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Toxicity of the Insecticides Fipronil and Endosulfan to Selected Life Stages of the Grass Shrimp (*Palaemonetes pugio*)

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Fipronil is an insecticide first registered in the US in 1996. A variety of fipronil formulations are presently used for pests of corn, rice and turf and for control of cockroaches, ants, termites and fleas. It is a member of a new class of insecticides called phenylpyrazoles and acts by blocking the chloride channels at the gamma-aminobutyric acid (GABA) receptor in the central nervous system, leading to neural excitation and eventually death of the organism. Use of this insecticide has been gradually increasing as it becomes commercialized around the world (Gant et al. 1998). Endosulfan, a cyclodiene insecticide, is used in commercial agriculture for both food and non-food crops. Endosulfan is neurotoxic and, similar to fipronil, inhibits neuronal function by blocking the GABA-gated chloride channels of the nervous system (Murray et al. 1993).

Studies by other investigators have shown endosulfan to be quite toxic to adult grass shrimp (*Palaemonetes pugio*; Scott et al. 1999), but there is no data for other grass shrimp life stages. There are also no similar data currently available for evaluating fipronil toxicity to nontarget estuarine crustaceans. With recent restrictions on organophosphate insecticides by the US Environmental Protection Agency, fipronil use may increase. The goals of this research were to 1) expose three life stages of the grass shrimp to fipronil to determine 96-hr LC50s; 2) expose larval and embryonic grass shrimp to endosulfan to determine 96-hr LC50s; and 3) compare endosulfan and fipronil toxicity to each other and to three common organophosphate insecticides which have been tested using the same protocol.

MATERIALS AND METHODS

Grass shrimp were collected from Leadenwah Creek (N 32º36'12" ; W 80º07'00"), a pristine tidal tributary of the North Edisto River Estuary, SC (Scott et al. 1999). Shrimp were acclimated in 76-L tanks at 25ºC, 20‰ salinity and 16-hr light:8-hr 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

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interference. Larvae from at least 10 females were pooled for all tests. Embryos (Stage VI - defined oval eye, body movement, rapid heartbeat) were excised from the gravid females.

All toxicity tests were 96-hr static renewal tests. Adult and larval tests were run in a Revco Environmental Chamber at 25°C, 20‰ salinity, and a 16-hr light:8-hr dark cycle. Adult shrimp were exposed in 4-L wide mouth glass jars containing 2-L of media and 10 shrimp/jar with two replicates/treatment, modified from Key et al. (1998a). A media change was made every 24 hr. Before each change, water quality measurements (dissolved oxygen, pH, temperature, salinity) were taken in the control test jars. Larvae used for all tests were one to two days old and exposed in 600-mL glass beakers containing 400 mL of media with 10 larvae/beaker and three replicates/treatment. Adults were not fed during the test. Larvae were fed newly hatched *Artemia* after daily media change. Adult and larval nominal fipronil concentrations were 0.13, 0.25, 0.50, 1.00, 2.00 µg/L and control. Larval nominal endosulfan concentrations were 0.32, 0.63, 1.25, 2.50, and 5.0 µg/L and control.

Embryo exposure chambers were 24-well plates with 2 mL media, one Stage VI embryo/well and one plate per control and concentration (Rayburn et al. 1996). Plates for all tests were placed on an orbital shaker at 80 rpm in a Revco Environmental Chamber at 27°C, 20‰ salinity, and a 24-hr dark cycle. After 96 hr, surviving embryos were allowed to hatch. In determining LC50s, the percent embryo survival reflected successful hatching. Nominal fipronil concentrations were 32.0, 64.0, 128.0, 256.0, 512.0 µg/L and control. Nominal endosulfan concentrations were 12.5, 25.0, 50.0, 100.0, 200.0 µg/L and control.

Pesticide grade acetone was used as a carrier (0.1%) in all tests. Acetone was added to the control groups equal to the amount of carrier solvent used for the toxicity tests. Median Lethal Concentrations (LC50) with 95% confidence limits were determined using the Trimmed Spearman-Karber Method (Hamilton et al. 1977). A Kruskal-Wallis nonparametric one-way ANOVA was performed followed by Dunn's Method to determine significant effects of each insecticide on embryo hatching time ($p \leq 0.05$). A Lowest Observed Effect Concentration (LOEC) was the lowest concentration that had statistically significant mortality. A No Observable Effect Concentration (NOEC) was the highest concentration that had no statistically significant mortality. The Threshold Concentration (TC) was an estimated effects level calculated from the geometric mean of the NOEC and LOEC.

