THE INFLUENCE OF MIREX BAIT ON PRODUCTION AND SURVIVAL OF LOUISIANA RED CRAWFISH, *PROCAMBARUS CLARKI* (GIRARD)

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ABSTRACT

An investigation was conducted concerning the effect of mirex bait on production and survival of red crawfish, *Procambarus clarki* (Girard), in rice fields of southern Louisiana. Twenty experimental plots were stocked with 2,000 brood crawfish (50 pairs per plot). Each plot was randomly assigned to one of four treatments: (1) conventional rice insecticides, malathion and carbofuran; (2) mirex alone; (3) mirex in conjunction with malathion and carbofuran, and; (4) untreated controls. Mirex bait (0.3 per cent technical material) was applied at the rate of 1.25 pounds per acre in three applications, approximately 90 days apart.

Statistical analyses of data revealed no significant differences among treatments in crawfish size, sex ratio, and weight yield, whereas the differences among treatments in number harvested were significant (P .05). Plots treated with mirex alone produced the fewest crawfish (3,642) and control plots the most (5,667). The treatment consisting of mirex, malathion, and carbofuran, however, produced the second highest number (5,637); slightly below the total collected from control plots. Orthogonal comparisons indicated that the disparity among treatments was not due to mirex activity. Mirex residues in crawfish from plots receiving mirex applications averaged 0.248 ppm (range 0.09 2.01 ppm) on a whole-body basis, which is above the tolerance established for mirex in fat of red meat (0.1 ppm).

INTRODUCTION

Persistent organochlorine insecticides have been variously described as actual or potential contaminants of aquatic ecosystems (Cope 1966, Newsom 1967, and Stickel 1968). It is, therefore, important to determine the impact on aquatic environments of any program authorizing widescale use of a stable organochlorine. Such a program has recently been adopted to control the red imported fire ant, *Solenopsis invicta* Buren, using mirex, an extremely stable chlorinated insecticide (USDA 1971). Prior to 1971, millions of acres in the southeastern United States had already been subjected to mirex treatment (1.25 pounds per acre of a bait formulation containing 0.3 percent mirex, 14.7 percent soybean oil, and 85 percent corncob granules).

In several studies with fish, mirex was reported to cause no mortality to such species as bluegill, *Lepomis macrochirus* (Van Valin *et al.* 1968, Jenkins 1963), pinfish, *Logodon rhomboides* (Lowe *et al.* 1971), spot *Leiostomus* spp. (Butler 1964), or channel catfish, *Ictalurus punctatus* (Collins and Davis 1971).

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However, goldfish, *Carassius auratus*, experienced mortality directly related to mirex exposure (Van Valin *et al.* 1968).

In acute toxicity studies on crawfish, *Procambarus clarki*, Muncy and Oliver (1963) observed no mortality from exposure to mirex (0.0004 - 0.1 ppm) for 24-72 hours. However, Ludke *et al.* (1971) observed that mortality of crawfish, *Procambarus blandingi*, was delayed, increased with concentration, and was related to crawfish age. They also reported that mirex accumulated on a whole-body basis at 27,210 times the level detected in test water and in digestive glands at 126,602 times the amount present in the water.

Crawfish farming has become an important industry in Louisiana(Lovell 1968, Avault 1970, LaCaze 1970, and Perry *et al.* 1970) and is especially prevalent in rice producing areas (Thomas 1963, Hendrick *et al.* 1966). Therefore, a study was initiated to investigate the effect of mirex on production and survival of crawfish grown in rice fields of southern Louisiana.

METHODS AND MATERIALS

Experimental Procedure

The study was conducted at the Rice Experiment Station, Crowley, Louisiana during 1971 and 1972, and was designed to adhere as closely as possible to normal rice-crawfish culture as described by Hill and Cancienne (1963). Twenty experimental plots, 25 x 88 feet (0.053 acre), were established on land planted to rice the previous year. Ten plots were located on each side of a central drainage lateral and conduits were arranged to permit flooding and draining of plots separately, thereby minimizing cross-contamination. Each plot was bordered by standard earthern levees approximately one and one-half feet high and five feet across at the base.

