BIOASSAY PROCEDURE TO EVALUATE THE ACUTE TOXICITY
OF SALINITY AND GEOTHERMAL POLLUTANTS (PESTICIDES)
TO GAMBUSIA AFFINIS

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ABSTRACT

The salinity tolerance of Gambusia affinis was determined in static bioassays. Gambusia easily tolerated salinity of 47.5‰ in 96 hour static bioassays. Survival at this level was 93.3% with the lowest survival being 68.% at 40.% salinity. The acute toxicities of endrin, DDT, aldrin, and dieldrin to Gambusia were determined by static and intermittent-flow bioassays. Toxicity was measured as the Median Lethal Concentration (TL_{50}) for 96-hr exposures. TL_{50} values were lower in the intermittent-flow bioassays than in static bioassays. We also compared residue concentrations in surviving and dead fish from the intermittent-flow bioassays. Residue concentrations in fish that died during tests were higher than those of fish that survived. However, the range of concentrations in dead and living fish overlapped.

INTRODUCTION

Geothermal brines from geothermal energy development efforts being pursued in the Imperial Valley of Southern California is a common pollutant and the potential hazard of the hot and highly saline brines pose to the aquatic fauna in the area is great. The salinity of the Salton Sea which is situated in the Imperial Valley is greater than that of the Pacific Ocean, and has been increasing due to the net effect of evaporation and influent tailgate irrigation water via the New and Alamo Rivers (4,000 to 6,000 ppm, TDS). The marine organisms have stable reproduction which maintains the population but the food chains are simple with one or two species per trophic level.

This project was concerned with the effects of geothermal brines, pesticides, herbicides and heavy metal concentrations on an important member of the aquatic community of the Imperial Valley, Gambusia affinis. This organism may possibly serve as an indicator of increased aquatic pollution in the Imperial Valley. The mosquitofish, as Gambusia is sometimes called, has proven to be an excellent indicator organism for study to detect dangerous levels of aquatic contaminants (Cully and Ferguson, 1969).

The mosquitofish, family Poeciliidae, was introduced into the Salton Sea sometime prior to 1929. It is native to the eastern United States, but was distributed widely during the early part of this century to aid in mosquito control. It is common in the fresh waters surrounding the sea and is often found in the shallow areas, particularly near freshwater inlets. This unique habitat make them ideal potential

indicators of geothermal brine salinity, heavy metal and pesticide contamination of not only the Salton Sea but also the New and Alamo Rivers.

Specifically the present contract study was undertaken to accomplish the following objectives:

1. Design and develop a series of static bioassay tests to accurately determine the salinity tolerance of Gambusia affinis.
2. Design and develop a series of static bioassay tests to accurately determine the acute (48 and 96 hr LC₅₀) toxicity the heavy metal components of the geothermal brines in the Imperial Valley to Gambusia affinis.
3. To evaluate the toxicity of several pesticides and herbicides used in the agricultural activities in the Imperial Valley to Gambusia affinis.
4. To test for synergram and antagonism by combining salinity, heavy metal, pesticides and fish density loading.

The information obtained from experiments designed to achieve these objectives is highly important in characterizing the tested material.

METHODS AND MATERIALS

Stock cultures of mosquito fish were collected for all experiments by the method outlined in the Appendix. Details on rearing and handling the organisms also are given. The present study was conducted in two phases to allow two teams of investigators to carry on most of the experimentation at the same time. The materials and methods used thus varied with each phase. Phase I was a study of the salinity tolerance of Gambusia affinis and Phase II focused on the acute toxicity of Gambusia to pesticides in varying salinity. Heavy metals were introduced but the results were inconclusive and no good data could be generated from these tests.

Phase I. Salinity Tolerance Tests

Gambusia were acclimated in the laboratory in 20 gallon holding units (aquariums) for 4 weeks in 37% salinity. Tests for tolerance were conducted in 1-gallon glass jars containing 2 liters of filtered estuarine water with NaCl added to achieve the desired salinity. Ten organisms were placed in each jar directly from the holding unit with no acclimation to hypersalinity. Controls were run concurrently in 1-gallon jars at 37% salinity and all tests were performed in duplicate or triplicate with each unit receiving aeration. Exposure time was 96 hr. The results of these tests are given in Table 1.