RESULTS AND DISCUSSION

For fipronil and endosulfan, adult grass shrimp were more sensitive than larvae. Results of the 96-hr fipronil toxicity test showed adult grass shrimp, with an LC50 of 0.32 µg/L, were significantly more sensitive to fipronil than larvae and embryos (Table 1). Adult mortality from fipronil exposure first occurred at 24 hr in the two highest concentrations and by 72 hr, the highest concentration had complete mortality. Larval mortality from fipronil exposure did not occur until 48 hr in the highest concentration with complete mortality at 96 hr. Larval mortality from endosulfan exposure first occurred in the highest concentration after 24-hr exposure. Comparisons between fipronil and endosulfan showed higher endosulfan 96-hr LC50s for both adult and larval grass shrimp (Table 1). Adult grass shrimp were more sensitive than larvae to both fipronil and endosulfan.

Table 1. Toxicity values (µg/L) for three life stages of the grass shrimp exposed to fipronil and endosulfan for 96 hr.

Insecticide	Adult	Larvae	Embryo
Fipronil			
LC50	0.32	0.68	>512.0
95% confidence limit	(0.24 – 0.41)	(0.57 – 0.80)	
LOEC	0.13	0.50	32.0
NOEC	<0.13*	0.25	<32.0*
Threshold Concentration	<0.13	0.35	<32.0
Endosulfan			
LC50	1.01+	2.56	117.0
95% confidence limit	(0.72 – 1.43)	(1.82 – 3.59)	(0.73 – 18,810.0)
LOEC	0.1+	1.25	12.5
NOEC	0.01+	0.63	<12.5*
Threshold Concentration	0.032+	0.887	< 12.5

*Estimate based on the lowest concentration which produced mortality.
+Scott et al. 1999

Embryonic grass shrimp were relatively insensitive to fipronil in terms of lethal toxicity at the exposures tested (Table 1). For fipronil, there was no more than 29% mortality at any concentration tested. For endosulfan, a

96-hr LC50 was obtained (117.0 μg/L) but with a large 95% confidence limit (0.73 – 18,810 μg/L; Table 1).

Low embryo toxicity could be partially explained by the presence of an embryonic coat (EC). The grass shrimp EC helps to protect the embryo during development from potentially harmful conditions of the ambient water. The greatest permeability of the EC is just before hatching (Glas et al 1997). Mortality that occurred in the fipronil and endosulfan tests was not observed until 96 hr - when embryos were hatching. This was also observed by Lund and Fulton (1999) after exposing embryos to chlorpyrifos. Low embryo toxicity could also be explained by the immaturity of the embryonic nervous system. The mode of action of fipronil, endosulfan and chlorpyrifos is generally disruptive to central nervous system activity. An increase in metabolic rate with embryonic development, and the development of phase I monooxygenases (cytochrome 450) may also play a role in the uptake, metabolism and toxicity of pesticides to grass shrimp embryos.

The LOEC and NOEC values for fipronil and endosulfan reiterate that adult shrimp are less tolerant than larvae and embryos to fipronil and endosulfan (Table 1). The Criteria Maximum Concentration (CMC; estimate of highest concentration of endosulfan in surface water to which an aquatic community can be briefly exposed without unacceptable effects) for endosulfan is 0.034 μg/L for saltwater (USEPA, 1999). The endosulfan TC of 0.032 μg/L for adults in Table 1 indicates that there could be an effect on adult grass shrimp if exposure is at least 96 hr in duration. At this time, no CMC value for fipronil has been established. However, an estimated peak surface water concentration of about 5.0 μg/L has been formulated for fipronil in rice culture (USEPA, 1997). This concentration is well above adult and larval LOEC, NOEC and TC values. An average estimated peak surface water concentration of 0.17 μg/L has been formulated for fipronil in fire ant control in the southeastern US (USEPA, 2001). While this is below the adult and larval fipronil LC50s it is still above the adult LOEC (Table 1).

