Toxicity of Mirex to Crayfish, *Procambarus blandingi*

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Little information exists describing the effects of the polycyclic chlorinated insecticide Mirex on non-target organisms. Mirex does not appear to be highly toxic to quail (1), mallards, and pheasants (2). Swiss mice fed a Mirex-impregnated diet (5.0 ppm Mirex) had significantly greater parent mortality and reduced litter size when compared with controls (3). Mirex was not acutely toxic to bluegill sunfish or goldfish, but did cause lesions in the gills and kidneys of chronically treated goldfish (4).

The Plant Pest Control Division of the Agricultural Research Service (USDA) and state agencies of 9 Southeastern states have proposed a 12-year plan to eradicate the imported fire ant (Solenopsis saevissima richteri Forel) by treating about 120-million acres three times with 1.25 pounds of Mirex bait per acre per treatment. In light of the economic importance of the crayfish in the Southeast (5), we undertook this study to determine the sensitivity of crayfish to low concentrations of Mirex in water and to Mirex bait granules.

MATERIALS AND METHODS

Crayfish were dipped from Chappapecla Creek, Tangipahoa Parish, Louisiana (Procambarus blandingi) and from Oktibbeha County, Mississippi (P. hayi). All animals were held overnight in the laboratory prior to bioassay.

Experimental procedures included the exposure of crayfish to several sublethal concentrations of technical Mirex solution for various periods of time, the determination of the toxicity to crayfish of Mirex leached from bait granules in water, and the feeding of Mirex bait (0.3% active ingredient) to crayfish. Mortality was recorded and dead crayfish were removed at 12-hr intervals. Individuals failing to respond to touch were considered dead.

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In all experiments the mortality caused by the insecticide was calculated as follows:

$$M_x = \frac{Sc - St}{Sc} \times 100$$

where M_x = % mortality caused by insecticide
Sc = % of surviving control animals
St = % of surviving treated animals

Sample Extraction and Cleanup

Water samples were extracted with hexane; 10:1 (v/v) water to hexane. Samples were shaken with the appropriate volume of hexane four times for 30 minutes on an Eberbach shaker. The hexane portions were separated from the water, percolated through sodium sulfate, and concentrated to 5 ml for analysis by GLC. Samples of water were fortified with known concentrations of Mirex and checked for recovery efficiency. Recovery ranged from 90 to 100 + %.

Crayfish (whole bodies and digestive glands) were weighed, ground with sodium sulfate, and extracted with hexane by shaking three times for 30 min each. The extract was filtered through a column of sodium sulfate (2 X 1 cm) and then concentrated to a volume of 5 ml from which an aliquot was taken for cleanup on a microcolumn (6).

Samples were analyzed on a model 5360 Barber-Colman pesticide analyzer equipped with an electron-capture detector and a mixed column (6' X $\frac{1}{4}$") consisting of OV-17 (1.5%) and QF-1 (1.95%) coated on 100/120 mesh chromosorb-WHP. Operating conditions were as follows: column temperature, 200°C; detector temperature, 205°C; injection temperature, 220°C; nitrogen flow about 85 ml per min. Retention time for Mirex was 27 min.

EXPERIMENTS AND RESULTS

Tolerance of Juvenile Crayfish to Mirex

Juvenile crayfish were bioassayed in static conditions to determine mortality when exposed to low Mirex concentrations. Technical grade Mirex was prepared in 1% acetone solution and diluted in tap water (pH 7.8, hardness 28 ppm) in a 10-liter glass mixing jar with about 3 ml of acetone per liter of final test solution. A saturated solution of sodium thiosulfate (0.3 ml/10 liters) was used for dechlorination of tap water. Controls were placed in tap water containing acetone and sodium thiosulfate solution.

Samples of 10 crayfish (avg length = 1.5 cm) were placed in 5 liters of test solution in 3-gal glass aquaria. Crayfish were exposed to Mirex concentrations (1 and 5 ppb) for 6-144 hr, transferred to tap water, and held in 15-gal aquaria for 10 days. All solutions were aerated and changed every two days.

Mortality approached 100% in 5 days in crayfish exposed for 144 hr to 1 ppb (Table 1). Exposure to 5 ppb for 6, 24, and 58 hr yielded 26, 50, and 98% mortality respectively 10 days after initial exposure. Delayed mortality was apparent in all tests.

