Journal of Environmental Science and Health Part B

ISSN: 0360-1234 (Print) 1532-4109 (Online) Journal homepage: https://www.tandfonline.com/loi/lesb20

Toxicity of the Mosquito Control Pesticide Scourge® to Adult and Larval Grass Shrimp (*Palaemonetes pugio*)

PETER KEY , MARIE DELORENZO , KRISTEN GROSS , KATY CHUNG & ALLAN CLUM

To cite this article: PETER KEY , MARIE DELORENZO , KRISTEN GROSS , KATY CHUNG & ALLAN CLUM (2005) Toxicity of the Mosquito Control Pesticide Scourge® to Adult and Larval Grass Shrimp (*Palaemonetes pugio*), Journal of Environmental Science and Health Part B, 40:4, 585-594, DOI: 10.1081/PFC-200061552

To link to this article: https://doi.org/10.1081/PFC-200061552

Published online: 06 Feb 2007.

Journal of Environmental Science and Health Part B, 40:585–594, 2005

ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1081/PFC-200061552

Toxicity of the Mosquito Control Pesticide Scourge® to Adult and Larval Grass Shrimp (*Palaemonetes pugio*)

Peter Key,[1] Marie DeLorenzo,[1] Kristen Gross,[2] Katy Chung,[1] and Allan Clum[1]

[1]National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, Charleston, South Carolina, USA
[2]Florida Agricultural and Mechanical University, Tallahassee, Florida, USA

This study investigated the toxicity of various concentrations of technical resmethrin and Scourge® on adult and larval *Palaemonetes pugio*, a common grass shrimp species. Two types of tests were conducted for each of the resmethrin formulations using adult and larval grass shrimp life stages, a 96-h static renewal aqueous test without sediment, and a 24-h static nonrenewal aqueous test with sediment. For resmethrin, the 96-h aqueous LC_{50} value for adult shrimp was 0.53 μg/L (95% confidence interval (CI): 0.46–0.60 μg/L), and for larval shrimp was 0.35 μg/L (95% CI: 0.28–0.42 μg/L). In the presence of sediment, technical resmethrin produced a 24-h LC_{50} value for adult shrimp of 5.44 μg/L (95% CI: 4.52–6.55 μg/L), and for larval shrimp of 2.15 μg/L (95% CI: 1.35–3.43 μg/L). For Scourge®, the 96-h aqueous LC_{50} for adult shrimp was 2.08 μg/L (95% CI: 1.70–2.54 μg/L), and for larval shrimp was 0.36 μg/L (95% CI: 0.24–0.55 μg/L).

The 24-h sediment test yielded an LC_{50} value of 16.12 μg/L (95% CI: 14.79–17.57 μg/L) for adult shrimp, and 14.16 μg/L (95% CI: 12.21–16.43 μg/L) for larvae. Adjusted LC_{50} values to reflect the 18% resmethrin concentration in Scourge® are 0.37 μg/L (adult), 0.07 μg/L (larvae) for the 96-h aqueous test, and 2.90 μg/L (adult), 2.6 μg/L (larvae) for the 24-h sediment test. Larval grass shrimp were more sensitive to technical resmethrin and Scourge® than the adult life stage. The results also demonstrate that synergized resmethrin is more toxic to *P. pugio* than the nonsynergized form, and that the presence of sediment decreases the toxicity of both resmethrin and Scourge®.

Key Words: Mosquito control insecticide; Grass shrimp; Estuarine; Synergist.

Received December 23, 2004.
*Address correspondence to Peter Key, National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC 29412, USA; E-mail: Pete.Key@noaa.gov.