Plots were randomly assigned to one of four treatments: (1) conventional rice insecticides, malathion and carbofuran (FuradanR); (2) mirex; (3) mirex in conjunction with malathion and carbofuran, and; (4) untreated controls. Three applications of mirex bait were made approximately 90 days apart. Application rates, methods, and dates of application of all materials used are presented in Table 1.

To prevent crawfish dispersal, a three foot, 14 mesh aluminum screen fence was erected around each plot. The fence was also entrenched approximately six inches below the levee surface to retard shallow burrowing from plot to plot.

All plots were planted with Saturn rice on April 20, and flushed on April 30 and again on May 18 to stimulate seed germination and growth. The plots were subsequently flooded on June 1, at which time 2,000 brood crawfish (50 pairs per plot) were stocked. The crawfish averaged 17.5 grams and the stocking rate was about 80 pounds per acre. Rice straw was left in the plots as a food source for the crawfish. All plots were reflooded on October 21 and maintained with about 6-8 inches of water until termination of the study on March 30, 1972.

Data Collection

To determine production from each plot, crawfish were harvested during February and March 1972. All plots were sampled five times with cylindrical funnel-cone traps or commercial lift nets. Nets were considered more efficient and were, therefore, used for the bulk of collecting and beef pancreas or "melt" was used exclusively as bait. The number and weight of crawfish harvested from each plot were recorded.

Over 2,500 crawfish were sexed during a two-week period to determine any differential mortality. All plots were sampled five times and crawfish sexed according to structural characteristics of the first pleopods (swimmerets) as described by Penn (1959).

Crawfish collected on February 6 and February 20, 1972 were measured to detect any differences in size due to treatments. Length was determined by measuring the carapace (cephalothorax), in millimeters, along the mid-dorsal line from the anterior tip of the rostrum to the posterior edge of the carapace. This measurement constitutes approximately one-half of total crawfish length (Van Deventer 1937, and Penn 1943).

Residue Determination

Crawfish were sampled on August 19 (1971), January 10, and March 16 (1972) for mirex residues. Collections of at least 20 crawfish from each treatment were made subsequent to the first two applications, and approximately 20 crawfish were obtained from each plot after the final mirex application.

Other samples subjected to residue analysis included soil and water (pre- and post-treatment analysis) and rice grain (at time of harvest). Snakes and turtles found in some of the plots were also sampled to detect any mirex biomagnification.

Extraction, clean-up, and confirmation of mirex residues followed the methodology used by Graves *et al.* (1969). Whole crawfish and reptile muscle were ground in a Hobart meat grinder and reptile liver and eggs were blended in a Waring blender. These samples, along with fat, were triturated with anhydrous sodium sulfate, extracted with petroleum ether, partitioned with acetonitrile, and chromatographed through florisil columns. A florisil recovery standard and reagent blank were included with each series of samples to determine the percent recovery and to monitor solvent interference. Percent recovery ranged from 95 to 107. Mirex was measured using Aerograph Pestilyzer gas chromatographs (Models 680 and 682) employing electron capture detectors. Mirex standards were injected with each set to determine elution time and also to establish base peaks. The minimum detectable amount was 0.01 ppm in tissue and eggs and 0.05 ppm in fat. Calculations were performed by direct linear comparisons with similar peaks of known value. Random samples were chosen for thin layer chromatographic analysis (Damaska 1964) for qualitative comparisons.

RESULTS AND DISCUSSION

Ludke *et al.* (1971) and Lowe *et al.* (1971) reported that crawfish and other crustaceans were highly susceptible to low levels of mirex; mortality approached 100 percent from exposure to concentrations as low as 0.1-1.0 ppb. Results of the present field study, however, did not confirm the degree of toxicity suggested by their laboratory observations.