TABLE 1

Percent mortality of P. blandingi and P. hayi (avg length = 1.5 and 0.6 cm, respectively) at 4, 5, and 10 days following initial exposure (6-144 hr) to various concentrations of Mirex.

Concentration (ppb)	Exposure Time	N	% Mortality			
			End of Exposure	Days Following End of Exposure		
				4	5	10
1+	144	30	0	--	95	95
5+	6	30	0	--	2	26
5+	24	30	6	--	13	50
5+	58	40	5	--	76	98
0.1*	48	30	19	65	--	--
0.5*	48	30	12	71	--	--

* - P. hayi
+ - P. blandingi

In a second series of tests, samples of 5 crayfish (avg length 0.6 cm) were exposed to 0.1 and 0.5 ppb Mirex in culture dishes containing 200 ml Mirex solution. The crayfish were transferred to tap water after a 48-hr exposure and mortality was recorded for an additional 4 days. In these tests crayfish exhibited 65 and 71% mortality respectively (Table 1).

Toxicity of Mirex from Leaching

Twenty crayfish (5/liter) were placed into two glass aquaria containing 2 liters of tap water and 10 granules of Mirex bait (0.3% active ingredient). The bait was enveloped in Whatman No. 2 filter paper (5.5-cm diameter) and in screen wire (1/16 in mesh) about 4 inches square successively. After 3 days of

exposure the mortality increased markedly (Table 2). After 7 days all but one Mirex-exposed crayfish were dead, and only one control of 20 had died.

TABLE 2

Percent mortality of juvenile crayfish (N = 20) through leaching during indirect exposure to Mirex bait enfolded in filter paper and screen wire.

	DAYS						
	1	2	3	4	5	6	7
% Mortality	0	0	0	30	55	80	95

In a similar experiment 35 crayfish (about 1 cm long) were exposed to 35 granules of Mirex bait enclosed in screen as described above. After 54 hours 33 of 35 crayfish were dead; only 2 of 13 controls had died. Duplicate water samples analyzed by gas-liquid chromatography contained 0.86 ppb Mirex. Triplicate samples of crayfish (6/sample) had body residues of 1.602, 1.409, and 1.339 ppm Mirex.

Toxicity of Mirex through Feeding

Each of 108 juvenile crayfish (avg length = 1.5 cm) was placed in a culture dish containing 200 ml of tap water and fed one granule of Mirex bait. Controls were treated similarly but received no Mirex.

Only 2% of the crayfish had died one day after feeding (Table 3). The greatest increase in mortality occurred during the 2nd and 3rd days. The actual time of feeding was not determined because of the small amount of the Mirex granule eaten. Three pooled samples of crayfish (4-14 individuals per sample) which died on the 2nd and 3rd days of exposure had body residues ranging from 3.9 to 6.1 ppm Mirex. All controls survived the 6-day test.

Two groups of 15 adult crayfish (avg 3.0 cm) were placed in 7.5 liters of tap water in 5-gallon aquaria. Two groups were fed Mirex at a rate of one and two granules per individual, respectively. Controls were held in tap water and not fed. Crayfish fed one and two granules suffered 55.5 and 100% mortality, respectively, in 4 days (Table 3).

TABLE 3

Daily % mortality in juveniles and adult crayfish fed one and two granules of Mirex bait.

Feeding Rate	N	Avg Length (cm)	% Mortality Days after Feeding					
			1	2	3	4	5	6
1 granule	108	1.5	2	32	55	61	69	77
1 granule	15	3.0	0	0	0	55.5	--	--
2 granule	15	3.0	0	13	40.6	100	--	--

Eight crayfish were placed individually in 250 ml tap water in quart jars with 2 granules of Mirex bait. Seven controls received no Mirex. Mortality was recorded and individuals were removed at death for residue analysis (Table 4). After 6 days of exposure 50% of the treated group had died, and the crayfish contained 8.680 ppm body residue. Analysis of a pooled sample of digestive glands of 4 treated animals revealed 62.795 ppm Mirex. The residue in water increased in the first 2 days of the test and then declined. Mirex residues in the bodies of crayfish increased initially, but remained fairly constant after the second day. There was no mortality nor detectable Mirex residue in the bodies or water of the control group.

