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Acute Toxicity of Emulsifiable Concentrations of Three Insecticides Commonly Found in Nonpoint Source Runoff into Estuarine Waters to the Mummichog, *Fundulus heteroclitus*

Alan H. Trim

South Carolina Department of Health and Environmental Control, Columbia, SC 29201

Protection of aquatic life from adverse effects of non-point source pollution is a recurring theme of current federal water pollution control legislation. Present concern regarding protection of aquatic life has prompted evaluation of effects of agricultural chemicals on aquatic invertebrates and fishes. More specifically, the introduction of insecticides into the aquatic environment through non-point source agricultrual runoff has received considerable attention from environmental regulatory agencies.

A major threat to environmental integrity of estuarine waters is related to insecticide concentrations discharged from adjacent agricultural lands. Varied land uses and different agricultural practices could be expected to contribute differing amounts and types of insecticides to the aquatic system (Wauchope 1978). In recent years, significant fish kills have been reported due to many insecticides used in agriculture for insect control. Each of these kills occurred following periods of high rainfall (Ware 1980; McDowell et al. 1981). The amount of an agriculturally-applied insecticide that drains into surface waters depends primarily on: 1) the intensity and duration of rainfall; 2) length of time between insecticide application and rainfall occurrence; 3) type and amount of ground cover and tillage practices; 4) percent slope of land; 5) soil type; 6) properties of the insecticide; and 7) method of insecticide application (Weber et al. 1980). In South Carolina a total of 128 coastal fish kills were recorded from 1977 - 1984. Over 56% of these kills were pesticide-related, primarily endosulfan and malathion. Additional concern has been expressed over the increasing use of fenvalerate, a synthetic pyrethroid, in the coastal zone and its implication in several fish kills. The objecitve of this study was to determine

Send reprint request to Alan Trim at the above address.

the acute toxicity as described by the 50 percent lethal concentration (LC 50) value of three commonly used agricultural insecticide formulations (malathion, endosulfan, fenvalerate) and an insecticide mixture (fenvalerate and endosulfan) on a key estuarine species, the adult mummichog, Fundulus heteroclitus.

MATERIALS AND METHODS

Adult Fundulus heteroclitus were collected by minnow trap during the summer and fall of 1984 from Bohicket Creek, a pristine tidal tributary of the North Edisto River estuary south of Charleston, South Carolina (32° 36'N, 80° 15'W) and transported to the laboratory. There, fish were acclimated for 2 weeks to a 12-h light:dark (L:D) cycle, 20 parts per thousand (ppt) salinity and 20° C exposure regime. The fish were fed Tetramin Staple Fish Food daily.

The acclimation tank used was a 300-L pool equipped with a Dynaflow 150 filter and sump pump (Little Giant) which circulated the water to provide a swimming current for fish, and with an air pump (DP model 770) to provide aeration. Periodic dissolved oxygen measurements indicated the water was at or near saturation throughout the experiment.

Static 96-h toxicity tests were conducted using three insecticide formulations: malathion (50% active ingredient (a.i.)), endosulfan (30% a.i.), and fenvalerate (24% a.i.), also an insecticide mixture of fenvalerate and endosulfan (3:1) was tested. The remaining percentages of each formulation used consisted of inert ingredients. Emulsifiable concentrates (EC) were used because they best reflect the formulations applied in the field which are subject to actual runoff providing direct challenge to estuarine organisms (Ware 1980; McDowell et al. 1981). Previous studies have shown that LC50 values obtained from EC formulations, calculated on the basis of percent active ingredient, were significantly lower than LC50's of technical products (Coats 1979). An insecticide mixture was included for analysis to allow an assesment of possible toxicological interaction between insecticides that would coexist in agricultural nonpoint discharges to the estuarine environment.

Each EC was dissolved in reagent grade acetone to produce a concentrated stock, which was analyzed by gas chromatography before each toxicity test. Test concentrations were nominal dilutions from this measured concentrated stock with 20° ppt seawater. Analytical standards were obtained from USEPA Pesticides Industrial Chemicals Repository, Research

Triangle Park, Raleigh, North Carolina. Analysis was performed on a Varion 3700 gas chromatograph with a Hewlitt Packard 3390 integrator and BPI bonded phase fused silica capillary column. Hydrogen was used as the carrier gas at a flow of 3 mL/min. The injection port temperature was 220° C and the detector temperature was 320° C. The temperature program ran from 150 -280° C at a rate of 5° C per minute. Samples were directly injected into the chromatograph.

Acute 96-h toxicity tests (LC50) were conducted under conditions similar to those described in the maintenance of the animals during acclimation (12 h L:D cycle, 20° C, and 20 ppt seawater). The goal of each test was to establish LC50 values for fenvalerate, endosulfan, malathion, and the fenvalerate/endosulfan mixture (3:1).

Fifteen fish (3 replicates/exposure) were exposed for each concentration tested. An additional group of 15 fish was exposed to the carrier (acetone 0.1%) without any insecticides. Another unexposed group was maintained as a seawater control. Fish were not fed during any of the tests. All fish used for assay purposes were no more than 1.5 times the size of other fish used (Sprague 1973).

