AN ABSTRACT OF THE THESIS OF

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(Name) (Degree) (Major)

Date thesis is presented May 9, 1966

Title TOXICITY OF SELECTED HERBICIDES TO FOUR SPECIES OF FRESHWATER FISH

Redacted for privacy

Abstract approved ______________________
(Major professor)

This study was primarily concerned with the toxicity of 13 herbicides to four species of freshwater fish -- rainbow trout (Salmo Gairdneri), coho salmon (Oncorhynchus kisutch), bluegill (Lepomis macrochirus), and guppy (Lebistes reticulatus).

The toxicity of the herbicides was studied by the five following methods: (1) static water bioassays for estimating median tolerance limits (TLm), (2) short-term (15- and 30-minute) exposures, (3) tests in which fish were made to exercise, (4) bioassays to determine the time necessary to produce loss of equilibrium (turnover), and (5) long-term (90-day) exposures.

There appeared to be some fairly consistent relationships between the results of the different methods for studying acute toxicity. Fish could withstand, with no observable effect, concentrations of herbicides up to five times the static water 24-hour TLm for short periods of time if they were then released into fresh

water. For many of the chemicals tested static water TLm and turnover TLm closely approximated each other. The exercise tests demonstrated that when fish were made to swim at .5 feet per second while being exposed to the 96-hour static water TLm for seven different herbicides, in only one instance did more than 50 percent of the fish survive for 48 hours. In two cases, rainbow trout exercising in the 96-hour static water TLm for Hyvar and coho salmon exercising in the 96-hour static water TLm for CIPC, no fish survived for 48 hours.

The long-term exposure tests with two chemicals showed two basic effects. Low concentrations of both Diquat and Tordon retarded growth of bluegills. In addition, the Diquat appeared to have a cumulative effect, and during the last four weeks of the experiment 50 percent of the fish died.

TOXICITY OF SELECTED HERBICIDES TO FOUR SPECIES OF FRESHWATER FISH

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1966

APPROVED:

Redacted for privacy

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Date thesis is presented May 4, 1966

Typed by Erma McClanathan

ACKNOWLEDGMENTS

Many thanks and sincere gratitude are extended to Dr. Carl Bond, Professor of Fisheries, for all of his help, time, and suggestions in both the experiments and the preparation of the thesis.

The author is indebted to Mr. Dean Shumway, Assistant Professor of Fisheries, for his help at the Oak Creek fisheries laboratory and for his many helpful suggestions on the writing of this thesis.

My appreciation and many thanks are offered to Mr. William Snow who gave unselfishly of his time in helping with the experiments.

Mr. Dennis Wilson, Mr. Randal Brown, Mr. Roger Paul, Mr. Logan Norris, Mr. Frank Oliver and my wife Francie are all thanked for their help and suggestions.

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INTRODUCTION

With the increasing use of herbicides for plant control, the effect of these chemicals on desirable non-target organisms can become an increasing problem. While some chemicals are effective on water plants and are purposely applied to aquatic environments, others, though used for control of terrestrial plants, may be accidentally introduced to the water by spray drift, runoff, or by washing equipment in streams or ponds.

Several workers have previously studied the toxicity of herbicides by various methods. Effects of 15-minute and 30-minute exposures of fish to herbicides have been studied by Fryer (1957) and Lewis (1959). The swimming ability of fish under stress from a toxicant has been investigated by Mount (1962), Leduc (1966), and Cairnes and Scheier (1963). Long-term effects of selected herbicides on farm ponds have been examined by Domogalla (1935), Harp and Campbell (1964), Cowell (1965), Walker (1959), Surber and Everhart (1950), and Lemke and Mount (1963). Numerous workers have estimated median tolerance limits (TLm) for some of the same chemicals reported here.

One objective of this work was to make comparative studies for Oregon and to establish safe limits for these herbicides in the state. A second major objective was to determine relationships between TLm values, turnover times, and effects of moderate exercise in toxicants. The third objective was to explore the effects of low concentrations of herbicides on the growth of fish.

In order to learn more about the impact of various herbicides on fish, studies were designed to show both long-term and short-term effects. Besides the acute toxicity method set forth by Doudoroff et al. (1951) for estimating TLm values, the toxicity of herbicides was studied by four additional methods: (1) exposure of the test animals to concentrations of herbicides for 15 and 30 minutes; (2) exposure for 90 days to low concentrations; (3) exposure of fish to herbicides during moderate exercise; and (4) tests showing time necessary to produce loss of equilibrium to the test fish (turnover time).

