TOXICITY OF MIREX TO POSTLARVAL AND JUVENILE FRESHWATER PRAWNS[1]

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Abstract: Postlarval and juvenile prawns (*Macrobrachium rosenbergii*) were exposed to 7 mirex concentrations plus control for 96 hours. Static acute toxicity tests were conducted at 28 C using deionized water reconstituted to hardnesses of 11, 42, 160 and 300 mg/l as $CaCO_3$ with postlarvae and 42 mg/l with juveniles. No statistical differences were observed in survivals of postlarvae or calculated median lethal mirex concentrations among hardnesses. A 96-hour LC_{50} for all postlarvae was 33.9 μg/l and for juveniles 900.8 μg/l mirex. Postlarvae and juveniles exposed to 1000 μg/l accumulated mirex up to 513× and 88× control levels. Mirex residues in juveniles were greater in nerve tissue than abdominal muscle or remaining carcass.

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Water quality is an important consideration in aquaculture operations, and use of certain pesticides can significantly affect production. Mirex, a stable chlorinated cyclodiene pesticide, was used extensively in the southeastern United States to control the imported fire ant, *Solenopsis invicta*. Many tropical and subtropical countries outside the United States still use mirex for control of insect pests. Mirex can leach out of corncob grit bait (Lowe et al. 1971), can be transported in surface water (Borthwick et al. 1973), and can be absorbed across the integument and gills of animals (Lowe et al. 1971, Schoor 1974). Mirex accumulates in animals and is biomagnified in higher trophic levels (Borthwick et al. 1973, Hyde et al. 1973, Tagatz et al. 1976).

Mirex is toxic to several decapod crustaceans (Lowe et al. 1971, Ludke et al. 1971, Bookhout et al. 1972, Naqvi and de la Cruz 1973, Summer and Eversole 1978, and others). Summer and Eversole (1978) also recorded a decrease in molt frequency and growth in postlarval *Macrobrachium rosenbergii* exposed to increasing concentrations of mirex.

Intensive culture of *M. rosenbergii* is a relatively new venture. Approximately 50 ha of managed prawn ponds were in operation in the United States in 1976, with an annual yield of nearly 2.2×10^3 kg/ha (Bennett 1977). Currently, 21 individual growers operate a total of 111 ha of private ponds in Hawaii, and more than 22 ha of ponds are involved in experimental or pilot commercial production of prawns in the southeastern United States (Joint Subcommittee on Aquaculture 1980). Increased growout efficiency and expansion to 1,000 ha worldwide could put production near 4.5 million kg (Glude 1978).

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Since the geographical area suited for pond culture of *M. rosenbergii* is within the existing range of the fire ant, and the most efficient means of controlling fire ants is mirex, a project was designed to determine effects of mirex on postlarval and juvenile *M. rosenbergii*, and tissue specific mirex accumulation.

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METHODS

Postlarval and juvenile *M. rosenbergii* were exposed to mirex in 96-hour static acute toxicity. A LD14:10 cycle and 28 C water temperature were maintained. Prawns were acclimated to test conditions 24 hours prior to mirex administration. Water was continuously aerated, and a glass tube substrate was provided. Tests were conducted with 10 l of water in 19 l glass jars.

Postlarvae (10 - 40 mm total length - TL, tip of rostrum to tip of telson) were exposed to mirex concentrations of 0.1, 10, 50, 100, 150, 500, 1000 μg/l and a control. Test water was reconstituted from deionized water to hardnesses of 11, 42, 160 and 300 mg/l as $CaCO_3$ (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). Nine hundred sixty postlarvae (10/jar) were tested in 3 96-hour toxicity tests.One hundred twenty-eight juveniles (41 - 100 mm TL) were exposed (1/jar) to the same mirex concentrations in deionized water reconstituted to 42 mg/l as $CaCO_3$ during 4 96-hour toxicity tests.

Serial dilution of a 1 mg/l stock solution of technical grade mirex (1,1a,2,2,3,-3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4 metheno-1H cyclobuta [c,d] pentalene - Allied Chemical, SN-1-104.3) in pesticide grade acetone was used to prepare test concentrations. Controls received 10 ml acetone without mirex. Observations were made at 12-hour intervals, and prawns which failed to respond to prodding were considered dead, removed, washed in acetone, placed in aluminum foil and frozen. Prawns remaining alive after 96 hours were sacrificed, washed and frozen. Water samples (100 ml) collected from each container at 0 hours and 96 hours were frozen in glass jars.

