RELATIONSHIP OF DIELDRIN TOXICITY TO CONCENTRATIONS OF DIELDRIN IN THE BLOOD AND BRAIN OF THE GREEN SUNFISH, LEPOMIS CYANELLUS

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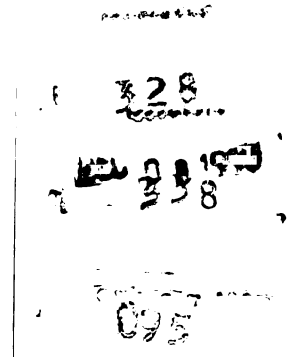

ABSTRACT

RELATIONSHIP OF DIELDRIN TOXICITY TO CONCENTRATIONS OF DIELDRIN IN THE BLOOD AND BRAIN OF THE GREEN SUNFISH, LEPOMIS CYANELLUS

by Roger L. Hogan

Green sunfish, Lepomis cyanellus, were exposed to concentrations of dieldrin which were maintained for periods over 124 hours. Blood and brain samples from the fish analyzed by gas chromatography indicated threshold toxic levels of approximately 6.0 micrograms per gram for blood and 9.0 micrograms per gram for the brain. Surviving fish had levels markedly lower than those experiencing mortality. Correlations were noted between dieldrin levels in blood and brain, and between blood and water dieldrin concentration. Surviving fish exhibiting severe pesticide poisoning symptoms had significantly higher blood and brain concentrations than did those showing moderate to minimal symptoms.

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By

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INTRODUCTION

With the current emphasis on the dangers of the chlorinated hydrocarbon pesticides it becomes increasingly important to extablish methods by which their presence and effects on fish can be assessed. There have been several studies done on levels of various pesticides and their distribution within the organs of fish, but very few of these have been of a toxicological nature. Mount, *et al.* (1966) established the long-term toxic level of endrin in the blood of the channel catfish, *Ictalurus punctatus* (Rafinesque), and thus suggested the use of blood as an indicator tissue for endrin-caused fish kills. Blood threshold levels of endrin were set for the gizzard shad, *Dorosoma cepedianum*, by Brungs and Mount (1967). Blood serves as the transporter for the chlorinated hydrocarbon pesticides and distributes them throughout the exposed fish.

It is generally accepted that the nervous system is the primary site of action for chlorinated hydrocarbon pesticides. Matsumura and O'Brien (1966[a]), working with DDT and the central nervous system of the American cockroach, *Periplaneta americana* (L.), found lipids in the nerve cord to be the primary binding substance. Using DDT and components of the American cockroach nerve,

Matsumura and O'Brien (1966[b]) suggested that the pesticide combines with the nerves to form a charge transfer complex which interferes with potassium ion efflux and nerve transmittance. Narahashi and Haas (1968) came to similar conclusions while studying the effects of DDT on components of lobster (Homarus sp.) nerve membrane. Some investigators, notably Dale, et al. (1962) have correlated clinical signs of DDT poisoning with concentrations of the pesticide in the brain of the rat and also noted sharp increases in plasma concentrations of DDT after administration. The plasma concentrations did not vary significantly, however, from the onset of symptoms, through convulsions, to death. Dale, et al. (1963) and Hayes and Dale (1964) found essentially the same brain-DDT level-symptom correlation in their studies with rats, and Stickel, et al. (1966), working with birds, observed essentially the same phenomenon. Using rats and the chlorinated hydrocarbon insecticide dieldrin, Heath and Vandekar (1964) concluded that the brain was the target organ upon which the dieldrin acted to cause death.

This study was initiated to determine the relationship of dieldrin toxicity to concentrations of dieldrin in the blood and brain of the green sunfish, Lepomis cyanellus.