The laboratory experiments reported here were conducted at the Oak Creek and South Farm fisheries laboratories of Oregon State University from January to October, 1965. The long-term exposure tests were conducted at the Soap Creek experimental ponds during the summer of 1965. These studies were an extension of the Oregon State University Agricultural Experiment Station

Project 294, "Limnology and Management of Oregon Farm Fish Ponds and Small Impoundments."

METHODS AND MATERIALS

Bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), and coho salmon (Oncorhynchus kisutch) were used as experimental animals because of their importance as game fish in the Pacific Northwest and their ready availability. Guppies (Lebistes reticulatus) were used so that possible relationships between the susceptibility of these readily available animals and that of the game species could be determined. The rainbow trout and coho salmon were obtained from Oregon State Game Commission hatcheries. The bluegills were seined from Willamette River sloughs and experimental ponds of Oregon State University. Guppies were raised in aquaria from brood stock obtained from Oak Creek fisheries laboratories. With the exception of fish used in long-term exposures, the sizes of experimental fish were as follows: guppies, 9 to 14 mm; coho salmon, 34 to 59 mm; rainbow trout, 18 to 30 mm; and bluegill, 39 to 57 mm. The fish in the long-term exposure tests ranged from 8 to 69 grams.

For estimation of TLm values bioassays were run using procedures similar to those outlined by Doudoroff et al. (1951). The bioassays were conducted in eight liters of water in disposable, polyethylene bags supported by cylindrical, cardboard containers.

Replicate runs were made using ten fish per container. Experimental fish were acclimated for five days in a constant-temperature room at experimental temperature (15° C for salmonids, 20° C for bluegills, and 22° C for guppies). The fish were not fed for 48 hours prior to the beginning of the experiments. Since tests were not being made for a specific body of water, either well water from Oak Creek laboratory or dechlorinated city water was used. Preliminary tests showed there were no differences in mortality of test fish due to the source of water. Prior to testing, the water was held at experimental temperature for a period of at least 48 hours. Except for the pH which was determined by using a Beckman pH meter, water analyses of the test solutions were run by the methods outlined in _Standard Methods for Examination of Water and Water Wastes_ (American Public Health Association, 1960). The dissolved oxygen which was measured daily was not allowed to fall below five ppm. The pH which was also measured daily varied within given tests from a minimum of 6.7 to a maximum of 7.7. The methyl orange alkalinity which was measured prior to the beginning of the bioassay and again at the conclusion of the experiment showed what appeared to be a seasonal fluctuation from 84 to 118 ppm.

When turnover times were noted the procedures were exactly the same as those described above for the static water acute toxicity tests. Individual fish were removed, weighed, and measured as soon as they lost equilibrium (turned over), except where preliminary tests indicated they might recover.

The methods used in the short-term exposures were as follows. The fish were handled in nearly the same manner as the fish used in the static water tests, except that 48 hours prior to the test the fish were held in small stainless steel wire baskets. This procedure was followed so the fish would not be as excited when placed into the test solution. The baskets were then dipped into test solutions for either 15 or 30 minutes. The fish were then released into eight liters of fresh water.

The methods used for activity tests were as outlined for the static water bioassays except that instead of eight liters of water, ten liters were used. The tests were run in an apparatus which was similar to that described by Chapman (1965). It consisted of three endless troughs each with two parallel straight sections connected by two semi-circles. A small paddlewheel placed in each trough was used to maintain a constant water velocity of .5 feet per second. The following modifications of Chapman's model were used for these experiments.

(1) Stainless steel screens were used instead of monofilament. (2) A nine-volt electrical current was used to keep the test animals from resting against the screens.

The method used to study the effects of long-term exposure of fish to low concentrations of herbicides consisted of exposing ten bluegills in each of nine 750-gallon tanks for 90 days. An additional tank was used to hold test fish for one week prior to initiation of the tests so that any ill effects of transportation could be noted. During the tests once each week 200 mosquito fish (Gambusia affinis) were fed to the bluegills. Every three weeks the test fish were seined, weighed, and measured. The tanks were approximately 7-1/2 feet in diameter and 3-1/2 feet high (Figure 1). The water depth was 2-1/2 to three feet. The tanks consisted of a liner of sheet polyethylene inside a sheet aluminum frame. A tar-impregnated burlap lining was inserted between the polyethylene and the aluminum for protection and for insulation from heat. A similar lining was placed under the tanks to prevent rocks from puncturing the polyethylene and to discourage mice from gnawing on the polyethylene.