INTRODUCTION

Resmethrin [5-(phenyl methyl)-3-furanylmethyl-2,2-dimethyl-3-(2-methyl-1-propenyl) cyclopropane carboxylate] is a synthetic pyrethroid used as a selective contact insecticide for a variety of insects. It is commonly used in mosquito control programs to kill adult mosquitoes.[1] This class of chemicals disrupts the permeability of nerve membranes to sodium ions resulting in the repeated release of neurotransmitters.[2] Resmethrin is available in a technical grade formulation (SBP-1382) and as an active ingredient in the commonly used pesticide Scourge®. In Scourge®, resmethrin toxicity is enhanced by the synergist piperonyl butoxide (PBO), a monooxygenase inhibitor that effectively prevents the microsomal monooxygenase system from detoxifying pyrethroids.[3] Resmethrin has a molecular weight of 338.4 and is moderately lipophilic ($K_{ow} \sim 2.66 \times 10^5$) with low water solubility and low volatility from water.[4]

Resmethrin is one of the three most important mosquito adulticides in the United States along with malathion and naled. With organophosphate insecticides such as malathion and naled possibly being phased out in the United States, the use of resmethrin has the potential to increase.[5] For mosquito control, Scourge is typically diluted with light mineral or soybean oil and applied by ultra low volume (ULV) ground or aerial equipment. Resmethrin concentrations in water would be expected to decrease quickly due to sorption to sediment, suspended particles, and plants, and it undergoes rapid degradation in the presence of sunlight. Based on these chemical characteristics, the potential for chronic toxicity is low and concern is primarily directed toward the impact of short pulses of resmethrin.[6]

While the use of mosquito control insecticides has increased recently,[7] toxicity data concerning estuarine invertebrates are lacking. Especially with resmethrin, the estuarine crustacean data available show much higher toxicity compared to estuarine fishes.[7] Brackish water grass shrimp, *Palaemonetes pugio*, are among the most widely distributed, abundant, and conspicuous of the shallow water benthic macroinvertebrates in the estuaries of the Atlantic and Gulf coasts.[8] Grass shrimp play a vital role in estuarine ecosystems, functioning as detritivores, primary consumers, and secondary consumers. They break down organic matter and serve as food for larger commercially and recreationally important estuarine species. The purpose of this study was to compare the toxicity of technical grade resmethrin and Scourge® on adult and larval estuarine grass shrimp (*Palaemonetes pugio*) in two exposure scenarios.

MATERIALS AND METHODS

Collection and Maintenance

Grass shrimp (*Palaemonetes pugio*) and sediment were collected from Leadenwah Creek (N 32°36′12″; W 80°07′00″), a pristine tidal tributary of the North

Edisto River, SC.[9] Shrimp were acclimated in 76-L tanks at 25°C, 20‰ natural seawater, and 16-h light:8-h dark cycle and fed a mixture of Tetramin® Fish Flakes and newly hatched *Artemia*. Gravid females were placed in brooding traps to allow larvae (zoea) to hatch and escape without interference. Larvae from at least 10 females were pooled for all tests.

Chemical Analysis

Technical resmethrin and Scourge® were both obtained from Bayer Environmental Science (Montvale, NJ). Technical resmethrin was composed of 91.1% resmethrin. The Scourge® formulation was SBP-1382/PB 18% + 54% Formula II, which is composed of 17.98% resmethrin and 54.42% piperonyl butoxide (PBO). Pesticide stock solutions were made in 100% acetone (pesticide grade). Acetone was also used as a carrier in all bioassays (0.1% final concentration in all treatments and controls). Analytical stocks were obtained from Ultra Scientific for resmethrin (PST-8706) and PBO (PST-8205) both at 100 μg/mL in MeOH. The analytical standards were used to prepare instrument calibration curves and verify the concentration of the technical stocks. Deuterated terbutylazine (terbutylazine-d5) was obtained from CDN Isotopes (D-5313) and used as the internal standard.