METHODS

Green sunfish with a weight range of 33.75 grams tO 70.62 grams and a mean weight of 47.20 grams, were obtained from a pond located on the Kellogg Bird Sanctuary near Hickory Corners, Michigan. They were held a minimum of three weeks in a 500-gallon holding tank with a carbon-filtered water re-cycling system. Six blood and six brain samples taken to determine the presence of pesticide residues, were negative. Following the holding period, 3 replications were run with 10 fish in each replication. The fish were placed in a 70-liter test tank to which was attached a pump to facilitate complete mixing of the water and dieldrin, and a glass wool filter to eliminate detritus and bacteria build-up. Water in the tank maintained a temperature of 18 degrees C. and had been previously carbon filtered to eliminate any traces of chlorinated hydro-carbons. Dieldrin was added to the tank by a marriotte bottle containing 4 liters of water and 2000 micrograms (500 ppb) of dieldrin. The pesticide had been added to the marriotte bottle with 5 milliliters of acetone. The bottle could be calibrated to add from 2 to 10 milliliters per minute with reliable long-term accuracy. Rates of addition during the 3 replications ranged from 4.0 to 6.5 milliliters per minute. Adsorption on glass walls of the

tank accounted for a large portion of dieldrin losses in the 70-liter test tank and filter. Three studies were carried out to determine the amount of dieldrin losses attributable to glass adsorption. In each study 1500 micrograms of dieldrin were added to the covered, non-aerated tank, and 6 water samples were taken at intervals between 1 and 24 hours. An average of 55% of the initial dieldrin dosage was adsorbed in 24 hours, with 45 % occurring within the first 8 hours (Figure 1). Decreases in water concentration from the 8-hour level to the 24-hour level, at which time the adsorption study was terminated, occurred at the average rate of 1.19% per hour. Aeration losses, as discussed by Mount (1962) and Bowman, et al. (1964), were computed in one of the adsorption studies by using the 24-hour dieldrin water level as the starting point. The water was then vigorously aerated for an additional 24 hours after which a water sample was taken to determine the dieldrin concentration. Allowing for the 1.19% per hour adsorption loss determined earlier, the 24-hour aeration loss was found to be 10.44%.

Twenty-four hours before the initiation of each replication, 1000 micrograms of dieldrin were added to the test tank to allow for glass adsorption losses. Water samples were taken at the end of the 24-hour period to