The effects of low concentrations of herbicides on fish were studied in these tanks. Three tanks receiving

Figure 1. Tanks used in long-term exposures.

no herbicides were used as controls; three tanks received enough Tordon to give a concentration of approximately five ppm active ingredient; three tanks contained a solution of Diquat of ten ppm active ingredient. In one of each of the above sets of three, one-half inch of soil was placed on the bottom to determine its effect on either of the herbicides.

After the weight of water in each tank had been calculated, enough herbicide was added to obtain the desired concentrations. In order to maintain original concentrations, additional formulated material was added every two weeks. The amount to be added was based on the difference in concentration between a sample taken at the time of treatment and one extracted two weeks later. Samples were then collected weekly and at the conclusion of the experiment were analyzed by chemists of the Department of Agricultural Chemistry, Oregon State University. In all instances samples were taken prior to the addition of herbicide except on June 28 for all tanks and September 3 for tanks 1, 3, 6, and 7 which were taken immediately after the addition of the herbicide. Table I shows the actual concentration of herbicide maintained during the summer. The variation from the desired concentrations and the accumulation of the chemical throughout the summer should be noted (Table I).

Table II shows the chemicals used in both long-term and short-term experiments.

Table I. Concentrations of Tordon and Diquat in ppm in Tanks During 90-day Exposure Tests.

Date	Tordon 1	Tordon 2	Tordon Mud 6	Diquat 3	Diquat 4	Diquat Mud 7
6-28	5.3*	5.2*	4.8*	9.8*	9.6*	9.9*
7-13	3.2	3.0	2.1	4.9	4.4	2.8
7-21	7.3	4.8	6.9	6.7	6.0	4.1
7-29	4.6	4.4	6.1	4.6	4.5	1.2
8-13	5.9	7.4	7.8	7.2	7.0	2.5
8-20	4.5	4.8	6.0	6.2	6.8	.85
8-27	5.9	7.4	6.9	8.9	8.3	2.9
9-3	12.5*	5.2	13.3*	13.0*	7.9	6.7*
9-9	9.4	9.1	9.0	11.5	12.5	3.0
9-17	8.6	7.5	7.7	10.0	11.7	1.0
9-25	8.9	8.0	8.8	12.7	12.6	2.7

* Indicates that samples were taken immediately after herbicides were added.

Table II. Common Name, Chemical Name, Percent Active Ingredient, Form and Use of Herbicides Tested.

Common Name	Chemical Name	% Active Ingredient	Form	Use
Diquat	6,7-dihydrodipyrido (1,2-a:2′,1′-c) pyrazidiinium salt	24.9	Liquid	Broadleaf & Aquatic weed control
Paraquat	1:1 dimethyl - 4,4-dipyridylium dichloride	24.9	Liquid	Aquatic weed, broadleaf plant, & grass control
Atrazine	2-chloro-4-ethylamino-6-isopylamino - s - triazine	80.0	Powder	Broadleaf weed, grass, & algae control
Simazine 80W	2-chloro - 4,6-bis (ethylamino) -s-triazine	80.0	Powder	Algacide & soil sterilant
Prometone	2-methoxy-4,6,-bis (isopropylamino)-s-triazine	25.0	Liquid	Soil sterilant
Kurosal SL (Silvex)	2 - (2,4,5, trichlorophenoxy propionic acid), potassium salt	60.0	Liquid	Aquatic weed control
Fenac	2,3,6, trichlorophenylacetic acid, sodium salt	16.1	Liquid	Aquatic & terrestrial weed control

Table II. (Continued)

Common Name	Chemical Name	% Active Ingredient	Form	Use
AmChem 64-296B	N.A.	24.9	Liquid	Experimental chemical
SD11831	4 - (methyl sulfonyl) - 2,6 - dinitro N,N dipropyl	80.0	Powder	Annual grass & broadleaf weed control, experimental chemical
Hyvar (Bromicil)	5-bromo - 3-secbutyl - 6 - methyluracil	80.0	Powder	Soil sterilant
IPC	Isopropyl N phenylcarbamate	75.0	Powder	Soil sterilant & grass control
CIPC	Isopropyl N - (3-chlorophenyl) carbamate	50.0	Powder	Soil sterilant & grass control
Tordon 22K	4 - amino - 3,5,6, trichloro-picolinic acid, potassium salt	24.9	Liquid	Broadleaf weed control

RESULTS

Because of the nature of the results obtained, they are presented here mainly in tabular form. The discussion section which follows will refer to these tables.