At the onset of each experiment, fish were collected from acclimation tanks and randomly distributed into each exposure regime. Testing was conducted under static conditions in 3.8-L glass jars. Water changes were made every 24-h to insure that the depletion of toxicant and oxygen did not occur. Test water was artificially aerated throughout each test by bubbling air through it at a uniform rate that maintained the dissolved oxygen levels near saturation for the test temperature. The method of aeration was similar to that described by Eisler (1970). The tops of test vessels were sealed to limit volatilization. Samples of the test waters were collected before aeration and after 24-h of aeration for chemical analysis. There was less than 15% change in concentration over each 24-h period in each test regime. Nominal concentrations were not corrected for these losses.

A minimum of eight concentrations were tested for each insecticide and the mixture. Insecticide seawater mixtures were changed daily, mortality recorded and dead animals removed when discovered. LC50 values and 95% fiducial limits were determined for each pesticide by using Logist and Probit Analysis Models with Log 10 transformation of dose according to procedures described in SAS (1982). Insecticide mixtures were tested for additive toxicity using methods described by Marking (1977).

RESULTS AND DISCUSSION

Endosulfan was the most toxic insecticide as based on a 96-h LC50 value of 1.15 ug/l (95% Fiducial Limits = 1.13 - 1.18 ug/l). Fenvalerate was the second most toxic insecticide tested with a 96-h LC50 value of 1.84 ug/l (95% FL = 0.13 - 13.41 ug/l). The fenvalerate/endosulfan mixture was the third most toxic insecticide tested based on a 96-h LC50 value of 3.06 ug/l (95% FL = 2.97 - 3.20 ug/l). Statistical analysis of the results for the fenvalerate/endosulfan mixture indicated less than additive toxicity when compared with individual endosulfan and fenvalerate LC50 values. Malathion was the least toxic of the insecticides tested with a 96-h LC50 value of 22.51 ug/l (95% FL = 16.01 - 31.24 ug/l).

Using the U.S. Fish and Wildlife Acute Toxicity Rating Scale the following ratings were assigned to each insecticide tested: endosulfan - supertoxic (96-h LC50 less than 10 ug/l); fenvalerate - supertoxic; fenvalerate/endosulfan mixture - supertoxic; and malathion - extremely toxic (96-h LC50 less than 100 ug/l).

The 96-h LC50 values for EC of endosulfan (1.15 ug/l) and fenvalerate (1.84 ug/l) measured in adult F. heteroclitus in this study were slightly higher than values reported by Schimmel et al. (1977, 1983) for technical grade endosulfan (0.09 - 0.38 ug/l) and fenvalerate (0.31 - 0.58 ug/l) in mullet (Mugil cephalus) and Atlantic silverside (Menidia menidia). Schimmel et al. (1983) reported a higher LC50 value of 5.0 ug/l for technical grade fenvalerate in sheepshead minnow (Cyprinodon variegatus). Whether these toxicity differences reflect species differences and/or insecticide formulation/technical grade chemical differences is not known. Bradbury (1985) compared the acute toxicity of technical grade (TG) and emulsifiable concentrations of fenvalerate with the fathead minnow (Pimephales promelas) in flow-thru toxicity tests. Results from these tests indicated slower uptake kinetics for the fenvalerate EC than TG, which resulted in an initial depression of 96-h acute toxicity in the EC. Earlier studies (Coats 1979) of other pesticides have indicated greater acute toxicity of EC than TG and enhanced uptake of insecticides due to emulsifiers in aquatic species tested. The fenvalerate/endosulfan EC mixture was also supertoxic to adult F. heteroclitus, but was less than additively toxic when compared to individual toxicity of endosulfan and fenvalerate. The EC of malathion in this study was extremely toxic to an adult F. heteroclitus with 96-h LC50 value of 22.51

ug/l. Eisler (1970) reported a 96-h LC50 value of 80 ug/l for F. heteroclitus exposed to TG malathion. Comparison of these results indicate increased toxicity for the EC formulation of malathion when compared to the TG formulation.

The toxicity of insecticides present in non-point source agricultural runoff to non-target aquatic species is of primary concern when evaluating a pesticide for environmental use. The acute toxicity of insecticides applied to agricultural fields that may drain into coastal estuaries must be considered when permitting a pesticide for use in coastal areas due to the often close proximity of the crop field to the estuarine water system. Other factors to be considered are environmental persistence; solubility; bioconcentration, bioaccumulation and biomagnification potential; bioavailability potential; carcinogenic, mutagenic, and teratogenic potential; and the potential for human health effects (USEPA 1982).

This study indicates that an insecticide mixture and three insecticides commonly used in the coastal zone of South Carolina are highly toxic to nontarget fish. Acute 96-h toxicity test using emulsifiable concentrations of malathion, endosulfan, fenvalerate, and a fenvalerate/endosulfan mixture indicated these three insecticides were super toxic to the estuarine fish Fundulus heteroclitus.

Future studies should be directed toward laboratory toxicity testing more attuned to realistic field-use characteristics (technical grade vs. emulsifiable concentrations) and toward field validation of the laboratory testing for better assessment of the suitability of insecticides used in areas in close proximity to estuarine habitats. This integrated approach of field and laboratory toxicity testing should employ both toxicity and sublethal parameters as indicators of actual biological impact. The addition of ecological field sampling to this integrated laboratory and field toxicity approach would provide field confirmation necessary to properly assess the ecological effects of insecticides used in the coastal zone and to determine the compatibility of the use of such insecticides there.

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