extract and washes were combined and evaporated to 5 ml under nitrogen gas in a 50°C water bath with a Zymark TurboVap II Evaporator (Hopkinton, MA, USA). The extract was solvent exchanged with 10 ml of hexane and further evaporated to 1 ml under nitrogen gas with a Pierce Model 1878 Reactivap (Rockford, IL, USA).

The cleanup procedure for the pyrethroid extracts consisted of solid phase extraction with a PrepSep vacuum manifold (Fisher Scientific) using a dual-column cartridge with 200 mg of graphitized carbon black and 600 mg of primary secondary amine. After conditioning the cartridge with 3 ml of hexane, the extract was loaded. The tube previously containing the extract was washed twice with 0.5 ml of hexane, and these rinses were added to the cartridge. The analytes were eluted with 7 ml of 30% dichloromethane in hexane. The eluent was evaporated to near dryness under nitrogen gas, and solvent was exchanged to 1.0 ml of 0.1% acetic acid in hexane before analysis.

The cleanup procedure for the fipronil, fipronil sulfone, and fipronil sulfide extracts consisted of solid phase extraction and additional filtering in preparation for gel permeation chromatography (GPC). The solid phase extraction method is the same as for pyrethroids with the exception of the extracts being eluted from a 250-mg graphitized carbon black cartridge with 3 ml of dichloromethane. After the eluent was evaporated to 1 ml, the concentrated extract was filtered through a 0.2-μm Whatman GD/X filter (Florham Park, NJ, USA). The test tube that contained the extract was washed twice with 0.5 ml of dichloromethane and filtered. The filter was washed three times with 2 ml of dichloromethane, and the extract was evaporated to 0.4 to 0.5 ml before injection into gel permeation chromatography. This was performed on an Agilent 1100 high-performance liquid chromatography (Agilent Technologies, Palo Alto, CA, USA). A Foxy Junior Fraction collector (Isco, Lincoln, NE, USA) was set to collect the analytes that eluted between 7.5 and 9.0 min. The separation was completed on a Waters 300 × 19 mm Envirogel gel permeation chromatography column with a 5 × 19 mm precolumn (Milford, MA, USA). The mobile phase (dichloromethane) was set at a flow rate of 5 ml/min. The extract was injected into the gel permeation chromatography with a Rheodyne 7225 injector with a 0.5-ml sample loop (Cotati, CA, USA). The fractions were evaporated to near dryness, and solvent was exchanged to 0.5 ml with hexane for analysis using a gas chromatograph and an electron capture detector.

Quality assurance and quality control consisted of a laboratory control blank, laboratory control sample, matrix spike, and a matrix spike duplicate every 20 samples. The laboratory control blank was an aliquot of clean sea sand with the surrogates only, while the laboratory control sample was an aliquot of clean sea sand containing the surrogates and the analytes of interest. The matrix spike and matrix spike duplicate contained the sediment used in the experiment spiked with the surrogates and analytes of interest. The matrix spike and matrix spike duplicate percent recoveries were 90 to 132% for the pyrethroids and were 84 to 119% for fipronil and its degradation products. All quality assurance and quality control samples were extracted in the same manner as the experimental samples, including same weight (10 g). As with experimental samples, accuracy of extractions is based upon percent recovery of the surrogates within a range of 80 to 120%.

Chemical analysis of the final extracts were performed on an Agilent 6890 series gas chromatograph (Agilent Technologies) equipped with an electron capture detector and an HP-5ms (30 × 0.25 m, film thickness 0.25 μm) column. Helium and nitrogen were used as the carrier and makeup gas, respectively, with the flow rate of the carrier gas at 3.5 ml/min. A 2-μl sample was injected into the gas chromatograph with a pulsed splitless mode. For pyrethroid analysis, the oven was set at 100°C, heated to 180°C at 10°C/min increments and then to 205°C at 3°C/min increments, held for 4 min, heated to

Table 1. Mortality, immobilization, and growth endpoints of pyrethroids, fipronil, and fipronil metabolites to *Chironomus tentans*[a]