Median Tolerance Limit

Table III shows the estimated TLm values, both in parts per million active ingredient and in parts per million formulated material.

Turnover Median Tolerance Limits

From the data presented in Table IV the relationship between the static water TLm and turnover TLm can be observed. Table V shows the median turnover time at the various concentrations of herbicides tested. Apparent detoxification of Hyvar is demonstrated. A concentration of 75 ppm caused 50 percent of the test animals to turn over in 15 to 18 hours. With additional exposure of fish to the above concentration of Hyvar, there were no more fish turned over during the next 54 hours. However, after 75 hours the test fish began to regain equilibrium, and a concentration of 100 ppm was necessary to keep 50 percent of the fish turned over.

Exercise Tests

The relation between the static water TLm and the

Table III. Estimated Median Tolerance Limits.

	Active Ingredient (ppm)				Formulated Material (ppm)			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs
CIPC								
Rainbow Trout	9.1	9.1	9.1	9.0	18.2	18.2	18.2	18.0
Coho Salmon	9.3	9.1	9.0	8.9	18.6	18.2	18.0	17.8
Guppy	9.1	8.9	8.8	8.7	18.2	17.8	17.6	17.4
IPC								
Coho Salmon	40.9	39.9	35.0	34.0	54.5	53.2	46.7	45.3
Guppy	40.5	39.5	39.0	37.0	54.0	52.6	52.0	49.0
PARAQUAT								
Rainbow Trout	56.0	30.2	26.0	22.0	224.0	120.8	104.0	88.0
Coho Salmon	49.0	28.5	25.0	22.5	196.0	114.0	100.0	90.0
Bluegill	49.0	42.0	35.0	27.5	196.0	168.0	140.0	110.0
Guppy	96.0	87.0	39.9	34.0	384.0	348.0	159.6	136.0
HYVAR X								
Rainbow Trout	102.0	75.0	42.0	29.0	127.5	93.7	52.5	36.2
Bluegill	120.0	116.0	113.0	107.0	150.0	145.0	141.2	133.7
SD11831								
Coho Salmon	64.0	49.0	42.0	35.0	80.0	61.2	52.5	43.7
Guppy	68.0	62.0	54.0	43.5	85.0	77.5	67.5	53.7
FENAC								
Bluegill	86.0	62.0	55.0	42.0	534.1	385.3	341.0	260.8
Guppy	105.0	93.0	75.0	59.0	652.1	577.5	465.7	366.4
TORDON								
Guppy	43.0	38.0	35.2	28.5	172.4	152.4	141.2	114.3
Coho Salmon	29.0	25.0	24.0	21.0	116.3	100.2	96.2	84.2
Bluegill	26.5	22.5	21.8	21.0	106.3	90.2	87.4	84.2

Table III. (Continued)

	Active Ingredient (ppm)				Formulated Material (ppm)			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs
AMCHEM 64-296B								
Guppy	.69	.67	.45	.30	2.76	2.68	1.8	1.2
Coho Salmon	.65	.58	.50	.49	2.6	2.32	2.0	1.96
Bluegill	1.03	1.00	.95	.92	4.12	4.00	3.80	3.68
SIMAZINE								
Guppy	No mortality at 32 ppm				No mortality at 40 ppm			
Coho Salmon	No mortality at 32 ppm				No mortality at 40 ppm			
Bluegill	No mortality at 32 ppm				No mortality at 40 ppm			
Rainbow Trout	No mortality at 32 ppm				No mortality at 40 ppm			
ATRAZINE								
Guppy	No mortality at 56 ppm				No mortality at 70 ppm			
Coho Salmon	No mortality at 56 ppm				No mortality at 70 ppm			
Bluegill	40% mortality-96 hrs-56 ppm				40% mortality-96 hrs-70 ppm			
PROMETONE								
Coho Salmon	9.6	9.6	9.6	9.6	38.4	38.4	38.4	38.4
Bluegill	8.7	8.7	7.0	7.0	34.8	34.8	28.0	28.0
Guppy	9.5	9.4	9.4	9.4	38.0	37.6	37.6	37.6
DIQUAT								
Coho Salmon	66.0	48.0	41.0	22.7	178.4	129.4	110.8	61.3
Bluegill	100.0	36.5	25.1	20.7	270.0	98.6	67.8	55.9
KUROSAL SL								
Bluegill	28.0	20.0	19.4	18.0	46.5	33.2	32.2	29.9