Water samples were extracted using a liquid-liquid extraction technique with dichloromethane as the extraction solvent. Quantification of target analytes was based on internal standard calculations using terbutylazine-d5. The internal standard was added to each sample prior to extraction and d-HCH was added as a recovery compound before instrument analysis to enable the calculation of internal standard recoveries. Analysis was performed on a capillary gas chromatograph/mass spectrometer (Agilent 6890n/5973) using electron impact ionization operating in selective ion monitoring (SIM) mode. The quantitation ions monitored for terbutylazine-d5, d-HCH, *trans*-resmethrin, and PBO were 178, 181, 123, and 176, respectively.

Instrument calibration curves were constructed for *trans*-resmethrin (t-resmethrin) and PBO. Reported resmethrin values are based on the t-resmethrin calibration curve after integrating the resolved *trans*- and *cis*-isomer peaks. For the Scourge® stock solutions this method indicated an 80:20 *trans-/cis*-ratio for resmethrin and a PBO/resmethrin ratio of 3:1. Instrument detection limits were 5 ng/mL *trans*-resmethrin and 15 ng/mL PBO. Based on 100 mL sample extracts the minimum method detection limits were calculated to be 0.05 ng/mL *trans*-resmethrin and 0.1 ng/mL PBO, assuming 100% extraction efficiency.

Adult Shrimp Toxicity Tests

Two types of tests with adult grass shrimp were conducted: a 24-h static test with sediment and a 96-h static renewal test with no sediment. All experiments were conducted in 4-L glass jars covered with Teflon-lined plastic caps. For the 24-h sediment tests, 340 g of thoroughly mixed, unsieved sediment was added to each jar followed by 2 L of seawater. The sediment was allowed to settle 24 h before dosing. Ten adult shrimp of similar size were added to each treatment jar following dosing. Jars were placed in an environmental chamber on a 16-h light, 8-h dark cycle at 25°C for the duration of the test and aerated. The test was composed of six treatment concentrations, including a control, with three replicates per treatment for a total of 18 jars. The treatment concentrations for Scourge® test were 6.25, 12.5, 25, 50, and 100 μg/L. The treatment concentrations for technical resmethrin test were 1.56, 3.125, 6.25, 12.5, and 25.0 μg/L.

For the 96-h aqueous tests, setup was as stated above except no sediment was added. The treatment concentrations for the Scourge® test were 0.2, 0.6, 1.8, 5.4, and 16.2 μg/L. The treatment concentrations for technical resmethrin test were 0.125, 0.25, 0.50, 1.0, and 2.0 μg/L.

Larval Shrimp Toxicity Tests

Two types of tests with larval grass shrimp were conducted; a 24-h static test with sediment and a 96-h static renewal test with no sediment. All tests were conducted as described above except 600 mL beakers were used. The test was composed of six treatment concentrations, including a control, with three replicates per treatment for a total of 18 beakers. For the sediment tests, 68 g of sediment was used with 400 mL of seawater. The treatment concentrations for the Scourge® sediment test were 5.1, 10.2, 20.25, 40.5, and 81.0 μg/L. The treatment concentrations for the resmethrin test were 0.625, 1.25, 2.50, 5.0, and 10.0 μg/L.

For the 96-h aqueous tests, 400 ml of seawater was used. The treatment concentrations for Scourge® were 0.20, 0.60, 1.8, 5.4, and 16.3 μg/L. The treatment concentrations for resmethrin were 0.125, 0.25, 0.5, 1.0, and 2.0 μg/L.

For all bioassays, concentrations given are nominal. Water quality parameters including temperature, salinity, dissolved oxygen, and pH were recorded daily from control jars and any dead shrimp were removed prior to renewal. In the 96-h tests, the treatments were renewed and mortality was recorded at 24-h intervals. No gravid females were used in these tests, and adult shrimp were not fed during testing. Larval shrimp were fed newly hatched *Artemia* after each media change.

