Effects of Mirex and Methoxychlor on Juvenile and Adult Striped Mullet, *Mugil cephalus* L.

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Mirex, a polycyclic chlorocarbon insecticide (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene), has been used many years for control of the imported fire ant (Solenopsis savissima richteri) in various southeastern U.S.A. states (COON and FLEET 1970). Accumulation of mirex residues by various species of aquatic organisms (BUTLER 1969; WOLFE and NORMENT 1973; BORTHWICK et al. 1973) is indicative of the widespread occurence of this insecticide in the environment.

Methoxychlor (1,1,1-trichloro-2,2-bis[p-methoxyphenyl] ethane) has been a commercial insecticide since discovery of its insecticidal properties (LAUGER et al. 1944), and has replaced DDT.

The purpose of this investigation has been to determine the acute toxicity of mirex and methoxychlor to striped mullet (Mugil cephalus L.) using continuous flow bioassay. The striped mullet have an extensive geographic distribution and can tolerate a wide range of physical parameters in both freshwater and marine environments (SYLVESTER et al. 1974; THOMPSON 1966).

Materials and Methods

Juvenile mullet used in this study were seined from coastal streams and bays around the island of Oahu, Hawaii. Young juveniles ranged in standard length from 20 to 43 mm and older juveniles ranged in standard length from 70 to 150 mm. Adult mullet, standard length 260 to 380 mm, were collected from the island of Hawaii. All fish were transported to the laboratory in aerated tanks and acclimated from ten days to two weeks in a 4,000 gallon vat or in ponds.

Bioassay Techniques

Four different concentrations of each insecticide (0.01, 0.1, 1.0, and 10.0 ppm) and controls were tested simultaneously. Total flow rate through glass test aquaria was 260 ml/min of insecticide solution and seawater. The number of mullet used in each test was 25 for young juveniles, 10 for older juveniles and two for adults. Tests were replicated four times at each concentration of insecticide. The dosing apparatus and procedures of the tests were similar to those described by BURKE and FERGUSON (1968) and SPRAGUE (1969). Quantitative analysis of insecticides were made by gas chromatography.

Mortalities were recorded daily during a 96-hour exposure period and percent mortality calculated according to the method given by LUDKE et al. (1971). Dead fish were removed, rinsed with acetone and frozen for tissue residue analysis. At the end of 96 hours, remaining live fish were sacrificed with acetone solution and frozen.

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Water samples were collected periodically from the test aquaria during tests and analyzed for insecticide concentration. Dissolved oxygen, temperature and salinity were monitored daily.

Residue Analysis

At the end of 96 hours, live fish were killed in acetone, then rinsed with acetone and frozen for residue analysis. Dead fish in mirex-treated tanks were removed as soon as they were observed, then rinsed in acetone and kept in the freezer. Methoxychlor was measured from fish killed by the insecticide and pooled at the end of the bioassays, except those in 0.01 mg/l methoxychlor tanks and controls (1-10 ppm). Frozen samples were minced in a laboratory blender. Approximately 5 grams of the minced sample (duplicates from individual test aquaria) were ground thoroughly with additions of anhydrous sodium sulfate in a mortar and pestle.

Results and Discussion

The results indicate that young striped mullet juveniles are more susceptible to mirex poisoning than either older juveniles or adults (Table 1). Over a 96-hour test period, no mortalities occured among older juveniles or adults. Among young juveniles highest mortalities of 32.1 percent and 26.9 percent occurred in mirex concentrations of 1.0 and 0.1 ppm. However mortalities were lower in 10.0 and 0.01 ppm. Mean deaths in these concentrations were 9.0 percent and 6.4 percent. The causes of the differences in mortality between concentrations of 10.0 and 0.01, and those of 1.0 and 0.1 are obscure. The behavior of the young juveniles was similar in all test tanks and control tanks.

TABLE 1

Percent Mortality of Striped Mullet for 96-Hour Continuous-flow Bioassay for Mirex

(Percent mortalities were calculated according to the method given by LUDKE et al. [1971]).

Sample (no. in each tank)	Mirex Level (ppm)	No. of test tanks	Mortality (%)
Young juvenile (25)	0.01	4	6.4
	0.10	4	26.9
	1.00	4	32.1
	10.00	4	9.0

Table 1 - continued

Sample (no. in each tank)	Mirex Level (ppm)	No. of test tanks	Mortality (%)
Juvenile (10)	0.01	4	0
	0.10	4	0
	1.00	4	0
	10.00	4	0
Adult (2)	0.01	4	0
	0.10	4	0
	1.00	4	0
	10.00	4	0

The anomalous results of these mirex experiments could be a result of its specific action on striped mullet and the physical parameters of the experimental conditions. The particular characteristics of the effects of mirex on fish has been reported elsewhere. In a study of the effects of mirex on bluegill, Lepomis macrochirus and goldfish, Carrasius auratus, VAN VALIN et al. (1968) reported that bluegill exposed to 0.0013 ppm and 1.0 ppm showed no relationship between mortalities and mirex exposure. However, they observed 68.8 percent mortality in goldfish in 0.1 ppm and 85.5 percent mortality in 1.0 ppm during a 308-day experimental period.