Endpoint[bc]	Bifenthrin	Lambda-cyhalothrin	Permethrin	Fipronil	Fipronil-sulfide	Fipronil-sulfone
LC50	6.2 (8.7–5.1)	2.8 (3.5–2.3)	24.5 (58.9–5.7)	0.13 (0.14–0.12)	0.16 (0.23–0.12)	0.12 (0.14–0.10)
$EC50_{Immob}$	2.2 (2.4–1.9)	0.3 (0.6–0.1)	11.5 (15.4–7.8)	0.10 (0.11–0.08)	0.06 (0.07–0.03)	0.04 —[d]
$IC50_{AFDM}$	2.4 (2.8–1.6)	1.8 —	27.4 (60.9–14.4)	0.17 —	0.10 —	>0.20[e] —
$IC20_{AFDM}$	1.0 (1.3–0.7)	1.0 (1.8–0.0)	12.6 (31.1–0.0)	0.11 (0.18–0.05)	0.06 (0.10–0.03)	0.05 —
$IC50_{IGR}$	1.5 (1.6–1.2)	1.9 —	27.2 (58.3–15.4)	0.12 (0.15–0.05)	0.09 (0.12–0.08)	0.04 (0.06–0.00)
$IC20_{IGR}$	0.6 (0.7–0.5)	1.1 —	13.7 (31.0–8.8)	0.06 (0.15–0.03)	0.06 (0.09–0.02)	0.02 (0.05–0.0)
LSR ($LC50/EC50_{Immob}$)	2.9	9.0	2.1	1.3	2.9	3.2
LSR ($LC50/IC20_{AFDM}$)	6.5	2.8	1.9	1.1	2.7	2.3
LSR ($LC50/IC20_{IGR}$)	10.7	2.6	1.8	2.2	2.8	7.3

[a] Concentrations are μg/g of organic carbon (OC), and for conversion to dry weight concentrations, sediment OC values were 0.69% for permethrin, fipronil, fipronil-sulfide, and fipronil-sulfone and 0.97% for bifenthrin and lambda-cyhalothrin.
[b] Growth endpoints consist of ash-free dry mass (AFDM) and instantaneous growth rate (IGR). For point estimates, 95% confidence intervals are in parentheses. Lethal to sublethal ratios (LSR) are also provided for selected point estimates.
[c] The lethal (LC), effective (EC), and inhibitory (IC) concentrations to either 20 or 50% of the test population are indicated.
[d] 95% confidence intervals not calculable.
[e] IC50 greater than highest concentration tested.

280°C at 20°C/min increments, and held for 15 min. For fipronil and its metabolites, the oven was set at 100°C, heated to 180°C at 10°C/min increments and then to 205°C at 3°C/min increments, held for 4 min, heated to 280°C at 20°C/min increments, and held for 7 min. Six external standards were used for linear calibration of all analytes at concentrations of 500, 250, 100, 50, 10, and 5 ng/ml. Qualitative identity of analytes was established using a retention window of 1%.

Method detection limits for bifenthrin, lambda-cyhalothrin, and permethrin were 0.26, 0.30, and 0.62 ng/g of dry mass (DM) for sediment, respectively. Method detection limits for fipronil, fipronil-sulfide, and fipronil-sulfone for these experiments were 0.172, 0.211, and 0.176 ng/g of DM for sediment.

Statistical analyses

Point estimates were determined for the following lethal and sublethal endpoints: survival, immobilization, head capsule width, head capsule length, and AFDM. For quantal responses such as survival and immobilization, LC50s and EC50s were estimated using maximum likelihood methodology. Data were fit using three models (probit, logit, and weibull) and the relative goodness of fit of these models was compared using χ^2 ratios [20]. The best-fit model was used for endpoint estimation analysis. Percent mortality and immobilization data were arcsine square root–transformed. For nonquantal responses, such as AFDM, head capsule width, head capsule length, and daily IGR, selected inhibition concentration point estimates were calculated using linear interpolation with 5,000 resamples for bootstrapping [21]. Abbott's correction was applied in all point estimate calculations. Hypothesis testing was conducted on these response variables to identify NOECs and lowest-observed-effect concentrations (LOEC) using Dunnett's test. Normal distribution and equal variances were examined with Shapiro-Wilk's and Bartlett's tests, respectively. For data that did not exhibit normal distribution or equal variances, means were compared using the Wilcoxon rank sum test.

Six exposure concentrations were tested for each compound during these experiments. However, one exposure concentration for fipronil-sulfide and two exposure concentrations for fipronil-sulfone were below method detection limits. These data were not included in analyses for determination of point estimates, LOECs, and NOECs.