Crawfish Production

The number and weight of crawfish harvested and a projection of yield per acre are presented in Table 2. There was considerable variation among treatments. The least number of crawfish (3,642) was collected from the mirex treated plots and the greatest number was obtained from the control plots (5,667) although only slightly fewer (5,637) were collected from the mirex, malathion, and carbofuran treatment. The number collected from the mirex treated plots represented a 35.7 percent reduction from that of the control plots. This difference was statistically significant (analysis of variance; $P<.05$. The 109.9 pounds harvested from the mirex treated plots was 25.0 percent less than that of the control plots (146.7 lbs) but was not significantly different ($P>.05$). A positive correlation was revealed between weight and number of crawfish collected from the rice plots, indicating a significant linear relationship between these two variables ($r = .87$).

Although the difference in number harvested between treatments was statistically significant, it cannot be entirely ascribed to mirex activity since the second highest number of crawfish came from the mirex, malathion, and carbofuran treatment. In order to more completely evaluate treatment differences, orthogonal couparisons were conducted. Results of these comparisons revealed that the disparity between treatments was not due to mirex exposure. The comparison of number of crawfish from mirex-treated versus non-treated plots was not significant (P>.05). Conversely, a significant difference (P<.05) was detected between mirex plus malathion and carbofuran treatments and the other two treatments in crawfish numbers. Malathon and carbofuran are not known to, nor would they be likely to, decrease the toxicity of mirex. Therefore, the difference between treatments in number of crawfish harvested cannot be explained entirely as a result of mirex toxicity. A more thorough examination of this interrelation is needed.

Sex Survival Rates

Male and female crawfish occur naturally in a 1 to 1 sex ratio (Penn 1943). Results obtained from sex determination of over 2,500 crawfish revealed 1,257 males and 1,262 females or 1.004 females per male. There was very little variation observed from treatment to treatment with the exception of the mirex treatments where a slightly greater percentage of males occurred (Table 3). These differences were not significant and were considered within the range of normal sampling error.

Crawfish Size

On February 6 and February 20, 1972, a total of 2,144 crawfish were collected and their lengths determined. The distribution of these crawfish into ten size classes and the overall mean carapace lengths are given in Table 4. A mean length of only 0.65 mm separated crawfish collected in mirex, malathion and carbofuran treated plots from the control crawfish, while crawfish from the mirex treatment had the second smallest mean length (36.68 mm), but neither of these differences of 1.74 and 1.66 percent, respectively, were significant (P> .05). Crawfish with cephalothorax measurements under 45 mm constituted at least 86 percent of the total sample from each treatment. A fairly constant percentage of crawfish was represented in each size class for each treatment, thus, mirex did not appear to effect a differential mortality on juvenile crawfish as expressed in laboratory studies (Ludke *et al.* 1971).

The overall mean increase in carapace length from February 6 to February 20 was only 1.56 mm. This growth was far below the 3 mm per week reported by Hendrick (1965) and 2-3 mm per week indicated by Penn (1943). Several factors could be responsible for a slow growth rate. Lack of food, overcrowding, poor water and soil quality, low ambient temperatures, and other physiological and environmental stresses (including insecticide exposure) may have contributed to an arrested rate of growth. However, further investigation is needed to clarify this point.

Residues

Mirex was not detected (0.01 ppm sensitivity level) in pretreatment samples of soil, water, and brood crawfish. In addition, none was detected in rice grain sampled during harvest (3 months after first mirex application).

Results of gas-liquid chromatographic analyses for mirex residues in crawfish subsequent to one, two, and three applications are presented in Table 5. Generally, the highest residues were obtained from plots treated with mirex, either alone or with conventional rice insecticides. Mirex accumulation in these treatments ranged from 0.09 to 2.01 ppm and was significantly higher than residues in crawfish from untreated plots (P<.01).

