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Contaminant Sensitivity of Freshwater Mussels

ACUTE AND CHRONIC TOXICITY OF PESTICIDE FORMULATIONS (ATRAZINE, CHLORPYRIFOS, AND PERMETHRIN) TO GLOCHIDIA AND JUVENILES OF *LAMPSILIS SILIQUOIDEA*

ROBERT B. BRINGOLF,*† W. GREGORY COPE,† M. CHRIS BARNHART,‡ SHAD MOSHER,† PETER R. LAZARO,† and DAMIAN SHEA†

†Department of Environmental and Molecular Toxicology, North Carolina State University, Campus Box 7633, Raleigh, North Carolina 27695-7633, USA

‡Department of Biology, Missouri State University, Springfield, Missouri 65804, USA

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Abstract—Freshwater mussels are among the most imperiled faunal groups in North America; approximately 67% of the nearly 300 native freshwater mussel species (family Unionidae) are listed as endangered, threatened, or of special concern. Despite evidence that glochidia and juvenile life stages are highly sensitive to some chemical contaminants, the effects of pesticides on early life stages of unionid mussels are largely unknown. In the United States, pesticide registration is based on toxicity data of the active ingredient, not formulations as they are sold and applied. Some pesticide formulations, however, are more toxic than their active ingredient (technical-grade pesticide) alone because of the presence of surfactants, adjuvants, or other ingredients in the formulation. The objective of the present study was to compare the toxicity of active ingredients of several current-use pesticides (atrazine, chlorpyrifos, and permethrin) to the toxicity of pesticide formulations to glochidia and juvenile life stages of a freshwater mussel (*Lampsilis siliquoidea*). The atrazine formulation (Aatrex) was more toxic than technical-grade atrazine in chronic tests with juvenile *L. siliquoidea*. For other pesticides, acute and chronic toxicity of technical-grade pesticides were similar to the toxicity of pesticide formulations. Median effective concentrations for chlorpyrifos were 0.43 mg/L for glochidia at 48 h, 0.25 mg/L for juveniles at 96 h, and 0.06 mg/L for juveniles at 21 d. Atrazine and permethrin as well as their formulations did not cause significant acute toxicity in glochidia or juveniles at exposure concentrations approaching water-solubility limits. Additional research is needed on other pesticides with different modes of action, on the role of different routes of exposure, and with other species of unionid mussels to evaluate similarities of toxic response.

Keywords—Herbicides Insecticides Early life stage Growth

INTRODUCTION

The widespread decline of native freshwater mussels (Bivalvia: Unionidae) during the past half-century in North America is well documented; nearly 70% of native unionids presently are listed as federally endangered, threatened, or of special concern in the United States [1]. Causes of some site-specific and catastrophic mussel die-offs have been identified [2,3]; however, the exact causes for the gradual loss of unionid abundance and diversity on a widespread scale have not been well characterized. Among the many anthropogenic stressors identified as potential factors in the chronic decline of unionids is chemical contamination of surface waters by domestic, industrial, and agricultural point as well as nonpoint sources [4]. The toxicity of pesticides generally is well described for common aquatic species (e.g., cladocerans and fish) found in toxicity databases, but little is known about the toxicity of many pesticides to unionids.

The unique life history of unionids makes them potentially susceptible to pesticides (and other stressors) at each developmental stage. Glochidia (the parasitic larval stage) are released into the water column by adult females for attachment to an appropriate fish host, and they may be viable in the water column for hours to days after release. Once attached to host tissue, the glochidia become encysted for a period of, typically, a few weeks while they transform into juveniles. The potential for exposure to contaminants while encysted in host tissue is not well described, but limited evidence indicates that encystment may provide a measure of protection from at least some waterborne contaminants [5]. Transformed juveniles detach from their host and settle to the sediment, where they burrow and subsist, primarily by pedal (foot) feeding. As the juveniles grow, they become filter-feeding adults capable of filtering as much as 110 L of water per day [6]. Thus, many routes (e.g., water, sediment, and diet) exist for potential exposure to toxicants for the various life stages of freshwater mussels, but the early life stages are especially sensitive to certain contaminants relative to other commonly tested aquatic species [7,8].