The principal component analysis to reduce morphological parameters (head capsule length and width) to a single size score was conducted using PC-ORD, Ver 4 (MjM Software Design, Gleneden Beach, OR, USA). A centered variance and covariance cross-products matrix was used, and the principal component analysis was not standardized.

Bartlett's and Shapiro-Wilk's goodness-of-fit tests indicated that *C. tentans* BCI data for some compounds did not have equal variances and were not normally distributed, despite $\log(x + 1)$ transformation. Thus, untransformed data for these endpoints were compared among contaminant concentrations and controls using a Kruskal-Wallis rank sum test and a Tukey-Kramer multiple comparisons test (SAS JMP®, Ver 4.0.4, SAS Institute, Cary, NC, USA). For all response variables, the negative and solvent controls were pooled if they were similar (t test; $p > 0.05$). Significance level for all hypothesis testing was $\alpha = 0.05$.

RESULTS

Lethal and sublethal toxicity endpoints

Pyrethroid LC50s were 6.2, 2.8, and 24.5 μg/g of OC for bifenthrin, lambda-cyhalothrin, and permethrin, respectively (Table 1). For fipronil, fipronil-sulfide, and fipronil-sulfone LC50 values were 0.13, 0.16, and 0.12 μg/g of OC, respectively (Table 1). Immobilization EC50s and 50% inhibition or reduction (IC50s) for IGR and AFDM were lower than LC50s for most endpoints and compounds (Table 1). Ratios of the LC50 to sublethal endpoints ranged from 1.1 to 10.7. The ratio of LC50 to IC20 for AFDM was the most consistent among compounds, ranging from 1.1 to 6.5 (Table 1).

For both AFDM and IGR, LOECs ($p < 0.05$) were 2.2, 2.0, 74.2, 0.2, 0.1, and 0.1 μg/g of OC for bifenthrin, lambda-cyhalothrin, permethrin, fipronil, fipronil-sulfide, and fipronil-