Phase II. Acute Toxicity of pesticides to Gambusia

The organisms were removed from the general holding tank (55-gallon aquarium) and acclimated in another holding unit (29 gallon aquarium) containing filtered estuarine water with a salinity of 28‰. Because of the oxygen demand of turbid, saline water, we had a little difficulty maintaining acceptable dissolved oxygen levels for 96 hr static bioassays. Therefore, we used only 2 or 3 fish per concentration to avoid driving the toxicant from solution by aeration. The salinity tests were run with 10 organisms per unit under mild aeration without appreciable alteration of salinity. Originally, we had hoped to compare toxicity data obtained by static and intermittent-flow bioassays. But because of the small numbers of fish used in the static tests, these data were primarily for establishing pesticide concentrations to be used in the intermittent-flow bioassays. Limited holding space and small numbers of fish of the same size prevented us from running replicates. Satisfactory levels of dissolved oxygen were easily maintained in intermittent-flow bioassays since the water is replenished regularly. Therefore, we exposed 5 fish at each concentration.

All bioassays in this phase were run in 65-liter glass aquaria. Test water was delivered through a sand filter, but no attempt was made to maintain a specific turbidity or salinity although the effects of these factors was part of the objective of the tests along with the toxicity of the pesticides. The dilution water was raw pond water treated with NaCl to give a salinity at or near 30-33‰. In the intermittent-flow bioassays, turbidities and salinities were determined with a Hach 2100 nephelometer and a Kahlsico salinity hydrometer,

respectively. Turbidity and salinity were determined only on the first day in the static tests. Temperature of neither the static or intermittent bioassays were controlled and fluctuated from 14 to 18°C. Test fish were not fed for 24 hours preceeding or during a bioassay.

Pesticides used in this study were supplied by Fisher Scientific, Raleigh, N. C. The pesticides were technical grade and the specific ones used in this study that produced the most definitive results in bioassay application consisted of aldrin, dieldrin, endrin, and DDT.

The proportional diluter we used for the intermittent-flow tests was described by Mount and Brungs (1967). All intermittent stock solutions had ethanol as the solvent. Stock solutions for static tests were prepared by dissolving the pesticide in Acetone. Serial concentrations, of the stock solution, were pepetted into the aquaria at least 2 hours after the fish had been introduced. The same amount of acetone used in the highest concentration was added to the control. Mortality was recorded daily, by only the 96-hour results are reported. The TL_{50} values (tolerance limits for 50% of the test animals) for static tests were determined graphically with logarithmic-probability paper. The diluter data were converted to logs and probits and a linear regression equation calculated according to a modification of Litchfield and Wilcokin's (1949) Method.

Whole body residue concentrations were determined for fish exposed in either preliminary or definitive diluter bioassays. The analytical procedures as described by Earnest and Benville (1971) were used. Mean residue concentrations were recorded for fish that either died during, or survived the 96-hr. bioassays.

RESULTS AND DISCUSSION

Phase I. Salinity tolerance: *Gambusia* tolerated extremely high salinities (Table 1). Survival was 93.3% at 47.5‰. The lowest survival was 68.0% at 40.0‰. However, it appears that in this instance mortality was due to causes other than salinity, since mortalities occurred in all concentrations, including the controls. Whether mosquitofish can tolerate salinities higher than 47.5‰ was not determined, although it appears that they could.

Phase II. Acute Toxicity of Pesticides to *Gambusia*: In static bioassays, endrin was the most toxic of the four pesticides tested (Table 2). Because of the small number of fish exposed at each concentration, little emphasis could be placed on the static bioassay data. The TL_{50} values as determined by intermittent-flow bioassays were much lower than those for the static tests (Table 2 and 3). For example, the static TL_{50} of DDT was 7.6 µg/l but the intermittent-flow value was 0.45. As in the static tests, endrin was significantly more toxic than aldrin or dieldrin.