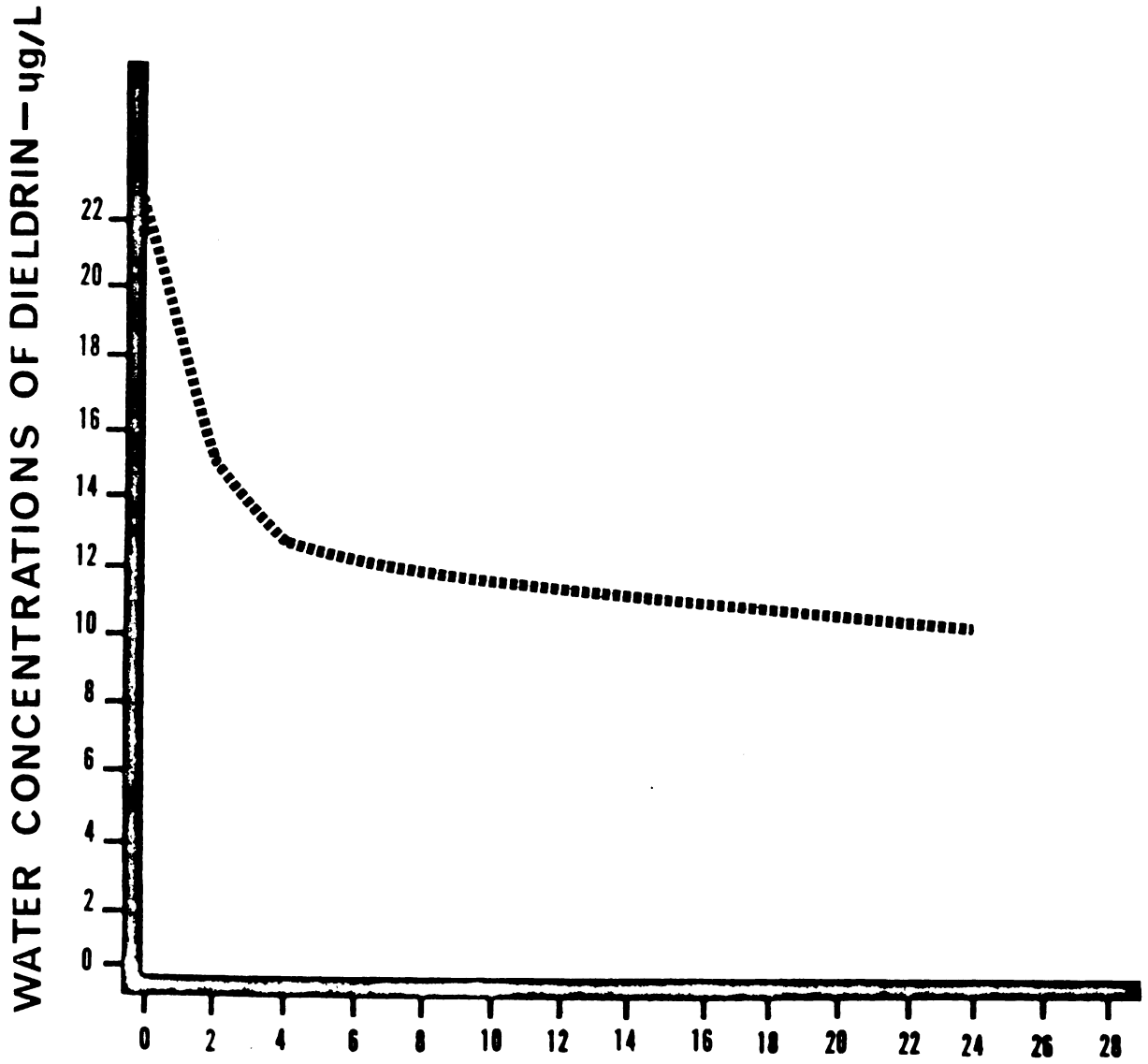

FIGURE 1. ADSORPTIVE LOSSES OF DIELDRIN IN TEST TANK AND FILTER

determine levels of dieldrin existing just prior to the addition of the fish. Three replications having a mean dieldrin concentration of 6 parts per billion were run, the duration of each being determined by the length of time required for 50% of the test fish to die (Figure 2). The criteria for death was set at that point where there was no longer opercular action and the fish could not be stirred to movement by its removal from the tank. All fish were removed immediately after death and blood samples taken by severing the caudal peduncle. Blood aliquots were then weighed and frozen. The brain cases were removed and fixed in a 10% formalin solution for 24 hours after which the brains were excised and weighed. Fixation facilitated the removal of the brains from the brain case and also provided a method of temporary storage. The surviving fish were sacrificed using a 10% tricaine methane-sulphonate solution; brain and blood samples were taken in the above described manner. Length, weight, sex, and previously noted symptoms of all specimens were recorded.

Blood and brain samples were assayed using a modification of the method described by Schafer, Busch, and Campbell (1963). The samples were saponified with alcoholic sodium hydroxide over a hot plate which allowed a slight refluxing action. After cooling, the samples were extracted