Mirex was detected in liver (0.20 ppm) and fat (0.01 ppm) of three garter snakes, *Thamnophis* spp., however, none was found in liver, fat or muscle of three water snakes, *Natrix* spp. or in muscle and fat of three turtles, *Chrysemys picta* (Table 6). Eggs from one water snake and from two turtles contained 0.02

and 0.03 ppm mirex, respectively, even though none was detected in other tissues.

The fact that mirex appeared in plots not subjected to direct applications reveals its potential to move in biotic and abiotic substances. The most likely method of cross-contamination between plots was by incorporation in or adherence to particulate matter (organic debris or microorganisms) suspended in the water. The entire study area was inundated during several heavy rainfalls and levees were damaged periodically by muskrats, *Ondatra zibethica* (L.). In both cases, mirex may have been transported in water, causing residues to appear in untreated plots. This may exemplify the capacity of mirex to move and concentrate in watersheds, thereby contaminating aquatic habitats not directly exposed to mirex treatment.

Considering the amount of mirex applied per plot (2.7 grams of total technical material for ten plots; three applications), residues detected in crawfish were not exceptionally high. The theoretical maximum accumulation of mirex (100 percent), based on the total amount applied (2.7 gms) and on the total crawfish weight harvested from mirex treated plots (260.27 lbs), was calculated at 22.85 ppm. This amount is 92 times the mean mirex residues obtained (0.248 ppm). It is particularly relevant to note, however, that residues accumulating in crawfish (whole-body) were often in excess of government established tolerances for mirex in fat of red meat (0.1 ppm).

Conclusions

Although the significance of chronic mirex exposure to crawfish is not entirely understood, the results of the present study support the following observations:

(1) Mirex, at the rate recommended in the imported fire ant program, poses no overt threat to crawfish production in rice fields.

(2) No mortality was directly related to mirex exposure. Crawfish were not observed exhibiting symptoms of mirex poisoning (irritability, undirected movement, loss of equilibrium, and paralysis) as reported by Lowe *et al.* (1971).

(3) There was no evidence that mirex influenced crawfish size, sex ratio, or weight yield.

(4) Mirex did not accumulate in detectable amounts in post-treatment bottom sediments, water, or in rice seed but did bioconcentrate in crawfish and reptiles from the study area. However, only a small portion of the total mirex applied was subsequently detected. The fate of mirex in aquatic ecosystems, thus, warrants further study.

(5) Residues accumulated in crawfish at levels above the established tolerance for mirex in fat of red meat (0.1 ppm).

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Table 1. Materials used in rice-crawfish study.

Material	Date of Application[1]	Rate of Application[2]	Method of Application
Saturn (Lot 2) rice	April 20	101.0 lbs/acre	Drill planted
Fertilizer (16-8-8)	April 20	525.0 lbs/acre	Aerial
Captan (Fungicide)	April 20	3.3[3]	Seed treatment
Propanil (Herbicide)	May 26	3.0 lbs/acre	Aerial
Carbofuran[4]	June 4	0.5 lbs/acre	Broadcast (3% granules)
Malathion[5]	August 2	0.5 lbs/acre	Pressurized spray
Mirex	June 15	1.7 g/acre	Broadcast
Mirex	October 21	1.7 g/acre	Broadcast
Mirex	January 24[6]	1.7 g/acre	Broadcast

[1]Applications during 1971 unless indicated otherwise.
[2]Rates for chemicals given in actual material applied.
[3]Ounces per 100 pounds of seed.
[4]Applied for control of rice water weevil *Lissorhoptrus oryzophilus* Kuschel.
[5]Applied for control of rice stink bug *Oebalus pugnax* (F.).
[6]1972.

Table 2. Summary of data on crawfish harvested from twenty experimental rice plots at the Rice Experiment Station, Crowley, Louisiana during February and March, 1972.