Because of the small number of fish, pesticide concentrations and their range are presented for all fish that were dead, or all fish that survived at the termination of the bioassay (Table 4). Residues of endrin found in the tissues of dead endrin-exposed fish were lower than those of DDT in dead DDT-exposed fish. In three of four samples, less aldrin than dieldrin was found in the tissue of the aldrin-exposed fish. Thus, a considerable amount of aldrin appears to have been metabolized to dieldrin by the time the fish had died or the bioassay

was terminated. Any effects that the aldrin-dieldrin metabolic relationship might have had on toxicity values is unknown. Residue levels in some fish that died during certain experiments were lower than the residual levels in fish that survived at 96 hours. This same relationship was observed in other work by Woodwell et al (1967).

The tremendous amount of pesticides used in the Imperial Valley along with the ever increasing salinity in the Salton Sea and the freshwater systems makes it almost mandatory that we have some knowledge of the salinity tolerance and the acute effects these compounds and conditions have on the aquatic fauna of the area. We found that less than $3\mu\text{g/l}$ endrin, DDT, aldrin, and dieldrin under the described experimental conditions, are lethal to the mosquitofish. The salinity tolerance experiments indicate that *Gambusia* will not be a good indicator of increased salinity in the Imperial Valley because this organism is unusually tolerant of high saline environments. Although our preliminary studies (see Appendix) indicate tolerance for relatively high levels of toxaphene, lindane, heptachlor and azodrin in freshwater bioassays. Eisler (1970) found that in static bioassays, that different salinities had little effect on the toxicity of DDT or endrin to the species of fish tested. Katz and Chadwick (1961) also reported that different salinity levels had little effect on the toxicity of endrin to stickleback, but temperature variations greatly effected the toxicity of endrin to bluegills.

The results obtained with a proportional diluter, for the size of the fish (2.0 to 5.8 cm in length) we tested, are more meaningful than those in the static tests. Under static conditions, oxygen and waste products are limiting factors. Also, pesticides may be

absorbed by the organism, glass and detritus, resulting in higher TL_{50} values.

Data on whole body residue concentrations in organisms have certain limitations since length of exposure and concentration of contaminant are unknown (Butter, 1966). However our results do suggest a correlation between residue concentration and mortality after relatively brief exposures, even though the effect of the residues at specific sites is unknown. Typically, when using estuarine water, similar to that shipped to us from the Imperial Valley, we experienced variations in salinity and turbidity in our test solutions. (The Imperial Valley samples had precipitates in large amounts in the bottom of each bottle when they arrived, thus we could only measure salinity and duplicate the saline properties in our dilution water, which was estuarine in origin). This may have contributed to some of the variation in our bioassay results. However, during the winter and spring runoff when turbidities are high, salinities are low, and estuarine fish are at the state, an estuary, such as the one at Piners Island is probably receiving its heaviest contaminant load. This condition probably exists in the Imperial Valley. Bioassays must be run under these conditions to best simulate the factors that are affecting estuarine organisms in their natural environment.

TABLE 1
PERCENTAGE SURVIVAL OF GAMBUSIA AFTER EXPOSURE TO HIGH SALINITIES
FOR 96 HOURS

TEST NUMBER	Controls		12.0%		24.0%		28.0%		32.0%		38.0%		40.0%		42.5%		47.5%	
	* NT	* PS	NT	PS	NT	PS	NT	PS	NT	PS	NT	PS	NT	PS	NT	PS	NT	PS
1	20	100	20	95.0	-	-	-	-	-	-	-	-	20	75.0	-	-	-	-
2	30	96.7	-	-	30	90.0	-	-	30	93.3	-	-	30	63.3	-	-	-	-
3	30	86.7	-	-	-	-	30	96.7	30	86.7	-	-	-	-	30	93.3	-	-
4	30	96.7	-	-	-	-	-	-	30	90.0	30	100	-	-	-	-	30	93.3
Totals	110	94.6	20	95.0	30	90.0	30	96.7	90	90.0	30	100	50	68.0	30	93.3	30	93.3

NT = Number Tested PS = Percent Survival

TABLE 2
TOXICITY OF FOUR PESTICIDES TO GAMBUSIA IN STATIC BIOASSAYS

Pesticide	Turbidity (JTU)	Salinity (0/%)	96-hr TL ₅₀ (µg/liter)
Endrin	5.0	26.0	0.8
Dieldrin	28.0	15.0	3.7
Aldrin	5.0	28.0	7.4
DDT	0.5	26.0	7.6