Statistical Analysis

The median lethal concentration, LC_{50}, was calculated with 95% confidence intervals using the trimmed Spearman–Karber method.[10] The lowest concentration that had statistically significant mortality, the lowest observable effect concentration (LOEC), and the highest concentration that had no statistically significant mortality, the no observable effect concentration (NOEC), were calculated. The threshold concentration (TC), an estimated effects level calculated from the geometric mean of the LOEC and the NOEC, was also determined.[11]

RESULTS

Technical Grade Resmethrin Toxicity Tests

The 96-h aqueous test with adult shrimp produced an LC_{50} of 0.53 μg/L (95% confidence interval (CI): 0.46–0.60 μg/L; Table 1). The highest treatment

Table 1: Toxicity values in μg/L for two grass shrimp life stages exposed to two formulations of resmethrin with 95% confidence intervals (CI) where appropriate.

Formulation	Test and lifestage	LC_{50} (95% CI)	LOEC	NOEC	TC
Scourge®	24-h adult (aqueous)	4.35 (3.49–5.43)	5.4	1.8	3.12
	96-h adult (aqueous)	2.08 (1.70–2.54)	1.8	0.6	1.04
	24-h larvae (aqueous)	1.99 (1.38–2.86)	5.4	1.8	3.12
	96-h larvae (aqueous)	0.36 (0.24–0.55)	0.2	<0.2	<0.2
	24-h adult (sediment)	16.12 (14.79–17.57)	25.0	12.5	17.68
	24-h larvae (sediment)	14.16 (12.21–16.43)	20.5	10.125	14.32
Technical Resmethrin	24-h adult (aqueous)	1.14 (0.93–1.40)	1.0	0.5	0.71
	96-h adult (aqueous)	0.53 (0.46–0.60)	0.5	0.25	0.35
	24-h larvae (aqueous)	>2.00*	—	—	—
	96-h larvae (aqueous)	0.35 (0.28–0.42)	0.5	0.25	0.35
	24-h adult (sediment)	5.44 (4.52–6.55)	6.25	3.125	4.42
	24-h larvae (sediment)	2.15 (1.35–3.43)	1.25	0.625	0.88

*Highest concentration tested (37% mortality).
LC_{50} = median lethal concentration.
LOEC = lowest observable effect concentration.
NOEC = no observable effect concentration.
TC = threshold concentration.

concentration, 2.0 μg/L, exhibited 100% mortality within 48 h. The 24-h sediment test with adult shrimp produced an LC_{50} of 5.44 μg/L (95% CI: 4.52–6.55 μg/L; Table 1). For larval grass shrimp, the 96-h aqueous LC_{50} was lower at 0.35 μg/L (95% CI:0.28–0.42 μg/L; Table 1). Larvae were also more sensitive in the 24-h sediment test with an LC_{50} of 2.15 μg/L (95% CI: 1.35–3.43 μg/L; Table 1).

Scourge® Toxicity Tests

The 96-h adult aqueous test produced an LC_{50} of 2.08 μg/L (95% CI: 1.70–2.54 μg/L; Table 1). In the first 24 h, the 16.2 μg/L treatment had 93% mortality. The 24-h adult sediment test produced an LC_{50} of 16.12 μg/L (95% CI: 14.79–17.57 μg/L; Table 1). The higher treatment concentrations, 50 μg/L and 100 μg/L, caused mortality within minutes of exposure.

With larvae, the 96-h LC_{50} was significantly lower at 0.36 μg/L (95% CI: 0.24–0.55 μg/L; Table 1). After 24 hours, mortality was 100% in the highest concentration (16.2 μg/L) and over 85% in the next highest concentration (5.4 μg/L). The 24-h sediment assay LC_{50} with larvae (14.16 μg/L; 95% CI: 12.21–16.43 μg/L) was not significantly different from the adult sediment assay LC_{50} (Table 1).