Table IV. Comparison Between Estimated Static Water TLm Values (Active Ingredient) and Turnover TLm Values (Active Ingredient).

	24 Hours		48 Hours		72 Hours		96 Hours	
	Static Water TLm	TO TLm	Static Water TLm	TO TLm	Static Water TLm	TO TLm	Static Water TLm	TO TLm
TORDON								
Bluegill	26.5	25.8	22.5	21.6	21.8	21.2	21.0	21.0
Coho Salmon	29.0	29.0	25.0	25.0	24.0	24.0	21.0	21.0
DIQUAT								
Bluegill	100.0	42.0	36.5	32.0	25.1	24.0	20.7	18.0
HYVAR								
Bluegill	120.0	62.1	116.0	61.8	113.0	75.0	107.0	100.0
SD11831								
Coho Salmon	64.0	56.0	49.0	40.0	--	--	--	--
CIPC								
Coho Salmon	9.3	9.2	9.1	8.7	--	--	--	--
FENAC								
Bluegill	86.0	84.0	62.0	58.0	--	--	--	--
PARAQUAT								
Bluegill	49.0	42.0	42.0	32.0	--	--	--	--
Coho Salmon	49.0	47.0	28.5	28.0	25.0	24.0	22.5	22.0
KUROSAL SL								
Bluegill	28.0	28.0	20.0	19.7	19.4	19.0	18.0	17.4
AMCHEM 64-296B								
Bluegill	1.03	1.02	1.00	.98	--	--	--	--

Table V. Median Turnover Time (50 Percent of Test Fish).

Chemical and Species	Concentration (ppm)	Hours 50% of test fish turned over
KUROSAL SL		
Bluegill	28	18-21
	21	42-45
	18	84-87
HYVAR X		
Bluegill	100	9-12
	75	15-18
TORDON		
Coho Salmon	32	12-15
	28	30-33
	24	63-66
	21	87-90
Bluegill	28	18-21
	24	33-36
DIQUAT		
Bluegill	42	21-24
	32	33-36
	24	63-66
	18	93-96
CIPC		
Coho Salmon	10	18-21
PARAQUAT		
Coho Salmon	49	18-21
	42	27-30
	24	66-69
SD11831		
Coho Salmon	56	21-24
FENAC		
Bluegill	100	21-24
	75	24-27
DIQUAT		
Coho Salmon	87	9-12
	56	27-30
AMCHEM 64-296B		
Bluegill	1.15	15-18
	1.0	36-39

percentage of fish that were able to swim at .5 feet per second for 48 hours can be seen from data in Table VI. Except for Tordon with a 55 percent survival, all fish exercised while being exposed to the 96-hour static water TLm values showed a mortality of 50 percent or greater in 48 hours.

Short-Term Exposures

The percentage of test fish that survived 48 hours in fresh water after a 15- or 30-minute exposure to various herbicide concentrations is contained in Tables VII and VIII. The results appear to be quite variable; at a concentration ten times the 24 hour TLm some chemicals produced no mortality while others produced 100 percent mortality.

Long-Term Exposures

When fish were exposed to a low concentration of herbicide for 90 days, there was little difference in growth among experimental lots of fish from replicate tanks without mud bottoms. Therefore, Figures 2 through 4 combine the results from the non-mud tanks. These figures show mean weights, range of weights, and percentage of population caught each sample period.

In addition to restricting growth, concentrations of Diquat shown in Table I resulted in mortalities of ten test fish (five in each pond).

Table VI. Survival of Exercised Fish Subjected to Concentration of Herbicide Equal to Static Water TLm.