TABLE 4

Percent mortality and Mirex residues in crayfish and tap water (1 crayfish/250 ml water) exposed to 2 granules of Mirex.

	Exposure (Days)				
	1	2	3	6	8
% Mortality	12.5	25.0	37.5	50.0	62.5
Crayfish residue (ppm)	--	1.900	8.773	8.680	5.947 (62.795)
Water residue (ppb)	0.073	2.021	1.489	0.319	0.496

( ) = pooled digestive glands
ppm = parts per million
ppb = parts per billion

For 6 days mosquitofish (Gambusia affinis) were fed Biorell food which was fortified with Mirex (10 ppm). Upon analysis the fish revealed a total body residue averaging 0.53 ppm Mirex. One mosquitofish per day was fed to each of 5 adult crayfish (Procambarus hayi) for 9 days. Control and treated crayfish were fed a diet of untreated mosquitofish for 50 days. After 50 days 4 of 5 treated adult crayfish had died; only 1 of 4 control individuals had died.

DISCUSSION

Crayfish were found to be extremely sensitive to Mirex through direct and indirect exposure. Mortality increased with time and Mirex concentration and appeared to be correlated inversely with animal size. Third instar crayfish were the most susceptible of all the size groups treated. When offered 1 granule of Mirex bait per crayfish (P. blandingi), individuals which averaged 1.5 cm in length were slightly more susceptible than individuals averaging 3.0 cm (Table 3). Crayfish (avg length 3.0 cm) fed 2 granules per individual suffered a 2-fold greater mortality after 4 days than did individuals offered 1 granule. Mortality from low or brief exposure was characteristically delayed (Tables 1 and 2).

Substantial amounts of Mirex leached from granular bait in water. When crayfish were placed into water in which Mirex bait was present, but inaccessible, the animals accumulated a residue (avg = 1.45 ppm) 16,860-fold greater than that in the water (avg = .86 ppb) into which the Mirex had leached.

Feeding tests also revealed Mirex to be extremely toxic in the bait granule form (Table 3). Juvenile and adult crayfish suffered high mortality after consuming extremely small amounts of granular Mirex. Smaller crayfish exhibited higher mortality and delayed mortality occurred in all size classes.

GLC analysis of bodies of exposed crayfish revealed an increase in residue with length of exposure (Table 4). Analysis of water in which the crayfish were held showed an initial rise in residue followed by a decrease. This relationship (increase in body residue with a decrease in water residue) indicates leaching of Mirex from the granules into the water followed by uptake of Mirex from the water by the crayfish. Mirex residues in crayfish bodies were from 940-fold to 27,210-fold greater than the concentration in the water in which the crayfish were held. The digestive glands from four individuals which were still living after 8 days of exposure contained a 126,603-fold greater quantity of Mirex than did the water. These data demonstrate the extent to which the crayfish may concentrate Mirex from bait granules. Although the crayfish were observed

feeding on the bait granules the residues were probably accumulated by the crayfish primarily by absorption through the respiratory apparatus. High residues in crayfish fed Mirex bait and mortality in individuals fed contaminated mosquitofish emphasize the potential hazard of biological magnification.

Indirect toxicity of Mirex was shown to be through leaching and/or consumption of Mirex bait. Both routes of exposure must be considered additive when determining the toxicity of Mirex in granular form to potential non-target consumers. Exposure to Mirex caused low percent mortalities initially. However, the continuous mortality exhibited in all tests shows the delayed toxicity of Mirex. All crayfish which showed symptoms of poisoning died. Treated individuals exhibited initial hyperactivity, followed by sluggishness, and loss of appetite and coordination.

Contamination of waters by chlorinated hydrocarbon insecticides through runoff from heavily treated soils has been reported by Finley, Ferguson, and Ludke (7). The resistance of Mirex to degradation (4) and movement into water through leaching would favor contamination of aquatic habitats surrounded by treated areas. The rapid accumulation of Mirex residues by fish (4) and crayfish through direct exposure in water and indirect exposures through the food chain shows the importance of Mirex as a potential pollutant of aquatic ecosystems. Mortality resulted from all concentrations to which crayfish were exposed. Residues of Mirex as low as 0.1 ppb, which might occur in aquatic environments, would produce mortality in crayfish, especially immature stages. Further study will be initiated to determine the lower limits of the tolerance range for crayfish to Mirex.

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