Additionally, toxicity values for Scourge® were calculated to reflect the 18% resmethrin formulation (Table 2). For the adult 96-h aqueous test, the LC_{50} calculated for 18% resmethrin was 0.37 μg/L (95% CI: 0.31–0.46 μg/L). For the adult 24-h sediment test, the LC_{50} calculated for 18% resmethrin was 2.90 μg/L (95% CI: 2.66–3.16 μg/L). The larval 96-h aqueous LC_{50} calculated for 18%

Table 2: Toxicity values in μg/L for two grass shrimp life stages exposed to Scourge® as calculated to reflect the 18% resmethrin formulation with 95% confidence intervals (CI) where appropriate.

Formulation	Test and lifestage	LC_{50} (95% CI)	LOEC	NOEC	TC
Adjusted Scourge® (18% resmethrin)	96-h adult (aqueous)	0.37 (0.31–0.46)	0.324	0.11	0.19
	96-h larvae (aqueous)	0.07 (0.04–0.10)	0.036	<0.036	<0.036
	24-h adult (sediment)	2.90 (2.66–3.16)	4.5	2.25	3.18
	24-h larvae (sediment)	2.55 (2.20–2.96)	3.64	1.82	2.57

LC_{50} = median lethal concentration.
LOEC = lowest observable effect concentration.
NOEC = no observable effect concentration.
TC = threshold concentration.

Table 3: Percent recovery (standard deviation (SD), $n = 3$) of nominal Scourge® (resmethrin and piperonyl butoxide (PBO)) concentrations for each adult grass shrimp experiment. Samples were collected immediately post-dose and 24 hours post-dose.

Scourge® dose analyzed	Time sampled	% nominal resmethrin (SD)	% nominal PBO (SD)
Scourge stock solution (100 mg/L in 100% acetone)	44 days after stock was made	98.6 (1.1)	106.7 (5.4)
96 hour adult nominal dose of 2 μg/L (aqueous)	0 h post-dose 24 h post-dose*	59.66 (5.89) 7.37 (0.60)	79.0 (6.0) 74.4 (7.28)
24 hour adult nominal dose of 16 μg/L (sediment)	0 h post-dose 24 h post-dose*	52.14 (7.89) 1.82 (0.06)	109.7 (4.93) 38.72 (7.89)

*24 h post-dose values are based on percent recovery of the 0 h measured concentrations.

resmethrin was 0.07 μg/L (95% CI: 0.04–0.10 μg/L). The larval 24-h sediment LC_{50} was 2.55 μg/L (95% CI: 2.20–2.96 μg/L).

Chemical Analysis

Gas chromatography analysis was performed to confirm the nominal concentration of the Scourge® stock solution. The stock was quantified to be 99% of nominal for resmethrin and 107% of nominal for piperonyl butoxide (PBO) (Table 3). Analytical chemistry results from the adult aqueous and sediment exposures indicate rapid adsorption and degradation of the Scourge® compound (Table 3). Recovery of the active pyrethroid ingredient resmethrin was approximately 60% of nominal in samples collected immediately post-dose, whereas approximately 80% of the PBO synergist was detected immediately post-dose. Only approximately 7% of the 0-h post-dose measured resmethrin concentration was recovered 24 h post-dose from the aqueous exposure. In the presence of sediment, approximately 1% of the initial measured concentration was detected 24 h post-dose. There was minimal loss of PBO from the aqueous exposure after 24 h, while there was an approximately 60% decrease in PBO after 24 h in the presence of sediment.

DISCUSSION

Larval grass shrimp were more sensitive than adults to Scourge® and resmethrin in the aqueous only exposures and in the resmethrin sediment exposure. In the Scourge® sediment exposure, larval and adult sensitivity were similar. However, the LOEC and NOEC values for all exposure scenarios show that larvae are more sensitive to the two resmethrin formulations than adults (Table 1). Increased sensitivity to pesticides in grass shrimp early life stages has been seen with the fungicide chlorothalonil,[12] and the insecticides azinphosmethyl,[13] chlorpyrifos,[14] and malathion.[15]

In a hazard assessment of resmethrin by Rand,[7] pink shrimp, *Penaeus* (*Farfantepenaeus*) *duorarum*, were the most sensitive of the marine organisms tested to technical resmethrin with a 96-h LC_{50} of 1.3 μg/L. Compared to this present study, grass shrimp adults and larvae are more sensitive to technical resmethrin than pink shrimp.