The present study is part of an effort to expand our knowledge regarding the hazards of current-use pesticides to early life stages of native freshwater mussels. Little data exist concerning the toxicity of pesticides to native freshwater mussels, likely because toxicity information for pesticides and mussels is not currently required by the U.S. Environmental Protection Agency (EPA) as part of the pesticide registration process and because, until recently [9], a standardized guideline for toxicity testing with freshwater mussels was not available. Additionally, the relative toxicity to mussels of pesticide formulations compared with toxicity of active ingredients is largely unknown, likely because U.S. EPA pesticide registration is based on toxicity data for the active ingredient of pesticides and not for pesticide formulations. Previously, however, we demonstrated that Roundup® (Monsanto, St. Louis, MO, USA), a

* To whom correspondence may be addressed (rbringolf@warnell.uga.edu).

formulation of the herbicide glyphosate, was significantly more toxic than technical-grade glyphosate to freshwater mussels [10]. Others have reported similar findings for glyphosate and Roundup with other aquatic organisms [11–14]. Specific objectives of the present study were to compare the acute and chronic toxicity of insecticide and herbicide formulations to the toxicity of the active ingredient only (technical-grade pesticide) in early life stages of freshwater mussels.

MATERIALS AND METHODS

Test chemicals

The pesticide formulations Aatrex 4L Herbicide® (40.8% active ingredient, atrazine; Syngenta, Greensboro, NC, USA), Lorsban® 4-E Insecticide (44.9% active ingredient, chlorpyrifos; Dow AgroSciences, Indianapolis, IN, USA), and Mosquito-B-Gone® Insecticide (2.5% active ingredient, permethrin [minimum, 35% *cis* isomers; maximum, 65% *trans* isomers]; Ortho, Marysville, OH, USA) were obtained from retail suppliers. Technical-grade atrazine (purity, 98%), chlorpyrifos (purity, 99%), and permethrin (44% *cis* isomers, 45% *trans* isomers) were purchased from Chem Service (West Chester, PA, USA). Atrazine, an agricultural triazine herbicide, is the most widely used herbicide in the United States and among the most frequently detected pesticides in surface and groundwater surveys [15,16]. Chlorpyrifos is a restricted-use, organophosphorous compound used as a broad-spectrum insecticide. Permethrin is an unrestricted synthetic pyrethroid compound used as a broad-spectrum insecticide. Test chemicals were chosen based on their widespread and frequent use in agricultural and other situations. Stock solutions of pesticide formulations were prepared weekly in distilled water and stored at room temperature. Stock solutions of technical-grade pesticides were prepared in acetone and stored at −20°C. Reconstituted hard water [17] was used as dilution water for all toxicity tests. The concentration of acetone in the solvent controls was equal to the greatest concentration of acetone in the treatments (≤0.02% for all tests).

Test organisms

Brooding adult female *Lampsilis siliquoidea* mussels were collected from April 2005 to June 2005 at Silver Fork of Perche Creek (Boone County, MO, USA), a largely forested watershed. Mussels were abundant at all sites from which they were collected. Roughly equal numbers of mature glochidia were obtained from the marsupial gills of at least three females by flushing the glochidia out of the gills using a syringe filled with water. Glochidia were shipped in coolers via overnight courier to North Carolina State University for toxicity testing. On arrival, the viability of all glochidia was assessed by exposing three subsamples of 50 to 100 glochidia (each) to a saturated NaCl solution, which initiates shell closure in viable glochidia. Glochidia were used for toxicity tests only if the initial viability exceeded 90%, in accordance with the standardized guidelines for toxicity tests with freshwater mussels [9]. Glochidia were acclimated to a 50:50 mixture of culture and dilution water for at least 2 h before toxicity tests began.

Juvenile *L. siliquoidea* were produced at Missouri State University as described by Bringolf et al. [18] and shipped via overnight courier to North Carolina State University for testing when the mussels were one to two months posttransformation. Juvenile mussels were acclimated to dilution water for 24 h before the start of toxicity tests. Suitability of juveniles for use in toxicity tests was evaluated by assessing foot movement outside the shell within a 5-min period [9].

