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Acute Toxicity of Temephos, Fenoxycarb, Diflubenzuron, and Methoprene and *Bacillus thuringiensis* var. *israelensis* to the Mummichog (*Fundulus heteroclitus*)

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The southeast United States has the single largest concentration of mosquito control efforts in the United States primarily due to the large concentration of fresh, brackish, and salt water marshes in these areas (NAS 1976). Salt marsh mosquitoes (Aedes sollicitans and Aedes taeniorhynchus) are the major mosquito pests along the entire Atlantic and Gulf Coast of the United States. Salt marsh mosquito control involves the application of chemical insecticides into breeding grounds, near estuarine tidal creeks, in an attempt to kill and control larval mosquitoes. The headwaters of many estuarine tidal creeks serve as nursery grounds for many fish species. The mummichog, Fundulus heteroclitus, is one of the dominant fish species present in these creeks. The application of chemical larvicides for mosquito control into salt marsh breeding grounds may pose a potential toxicity hazard to nontarget aquatic organisms. The larvicides generally recommended for use in South Carolina include: Abate (temephos), Dursban (chloropyrifos), Malathion, Altosid (methoprene), Pyrethrins, and Vectobac (Bacillus thuringiensis var. israelensis, Bti). Altosid and abate are among the most widely used larvicides in South Carolina and may be potentially toxic to nontarget species.

In addition to these chemicals, vectobac, a microbial larvicide, has been recently introduced as a larval mosquito control agent. This pesticide is characterized as not being persistent, bioconcentrated, bioaccumulated, or biomagnified in the estuarine ecosystem; however very little information exist on its acute toxicity to estuarine organisms. Therefore, the purpose of this report was to investigate and compare the acute toxicity of four chemical mosquito larvicides (methoprene; diflubenzuron; temephos; and fenoxycarb-an unregistered carbamate) and Bti to the mummichog (Fundulus heteroclitus). Based upon this comparison, recommendations can be made in terms of the safety of these compounds for mosquito control in estuarine tidal creeks.

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MATERIALS AND METHODS

Fundulus heteroclitus, adult mummichogs, were collected by a wire mesh minnow trap from the North Edisto River estuary, South of Charleston, South Carolina (Coordinates: latitude N32° 36'; longitude W80° 15') and transported back to laboratory in Columbia, South Carolina. In the laboratory, fish were placed in a 300-L circular tank (143 cm diameter 27 cm depth) and acclimated to 20 ppt salinity (seawater from Charleston) 12 hr light:dark cycle and 20°C exposure regime. Circulation in each tank was provided by a submersible pump (Little Giant, Oklahoma City, Oklahoma). The water in each tank was filtered through a large bore (12 cm diameter) column of oyster shell, charcoal, and filter floss. Additional filtration was provided by a dynaflo and tanks were continuously aerated. Fish were fed Tetramin fish flake daily, ad libitum.

All acute 96-hr toxicity tests were static renewal bioassays in which the test organisms were exposed to a fresh solution of each at a range of concentrations every 24 hr. Fish were exposed to each larvicide in 4-L grass aquaria with 2 replicate tanks per concentration tested. Toxicity tests were conducted under conditions those described during acclimation period (12 hr light:dark cycle, 20 ppt salinity, 23.9±1.4°C and 8.03±0.26 pH). A total of 6 fish/replicate and 12 fish/concentration were used in the tests with temephos (TG: technical grade), methoprene (EC: emulsifiable concentrate), diflubenzuron (WP: wettable powder (25%)), fenoxycarb (TG), and Bti (EC), and fenoxycarb/Bti mixture (1:1). A total of 10 fish/replicate (20 fish/ concentration) were used with the fenoxycarb test. Five to 19 concentrations of each insecticide were used in each toxicity test. An additional group of fish were exposed to the carrier (acetone), used for all tests except vectobac, diflubenzuron, and methoprene, without the insecticide and an unexposed group of fish were maintained as a seawater control at the same time. All fish were within the standard length (tip of snout to end of caudal peduncle) such that longest fish was no more than 2 times the size of the shortest fish (average length of 5.1 cm, ranging in size from 4.2 to 5.8 cm; average weight of 2.7 g, ranging from 1.99-3.92 g).