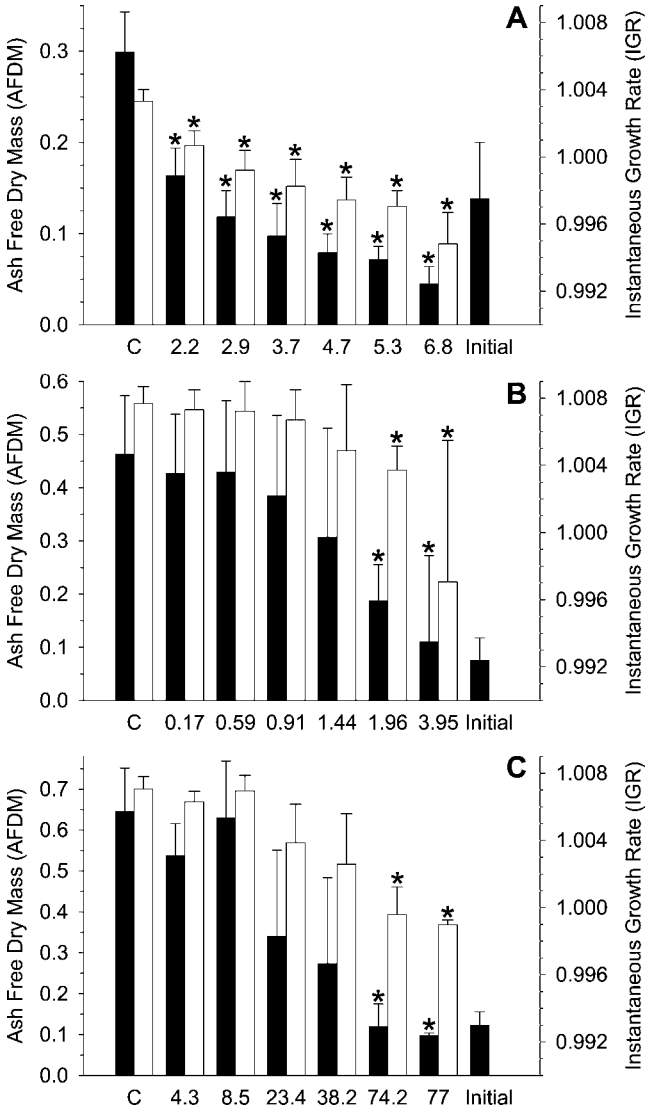

Fig. 1. Mean *Chironomus tentans* ash-free dry mass (AFDM) (solid bars) and instantaneous growth rate (IGR) (open bars) for controls and six bifenthrin (**A**), lambda-cyhalothrin (**B**), and permethrin (**C**) concentrations after a 10-d exposure period. Data on initial AFDM were obtained from a subset of *C. tentans* that was measured immediately before the exposure period for each compound and represent an estimate of starting mass for exposed organisms. Error bars are standard deviation, and asterisks indicate a significant difference from the control ($p < 0.05$). Concentrations are μg/g of organic carbon (OC) and time weighted (average of measured concentrations from the beginning and the end of the 10-d exposure period). For conversion to dry weight concentrations, sediment OC was 0.97% for the bifenthrin and lambda-cyhalothrin experiments and 0.69% for the permethrin experiment.

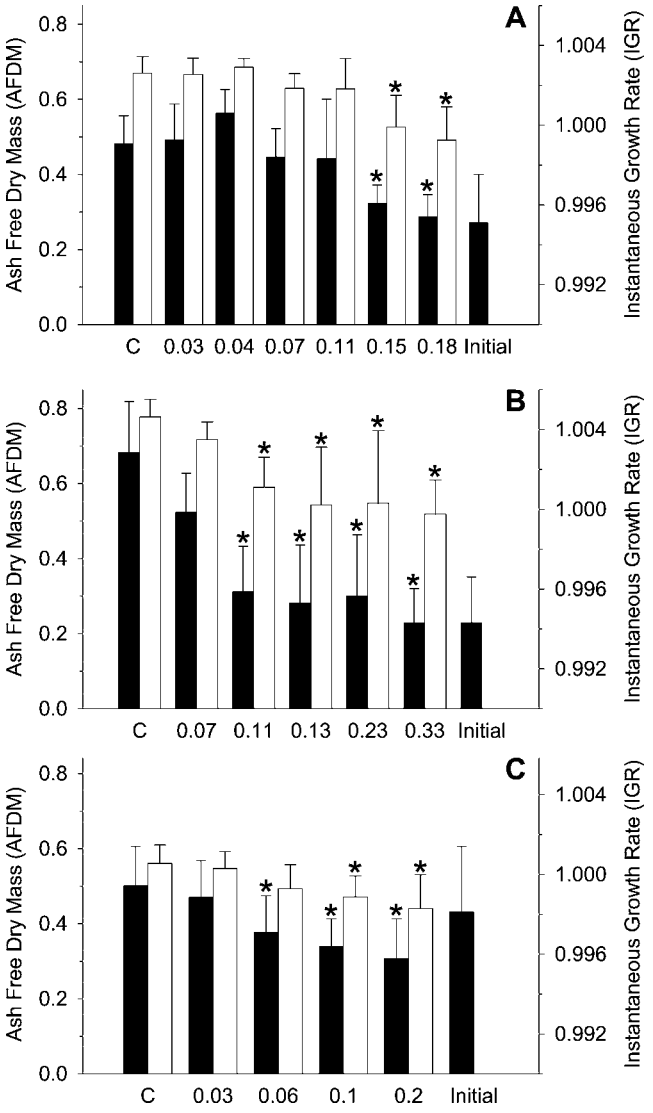

Fig. 2. Mean *Chironomus tentans* ash-free dry mass (AFDM) (solid bars) and instantaneous growth rate (IGR) (open bars) for controls and fipronil (**A**), fipronil-sulfide (**B**), and fipronil-sulfone (**C**) concentrations after a 10-d exposure period. Data on initial AFDM were obtained from a subset of *C. tentans* that was measured immediately before the exposure period for each compound and represent an estimate of starting mass for exposed organisms. Error bars are standard deviation, and asterisks indicate a significant difference from the control ($p < 0.05$). Concentrations are μg/g of organic carbon (OC) and time weighted (average of measured concentrations from the beginning and the end of the 10-d exposure period). For conversion to dry weight concentrations, sediment OC was 0.69%.

sulfone, respectively (Figs. 1 and 2). The BCIs were highly variable and for most compounds tested did not follow the expected pattern of reduced BCI with increasing exposure concentration (data not shown).