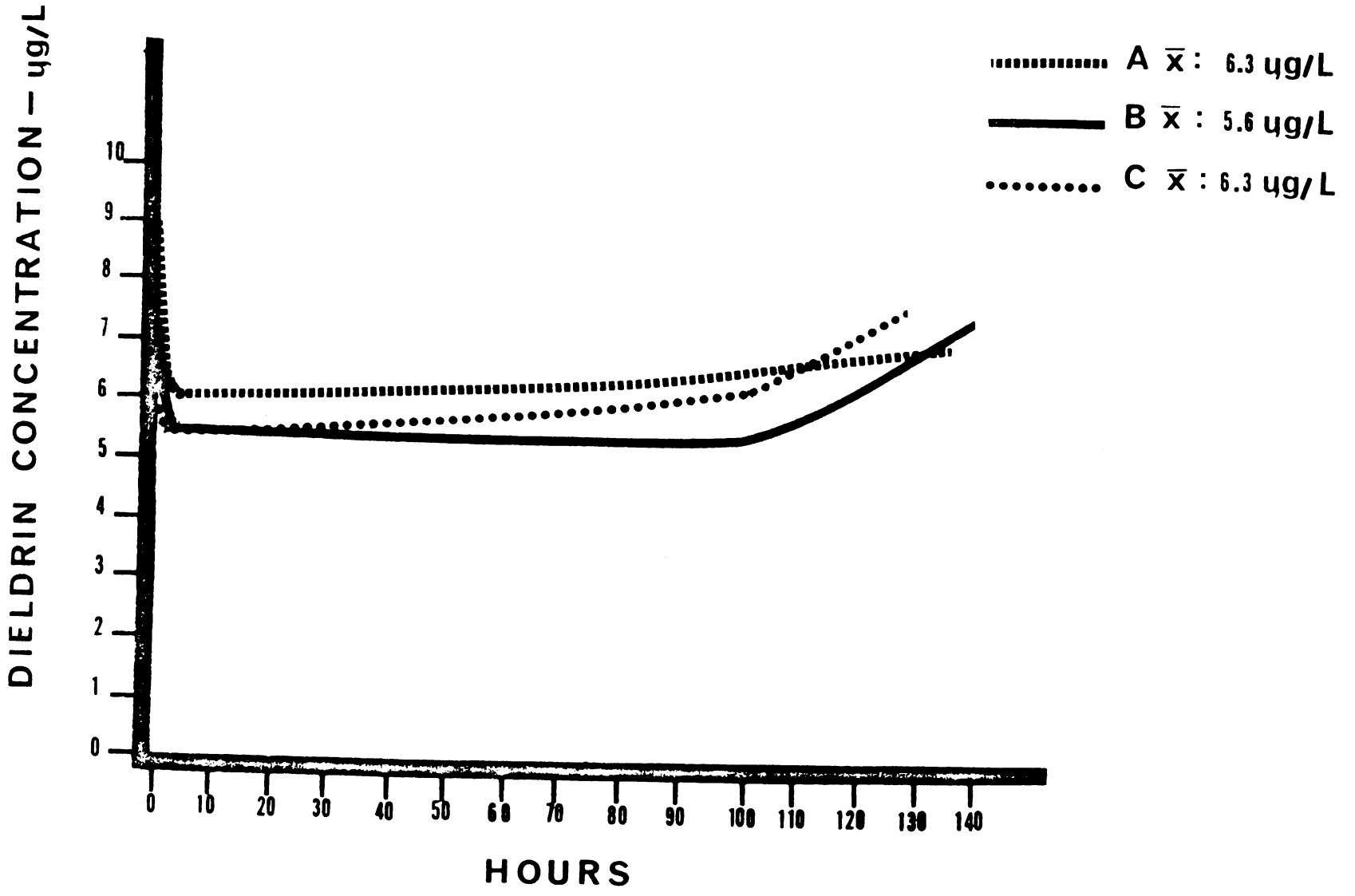

FIGURE 2. TEST TANK WATER CONCENTRATIONS OF DIELDRIN

into petroleum ether and concentrated to 15 milliliters. Measured volumes were then injected into an Aerograph 550B electron capture gas chromatograph equipped with a column of Chromosorb w packing coated with a 4% QF-1 liquid phase. Operating temperature was 175 degrees C and the nitrogen flow, 35 milliliters per minute. Three aliquots of each sample were injected and the resultant peaks compared to .1 microgram per milliliter standards which were run after each sample injection. Standards were prepared with Shell analytical standard 99+% HEOD (dieldrin). Reagent blanks and blood and brain samples from the control fish were consistently negative. Five samples each of blood and brain were fortified with dieldrin and interspersed with the normal samples in order to determine efficiency of extraction. Average extraction efficiencies were noted of 94.92% with a standard deviation of 8.8% for the blood, and 92.84% with a standard deviation of 9.7% for the brain.

An average of twenty-five 100-milliliter water samples were taken during each of the replications. These 100-milliliter aliquots were extracted into 20 milliliters of petroleum ether by shaking for 4 minutes and collecting and concentrating the supernatant. Average extraction efficiency was determined by the method described above and found to be 97.46% with a standard deviation of 5.2%.

RESULTS

During the experiments determinations of dieldrin uptake rates were made during periods when water concentrations of the pesticide remained at the same level for durations ranging from 5 to 8 hours, after fish had been exposed from 70-110 hours. At least one sample was taken from each of the 3 replications and micrograms of dieldrin uptake/gram of fish/hour were computed. These computations were made under the assumption that all dieldrin losses not due to glass adsorption and aeration could be attributed to uptake by fish. These losses were determined by comparing the amount of pesticide present at the beginning of each time period, and the amount added by the marriotte bottle, to the dieldrin level at the end of the time periods. After allowance was made for aeration and adsorption losses, values were calculated (Table 1).

Table 1. Hourly rate of dieldrin uptake per gram of 10 green sunfish.