Glochidia acute toxicity tests

Acute toxicity tests with glochidia were conducted according to standardized guidelines [9]. Briefly, test chambers were 90- × 50-mm, glass crystallizing dishes containing 100 ml of test solution and 150 to 200 glochidia. Three replicates were used for each of five or six test concentrations, dilution-water controls, and solvent controls. Glochidia toxicity tests were conducted for 48 h, and test solutions were not renewed. Viability was evaluated on subsamples of 50 to 75 glochidia from each replicate at 24 and 48 h by transferring the glochidia and approximately 2 ml of dilution water to one of the wells in a 12-well microplate for observation under magnification (×40). The total number of open and closed glochidia was recorded. Several drops (n = 3–4) of a saturated NaCl solution were added, and glochidia that did not respond (by closing their valves) were recorded as nonviable. Glochidia that closed before the addition of NaCl also were classified as nonviable based on the premise that they would not be able to attach to host fish for transformation into juveniles [9]. Digital photographs of glochidia were obtained before and after the exposure to NaCl to document glochidia response, thereby providing an additional measure of quality assurance/quality control for evaluation of glochidia survival in toxicity tests.

The test of each technical-grade pesticide was run concurrently with its respective formulation. Atrazine and Aatrex 4L test concentrations were 1.9, 3.8, 7.5, 15, and 30 mg/L. Chlorpyrifos and Lorsban test concentrations were 0.13, 0.25, 0.5, 1.0, and 2.0 mg/L. Permethrin and Mosquito-B-Gone test concentrations were 0.01, 0.03, 0.05, 0.1, and 0.2 mg/L. Consistent with standard methods [9], the test acceptability criterion was 90% or greater glochidia survival in the control and, when appropriate, solvent control treatments.

Juvenile acute toxicity tests

Acute toxicity tests with juvenile (one to two month old) *L. siliquoidea* were conducted according to standardized guidelines [9], with the exception that test chambers were 90- × 50-mm, glass crystallizing dishes containing 100 ml of test solution rather than 50-ml beakers with 30 ml of test solution, as suggested in the American Society for Testing and Materials guideline [9]. Three replicates, each with seven mussels, were used for each of five or six test concentrations, dilution-water controls, and solvent controls (described previously). Test duration was 96 h, and test solutions were renewed (95%) at 48 h. Survival (based on movement inside or outside the shell) was evaluated at 48 and 96 h. The test chamber (dish) was gently swirled to concentrate the mussels in a small area so that all the organisms in a dish could be observed simultaneously in the field of view under magnification (×15). Test concentrations for acute tests with juvenile mussels were the same as described above for acute tests with glochidia. The test acceptability criterion was 90% or greater survival in the control and solvent control (when appropriate) treatments.

Juvenile chronic toxicity tests

We adapted standardized guidelines for mussel toxicity testing [9] to conduct 21-d chronic toxicity tests with juvenile (one to two month old) *L. siliquoidea* exposed to technical-grade pesticides and their formulations. Length of the mussels (n = 135) at test initiation was 1,475 ± 306 μm (mean ±

standard deviation). Test solutions were renewed (95%) every 48 or 72 h, and survival (based on movement inside or outside the shell) and was assessed on days 7, 14, and 21. Juvenile mussels were fed daily with a commercial mixture of microalgae prepared from Instant Algae® Shellfish Diet 1800 and *Nannochloropsis* (Nanno 3600) concentrate (Reed Mariculture, Campbell, CA, USA) according to standardized guidelines for conducting chronic tests with juvenile mussels [9]. At initiation of the chronic test, the length of juveniles in each experimental unit was measured using QCapture PRO™ image analysis software (Ver 5.0; QImaging, Burnaby, BC, Canada) in conjunction with a stereomicroscope equipped with a digital camera. Lengths of surviving juvenile *L. siliquoidea* were measured on day 21, and growth was calculated for each replicate based on change in length (as the mean of all individuals in a replicate) from the start of the test. Mean ($n = 3$) growth as a percentage change in shell length was determined for each treatment with 30% or greater survival; growth was not calculated for replicates with less than 30% survival. Test concentrations for atrazine, Aatrex 4L, permethrin, and Mosquito-B-Gone were as described above for acute tests with glochidia and juveniles. Lower concentrations of chlorpyrifos and Lorsban were used in chronic toxicity tests (0.015, 0.03, 0.06, 0.13, and 0.25 mg/L). The test acceptability criterion was 80% or greater survival of mussels in the control and solvent control (when appropriate) treatments.

