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Trifluralin and Oryzalin Herbicides Toxicities to Juvenile Crawfish (*Procambarus clarkii*) and Mosquitofish (*Gambusia affinis*)

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Trifluralin and oryzalin are commercial herbicides sold as Treflan® and Surflan,® respectively. Both are dinitro compounds. Chemically, the former is alpha, alpha-2, 6-dinitro-N, N-dipropyl-p-toluidine and the latter is 3,5-dinitro N^4-dipropylsulfanilamide. The usual carrier for both herbicides is water. Trifluralin's solubility in water is 0.0001 ppm and for oryzalin it is 0.00024 ppm by weight. The recommended trifluralin usage for Louisiana's vegetable crops, nursery stocks, annual grasses and weeds of cotton and sugarcane is 227-907 g/A active ingredient; and oryzalin for soybean and crop weeds is 340-1814 g/A a.i. (LSU COOP. EXT. SERVICE 1983).

Toxicities of these herbicides to crawfish and mosquitofish are not known. However, trifluralin's toxicity to other fish species was reported by COUCH et al (1979) and PARKA and WORTH (1965). Although these herbicides are very little soluble in water, and are strongly adsorbed with soil particles thus leaching minimally, their potential hazard to especially crawfish is important because of their extensive use as human food in Louisiana and several other states as well as some foreign countries. The year round abundance of mosquitofish in ponds and lakes was the reason for choosing them for bioassays.

MATERIALS AND METHODS

Juvenile crawfish (3-4 cm) were collected from an experimental pond located at Louisiana State University, Ben Hur Experiment Station. Adult mosquitofish (2 - 2.5 cm) were obtained from Lake Kernan at Southern University. They were acclimatized in a wooden vat (2.4 X 1.2 X 0.3 m) containing tap-water (temperature 23-25°C dissolved oxygen 5.8 - 6.5 ppm). Test animals were fed a chow (Food®) during acclimatization period but not during testing.

Static fish bioassays were conducted in glass aquaria (5.7 L) containing 2 L of test solution and 5-10 fish. Freshly prepared 1% aqueous stock solutions were serially diluted to desired concentrations (ppm). No solvents were used to dissolve these compounds in water. Range-finding tests were conducted prior to testing for

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finding suitable concentrations which will cause 0-100% mortalities of test animals. Mosquitofish were exposed to 12 different concentrations of trifluralin ranging from 1-100 ppm. The dead fish were promptly removed from test containers at 24 h intervals and their mortality was recorded up to 96 h.

Crawfish were individually placed in 3.8 L wide-mouthed glass containers to avoid cannibalism. Twenty-four and 96 h mortalities were recorded in 10 different concentrations of trifluralin (1-20 ppm) and 9 of oryzalin (10-10,000 ppm). To test the combined effects of these herbicides, stock solutions were mixed in a 1:1 ratio. Crawfish mortality was recorded up to 96 h in concentrations ranging from 5-500 ppm oryzalin.

Each test was replicated 3 times and up to 65 animals were tested. The weight, length and sex of 50 randomly selected crawfish and mosquitofish were recorded to compute standard deviation and means. The LC_{50} values were determined by an IBM probit analysis computer program. Acute poisoning symptoms in test animals were recorded.

RESULTS AND DISCUSSION

Table 1 gives percentage mortalities of mosquitofish and crawfish exposed to trifluralin. Absolute mortalities (100%) of mosquitofish and crawfish in 24 h were 30 and 40 ppm, respectively. The 24 h LC_{50} for mosquitofish and crawfish were 28 and 13 ppm, respectively (Table 2). The 96 h LC_{50} was 12 ppm for both herbicides. No differences in 24 and 96 h mortalities were observed for crawfish at the concentrations used in this study. Differences were, however, observed for the mosquitofish as indicated in Tables 1 and 2.

Table 1. Percentage mortalities of juvenile crawfish (*Procambarus clarkii* and mosquitofish (*Gambusia affinis*) exposed to various concentrations (ppm) of trifluralin herbicide.

Conc. (ppm)	Mosquitofish			Crawfish		
	N	24 h	96 h	N	24 h	96 h
0	18	0	0	30	0	0
1	65	0	3	30	0	0
3				30	0	0
5	50	12	24	35	3	6
6				40	12	15
7				30	17	17
9				40	30	32
10	52	17	38			
12				30	37	40
15	61	39	69	40	57	62
18				30	80	93
20	54	48	81	40	100	100

Table 1 (Cont'd)

25	30	58	97
30	30	70	100
50	30	77	100
60	62	88	100

Table 2. Probit analysis LC_{50} values (Upper and lower limits) in ppm for juvenile crawfish and mosquitofish exposed to trifluralin and oryzalin herbicides and 1:1 mixture.