Time Period (hours)	$\bar{x}$ water concentration for the period (ppb)	Dosage rate ug/min.	Total Weight of 10 green sunfish (s)	Rate of uptake (ug/g/hr)
8	5.98	1.59	460.20	.198
8	6.28	1.59	460.20	.213
6	5.94	1.88	458.37	.253
8	6.32	1.97	497.41	.251
5	6.51	1.87	497.41	.248

As is shown by Table 2, with two exceptions in each instance, all fish that died had blood dieldrin levels of above 5.17 micrograms per gram, and all that survived had levels less than this amount. Comparing the means of the two groups of blood levels using the Mann-Whitney U test with $N_2 = 16$, significance was shown at the .001 level (Siegel, 1956). The variance and standard deviation for blood dieldrin concentrations of fish that died were 1.180 and 1.086 respectively. Concentration of dieldrin in the water at time of death influenced the pesticide blood level (Figure 3). Regression analysis of blood on water gave significance at the .01 level with 13 degrees of freedom.

As was the case with the blood, higher concentrations of dieldrin were found in the brains of the fish that died. With one exception, all mortalities had brain concentrations above 8.70 micrograms per gram, and all that survived, again with one exception, had brain levels below this figure (see Table 2). The Mann-Whitney U test indicated differences between the means of the two sample groups ($N_2 = 16$) to be significant at the .001 level. Values for the variance and standard deviation of the dieldrin concentrations in the brains of the dead fish were 4.049 and 2.012 respectively. Blood to brain correlation was indicated by a correlation coefficient of .95

Table 2. Relationship of blood and brain dieldrin concentrations to death and to symptoms in surviving green sunfish.

Blood dieldrin concentration (ug/g)	Brain dieldrin concentration (ug/g)	Symptoms
3.153	12.183	Death
5.738	10.789	
5.167	11.537	
6.446	13.350	
6.714	11.988	
5.381	8.900	
6.055	9.090	
6.015	8.718	
6.384	9.420	
3.740	8.706	
5.308	10.477	
6.521	8.602	
5.554	10.108	
6.931	10.461	
3.659	9.740	Hyperactive - some equilibrium loss
5.147	7.998	Hyperactive - some equilibrium loss
4.878	7.398	Hyperactive - erratic darting
4.740	7.147	Hyperactive
5.616	8.654	"
5.137	6.999	"
4.646	6.059	"
3.935	8.212	General darkening-vertical bars of
5.787	6.880	dark coloration-some hyperactivity
4.685	3.889	General darkening
4.674	3.763	" "
4.967	6.052	" "
3.526	3.811	" "
3.256	6.086	None
3.641	6.623	"
2.786	5.403	"