Pesticide exposure analysis

Pesticide exposure concentrations were determined for at least three treatment concentrations of each pesticide in acute and chronic toxicity tests. At the start of an experiment ($t = 0$), a 30-ml aliquot from each replicate within a given treatment was collected and combined to form a composite water sample. Water samples were extracted by liquid–liquid extraction with methylene chloride. Extracts were dried with anhydrous sodium sulfate and concentrated by use of rotary and nitrogen evaporation techniques. The concentrated extracts were analyzed on an Agilent 6890 gas chromatograph equipped with a Restek RTX-5MS capillary column (Bellefonte, PA, USA) and a 5973n mass-selective detector. Extraction efficiency for individual pesticides was determined from spiked samples. Final analyte concentrations were not corrected for extraction efficiency.

Exposure accuracy was calculated for each pesticide as

$$\text{Exposure accuracy} = (P_m/P_t)\cdot 100$$

where P_m is the measured pesticide concentration and P_t is the target concentration.

Water chemistry

Standard methods [17] were used for measurement of all water-quality parameters. Dissolved oxygen, conductivity, and temperature were analyzed with a YSI Model 556 MPS (Yellow Springs Instruments, Yellow Springs, OH, USA) calibrated multiprobe meter, and analysis of pH was performed with a Beckman Model Φ 240 (Beckman Instruments, Fullerton, CA, USA) calibrated meter. Alkalinity was determined by titration with 0.02 N H_2SO_4 to pH 4.5 and hardness by titration with 0.01 M ethylenediaminetetra-acetic acid.

Statistical analysis

Median effective concentration (EC50) estimates and 95% confidence intervals were calculated based on measured pesticide concentrations by the trimmed Spearman–Karber method [19] with ToxCalc™ statistical software (Ver 5.0.231; Tidepool Scientific Software, McKinleyville, CA, USA). The EC50s were considered to be significantly different when 95% confidence intervals did not overlap [17]. Statistical analysis of growth was performed with JMP Statistical Analysis® software (Ver 5.1; SAS Institute, Cary, NC, USA) by use of analysis of variance followed by Dunnett's test for means comparison ($\alpha = 0.05$). Chronic values were calculated as the geometric mean of the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC) for viability during the 21-d tests with juvenile *L. siliquoidea*. Acute to chronic ratios (ACRs) were based on the chronic values and 96-h EC50s for a given pesticide.

RESULTS AND DISCUSSION

General conditions and water chemistry

Viability of control (and solvent control) mussels met test acceptability criteria for all toxicity tests. Control viability was greater than 90% for all glochidia tests at 48 h and for all juvenile tests at 96 h. Control viability of juveniles in all chronic tests was greater than 80% at 21 d. No differences in survival or growth were found between the controls and solvent controls; therefore, solvent controls and controls were combined into a single control group for calculation of EC50s and growth.

Water chemistry values for test water were similar both within treatments and between tests and also to the values in the standardized guide for toxicity tests with mussels [9]. Temperature ranged from 20.7 to 21.7°C ($n = 36$), pH from 8.32 to 8.84 ($n = 36$), conductivity from 547 to 634 μS/cm ($n = 36$), alkalinity from 114 to 134 mg/L as $CaCO_3$ ($n = 36$), and hardness from 160 to 184 mg/L as $CaCO_3$ ($n = 36$). Dissolved oxygen was greater than 80% of saturation at all times.

Pesticide exposure validation

Measured concentrations of technical-grade pesticides were 105 to 116% of target, and measured concentrations for formulations were 111 to 152% of target (Table 1). Target analyte extraction efficiency was 72.6 to 92.1% for spiked samples of atrazine ($n = 6$), 80.2 to 89.8% for chlorpyrifos ($n = 3$), and 79.6 to 90.1% for permethrin ($n = 6$). Measured pesticide concentrations were not corrected for extraction efficiency. None of the three pesticides used in toxicity tests were detectable in water samples of the control ($n = 6$) and solvent control ($n = 3$) treatments.

Acute glochidia and juvenile toxicity tests

In acute tests, the sensitivity of *L. siliquoidea* glochidia and juveniles to pesticide formulations was similar to their sensitivity to the active ingredient of those pesticides. Early life stages of *L. siliquoidea* were not acutely sensitive to atrazine or permethrin or their formulations (Aatrex 4L and Mosquito-B-Gone, respectively). Acute toxicity of chlorpyrifos and Lorsban was similar for glochidia and juveniles, particularly at 48 h for glochidia and 96 h for juveniles (Table 2). These results suggest that formulations of atrazine, chlorpyrifos, and permethrin do not contain other ingredients (e.g., surfactants) that increase the toxicity of the formulation, unlike Roundup, which is substantially more toxic to aquatic organisms than its active ingredient, glyphosate [10–12].