Water quality, control survival, and control growth

Ranges of water quality parameters for the bifenthrin and lambda-cyhalothrin experiments were as follows: conductivity, 275 to 396 μS/cm; pH, 6.61 to 6.74; temperature, 22.3 to 23.0°C; and dissolved oxygen, 6.12 to 6.78 mg/L. For the fipronil-sulfide and fipronil-sulfone experiments, ranges of water quality parameters were as follows: Conductivity, 342 to 399 μS/cm; pH, 6.67 to 6.96; temperature, 22.3 to 25.3°C; and dissolved oxygen, 6.40 to 7.34 mg/L. The ranges of water quality parameters during the fipronil experiment were as follows: conductivity, 255 to 440 μS/cm; pH, 6.91 to 7.10; temperature, 21.8 to 23.5°C; and dissolved oxygen, 5.32 to 7.94 mg/L. Water quality data are grouped for more than one compound for those experiments conducted simultaneously using the same source of synthetic water. Water quality was monitored but not recorded during the permethrin experiment. Control survival during the toxicity experiments was 84.0, 88.9, 92.2, 95.0, 95.0, and 88.0% for bifenthrin, lambda-cyhalothrin, permethrin, fipronil, fipronil-sulfide, and fipronil-sulfone experiments, respectively. For all compounds and responses (except survival and immobilization for fipronil-sulfone), all le-

Table 2. Mean percentage ± standard deviation of initial concentrations remaining in spiked sediment after 14 d of aging in the dark at 4°C and after 24 d[ab]

Compound	% Remaining after 14 d	% Remaining after 24 d[c]
Bifenthrin	103.7 ± 9.2	95.5 ± 8.6
Lambda-cyhalothrin	83.4 ± 16.7	77.5 ± 16.8
Permethrin	98.0 ± 9.3	72.9 ± 6.0
Fipronil	55.7 ± 20.7	33.9 ± 11.0
Fipronil-sulfide	83.6 ± 11.1	102.5 ± 20.7
Fipronil-sulfone	106.0 ± 10.9	95.1 ± 21.6

[a] Data are percentage of compound remaining based on measured environmental concentration immediately after spiking and mixing.
[b] Means were calculated from measured values for each concentration used for the dose–response experiments (n = six concentrations for bifenthrin, lambda-cyhalothrin, permethrin, and fipronil; five concentrations for fipronil-sulfide; and four concentrations for fipronil-sulfone).
[c] Environmental conditions for the 24-d period included the 14-d aging period at 4°C in the dark and the 10-d toxicity test at 23 ± 1°C, with a 16:8-h light:dark photoperiod.

thal and sublethal response variables were not significantly different ($p > 0.05$) between solvent and negative controls and were combined. Survival and immobilization responses in the fipronil-sulfone experiment were different between negative and solvent control ($p < 0.05$); subsequently, solvent controls were used for analyses.

For organisms measured before the experiment (i.e., initial organisms), AFDM was significantly smaller than for control organisms measured after 10-d exposures (t test; $p < 0.0002$) for the bifenthrin, lambda-cyhalothrin, permethrin, fipronil, and fipronil-sulfide experiments, indicating that growth occurred during the experimental period. Initial AFDM did not differ from control AFDM for fipronil-sulfone (t test, $p = 0.263$) and is likely due to the use of early-fourth instar larvae (as described earlier). For the pyrethroids, initial AFDM was not significantly different among experiments (analysis of variance, $p < 0.001$) and facilitates comparing effects on growth among compounds.

Stability of chemicals in the sediment system

Pyrethroids were relatively stable over the 14-d aging period in the dark at 4°C (Table 2). Some degradation occurred during the 10-d organism exposure period, which was conducted at a warmer temperature (23 ± 1°C) and with light. Bifenthrin appeared to degrade at a slower rate than lambda-cyhalothrin and permethrin (Table 2). Fipronil degraded substantially over the 24-d period; however, the fipronil metabolites were more persistent (Table 2). Because these compounds degraded at different rates, all LC50s, EC50s, and IC50s, as well as LOECs and NOECs, were calculated based on the time-weighted average during the organism exposure period, that is, the average of measured concentrations at time = 14 d and time = 24 d.