Herbicide	Mosquitofish		Crawfish	
	24 h	96 h	24 h	96 h
Trifluralin	28 (30-35)	12 (11-13)	13 (12-14)	12 (11-13)
Mixture			266 (260-278)	210 (202-219)

Crawfish and mosquitofish mortalities were dosage dependent. Mosquitofish mortality had positive correlation with exposure time but not in case of crawfish (Tables 1 and 3). Crawfish mortality was greatly reduced in a 1:1 mixture of these herbicides. The 24 and 96 h values for crawfish were consequently increased from 13 to 266 ppm and from 12 to 210 ppm, respectively (Table 2). Oryzalin herbicide was practically non-toxic to crawfish. It failed to cause 100% death of crawfish even in 10,000 ppm exposure for 96 h (Table 3).

Table 3. Percentage mortality of juvenile crawfish (Procambarus clarkii) exposed to various concentrations of oryzalin and a 1:1 mixture of trifluralin and oryzalin herbicides.

Conc.	Oryzalin			Mixture		
(ppm)	N	24 h	96 h	N	24 h	96 h
0	40	0	0	40	0	0
5				40	0	0
10	40	0	0	40	0	0
50				40	0	0
100	40	0	0	40	0	0
150				40	15	20
200	40	0	0	40	20	25
300	40	0	0	40	55	65
350				40	75	90
400	40	0	0	40	100	100
500	40	0	10			
1000	40	6	16			
1500	40	6	20			
2000	40	19	38			
5000	40	20	40			
8000	40	20	46			
10000	40	20	60			

Mixture of oryzalin and trifluralin killed all crawfish at a concentration of 400 ppm. Oryzalin alone was not tested on mosquitofish. Crawfish mortality in herbicide mixture and oryzalin alone was dose dependent. Mortality of crawfish increased significantly during 96 h exposure to oryzalin, however, it was not so significant in herbicide mixture. Crawfish did not die up to 400 ppm oryzalin but more than 100 ppm herbicide mixture caused deaths. The 24 and 96 h LC_{50} values for oryzalin could not be computed from the data.

The mean weight and length of crawfish indicate that the males were longer (4.2 cm) than females (3.5 cm) with a standard deviation of 0.74 and 0.71, respectively. The female crawfish were slightly heavier than males 1.4 and 1.3 g, respectively (S.D. = 0.55 and 0.43). The 24 and 96 h LC_{50} values for trifluralin clearly show that mosquitofish and crawfish had similar tolerances to this herbicide. However, juvenile crawfish were far more susceptible to trifluralin than oryzalin.

Acute poisoning symptoms in crawfish were erratic movements, supination and appendage paralysis. Mosquitofish gill region and tail were observed to have hemorrhage. These symptoms may have some significance in relation to the mode of action for these herbicides. Presently it is not known how these herbicides actually kill crawfish and mosquitofish.

Bluegill and fathead minnow have been known to be very susceptible to trifluralin. PARKA and WORTH (1965) reported the 96 h LC_{50} values for the above named fish as 0.094 and 0.058 ppm, respectively. The estimated EC_{50} values for bluegill and channel catfish were 0.1 (24 h) and 0.4 ppm (96 h) trifluralin, respectively (BAILEY and LIU 1980). COUCH et al (1979) found that trifluralin herbicide is very toxic to the developmental stages of sheephead minnow. It caused vertebral hyperplasia when minnows at the zygote to the 28th day developmental stages were exposed to concentrations of 5.5 - 31.0 ppb. Additionally, the adults had higher serum calcium levels at 16.6 ppb trifluralin. However, mosquitofish in our study were much tolerant to this herbicide which is depicted by the 96 h LC_{50} of 12 ppm. This may be explained on the basis that different fish species might vary greatly in their tolerance to the same herbicide. SANDERS and COPE (1966) also observed similar phenomenon for some crustaceans which had a wide range of tolerances to insecticides. Trifluralin's toxicity to crawfish was not available in the literature. The only report for another crustacean, Gammarus lacustris listed the 24 h EC_{50} as 8.8 ppm (BAILEY and LIU 1980).

Although crawfish and mosquitofish are far apart taxonomically, they had similar tolerances to trifluralin herbicide. On the other hand, crawfish differed immensely in their susceptibility to closely related herbicides oryzalin and trifluralin. This is also exemplified by only 50% crawfish mortality in 12 ppm trifluralin,

while up to 8,000 ppm oryzalin failed to cause similar mortality. The apparent lack of oryzalin's toxicity is perplexing. Perhaps the photolability of this compound in laboratory conditions might be a possible factor (HERBICIDE HANDBOOK 1976).

It is difficult to precisely predict the possible adverse effects of these herbicides under field conditions, because they are adsorbed strongly to soil particles and leach minimally under normal rainfall. This also suggests that these herbicides, particularly oryzalin, might pose less danger to the aquatic fauna if recommended application rates are strictly followed. Unequivocally, many pesticides reach an aquatic ecosystem if abnormal amounts are released in the environment. Accidental mixing of two compounds may potentiate or negate their toxic effects and this may be critical, especially for non-target organisms.

We have found that mixing trifluralin and oryzalin herbicides in a 1:1 ratio caused a 17 - 20 fold decrease in their toxicities to juvenile crawfish. Therefore, we suggest that if these herbicides are used near rice fields or crawfish ponds, they should be applied in a mixture. We need to investigate whether such a combination will also decrease their herbicidal properties.

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