Chlorpyrifos acute toxicity values for *L. siliquoidea* glochidia (0.50 mg/L) and juveniles (0.25 mg/L) suggest that

Table 1. Summary of exposure accuracy (ratio of measured concentrations to target concentrations) for acute and chronic toxicity tests with early life stage mussels (*Lampsilis siliquoidea*)[a]

Pesticide	Chemical grade	Exposure accuracy[b] (%)
Atrazine	Active ingredient	104.7 ± 19.0
	Formulation (Aatrex 4L®[c])	110.9 ± 14.6
Chlorpyrifos	Active ingredient	112.0 ± 48
	Formulation (Lorsban®[d])	122.8 ± 6.9
Permethrin	Active ingredient	116.2 ± 8.2
	Formulation (Mosquito-B-Gone®[e])	151.6 ± 5.0

[a] Data are presented as the mean ± standard deviation ($n = 6$).
[b] Exposure accuracy = (measured concentration/target concentration)·100.
[c] Syngenta, Greensboro, NC, USA.
[d] Dow AgroSciences, Indianapolis, IN, USA.
[e] Ortho, Marysville, OH, USA.

chlorpyrifos may be more toxic to early life stages of mussels than to adults. Hemming and Waller [20] reported a 96-h median lethal concentration (LC50) of 0.96 mg/L for adult hooked mussel (*Ischadium recurvum*), and Doran et al. [21] reported that chlorpyrifos was not lethal to adult threeridge mussel (*Amblema plicata*) at concentrations up to 1.2 mg/L after 96 h. Saltwater bivalves may even be less sensitive to chlorpyrifos: Borthwick and Walsh [22] reported a LC50 for eastern oyster (*Crassostrea virginica*) of 2.0 mg/L, and Serrano et al. [23] reported LC50s of 22.5 and greater than 56 mg/L for *Mytilus galloprovincialis* and *Venus gallina*, respectively.

Results of the present study suggest that mussel glochidia and juveniles are less sensitive to chlorpyrifos than other aquatic invertebrate organisms commonly used for standard toxicity tests. For example, Moore et al. [24] reported 48-h LC50s for *Hyalella azteca* (0.1 μg/L), *Daphnia magna* (0.6 μg/L), and *Chironomus tentans* (0.3 μg/L). Similarly, Conners and Black [25] reported that freshwater mussels were relatively insensitive (compared to other aquatic invertebrates) to diazinon, another organophosphate pesticide similar to chlorpyrifos that requires bioactivation for adverse effects. Therefore, chlorpyrifos water-quality criteria based on data from these species (amphipods, cladocerans, and chironomids) likely would be protective of *L. siliquoidea* glochidia and juveniles; however, a paucity of data exists regarding the relative sensitivity of *L. siliquoidea* and other unionid species to pesticides. Bringolf et al. [18] reported that *L. siliquoidea* glochidia were the most sensitive of five unionid glochidia species to a reference toxicant. In that same study, Bringolf et al. reported that *L. siliquoidea* juveniles responded to a reference toxicant in a manner similar to two other species of unionid juveniles, suggesting that *L. siliquoidea* may be suitable as a surrogate species for other unionids.

Previous studies indicate that freshwater mussel species vary widely in their sensitivity to permethrin. Milam et al. [8] reported LC50s for permethrin ranging from 14.9 to 3,515 μg/L for four species of unionid glochidia. Results of the present study were consistent with those of Bringolf et al. [18], who reported that permethrin LC50s were greater than 200 μg/L for five species of glochidia and two species of juveniles. Milam et al. [8] also reported permethrin LC50s for other aquatic invertebrate species commonly used for standard toxicity testing, such as *Ceriodaphnia dubia* (8.7 μg/L) and *D. magna* (12.4 μg/L). Results of the present study and those of

Table 2. Median effective concentrations (EC50s) and 95% confidence intervals (in parentheses) based on immobility of test organisms, chronic values (ChV), and acute to chronic ratios (ACR) for early life stages of *Lampsilis siliquoidea* exposed to technical-grade pesticides and a commercial formulation of each