TABLE 3
TOXICITY OF FOUR PESTICIDES TO GAMBUSIA IN INTERMITTENT-FLOW BIOASSAYS

Pesticide	Turbidity (JTU) Mean (range)	Salinity (0/00) Mean (range)	96-hr TL ₅₀ (95%CL) (µg/liter)
Endrin	7 (2-8)	28 (27-28)	0.12 (0.06-0.25)
DDT	12 (9-16)	18 (13-23)	0.45 (0.21-0.94)
Dieldrin	6 (5-8)	28 (26-28)	1.50 (0.73-3.20)
Aldrin	7 (5-10)	25 (25-26)	2.26 (1.08-4.74)

TABLE 4
RESIDUE CONCENTRATION OF FOUR PESTICIDES IN DEAD
AND SURVIVING GAMBUSIA AFTER 96 HOURS OF EXPOSURE
IN INTERMITTENT-FLOW BIOASSAYS

	Dead	Alive	Control
Pesticide	mean (range)	mean (range)	
Endrin	0.13 (0.02-0.27)	0.07 (0.03-0.09)	ND
DDT	0.55 (0.44-0.65)	0.35 (0.24-0.48)	0.040
Dieldrin	2.33 (1.68-3.07)	1.34 (0.67-2.24)	0.027
Aldrin	1.00 (0.23-3.40)	0.42 (0.12-0.92)	ND
Dieldrin*	0.78 (0.19-1.02)	0.58 (0.24-1.00)	

*Dieldrin Concentrations in fish exposed to aldrin.

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APPENDIX A

METHODS AND PROCEDURES FOR COLLECTION AND MAINTENANCE

Gambusia were seined from shallow brackish and freshwater ponds around Beaufort and Durham, N. C. After collection, the fish were placed in holding tanks for transport to laboratory facilities. The size of the individuals collected ranged from 2.0 to 5.8 cm in length. After collection and transfer to laboratory tanks, the fish that were taken from freshwater ponds required 24 hour treatment in 2.5% nonionized table salt (NaCl) to rid them of fungus and tail rot. Those collected in brackish water required no treatment. After the fungus treatment all were held in freshwater (dechlorinated tap water) from 1 to 5 days before preliminary testing. During the treatment and the post treatment periods Gambusia was fed either rolled oats, dried Daphnia or a synthetic diet. No food was supplied to fish 24 hours before test. Water temperature in holding tanks and the test solutions were allowed to adjust to room temperature before experiments were conducted.

TEST PROCEDURES (PRELIMINARY)

For each test five fishes of uniform size (3.0 to 4.0 cm long) were transferred to a 1000 ml beaker containing 500 ml of the diluted pesticide (The pesticides and herbicides were diluted with either acetone or ethanol for the appropriate serial dosage). Five replicates represented each concentration. The control beakers received

500 ml of tap water and acetone or ethanol. These tests were repeated four times.

The results indicating death and loss of balance were recorded every 30 minutes until the end of 8 hours. When the fish were examined after 24 hours, the results remained almost the same. Death during this period may be due to a lack of oxygen in the test water.

RESULTS (PRELIMINARY TESTS)

The fish did not show any sign of being adversely effected in Azodrin at concentrations of 250 ppm after 8 hours of exposure. Data obtained from other pesticides, including a mixture of heptachlor and toxaphen (10:1) are summarized in Tables 1, 2, 3, and 4. The LC_{50} and LB_{50} (loss of balance 50) were determined and data are presented in Table 5.

The results of the bioassays performed during the past three months tend to indicate that *Gambusia* is well suited for use as an indicator of both chlorinated aryl hydrocarbon and organophosphorus pesticides in water.