The 14-d aging period in the present study addresses a scenario of aqueous conditions receiving pesticide-sorbed sediment shortly after both the application and the runoff event. It should be noted that aging period can directly influence phase equilibration of pyrethroids, which can be observed in differences in partition coefficients (e.g., K_d and K_{OC}), as well as reduced sediment toxicity [9].

DISCUSSION

Relationship of toxicity endpoints to previously published data

Toxicity of permethrin to *C. tentans* observed in the current study (LC50 = 24.5 μg/g of OC) was similar to previously published accounts for *C. tentans* or a conspecific under similar experimental conditions: Conrad, Fleming, and Crane [7] reported a *C. riparius* LC50 of 21.9 μg/g of OC, and Weston, You, and Lydy [8] estimated a *C. tentans* LC50 of 23 μg/g of OC. Alternatively, the bifenthrin LC50 of 6.2 μg/g of OC determined in the current study was 2.9 to 4.8 times lower than recently published values. Xu et al. [9] reported LC50s of 18.3, 29.0, and 29.8 μg/g of OC for sediments containing 5.0, 1.4, and 1.9% OC, respectively. Likewise, the LC50 for lambda-cyhalothrin (2.8 μg/g of OC) was lower than that reported for the 28-d *C. riparius* emergence EC50 of 6.8 μg/g of OC [5,8].

Typically, toxicity data are normalized to the OC content within the sediment to minimize variation among studies and among sediments. However, for hydrophobic organic contaminants, OC normalization does not always remove this variation, and that may be partly because of the presence of carbonaceous materials such as black carbon within sediments [22]. The role of black carbon may present a different sorption mechanism than organic matter and influence the bioavailable fraction of hydrophobic compounds [22,23]. Black carbon content was not quantified for the test sediments used in the current study, and this factor may explain the LC50s observed to be lower than those previously reported in the literature. It should also be noted that black carbon and other carbonaceous geosorbents could have the greatest concentrations in urban areas, presenting important implications for those pyrethroids often used for urban applications.

Toxicity estimates of sediment-sorbed fipronil for *Chironomus* spp. were not available in the literature; however, aqueous LC50 for *C. crassicaudatus* was 0.42 μg/L [24]. Fipronil sediment concentrations of 65 ng/g of DM resulted in significantly reduced copepod (*Amphiascus tenuiremis*) reproduction rates [25]. This value is 74 times higher than the fipronil LC50 for *C. tentans* determined in the current study (i.e., 0.88 ng/g of DM) and supports the pattern of high variability of fipronil sensitivity among aquatic organisms [26]. Data from the current study and Chandler et al. [25] and those summarized in Gunasekara et al. [26] suggest that dipterans, including *Chironomus* spp. and the mosquito genera, may be among the most sensitive aquatic organisms to fipronil and its metabolites. Because dipterans are important components of benthic macroinvertebrate communities, future estimates of risk of fipronil to aquatic communities should consider these taxa.

Comparisons among sublethal endpoints

In general, *C. tentans* immobilization and growth-based endpoints were more sensitive than lethal responses, and for most compounds immobilization EC50s and AFDM and IGR IC50s were within similar ranges. Several growth endpoints were examined in the present study (AFDM, BCI, and IGR). The BCI endpoint is unique in that it incorporates a composite score of body size (morphology) and organism mass, with a goal of partitioning structural contributions to mass from fats, muscle, and other energy reserves [13]. However, *C. tentans* head capsule growth was inhibited proportionally and linearly to AFDM with increasing exposure concentrations (Fig. 3).

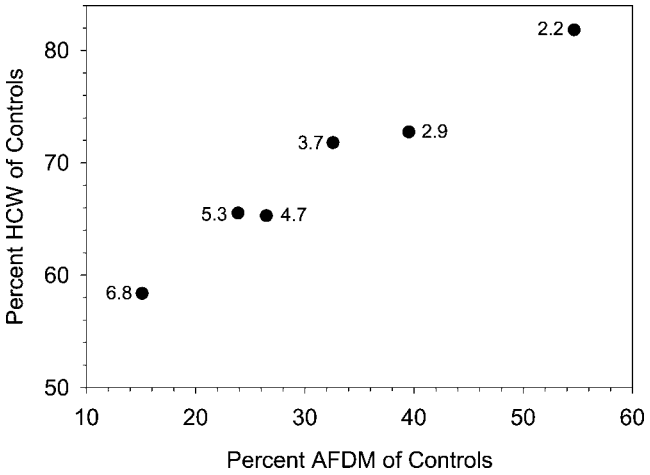

Fig. 3. The relationship between percent reduction in ash-free dry mass (AFDM) of controls and percent reduction in head capsule width (HCW) from controls for six bifenthrin concentrations. The data demonstrate the proportional reductions upon bifenthrin exposures in both the morphological measurement HCW and mass. Points are labeled with corresponding bifenthrin concentrations in μg/g of organic carbon.