	Glochidia EC50 (mg/L)		Juvenile EC50 (mg/L)					
Pesticide	24 h	48 h	96 h	7 d	14 d	21 d	ChV[a] (mg/L)	ACR[b]
Atrazine	>30	>30	>30	>30	17.1 (9.1–32.0)	10.1 (4.8–21.2)	5.3	>5.7[c]
Aatrex 4L®[d] (atrazine)	>30	>30	>30	>30	4.4 (1.9–10.3)	3.1 (2.2–4.4)	2.7	>11.1[c]
Chlorpyrifos	0.50 (0.32–0.78)	0.43 (0.29–0.63)	0.25 (0.17–0.37)	0.21 (0.11–0.40)	0.08 (0.04–0.15)	0.06 (0.03–0.14)	0.02	12.5
Lorsban®[e] (chlorpyrifos)	0.73 (0.49–1.10)	0.60 (0.40–0.90)	0.33 (0.26–0.42)	0.19 (0.15–0.25)	0.05 (0.03–0.11)	0.05 (0.03–0.08)	0.02	9.5
Permethrin	>0.2	>0.2	>0.2	>0.2	0.11 (0.06–0.21)	0.03 (0.005–0.20)	0.01	>20[c]
Mosquito-B-Gone®[f] (permethrin)	>0.2	>0.2	>0.2	>0.2	0.05 (0.02–0.09)	0.03 (0.02–0.04)	0.01	>20[c]

[a] Geometric mean of the no-observed-effect concentration and lowest-observed-effect concentration for viability at 21 d.
[b] Based on ChV and 96-h EC50.
[c] Based on EC50 for the highest concentration of pesticide tested.
[d] Syngenta, Greensboro, NC, USA.
[e] Dow AgroSciences, Indianapolis, IN, USA.
[f] Ortho, Marysville, OH, USA.

Milam et al. [8] suggest that like chlorpyrifos, permethrin generally is less toxic to unionid glochidia and juveniles than other common aquatic invertebrate test species. Furthermore, permethrin water-quality criteria derived from data with nonunionid aquatic invertebrates likely will be protective of early life stages of unionids.

Conners and Black [25] reported a 24-h LC50 for atrazine of 241.3 mg/L for *Utterbackia imbecillis* (a native unionid) glochidia exposed to Aatrex 4L. The highest test concentration in the present study was 30 mg/L of active ingredient, based on the water solubility of technical-grade atrazine (33 mg/L). These results suggest that the risk of acute effects from atrazine exposure is low for early life stages of unionids.

Chronic 21-d juvenile toxicity tests

Chronic toxicity of chlorpyrifos and permethrin formulations to mussels was similar to that of their respective technical-grade active ingredients; however, the 21-d EC50 for Aatrex 4L (3.1 mg/L) was significantly less than the 21-d EC50 for technical-grade atrazine (10.1 mg/L) (Table 2). It is uncertain whether the difference in toxicity between the technical-grade atrazine and the atrazine formulation was caused by inherent variability in test organism sensitivity to atrazine or whether a component of the formulation, such as a surfactant, may have been responsible for the increased toxicity of Aatrex 4L. In a separate study, Bringolf et al. [18] reported a 21-d EC50 of 4.3 mg/L for *L. siliquoidea* juveniles exposed to technical-grade atrazine. Although the 21-d EC50 (10.1 mg/L) for technical-grade atrazine in the present study was greater than that reported in our previous study (4.3 mg/L) [18], the 95% confidence intervals for these two EC50s overlap; therefore, they are not statistically different. Considered together, these results suggest variability among bioassays in the LC50s for technical-grade atrazine estimated for juvenile *L. siliquoidea*. Surfactant blends have been identified previously as a toxic component of other herbicide formulations, particularly glyphosate formulations. The commercial surfactant blend in Roundup, MON 0818, was highly toxic to aquatic organisms, including early life stages of unionids [10,11,14], whereas the active ingredient, glyphosate acid, was relatively nontoxic to the same organisms. Nonetheless, the 21-d NOEC of atrazine for viability (1.9 mg/L) and the LOEC (3.75 mg/L) suggest that the likelihood of toxicity to freshwater mussels from subacute atrazine exposure is low given that sustained, measured concentrations of atrazine in surface waters within this range would be unlikely [15,16].