Residue analyses were run on whole postlarvae, nerve tissue (supraesophageal ganglion, circumesophageal commissures and ventral nerve cord), abdominal muscle and the remaining carcasses of dissected juveniles. Postlarvae were grouped according to mirex concentration and water hardness, and juveniles were grouped by mirex concentration. Samples were ground and extracted in hexane. Florisil cleanup of residues in hexane was similar to the procedure used by Summer (1978).

Water samples extracted twice with hexane required further cleanup. Thin layer chromatography plates (20 $\times$ 20 cm $\times$ 250 μ Absorbosil-5) were spotted with 100 μl residues and concentrated standards (2 mg mirex) using a Kontes chromaflex spotter. Plates were developed in hexane, and standards were visualized with iodine gas. Residue areas adjacent to standard areas (R_f = 0.65) were scraped into centrifuge tubes and extracted with hexane.

Residue levels were determined on a Tracor MT-220 gas chromatograph with a ^{63}Ni electron capture detector. The glass column (183 cm × 0.64 cm OD) was packed with 1.5% OV-17 and 1.95% OV-210 on 100/120 mesh Chromosorb W. Nitrogen gas flow was 80 ml/min, with purge rate of 15 ml/min, and temperatures for oven, inlet and detector were 210, 220 and 300 C, respectively. Detector limit was 1 pg and the linear range was approximately 1 pg to 100 pg. Residues were quantified by comparing sample peak heights to standard peak heights. Mean retention times for mirex were 8 min in experimental samples and mirex standards. Percent recovery for tissues and water samples spiked with known amounts of mirex ranged from 82 - 95% (Summer 1978). Residue values obtained were not corrected for percent recovery.

Mortality responses in 96-hour static acute toxicity tests were computed with a maximum likelihood program for estimating the logistic model of quantal response to continuous stimulus (Burrows 1979). All calculated 96-hour median lethal concentrations (LC_{50}) were based on nominal concentrations of mirex. Measured concentrations were not used for LC_{50} calculations because water samples were pooled for residue analysis.

RESULTS

Calculated 96-hour LC_{50} values (±SE) for postlarvae exposed to mirex in water hardnesses of 11, 42, 160 and 300 mg/l $CaCO_3$ were 37.3 ± 6.37, 31.7 ± 6.83, 35.3 ± 6.70 and 31.5 ± 6.15 µg/l mirex, respectively. Since no statistical differences (ANOVA) were observed in survival of postlarvae or in these LC_{50} values among water hardnesses, data were combined across hardnesses for further computations and presentation. Survival of postlarvae was generally lower in higher mirex concentrations, but mortality in 10 µg/l mirex concentration was higher than expected in each of the 4 water hardnesses (Table 1). Water became cloudy at this concentration after 24 hours. After 48 hours, there were no surviving postlarvae in 500 and 1000 µg/l mirex concentrations, and 100 and 150 µg/l mirex treatments averaged about 15% survival. In mirex concentrations less than 100 µg/l, postlarvae exhibited a mean survival of 88% after 48 hours and 66% after 96 hours. The combined 96-hour LC_{50} value (±SE) for postlarvae was 33.9 ± 3.25 µg/l mirex (Table 1).

Table 1. Survival and LC_{50} value (±SE) for *Macrobrachium rosenbergii* postlarvae exposed to mirex in 96-hour static toxicity tests.

Treatment		%Survival			LC_{50} ± SE
(µg/l mirex)	N[a]	24 hr	48 hr	96 hr	(µg/l mirex)
0	120	98.3	96.7	89.2	
0.1	120	96.7	90.8	86.7	
10	120	95.9	70.8	0.8	
50	120	98.4	95.0	89.2	33.9
100	120	70.0	6.7	0.8	± 3.25
150	120	74.2	24.2	0	
500	120	43.4	0	0	
1,000	120	28.4	0	0	

[a] Three tests each at 4 water hardnesses with 10 postlarvae per treatment.

Cannibalism occurred in all treatments and are included in the survival patterns (Table 1). Cannibalism appeared to contribute to 12 of 13 mortalities in control treatments, and 11 of 13 in 50 μg/l concentrations of mirex. Cannibalism was substantially lower in other concentrations.Swimming postlarvae were disoriented in mirex concentrations of 500 and 1000 μg/l. Abdominal muscle appeared to become necrotic in most postlarvae near death, and muscle spasms were noted. Excitability and loss of equilibrium occurred 12 to 24 hours before death. No differences in behavior were noted among water hardnesses. Molting was not observed in 96-hour tests with postlarvae.

Survival of juveniles was higher than that of postlarvae exposed to the same mirex concentrations for 96 hours (Table 2). One hundred percent survival occurred in all mirex concentrations at 48 hours and in concentrations less than 100 μg/l at 96 hours. The calculated 96-hour LC_{50} value (±SE) for juveniles was 900.8 ± 1.31 μg/l mirex.