Growth was delayed or suspended in both size and mass dimensions for many of these compounds and explains why BCI at high sediment exposure concentrations was similar to controls or, for the case of bifenthrin and lambda-cyhalothrin, was greater than controls. These patterns would suggest that BCI might not be a good indicator of sublethal pyrethroid and phenylpyrazole insecticide exposure or for other chemical stressors in which both structural size and mass are severely inhibited. Thus, BCI may be an endpoint limited to effect assessments at very low exposure concentrations or in exposures to fully developed organisms.

In terms of individual endpoint sensitivity, mass-driven growth endpoints were more sensitive than morphological endpoints such as head capsule width and length. The AFDM and IGR measurements responded similarly, and LOECs and NOECs for these two growth endpoints were identical for five of the six compounds tested (Figs. 1 and 2).

Population and community-level implications of growth effects

In *Chironomus* spp., smaller body sizes during a short-term exposure, such as the 10-d experiments in the current study, can lead to implications at the population level. For example, reduced larval growth due to food restriction in *Chironomus* spp. was shown to negatively affect emergence [27–29], adult female size, number of eggs per female [29,30], and fecundity [29]. In addition, demographic modeling indicated a decline in *C. tentans* population size with reduced larval growth [29]. Widespread reduction in larval size of benthic macroinvertebrates can also affect trophic interactions [31] and result in increased predation risk due to lengthened time to attain refuge body sizes in aquatic communities dominated by gape-limited predators [32].

Growth inhibition was dramatic for the compounds examined in the current study, with much-reduced to no significant growth observed for some concentrations tested. Pyrethroid concentrations that resulted in IC50s for the growth endpoints of AFDM and IGR, were 0.9 to 4.3 times lower than the respective LC50 and within pyrethroid concentration ranges reported for agricultural and urban creek sediments [8,10]. Liber et al. [27] reported that greater than 64 and 73% reductions in growth resulted in 86 to 100% reduction in emergence and 0% of larvae pupating, respectively. Thus, it is likely that *C. tentans* exposures to concentrations at or greater than the IC50 would significantly affect pupation, emergence, or both. Because *C. tentans* reproduction occurs postemergence during the winged adult stage, individuals that fail to emerge do not contribute to population growth.

Another perspective, ecologically, for growth effects are larval DM benchmarks. It has been suggested that for larvae below 0.8 mg of DM/individual emergence begins to decline and that *C. tentans* larvae at or below 0.5 mg of DM/individual do not undergo pupation and emergence [27,29,33]. In the current study, an average of 0.8 mg of DM/individual occurred at or above the following concentrations: permethrin, 8.5 μg/g of OC; bifenthrin, 2.2 μg/g of OC; lambda-cyhalothrin, 1.4 μg/g of OC; and fipronil, 0.154 μg/g of OC.

These patterns indicate that although *C. tentans* growth endpoints in many cases may be more sensitive than lethal endpoints (as would be expected), resulting population-level effects from reduced growth may be similar to lethality (i.e., growth-inhibited individuals are eliminated from the reproducing population and do not contribute to population growth) as suggested by Sibley, Benoit, and Ankley [29]. This has implications for some recent surveillance work on agriculture-impacted sediments in California, USA that indicated that 40% of samples were toxic to *C. tentans* based on lethality responses. These percentages may be greater when considering toxicity based on growth endpoints. It is generally accepted that *C. tentans* are less sensitive to pyrethroids than other aquatic species such as *Hyalella azteca* [5,8,34]. Considering the growth-related effect concentrations reported here and their potential implications for population-level effects [27,29], the sensitivity gap between *C. tentans* and *H. azteca*, in an ecological sense, may be smaller than previously suggested. This idea is supported by results of several field and mesocosm studies. For example, biomonitoring of *Hyalella* spp. and chironomid abundances in aquatic systems in which organophosphate and pyrethroid insecticide stressors were present suggested that these two taxonomic groups showed similar proportional reductions in abundance at an impacted site compared to an upstream location [35]. Also, in a mesocosm study on esfenvalerate, both chironomid and amphipod abundance reductions (without recovery) followed similar patterns among treatment concentrations [34].