When the Scourge® formulation (18% resmethrin + 54% PBO) is adjusted for resmethrin, this synergized resmethrin is seen to be more toxic to adult and larvae grass shrimp than the nonsynergized formulation except for the larval sediment exposure (Table 2). These results support those reported by Singh and Agarwal[16] with synergized pyrethroids. A study exposing snails (*Lymnaea acuminata*) to permethrin and cypermethrin for periods ranging from 24 h to 96 h showed that the addition of the synergist PBO lowered the LC_{50} of both insecticides.[16] In nonlethal studies with PBO, the swimming stamina of trout (*Salvelinus fontinalis*) significantly decreased when exposed to synergized resmethrin as compared to nonsynergized resmethrin.[6] For pink shrimp, synergized resmethrin was also more toxic with a 96-h LC_{50} of 0.23 μg/L as compared to the technical resmethrin 96-h LC_{50} of 1.3 μg/L.[7]

Differences in toxicity of both the technical resmethrin and Scourge® were observed between the aqueous and sediment tests. The presence of sediment raised the LC_{50} concentration for both resmethrin and Scourge® (Table 1). Resmethrin has a very short half-life in direct sunlight (<1 h); but when it binds to soil, it is more persistent with a half-life of 30 days.[4] However, due to resmethrin's sorption to sediment and suspended particles, its lower toxicity to grass shrimp when sediment is present is not unexpected. Rapid sorption to sediment was observed in this study, with only 1–2% of the initial dose detected 24 h post-dose. The sediment in this study was fine-grained silt-clay, which would have greater contaminant binding capacity than more sandy sediments. When assessing the ecotoxicological risk of resmethrin, therefore, factors such as the turbidity and sediment composition of the exposed ecosystem should be considered because they may significantly affect the bioavailability of these compounds.

An estimated exposure concentration (EEC) for resmethrin was calculated based on a highest recommended application rate of 0.007 lbs AI/acre. At this application rate, the EEC in one foot of water/acre would be 2.57 μg/L. This represents an amount of resmethrin that could possibly contaminate a water system in the event of direct application, and it would result in 100% grass shrimp mortality. These values were then compared against the LC_{50}, LOEC, NOEC, and TC obtained from the resmethrin and Scourge® bioassays (Table 1). Rand[7] calculated an EEC for resmethrin with 0.1% runoff and 5% drift of

0.14 μg/L (in one foot of water), which would cause mortality in grass shrimp larvae. Rand[7] also calculated an EEC with 1.5% runoff and 10% drift of 0.45 μg/L (in one foot of water), which would cause mortality in both larval and adult grass shrimp. Rand[7] notes, however, that mosquitocide applications in the form of ULV sprays may only amount to a fraction of the EEC. Also, as seen in this present study, the presence of sediment can raise the toxic thresholds of resmethrin to grass shrimp.

This research demonstrates that both technical resmethrin and its synergized form, Scourge®, are highly toxic to grass shrimp and that larval grass shrimp would be the most appropriate life stage to use for resmethrin risk assessments.

ACKNOWLEDGMENTS

Financial support for Kristen Gross was provided through the U.S. National Oceanic and Atmospheric Administration's Educational Partnership Program with Minority Serving Institutions. The National Ocean Service (NOS) does not approve, recommend, or endorse any proprietary product or material mentioned in this publication. No reference shall be made to NOS, or to this publication furnished by NOS, in any advertising or sales promotion which would indicate or imply that NOS approves, recommends, or endorses any proprietary product or proprietary material mentioned herein or which has as its purpose any intent to cause directly or indirectly the advertised product to be used or purchased because of NOS publication.