	Number Harvested		Weight Harvested (lbs)		
Treatment	Total	Average/Plot	Total	Average/Plot	lbs/acre[a]
Malathion and Carbofuran	4,410	882.0	130.24	26.05	521
Mirex	3,642	728.4	109.88	21.98	440
Mirex, Malathion, and Carbofuran	5,637	1,127.4	150.39	30.08	602
Control	5,667	1,133.4	146.68	29.34	587
Total	19,356		537.19		

[a]Projected lbs/acre based on total weight per treatment.

Table 3. Percent male and female crawfish collected from January 31 to February 14, 1972, from experimental rice-crawfish plots at the Rice Experiment Station, Crowley, Louisiana.

	Treatment							
	Malathion, Carbofuran		Mirex, Malathion, Carbofuran		Mirex		Control	
Replication	% M	% F	% M	% F	% M	% F	% M	% F
1	43.2	56.8	44.1	55.9	51.7	48.3	51.2	48.8
2	45.0	55.0	61.2	38.8	50.8	49.2	48.9	51.1
3	45.5	54.5	56.7	43.3	50.0	50.0	46.1	53.9
4	53.4	46.6	52.4	47.6	43.9	56.1	46.8	53.2
5	49.7	50.3	52.1	47.9	55.8	44.2	52.8	47.2
Mean	47.4	52.6	53.3	46.7	50.4	49.6	49.2	50.8

Table 4. Number and percent of crawfish occurring in each of ten size classes from samples collected on February 6, and February 20, 1972, from experimental rice-crawfish plots at the Rice Experiment Station, Crowley, Louisiana.

Size Class	Treatment							
	Malathion, Carbofuran		Mirex, Malathion Carbofuran		Mirex		Control	
(mm)	Number	Percent	Number	Percent	Number	Percent	Number	Percent
15-19	3	0.6	4	0.7	0	0	0	0
20-24	6	1.2	16	2.7	4	0.9	14	2.4
25-29	28	5.4	48	8.2	29	6.2	22	3.8
30-34	125	24.2	172	29.4	135	29.2	136	23.5
35-39	164	31.7	145	24.8	161	34.7	186	32.2
40-44	119	23.0	138	23.5	86	18.6	158	27.3
45-49	59	11.4	61	10.4	42	9.1	53	9.2
50-54	11	2.1	2	0.3	4	0.9	9	1.6
55-59	1	0.2	0	0	0	0	0	0
60-64	1	0.2	0	0	2	0.4	0	0
Total	517		586		463		578	
Overall Mean Carapace Length (mm)	37.41		36.65		36.68		37.30	

Table 5. Mirex residues (ppm) in crawfish collected subsequent to the 1st, 2nd, and 3rd mirex applications.

	Treatment			
Collection Date[a]	Malathion, Carbofuran	Mirex, Malathion, Carbofuran	Mirex	Control
August 19, 1971	0.21	2.01	0.35	ND[b]
January 10, 1972	0.04	0.22	0.09	0.19
March 16, 1972[c]	0.014	0.214	0.282	0.044

a/ Dates represent collections subsequent to mirex applications on June 15, 1971, October 21, 1972, and January 24, 1972.
b/ No mirex detected at 0.01 ppm.
c/ Average of residues obtained from each replication.

Table 6. Mirex residues (ppm) detected in reptiles collected from rice-crawfish plots at the Rice Experiment Station, Crowley, Louisiana during March 1972.

Sample	Number Collected	Tissue Analyzed	Mirex Residues
Garter snake, *Thamnophis* spp.	3	liver	0.20
		fat	0.01
Water snake, *Natrix* spp.	3	liver	ND[a]
		fat	ND
		muscle	ND
		eggs[b]	0.02
Turtle, *Chrysemys picta*	3	fat	ND
		muscle	ND
		eggs[c]	0.03

a/ No mirex detected at 0.01 ppm sensitivity level.
b/ Eggs obtained from one *Natrix* 38 inches in length.
c/ Composite sample of eggs obtained from two turtles.