Decreased IGR for *C. tentans* larvae can also influence time to emergence in a sex-biased manner, with greater delays in female emergence than in males [27]. This sex-biased disruption of temporal emergence patterns could have significant population-level effects on insects that exhibit protandry such as chironomids and other dipterans [36] and presents an important research area for future investigation.

It is unclear whether the reduced growth observed in the current study is a result of food avoidance due to insecticide-bound organic material or whether feeding rates are maintained and reduced growth is a direct effect of these insecticides (e.g., energetic reserves are allocated toward detoxification). The latter scenario was reported in a study on larval damselflies (*Coenagrion puella*); aqueous exposures to carbaryl and endosulfan (organophosphorous and organochlorine class compounds, respectively) did not have an effect on food ingestion but did affect food assimilation efficiency [37]. Another pos-

sible mechanism would be behavioral modifications that lead to reallocation of energetic reserves from growth to other activities. For example, sublethal esfenvalerate exposures to the caddisfly *Brachycentrus americanus* induced a behavioral response (case abandonment) that leads to energetically costly activities (case-rebuilding) [38]. Such induced case-rebuilding in the caddisfly *Odontocerum albicorne* has been shown to reduce morphological growth [39].

Toxicity endpoints and reported environmental concentrations

To understand ecological risk, it is important to compare toxicity endpoints relative to sediment concentrations reported for aquatic systems in ecosystems in which these compounds are applied. In the first of two studies on pyrethroids in urban California creeks, 28.6, 57.1, and 0.0% of sediment samples ($n = 21$) exceeded the DM benchmark LOEC from the current study (0.8 mg of DM/individual) for concentrations of permethrin, bifenthrin, and lambda-cyhalothrin, respectively [11]. In the second study, 27.8, 33.3, and 0.0% of sediment samples ($n = 18$) exceeded the DM benchmark LOEC for permethrin, bifenthrin, and lambda-cyhalothrin, respectively [10]. In sediments from an agricultural landscape, 7.2, 2.8, and 1.4% of sediment samples ($n = 69$) exceeded the DM benchmark LOEC for permethrin, bifenthrin, and lambda-cyhalothrin, respectively [8]. This suggests that reported pyrethroid concentrations in creek sediments from urban landscapes could negatively affect chironomid populations through reduced growth and emergence and through elimination from reproducing populations. Furthermore, the previously described percentages are conservative because this assessment does not consider mixtures of the compounds in which lower concentrations of multiple pyrethroids in the same sediment might contribute to a toxic unit that exceeds LC50s and growth LOECs.

Fewer reports exist on fipronil occurrence in sediments of receiving aquatic systems in the environment. In two of three oxbow sediments within agricultural watersheds, fipronil concentrations were 1.7 and 3.2 ng/g of DM [40]. In river sediments, fipronil-sulfide ranged from 0.64 to 24.8 ng/g and fipronil-sulfone was reported as 10.5 ng/g [26]. The reported fipronil concentrations are above both the *C. tentans* LC50 (0.88 ng/g of DM or 0.13 μg/g of OC) and the 0.8 mg of DM/individual LOEC (1.06 ng/g of DM or 0.15 μg/g of OC) determined in the current study.

CONCLUSIONS

The results of the present study demonstrate that sublethal responses in *C. tentans*, particularly growth-related endpoints, are typically more sensitive than the lethal endpoint; for some compounds effects on growth were profound. This is important because of the unique life cycle of *C. tentans*, which involves pupation and emergence events in which effects on growth observed here would likely lead to ecological effects similar to what would be expected if mortality occurred, that is, inhibition of emergence and thus not being reproductively viable. Thus, previous assessment of ecological risk of sediment-associated pyrethroid and fipronil to chironomids, and potentially to other dipterans that are important to aquatic invertebrate communities, may have been under estimated. In addition, these results add to the growing evidence that fipronil and metabolite toxicity is highly variable among taxa and that dipterans may be a sensitive group of aquatic organisms to phenylpyrazole-class insecticides.

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