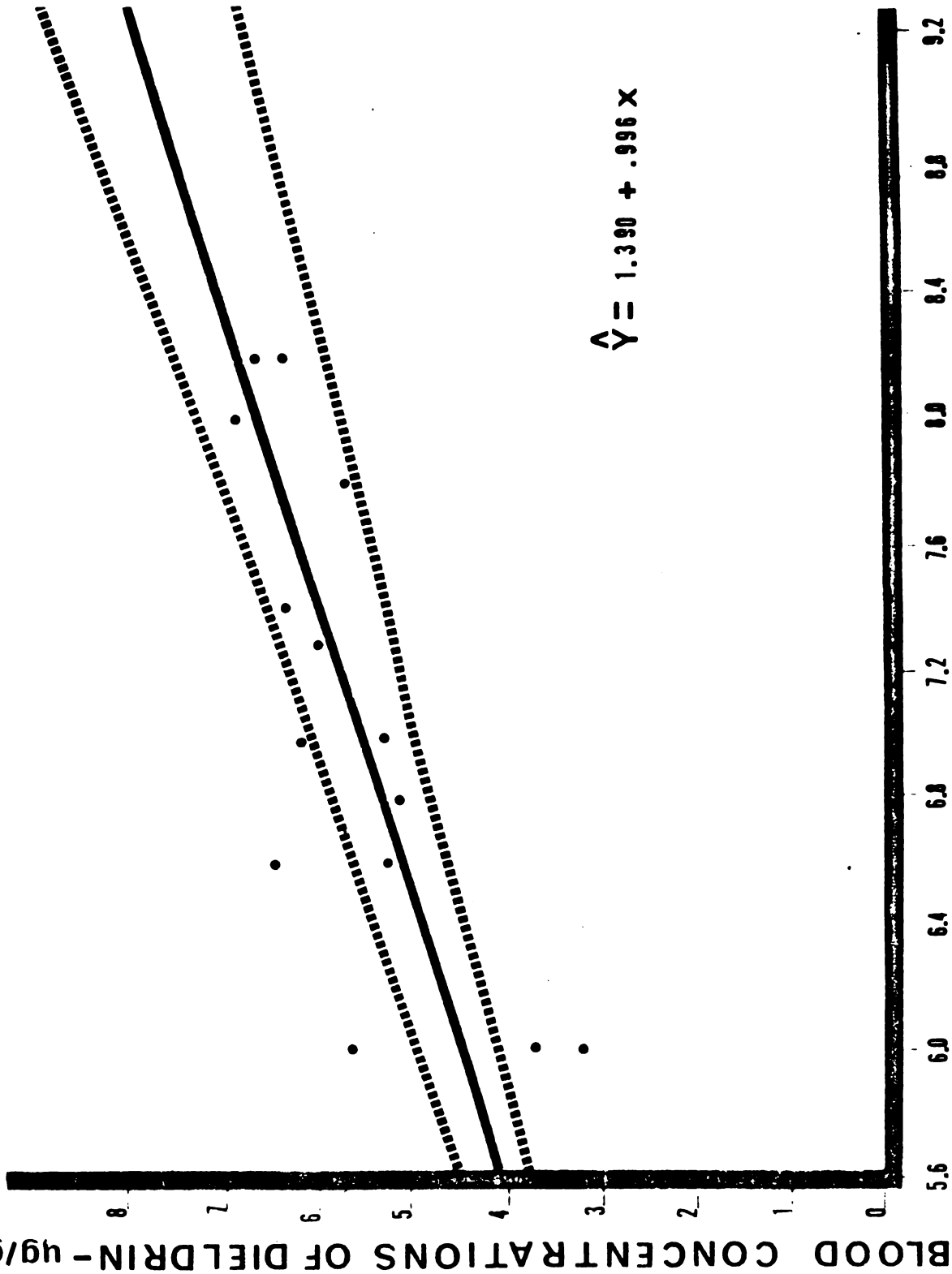

FIGURE 3. REGRESSION OF BLOOD DIELDRIN LEVEL OF FISH ON WATER CONCENTRATIONS OF DIELDRIN AT DEATH-ug/L

with 29 degrees of freedom. Regression analysis of brain on blood gave significance at the .05 level.

Symptoms of pesticide poisoning observed during the study were similar to those described by Ferguson, et al. (1964). The first noticeable symptom was a rising toward the surface of the water. A general darkening of body coloration accompanied by formation of dark, vertical bars occurred, followed by a period of hyperactivity characterized by rapid pectoral and opercular action, erratic swimming or darting, a gradual loss of equilibrium, and death. Using the fish that survived, and dividing them into two groups based on the severity of their symptoms (described in Table 2), differences between the blood and brain levels of the two groups were tested using the Mann-Whitney U test. Blood concentration difference between the two groups was significant at the .025 level ($N_2 = 9$) with the most severe symptoms having the highest dieldrin concentration. Brain level difference was significant at .001 with the same relationship of dieldrin to symptoms noted above.

DISCUSSION

Data on blood levels of dieldrin at death indicate a blood threshold level for the green sunfish of approximately 6.0 micrograms per gram.

The blood concentrations appear high when compared to the long-term work done with endrin. Mount, et al. (1966) in a 44-day study, using the channel catfish found toxic blood levels of endrin to be .28 micrograms per gram and above. In a 8-day study, Brungs and Mount (1967) working with gizzard shad found the critical level to be .10 micrograms per gram. However, Henderson (1959) and Katz (1961) studying 96-hour TLM values for dieldrin and endrin, found endrin toxicity to be 10-20 times as great as that of dieldrin. This fact when combined with the hardiness of the green sunfish and the short-term nature of the study indicates the dieldrin blood levels to be within expected limits.

Water concentration of dieldrin appears to exert a slight influence on the critical levels of the pesticide in the blood of fish experiencing mortality. This effect should be expected in short-term studies when blood is viewed as a transporter dependent upon the amount of pesticide passed over the gill surfaces to determine amounts picked up by blood proteins (Moss and Hathaway,

1964). Mount, et al. (1966) working with long-term endrin blood thresholds, found them to be essentially independent of concentrations of endrin in the water.