During each toxicity test, fish were not fed. Test solutions were gently aerated by bubbling air through 1-mL glass pipet to maintain 60-100 % dissolved oxygen saturation. Water changes were made every 24 hr to insure that the depletion of dissolved oxygen and toxicant should not occur. To prevent toxicants' volatilization the tops of test vessels were sealed. The experimental fish were collected from acclimation tanks and randomly distributed to the bioassay aquaria at the equal sex ratio (3-5 males/3-5 females) per tank. The 96-hr median lethal concentration (LC50) was determined using the Trimmed Spearman-Karber Method which was overall a better procedure (due to the small sample size/concentration) than the methods based on the probit and logit models (Hamilton et al. 1978). The No Effect

Concentration (NOEC) was also calculated for each larvicide by taking the geometric mean between the highest concentration in which no acute toxicity occurred and the subsequent concentration in which significant acute toxicity occurred. The additive toxicity of the fenoxycarb/Bti mixture was estimated based upon the method described by Marking (1977).

RESULTS AND DISCUSSION

Results of acute toxicity for all chemical or microbial larvicides tested are listed in Table 1. The 96-hr LC50 for temephos, the most toxic compound among the five tested larvicides, was 0.04 mg/L with 95 % confidence limits (CL) ranging from 0.02 to 0.05 mg/L. The lowest concentration of temephos tested (0.018 mg/L) caused some partial mortality (25 % out of 12 fish). Additionally, blisters containing blood on the fish skin were observed during bioassays.

The 96-hr LC50 for fenoxycarb was 2.32 mg/L with CL ranging from 2.15 to 2.50 mg/L indicating that fenoxycarb was the second most toxic in order of toxicity compared with results of other larvicides. The NOEC for fenoxycarb was 1.41 mg/L of fenoxycarb. Additionally, an anesthetic effect (loss of movement) was also remarkably observed on the fish from 2 mg/L to 5 mg/L of fenoxycarb within few minutes after exposure and fish exposed to 1 mg/L swam regularly.

For diflubenzuron, the 96-hr LC50 was 32.99 mg/L with CL ranging from 29.01 mg/L to 37.52 mg/L. Among the five insecticides tested, diflubenzuron was the third most toxic to Fundulus. The NOEC was 29.86 mg/L.

The 96-hr LC50 for methoprene was 124.95 mg/L with CL ranging from 90.97 mg/L to 171.64 mg/L and methoprene was the fourth most toxic larvicide tested. The NOEC was 24.68 mg/L for methoprene.

The 96-hr LC50 for the microbial larvicide vectobac was 980 mg/L (1,176,000 international toxic unit (ITU)/L) with CL ranging from 730 mg/L to 1330 mg/L (876,000 to 1,596,000 ITU/L) and this was the least toxic among five tested larvicides. The NOEC was 22.36 mg/L for vectobac.

In order to compare potential additive toxicity between two of chemical larvicides, (fenoxycarb and vectobac), a mixture (1:1) of two larvicides was additionally tested. The 96-hr LC50 value based upon fenoxycarb was 1.55 mg/L with CL ranging from 1.40 mg/L to 1.72 mg/L. The 96-hr LC50 based upon total toxicant units (fenoxycarb/vectobac) was 3.1 mg/L with CL ranging from 2.80 mg/L to 3.44 mg/L. These results indicated that the mixture of fenoxycarb/vectobac was more toxic than fenoxycarb to Fundulus. According to Marking's technique (1977) for assessment of additive toxicity, the sum of biological activity was 0.67 (<1.0), which indicated that the mixture of fenoxycarb and vectobac was greater than simple additive toxicity (=1.0).

Table 1. Acute Toxicity of Mosquito Larvicides to Fundulus

Insecticides	Mean 96-hr LC50 (mg/L)	95% Confidence Limits (mg/L)	NOEC[2] (mg/L)
Temephos[1] (TG)	0.04	0.02-0.05	0.02
Fenoxycarb[1] (TG)	2.14	2.01-2.27	1.41
Diflubenzuron[1] (WP)	32.99	29.01-37.52	29.86
Methoprene[1] (EC)	124.95	90.01-171.64	24.68
Vectobac (EC)	980.00	730.00-1330.00	22.36
Fenoxycarb[1] / Vectobac	1.55	1.40-1.72	1.41

[1] 96-hr LC50 rates and 95 % CL are based upon Nominal Concentration from a measured stock with the exception of vectobac which was reported as nominal concentration.

[2] NOEC (no effect concentration) was calculated as the geometric mean of the highest concentration in which no mortality was observed and the subsequent concentration causing significant toxicity.

Subsequently, the additive index and the magnification factor were obtained, 0.49 and 1.49, respectively. The toxicity of mixture (fenoxycarb/vectobac = 1:1) increased by a factor of 1.49 (magnification factor = 1.49) over the expected simple additive toxicity (magnification factor = 1).