Chronic values for permethrin, Mosquito-B-Gone, chlorpyrifos, and Lorsban (Table 2) indicate that juvenile mussels likely are at low risk of toxicity from chronic exposure to environmentally relevant concentrations of these insecticides (generally <0.5 μg/L) because of their relatively low use, low persistence, and low water solubility [26,27]. Water-quality criteria for chlorpyrifos and permethrin based on results of toxicity tests with standard aquatic test species likely would be protective of unionids. The chronic value for permethrin (0.01 mg/L) was similar to the 21-d EC50 (0.03 mg/L), indicating that a lower range of test concentrations may have allowed greater resolution of these values.

Acute to chronic ratios commonly are used to estimate chronic toxicity from acute data, because chronic tests are time and resource intensive. The U.S. Office of Pollution Prevention and Toxics has defined criteria of concern that correspond to an ACR of 10 [28]. In the present study, ACRs for chlorpyrifos and Lorsban were 12.5 and 9.5, respectively. We were unable to establish ACRs for atrazine, Aatrex 4L, permethrin, and Mosquito-B-Gone, because the EC50s for these compounds were greater than our highest test concentrations. Therefore, to estimate an ACR for these compounds, we assumed an EC50 equal to the highest test concentration (Table 2). This is a conservative approach, because the actual EC50 likely is much greater. Thus, the actual ACR is greater than what we have estimated. The approximated ACRs for permethrin and Mosquito-B-Gone in the present study were greater than 20; however, because the toxicity of these compounds to *L. siliquoidea* is relatively low compared to that of aquatic species commonly used for toxicity testing, water-quality criteria based on the sensitivity of standard test species likely would be protective of *L. siliquoidea* and, possibly, other unionids.

Growth was variable both within and among treatments and generally was not a sensitive indicator of chronic, sublethal effects of pesticide exposure (Fig. 1). Significant differences in growth between mussels exposed to permethrin and controls, however, was found, but the same trend was not evident for the permethrin formulation, Mosquito-B-Gone. Mean growth of controls in the six chronic tests ranged from 1.9 to 3.7 μm/d and was similar to growth in previous tests with juvenile *L. siliquoidea* [10,18] in which growth of controls ranged from 2.7 to 5.0 μm/d. Growth rates of 5.9 μm/d [29] and 6.6 μm/d [30] have been reported for juvenile *Lampsilis cardium* in the control treatments of their respective studies. Other studies have demonstrated that growth can be a sensitive indicator of sublethal toxicity to ammonia [29,31] and mercury [32], but sediment was present in test chambers during these studies and might have provided suitable substrate for growth of microorganisms to supplement the mussels' diet or simply allowed the mussels to feed more efficiently (e.g., via pedal feeding). Additionally, Wang et al. [33] reported substantially greater growth rates for juvenile (age, two months) *L. siliquoidea* during 21- and 28-d tests in a flow-through system. The current guideline for chronic toxicity tests with juvenile freshwater mussels [9] recommends water-only, flow-through exposures. The utility of growth rate as an end point for chronic studies with juvenile unionids in static-renewal tests will require additional evaluation. Additional research is needed to optimize the dietary constituents and feeding rate for juvenile mussels in chronic static-renewal tests.

CONCLUSIONS

Little difference was found between formulations of atrazine, chlorpyrifos, and permethrin and the technical grades of those pesticides in terms of acute toxicity to early life stages of native freshwater mussels. Aquatic invertebrate species commonly used for toxicity testing likely are suitable surrogates for *L. siliquoidea* glochidia and juveniles and the pesticides tested here; however, previous studies have shown that unionids may be more sensitive to some pesticides (e.g., 2,4-dichlorophenoxyacetic acid and glyphosate). Additional research is needed to determine if this also applies to other pesticides and if suitable surrogate species of unionid mussels exist for specific chemical classes. Although *L. siliquoidea* generally are not acutely sensitive to the pesticides in the present study, these tests involved water-only exposures and acute effects; additional research is needed to determine the role of sediment exposure and sublethal effects. Chronic toxicity tests suggest that juvenile mussels may be at risk from prolonged exposure to environmentally relevant concentrations of chlor-