Table 2. Survival and LC_{50} value (±SE) for *Macrobrachium rosenbergii* juveniles exposed to mirex in 96-hour static toxicity test.

Treatment		%Survival			LC_{50} ± SE
(μg/l mirex)	N[a]	24 hr	48 hr	96 hr	(μg/l mirex)
0	16	100.0	100.0	100.0	
0.1	16	100.0	100.0	100.0	
10	16	100.0	100.0	100.0	
50	16	100.0	100.0	100.0	900.8
100	16	100.0	100.0	93.8	± 1.31
150	16	100.0	100.0	93.8	
500	16	100.0	100.0	75.0	
1,000	16	100.0	100.0	43.8	

[a] Four tests with 4 replicates of 1 juvenile per jar.

Juveniles appeared excitable and often exhibited equilibrium loss while swimming or crawling after 24 hours in 500 and 1000 μg/l mirex. Abdominal muscles appeared necrotic in 2 juveniles exposed to 1000 μg/l mirex, and most juveniles exposed to 500 and 1000 μg/l mirex exhibited muscle spasms. Molting was observed in 23 juveniles, none of which died. With increasing mirex concentrations the number of molting juveniles decreased.

Postlarvae appeared to concentrate mirex (Table 3). Residues detected in controls and 10 μg/l mirex treatments were higher than expected; otherwise, mean values generally increased with increasing mirex concentrations. Residues in postlarvae ranged from nondetectable levels to 13,231 μg mirex per kg wet weight, with experimental residue levels up to 513× control level.

Juveniles appeared to accumulate less mirex than postlarvae. Mirex residues were generally higher in nerve tissue than abdominal muscle and remaining carcass (Table 4). Nerve tissue residues reached 250× control level, while abdominal muscle, remaining carcass and calculated whole body residues were 139×, 65× and 88× control levels, respectively.

Residue analyses of test water revealed that measured concentrations were close to nominal concentrations except for 0.1 μg/l mirex treatment. Mirex residues

Table 3. Mirex residues (μg/kg wet wt) of *Macrobrachium rosenbergii* postlarvae exposed in 96-hour static toxicity tests.

Treatment (μg/l mirex)	Postlarvae extracted[a]	Mean residue concentration[b]	Multiple[c] of control
0	36	25.8	
0.1	73	ND[d]	
10	92	80.8	3.2
50	109	1.5	0.1
100	108	11.6	0.4
150	106	414.6	16.1
500	105	1,285.6	49.8
1,000	114	13,231.4	512.8

[a] Four extractions per mirex treatment, corresponding to the 4 water hardnesses.
[b] Averages from 4 chromatograph trials per water hardness.
[c] Based on mean control mirex residue concentration.
[d] ND not detected.

Table 4. Mirex residues (μg/kg wet wt) of *Macrobrachium rosenbergii* juveniles exposed in 96-hour static toxicity tests, and residue concentrations as multiples of control levels in parentheses.

Treatment (μg/l mirex)	Nerve tissue[a]	Abdominal muscle[a]	Remaining carcass[a]	Whole animal[b]
0	125.4[c]	10.5[c]	19.5[c]	16.8
0.1	114.8	178.2	170.0	173.2
	(0.9)	(17.0)	(8.7)	(10.3)
10	123.5	243.9	66.9	142.3
	(1.0)	(23.2)	(3.4)	(9.5)
50	1,944.9	590.4	160.8	361.2
	(15.5)	(56.2)	(8.2)	(21.5)
100	459.3	673.1	117.8	380.1
	(3.7)	(64.1)	(6.0)	(22.6)
150	2,220.0	654.3	133.6	381.9
	(17.7)	(62.3)	(6.9)	(22.7)
500	4,067.7	220.6	198.8	230.5
	(32.4)	(21.0)	(10.2)	(13.7)
1,000	31,342.1	1,461.4	1,260.1	1,478.3
	(249.9)	(139.2)	(64.6)	(88.0)

[a] Average wet weight of nerve, 309.7 mg, muscle, 28.7 g, and remaining carcass, 38.3 g.
[b] Calculated total mirex residues based on proportional values in nerve, muscle and remaining carcass.
[c] Average mean residues from 4 chromatograph trials.

in water declined 10-fold over 96 hours (Table 5), and detectable concentrations were only found in 500 and 1000 μg/l mirex treatments.

Table 5. Mirex residues (μg/l) of test water used in 96-hour static toxicity tests with postlarval and juvenile *Macrobrachium rosenbergii*.