REFERENCES

1. U.S. Environmental Protection Agency (USEPA). *Pesticides: Topical and Chemical Fact Sheets. Synthetic Pyrethroids for Mosquito Control*. EPA 735-F-02-009, USEPA: Washington, DC, 2002.

2. Miller, T.A.; Salgado, V.L. The mode of action of pyrethroids on insects. In: *The Pyrethroid Insecticides*; Leahey, J.P., Ed. Taylor and Francis: London, **1985**; 43–89.

3. Ecobichon, D.J. Toxic effects of pesticides. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*; Klaassen, C.D., Ed. McGraw-Hill: New York; **2001**, 763–810.

4. EXTOXNET. *Resmethrin*. Extension Toxicology Network. Pesticide Information Profiles. http://extoxnet.orst.edu/pips/resmethr.htm (accessed September 21, 2004), 1996.

5. Clemson University Pesticide Information Program (CUPIP). *Potential Effects of FQPA on South Carolina Farmers*. Clemson University Cooperative Extension Service, Clemson University, Clemson, SC, 2003.

6. Paul, E.A.; Simonin, H.A. Effects of naled, synergized, and nonsynergized resmethrin on the swimming performance of young trout. Bull. Environ. Contam. Toxicol. **1996**, *57*, 495–502.

7. Rand, G.M. Hazard assessment of resmethrin: I. Effects and fate in aquatic systems. Ecotoxicol. **2002**, *11*, 101–111.

8. Anderson, G.A. *Species Profile: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (Gulf of Mexico)—Grass Shrimp*. U.S. Fish and Wildlife Service Biological Report 82(11.35), U.S. Army Corps of Engineers, TR EL-82-4, 1985.

9. Scott, G.I.; Fulton, M.; Moore, D.; Wirth, E.; Chandler, G.; Key, P.; Daugomah, J., Strozier, E.; Devane, J.; Clark, J.; Lewis, M.; Finley, D.; Ellenberg, W.; Karnaky, K. Assessment of risk reduction strategies for the management of agricultural nonpoint source pesticide runoff in estuarine ecosystems. Toxicol. Ind. Health **1999**, *15*, 200–213.

10. Hamilton, M.; Russo, R.; Thurston, R. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. **1977**, *11*, 714–719.

11. Key, P.B.; Chung, K.W.; Opatkiewicz, A.D.; Wirth, A.F.; Fulton, M.F. Toxicity of the insecticides fipronil and endosulfan to selected life stages of the grass shrimp (*Palaemonetes pugio*). Bull. Environ. Contam. Toxicol. **2003**, *70*, 533–540.

12. Key, P.B.; Meyer, S.L.; Chung, K.W. Lethal and sublethal toxicity of the fungicide chlorothalonil on three life stages of the grass shrimp, *Palaemonetes pugio*. J. Environ. Sci. Health **2003**, *B38*, 539–549.

13. Key, P.B.; Fulton, M.H.; Layman, S.L.; Scott, G.I. Azinphosmethyl exposure to grass shrimp (*Palaemonetes pugio*) life stages with emphasis on larval acetylcholinesterase activity. Bull. Environ. Contam. Toxicol. **1998**, *60*, 645–650.

14. Key, P.; Fulton, M. Lethal and sublethal effects of chlorpyrifos exposure on adult and larval stages of the grass shrimp, *Palaemonetes pugio.* J. Environ. Sci. Health **1993**, *B28* (5), 621–640.

15. Key, P.; Fulton, M.; Scott, G.; Layman, S.; Wirth, E. Lethal and sublethal effects of malathion on three life stages of the grass shrimp, *Palaemonetes pugio*. Aquat. Toxicol. **1998**, *40*, 311–322.

16. Singh, D.K.; Agarwal, R.A. Piperonyl butoxide synergism with two synthetic pyrethroids. Chemosphere **1986**, *15*, 493–498.