Loss of balance was a common response after Gambusia was exposed to the higher concentrations of the pesticides. This response can be unmistakingly recorded when the fish loses its ability to swim parallel to the bottom of the beaker or become unable to maintain its body in the proper vertical plane with the container. Loss of balance always preceded death, and a sublethal concentration (Less than 5 ppm) the fish rarely recovered their balance, after being transferred to

TABLE 1 - Toxicity of Toxaphen to Gambusia during 8 hrs of Exposure

Time in Minutes	Percent Mortality for varying ppm.						Percent Loss of Balance for varying ppm.					
	54.4	27.2	13.6	6.8	3.4	0	54.4	47.2	13.6	6.8	3.4	0
30							48	24				
60							92	36				
90							96	44	8	8		
120							96	56	12	12	2	
150							96	60	32	20	4	
180							100	68	32	20	4	
210	88						100	76	46	28	12	
240	92	12					100	80	44	28	12	
300	92	80	40	25	4		100	88	48	32	12	
360	96	88	48	25	4		100	92	52	32	12	
480	100	96	50	25	4		100	96	56	32	16	

TABLE 2 - Toxicity of Lindane to Gambusia During 8 Hours of Exposure

Time in Minutes	Percent Mortality for varying ppm.						Percent Loss of Balance in Varying ppm					
	20.0	10.0	5.0	2.5	1.25	0	20.0	10.0	5.0	2.5	1.25	0
30							84	12				
60							92	24				
90							92	32				
120							96	36				
150	8						96	36				
180	16						96	40				
210	28						100	40				
240	36						100	44				
300	52	12					100	44	4			
360	68	40					100	44	4			
480	96	44	4				100	44	4			

TABLE 3 - Toxicity of Heptachlor to Gambusia During 8 Hours of Exposure

Time in Minutes	Percent Mortality for Varying ppm.							Percent Loss of Balance for Varying ppm.						
	320	160	80	40	20	10	0	320	160	80	40	20	10	0
30								8	4	4	8	4		
60								40	4	4	8	4		
90								88	20	8	8	4		
120								92	40	12	8	4		
150								96	60	12	12	4		
180	96							100	68	12	12	4		
210	96							100	76	20	12	8		
240	96							100	76	24	12	8		
300	100	76						100	76	24	24	8		
360	100	84	36					100	88	36	32	12	4	
480	100	84	48	40	12	4		100	92	64	40	76	8	

TABLE 4 - Toxicity of A Mixture of Heptachlor + Toxaphene (10:1) to Gambusia
During 8 Hours of Exposure

Time in Minutes	Percent Mortality for Varying ppm.							Percent Loss of Balance for Varying ppm.						
	320	160	80	40	20	10	0	320	160	80	40	20	10	0
	+	+	+	+	+	+		+	+	+	+	+	+	
	34	17	8.5	4.25	2.12	1.06		34	17	8.5	4.25	2.12	1.06	
30								4						
60								10	20					
90								28	24	4				
120								28	24	4				
150								36	24	4				
180								40	36	4				
210	4							40	56	4				
240	8	4	4					56	56	4				
300	16	4	4					72	60	12				
360	36	4	4					84	60	12				
480	60	28	8					100	84	32				

TABLE 5
TOXICITY OF CERTAIN PESTICIDES TO GAMBUSIA AFFINIS
AFTER 8 HOURS OF EXPOSURE

Pesticide	LC ₅₀ in ppm.	LB ₅₀ in ppm.
Toxaphene	10.0	12.0
Lindane	10.2	11.2
Heptachlor	54.2	9.0
Heptachlor + Toxaphene (10:1)	260.5	281.0
Azodrin	-	-

stock holding tanks. Azodrin, up to 400 ppm did not effect either the balance or mortality of Gambusia during the 8 hours of exposure.

The resistance of Gambusia to this level of Azodrin is unclear. This is a very effective and potent organophosphate pesticide and we have no reason to believe our shipment is in any way lost its potency. There is some evidence in the literature that Gambusia (Culley and Ferguson, 1969) does develop resistance to pesticide toxicity and thus exists the possibility that our populations have developed resistance to Azodrin.

Laboratory selection studies are underway and upon completion should provide further insight into the resistance of Gambusia populations in our area to Azodrin. These fish are developing into very versatile and valuable assay organisms. Their presence in the Imperial Valley will make water quality monitoring for pesticide and herbicide contamination a realistic and practical program.

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