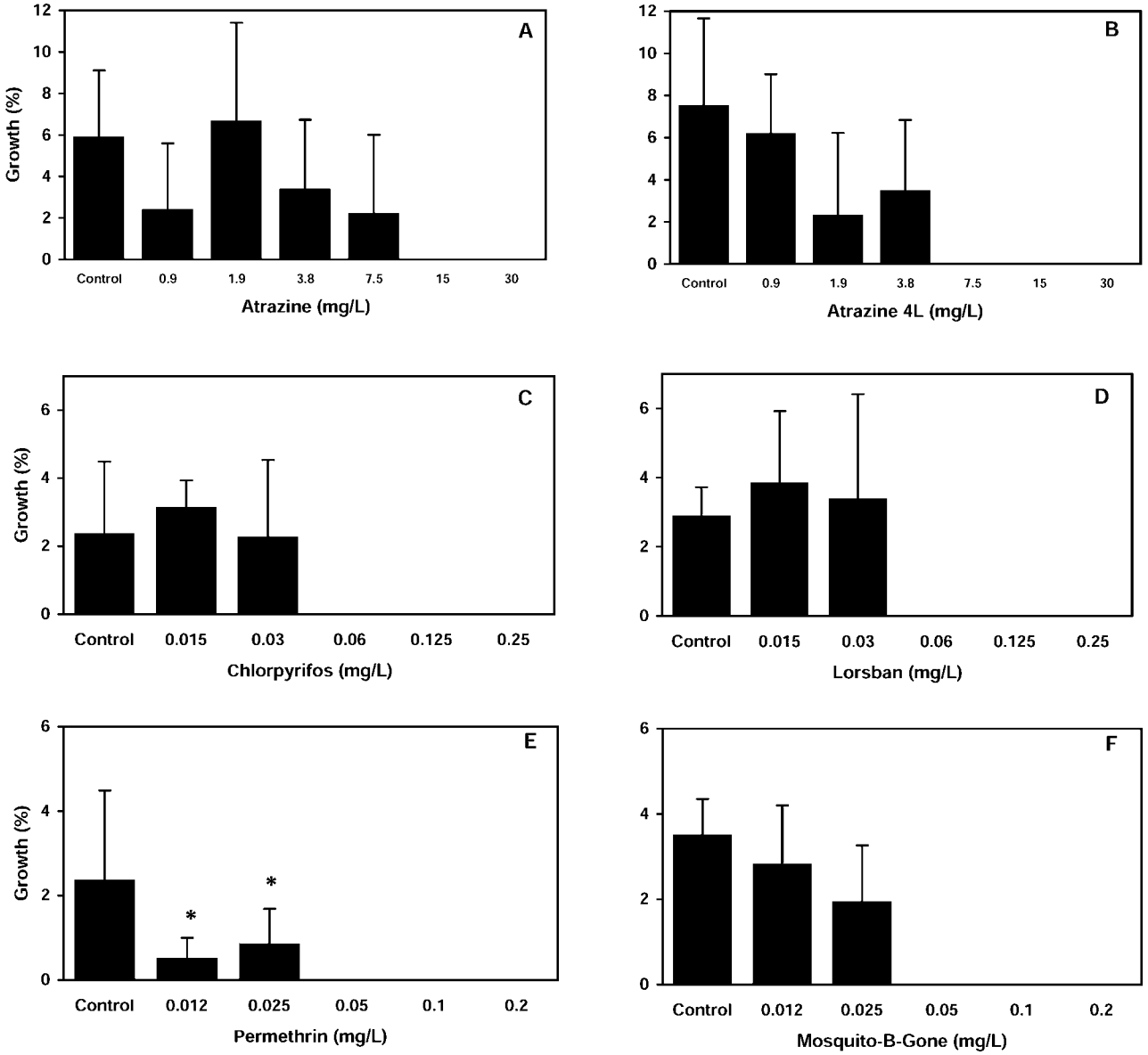

Fig. 1. Percentage growth (change in shell length) of juvenile *Lampsilis siliquoidea* after a 21-d exposure to technical-grade pesticides (**A**, **C**, and **E**) and pesticide formulations (**B**, **D**, and **F**). Data are presented as the mean ± standard deviation ($n = 3$). Missing data indicate less than 30% survival within a treatment. An asterisk (*) indicates a treatment mean that is significantly different from the controls (Dunnett's test, $\alpha = 0.05$). Pesticide formulations included Aatrex 4L Herbicide® (Syngenta, Greensboro, NC, USA), Lorsban® 4-E Insecticide (Dow AgroSciences, Indianapolis, IN, USA), and Mosquito-B-Gone® Insecticide (Ortho, Marysville, OH, USA).

pyrifos and permethrin (and their formulations). Additional research on this topic also is warranted.

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