The results of the mirex residue analyses and the measured mean concentrations of mirex in the test water are presented in Table 2. The largest amounts of mirex residue were found in the adults. These large amounts could be a result of the possibility that the adults may have a relatively higher proportion of body fat compared with the juveniles. The data further indicate that mirex residue levels in the test fish increased with increasing insecticide concentration.

TABLE 2

Residue Analysis of Striped Mullet Exposed to Mirex During 96-Hour Continuous-flow Bioassay

Sample	Nominal Conc. (ppm)	Measured, Mean Conc. (ppm)	Sample Size			Mirex Residue (μg/g)	
			#	Mean	Range	Mean	SD
Young Juvenile	0.01	0.009	100	30.2	22 - 37	0.18	0.01
	0.10	0.102	100	29.1	20 - 37	0.82	0.29
	1.00	0.989	100	26.1	20 - 36	3.50	0.46
	10.00	9.524	100	30.4	20 - 38	22.51	4.30
Juvenile	0.01	0.010	40	105.0	83 - 150	0.17	0.02
	0.10	0.104	40	88.8	70 - 125	0.85	0.31
	1.00	1.108	40	108.8	83 - 137	3.89	0.16
	10.00	9.518	40	106.8	79 - 148	17.80	2.47
Adult	0.01	0.012	8	292.4	260 - 315	0.38	0.06
	0.10	0.107	8	303.4	290 - 315	1.02	0.17
	1.00	1.033	8	303.5	260 - 325	6.14	1.91
	10.00	9.540	8	309.3	274 - 333	37.29	4.82

The results of the present study indicate that methoxychlor was more toxic to juvenile and adult mullet than mirex. Young mullet were more susceptible to methoxychlor than adults. Young juveniles exposed to 10.0 ppm sustained 100 percent mortality within three hours of initial exposure while all adults exposed to the same concentration died within six hours. In 1.0 ppm all juveniles were killed in nine hours and adults in 15 hours. At a concentration of 0.1 ppm about 95 percent mortality in the juveniles occurred within 48 hours and 63 percent mortality in the adults for the same time period (Table 3). During the experiments, affected mullet in the experimental tanks showed stress behavior apparently caused by insecticide poisoning. This behavior included sudden or rapid random movements, attempts to jump out of the test tanks, gradual loss of equilibrium and cessation of respiratory movements.

TABLE 3

Percent Mortality of Striped Mullet for 96-Hour Continuous-flow Bioassay for Methoxychlor

(Percent mortalities were calculated according to the method given by LUDKE et al. [1971])

Sample (no. in each tank)	Methoxychlor level (ppm)	No. of test tanks	Mortality %	
			48-hour	96-hour
Young juvenile (25)	0.01	4	4.0	5.1
	0.10	4	94.8	97.0
	1.00	4	100	---
	10.00	4	100	---
Adult (2)	0.01	4	0	0
	0.10	4	62.5	100
	1.00	4	100	---
	10.00	4	100	---

Table 4 indicates that relatively small amounts of methoxychlor residues accumulated in the mullet tissues. Juveniles exposed to 0.01 ppm accumulated a mean of 0.06 μg/g methoxychlor over a 96-hour period and adults accumulated 0.2μg/g over the same time period.

TABLE 4

Residue Analysis of Striped Mullet Exposed to Methoxychlor During 96-Hour Continuous-flow Bioassay

Sample	Nominal Conc. (ppm)	Measured, Mean Conc. (ppm)	Sample Size (mm)			Residue (μg/g)	
			#	Mean	Range	Mean	SD
Young juvenile	0.01	0.009	100	34.3	27 - 40	0.06	0.015
	0.10	0.104	100	34.3	29 - 41	1.64	0.572
	1.00	1.018	100	34.6	37 - 42	2.40	0.589
	10.00	9.361	100	35.8	25 - 43	1.69	0.296
Adult	0.01	0.010	8	297.0	258 - 380	0.20	0.038
	0.10	0.108	8	312.3	294 - 342	11.92	1.999
	1.00	1.022	8	296.3	205 - 328	11.83	2.398
	10.00	9.690	8	321.5	298 - 363	11.12	2.724

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