Brain levels for fish that died were more variable than those for the blood; however, a clear cut off point is evident between dieldrin concentrations in mortalities and survivors. The data indicate this threshold to be approximately 9.0 micrograms per gram. Blood level correlates with brain levels and both blood and brain concentrations showed a relationship to categories of observed symptoms in the test fish. In the two instances where fish died with blood levels below the established threshold, brain residues were above their critical concentration (Table 2). The remaining exception was a surviving specimen which had low blood dieldrin concentration but a brain concentration over the threshold level. This individual was also exhibiting strong pesticide poisoning symptoms.

Gakstatter (1966) working with channel catfish and bluegills, Lepomis macrochirus, indicated that the brain did not serve a storage function but that dieldrin residues decline rapidly following exposure. This same phenomenon was also noted for blood. Heath and Vandekar (1964) studying effects of dieldrin on rats, found that the faster the pesticide was added to the test animals,

the higher was their brain concentration. The investigators felt dieldrin, when added slowly, was being carried by the soluble blood proteins and was absorbed by body fat, but when added rapidly the body fat was not able to take it up rapidly enough, with the result that the pesticide reached the central nervous system and eventually caused death. Gakstatter (1966) found starved test fish to have a higher blood and brain dieldrin level than normal fish, and he suggested that the inherent protective system provided by the body fat can be overloaded by exceeding its storage capacity. This could conceivably be accomplished by long-term exposure to pesticides at moderate levels and would have the same end effect as exposure to high lethal concentrations. In each of two prior experiments by the author, 10 bluegills exposed to 41 ppb and 28 ppb of dieldrin, suffered a 50% mortality within 14 hours. Dieldrin concentration in the brains of fish that died ranged from 6.5 to 8.4 micrograms per gram with a mean of 7.78, and those surviving, 1.03 to 6.03 micrograms per gram with a mean of 3.27. A strong symptomatic correlation with brain concentration was also noted.

Ferguson, et al. (1964) working with endrin and dieldrin found a definite difference in 36-hour TLM limits between resistant and non-resistant fish. Ludke, et al. (1968) studying endrin and its effects on resistant golden

shiners, Notemigonus crysoleucas, found whole body residues 82 times and blood residues 64 times as great in resistant fish as those found in non-resistant fish killed by the exposure. The investigators, therefore, indicate a limitation which must be considered when using blood and brain thresholds as a diagnostic tool in fish-kill determinations.

Evidence indicates the brain to be a primary site of action for dieldrin. Additional research needs to be undertaken before being able to determine definitely dieldrin's mode of action and the target site or sites upon which it acts. Hayashi and Matsumura (1967) have initiated a line of research which could productively be applied to other organisms. Using two species of cock-roaches, the investigators compared the effects of dieldrin on ion movement in the nervous tissue of susceptible and resistant specimens. Initial response to dieldrin appeared to be much the same as was observed for DDT. More detailed studies patterned after work done by Ludke, et al. (1968) could prove valuable in answering many of the questions regarding blood and its transport function, and modes of action of the pesticide at its target site. Blood and tissue chemistry investigations such as under-taken by Eisler and Edmunds (1966) should be initiated on resistant organisms to determine differences between

resistant and susceptible forms.

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APPENDIX

Relationship of length, weight, and sex of green sunfish to mortality.

	Length	Weight	Sex
	131	41.60	Male
	147	60.08	Male
	125	39.80	Female
	115	33.39	Male
	141	60.02	Male
	135	47.56	Male
	128	38.52	Female
Died	136	45.70	Male
	131	42.90	Male
	130	41.20	Male
	140	45.00	Male
	156	70.62	Male
	126	39.22	Female
	145	38.30	Male
	125	33.35	Male
	122	44.20	Male
	134	55.85	Male
	141	53.61	Male
	136	48.51	Female
	136	45.76	Male
	136	50.07	Male
Survived	135	47.31	Male
	139	54.74	Male
	140	59.72	Male
	134	42.30	Male
	141	51.10	Male
	124	36.89	Female
	145	56.86	Male
	140	54.50	Male
	130	37.30	Female