Embryonic time to hatch after 96 hr Stage VI exposure to fipronil showed each concentration with hatch times higher than the control (Table 2). Statistically, only the lowest, median and highest concentrations were significantly higher. Endosulfan's effect on grass shrimp hatching time resulted in a significant increase for the highest concentration of 200 μg/L compared to the control (Table 2). Research with blue crab (*Callinectes sapidus*) embryos found endosulfan did lengthen hatching times at concentrations higher than 200 μg/L (Lee et al. 1996). In a study with chlorpyrifos, 96-hr exposure to embryos caused a significant increase in hatching times compared to controls at all concentrations except the two lowest (Lund and Fulton 1999). The ecological significance of increased hatch time is not known but it may have the potential for increased stress on the female due to the extended brood time.

In comparison to three organophosphate insecticides (malathion, azinphosmethyl and chlorpyrifos), which have been tested with grass shrimp using the same protocol, fipronil was either as toxic or more toxic to adult grass shrimp. Larval toxicity comparisons varied. Endosulfan was more toxic to adults and larvae than malathion and less toxic than chlorpyrifos (Table 3). Chlorpyrifos toxicity to larvae was significantly higher than any of the five insecticides and three life stages tested. Malathion was the least toxic compound for any life stage.

Table 2. Mean time to hatch (hr) with standard error for grass shrimp embryos exposed to fipronil and endosulfan.

Insecticide	Concentration (µg/L)	Mean Hatch Time (hr)
Fipronil		
	Control	97.0 (1.04)
	32.0	107.2 (3.2)*
	64.0	104.6 (3.2)
	128.0	114.8 (2.7)*
	256.0	105.3 (2.8)
	512.0	111.0 (3.0)*
Endosulfan		
	Control	98.1 (1.4)
	12.5	97.1 (1.1)
	25.0	96.0 (0)
	50.0	99.8 (2.1)
	100.0	103.4 (3.2)
	200.0	115.2 (4.8)*

*Significantly higher than its respective control.

Due to the relatively recent introduction of fipronil, toxicity to other organisms is not well known. Of those found in the literature, crustaceans are the most sensitive to fipronil exposure with a mysid (*Americamysis bahia*) 24-hr EC50 of 0.14 µg/L (US EPA 1996). Two crayfish species (*Procambarus clarkii* and *P. zonangulus*) had higher nominal 96-hr LC50s of 64 and 65 µg/L, respectively (Schlenk et al. 2001). Molluscs and fish were less sensitive to fipronil, but the insecticide was considered at least highly toxic to oysters (*Crassostrea virginica*) and sheepshead minnows (*Cyprinodon variegatus*) with 96-hr LC50s of 770 µg/L and 130 µg/L, respectively (US EPA 1996). Compared to this present research, grass shrimp toxicity to fipronil was higher than all but mysids.

These results indicate that fipronil is highly toxic to grass shrimp and represents one of the more toxic compounds tested in this organism. It is also important to consider that adult grass shrimp would be the most appropriate life stage to use for fipronil and endosulfan risk assessments since that stage is the most sensitive. These results also clearly indicate that fipronil has the potential to be highly toxic to estuarine crustaceans and care should be taken to ensure that its registered uses do not result in contamination of the estuarine environment.

Table 3. 96-hr LC50 values (µg/L) with 95% confidence limits for three grass shrimp life stages exposed to fipronil and endosulfan with comparisons to three other insecticides.

Insecticide	Adult	Larvae	Embryo	Reference
Fipronil	0.32 (0.24 - 0.41)	0.68 (0.57 - 0.80)	>512.0	Present research
Endosulfan	1.01 (0.72 - 1.43)	2.56 (1.82 - 3.59)	117.0 (0.73 – 18,810.0)	Scott et al. 1999 (adult) Present research (larvae, embryo)
Malathion	38.19 (31.91 - 45.69)	9.06 (7.65 - 10.73)	--	Key et al. 1998a
Azinphosmethyl	1.64 (1.27 - 2.12)	0.52 (0.45 - 0.61)	--	Key et al. 1998b
Chlorpyrifos	0.37 (0.30 - 0.44)	0.16 (0.14 - 0.18)	>40.0	Key and Fulton 1993 (adult) Key 1995 (larvae) Lund and Fulton 1999 (embryo)

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