Results from this study have indicated that temephos, an organophosphate insecticide, was the most toxic among the five mosquito larvicides tested. Temephos was followed in order of decreasing toxicity by fenoxycarb, fenoxycarb/Bti mixture (1:1), diflubenzuron, methoprene, and Bti. From the acute toxicity test with temephos, the 96-hr LC50 on Salmo gairdneri was reported as 0.158 mg/L (Verschueren 1983). Tsai(1978) has also reported the 72-hr LC50 (mg/L) values for temephos in several species of fish: Anguilla japonica-7.5; Tilapia mossambica-3.5; Mugil cephalus-0.6; Mugil carinatus-0.023. The LC50 of technical temephos exceeded 200 mg/L for four species of fish, but 2.5 mg/L of the emulsion produced 100 % mortality in all four species after 24 hr exposure. This indicated that the emulsifiable concentrate containing both temephos and inert ingredients were more toxic than the pure insecticide to fish (Petterson et al. 1966). Fenoxycarb, a new juvenile hormone mimic insect growth regulator (IGR), has demonstrated excellent potential in operational mosquito control programs in the laboratory and field studies. Maag Co. (1985) listed the 96-hr LC50 values(mg/L) for fenoxycarb

on several species of freshwater fish: rainbow trout (Salmo gairdneri)-16; blue gill (Lepomis macrochirus)-2.9; carp-10.2. The 96-hr LC50 from this study (2.32 mg/L) for the estuarine fish Fundulus heteroclitus falls between that on rainbow trout (1.6 mg/L) and blue gill (2.9 mg/L). In addition, unlike other types of carbamates, fenoxycarb does not inhibit cholinesterase as the mechanism of action (Maag Co 1985).

Another type of IGR, Diflubenzuron, is highly effective for controlling organophosphorus-resistant strains of mosquitoes. In California, 0.025 lb diflubenzuron/acre was applied at the concentration of 2.4 g/L by aircraft in which resulted excellent control against organophosphorus-resistant mosquitoes (Schaefer et al. 1975). Julin and Sanders (1978) reported the 96-hr LC50 (mg/L) for diflubenzuron on several fish species. The 96-hr LC50 value of this study (32.99 mg/L) was smaller than those of other studies which ranged from >150 mg/L to 660 mg/L. However, these differences in LC50 values may have resulted from both species differences and the different experimental conditions such as pH and temperature which might affect the toxicity and persistence of diflubenzuron.

McKague and Pridmore (1978) reported a 96-hr LC50 value of 86-106 mg/L for methoprene to juvenile coho salmon (Oncorhynchus kisutch) and juvenile rainbow trout. The 96-hr LC50 for Fundulus exposed to methoprene was 124.95 mg/L which was very similar to the LC50 value for other anadromous fish. Studies of other nontarget orgarnisms (snails, frogs, etc.), have indicated no deleterious effects of methoprene when recommended application rates were used (Miura and Takahashi 1974).

Bacillus thuringiensis var. israelensis are protein inclusion bodies produced during Bti sporulation and responsible for mosquitocidal activity with a 65,000 dalton protein (Lee et al. 1985). The environmental impact of this type of integrated chemical-microbial larvicide has not been assessed until this study. Vectobac appears to be less toxic to nontarget fish Fundulus heteroclitus than the other chemical larvicides. The acute toxicity of the fenoxycarb/vectobac mixture was increased by the factor of 1.38 over the simple additive toxicity. The increased toxicity may have resulted from ingestion of the vectobac, which may have resulted in increased uptake of both vectobac and fenoxycarb. Additional studies of fenoxycarb uptake/depuration kinetics are needed to better understand factors enhancing the toxicity of these compounds.

As this is shown, 96-hr LC50 values for Fundulus exposed to various mosquito control larvicides are highly variable. A comparison of NOEC obtained in this study for each larvicide with the expected water concentration (based upon 1 X surface application at recommended application rate for each larvicide) rather provides a measure of relative safety factor (NOEC/expected pesticide concentration in 12 in. of water). The safety factors are ranged from 3 to 3516 X (3 X; temephos, 78 X; fenoxycarb, 78 X;

fenoxycarb/Bti, 1242 X; Bti, 1788 X; diflubenzuron, and 3516 X; methoprene). Therefore, it is imperative that environmental managers select insecticides which are the most appropriate for use in the habitats where they apply. Additional toxicological consideration must also be given to other aquatic organisms, particularly crustaceans, when selecting the most appropriate larvicides.

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