Treatment	Initial Sample	Post 96 hours
0	ND[a]	ND
0.1	11.3	ND
10	42.7	ND
50	120.5	ND
100	150.7	ND
150	263.3	ND
500	534.4	125.9
1,000	1,058.1	165.3

[a] ND not detected.

DISCUSSION

Naqvi and de la Cruz (1973) determined a 24-hour LC_{50} value for the water strider *Gerris remigis*, a 72-hour LC_{50} for the gyrnid beetle *Dinutes americanus*, and a 96-hour LC_{50} for the shrimp *Palaemonetes kadiakensis*, to be 130, 40 and 510 μg/l mirex, respectively. Postlarval *M. rosenbergii* may be more sensitive to mirex than other aquatic arthropods. Summer and Eversole (1978) determined a LC_{50} of 104 μg/l mirex for *M. rosenbergii* postlarvae, with 24-hour exposure and 72-hour post exposure period. This corroborates the present study and is expected since over shorter test periods it should take more toxicant to kill the same number of animals. It is important to note that all 3 studies were done under static conditions and LC_{50} values were calculated using nominal concentrations. Since water-borne mirex has been shown to decline in concentration over the exposure period, these values are over estimations of actual LC_{50} values.

Postlarvae exposed to 10 μg/l mirex exhibited extremely high mortality, perhaps as a result of the additional stress imposed when water became cloudy at this concentration.

Cyclodienes (e.g. mirex) are neuroactive agents which probably affect ion permeability and cause repetitive nerve impulses in insects and other arthropods (Matsamura 1976). Mirex causes increased spontaneous activity and affect synaptic transmission in the central nerve cord of crickets, *Gryllus pennsylvanicus* (MacFarlane et al. 1975). Yamaguchi et al. (1979) observed that various ATPases in the synaptic region of the rat brain were susceptible to cyclodienes, which may eventually result in the release of neurotransmitters (e.g. acetylcholine, a major excitatory neurotransmitter). Crustaceans exposed to mirex exhibited increased aggressiveness (Schoor 1974), elevated irritability, increased metabolism and convulsions (Leffler 1975), and loss of equilibrium, paralysis and death (Tagatz et al. 1976). *M. rosenbergii* exposed to mirex exhibited similar symptoms.

Low concentrations of mirex have been shown to exert delayed toxicity and sublethal effects in marine and estuarine organisms (Bookhout 1972, Bookhout and Costlow 1975, Schoor and Newman 1976). Sublethal effects of mirex include

lengthening of zoeal development of the mud crab, *Rithropanopeus harrissi* (Bookhout et al. 1972) and megalop development of the blue crab, *Callinectes sapidus* (Bookhout and Costlow 1975), and increased molt interval length of postlarval *M. rosenbergii* (Summer and Eversole 1978). In this study, juveniles molted fewer times in increasing mirex concentrations. Mirex may have the general effect of lengthening molt interval of decapods, with the more dramatic responses occurring in the earlier developmental stages.

Mirex accumulated more in nerve tissue than in other tissue components analyzed from juveniles. Higher concentrations in nerve tissue indicate that mirex moved within organisms to sites on axonal membranes. These sites may possess myelinated areas or fatty sheaths. Mirex, like many other chlorinated hydrocarbons, would be expected to enter membranes and fats because it is nonpolar and lipophilic in nature. Adult blue crabs exposed to 0.05 - 0.25 μg/l mirex from 15 min to 16 hours, accumulated 0.75 - 19 μg/kg mirex in brain and thoracic ganglia, 2nd highest to concentration in hepatopancreas with 1.6 - 31 μg/kg mirex (Schoor 1974). Lower mirex residues were found in hemolymph serum and muscle by Schoor (1974). High levels are expected in the hepatopancreas, which removes selected substances from hemolymph.

Mirex was present in control prawns, but not in control water. Prawns may have been previously contaminated with mirex from water or food in hatcheries, growout ponds, or holding tanks prior to testing. Mirex residues in water from 0.1 μg/l mirex treatments were approximately 100× higher than expected. Mirex may not have adequately dispersed in these test containers before water samples were taken, resulting in a proportionately greater amount of mirex being sampled.

Mirex residues in test water declined dramatically over 96 hours. Stein and Pittman (1978) indicated that losses of mirex in aqueous solution can be expected if water is allowed to evaporate. Adsorption and deposition on test container walls probably also accounts for some decrease.

Information presented here and in other studies indicates that severe ecological implications can result from using mirex near freshwater and estuarine systems. Mirex is a persistent pesticide that can be transported in ground water, is toxic to postlarvae at low concentrations, may influence growth at sublethal concentrations, and bioaccumulates to high levels. Therefore, mirex should not be applied to watershed areas or ponds used for *Macrobrachium* culture.

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