Chemical and Species	Number of Fish per Concentration	Hours	Static Water TLm	Percent Survival 24 Hours	Percent Survival 48 Hours
CIPC					
Coho Salmon	20	24	9.3	0	0
		48	9.2	0	0
		72	9.0	0	0
DIQUAT					
Coho Salmon	20	24	66.0	10	0
		48	48.0	55	0
		72	41.0	65	10
		96	23.0	70	45
PARAQUAT					
Rainbow Trout	20	24	56.0	0	0
		48	30.0	40	0
		72	26.0	55	10
		96	22.0	75	45
Coho Salmon	20	24	49.0	5	0
		48	29.0	35	0
		72	25.0	55	5
		96	22.0	75	40
SD11831					
Coho Salmon	20	24	64.0	0	0
		48	49.0	50	0
		72	42.0	60	10
		96	35.0	70	50
HYVAR X					
Rainbow Trout	20	24	102.0	0	0
		48	75.0	5	0
		72	42.0	10	0
		96	29.0	25	0
TORDON					
Coho Salmon	20	24	29.0	0	0
		48	25.0	50	0
		72	24.0	65	10
		96	21.0	70	55
AMCHEM 64-296B					
Coho Salmon	20	24	.65	0	0
		48	.58	45	0
		72	.50	70	20
		96	.49	75	35
Control	310 (31 runs)			99.3	98.4

Table VII. Survival of Experimental Fish After A 15-Minute Exposure to Concentrations of Herbicides.

	Conc. (ppm)	0	1	2	6	12	18	24	48
		Observation periods in hours							
CIPC									
Coho Salmon	18	100	100	100	100	100	100	100	100
	45	100	100	100	100	100	100	100	100
	100	90	90	90	90	90	80	80	80
FENAC									
Bluegill	80	100	100	100	100	100	100	100	100
	160	100	100	100	100	100	90	90	80
	240	90	90	80	80	80	80	70	70
	800	10	0	0	0	0	0	0	0
AMCHEM 64-296B									
Coho Salmon	6	100	100	100	100	100	100	100	100
KUROSAL SL									
Bluegill	65	100	100	100	100	100	100	100	100
	100	100	100	100	95	95	95	95	95
	200	100	100	90	80	75	75	70	70
PARAQUAT									
Coho Salmon	100	100	100	100	100	100	100	100	100
	200	100	100	100	100	100	90	90	80
	300	100	50	35	20	20	20	0	0
Bluegill	40	100	100	100	100	100	100	100	100
	70	100	100	100	100	100	100	100	100
	140	100	100	100	80	80	80	60	50
	350	100	90	80	50	40	30	10	10
DIQUAT									
Coho Salmon	75	100	100	100	100	100	100	100	100
	150	100	100	100	100	100	100	100	100
	350	100	90	90	80	70	60	60	50
	750	100	90	80	60	50	40	30	30
Bluegill	100	100	100	100	100	100	100	100	100
	500	100	100	100	90	90	90	90	90
	1000	20	20	20	0	0	0	0	0
TORDON 22K									
Coho Salmon	60	100	100	100	100	100	100	100	100
	150	100	100	100	100	100	100	90	90
	300	100	100	90	90	90	90	80	70
Bluegill	270	100	100	100	100	100	100	100	100

Table VIII. Survival of Experimental Fish After A 30-Minute Exposure to Concentrations of Herbicides.

	Conc. (ppm)	Observation periods in hours 0	1	2	6	12	18	24	48
CIPC									
Coho Salmon	18	100	100	100	100	100	100	100	100
	45	100	100	100	100	100	100	90	80
	100	90	80	80	70	70	70	60	60
FENAC									
Bluegill	80	100	100	100	100	100	100	100	100
	160	100	100	100	100	100	100	90	80
	240	80	70	60	60	50	50	50	40
	800	0	0	0	0	0	0	0	0
AMCHEM 64-296B									
Coho Salmon	6	100	100	100	100	100	100	100	100
KUROSAL SL									
Bluegill	65	100	100	100	100	100	100	100	100
	100	100	90	90	80	80	80	80	80
	200	90	70	40	40	40	40	40	40
PARAQUAT									
Coho Salmon	100	100	100	100	100	100	100	100	100
	200	90	70	60	60	60	60	60	60
	300	10	0	0	0	0	0	0	0
Bluegill	40	100	100	100	100	100	100	100	100
	70	100	100	100	100	100	90	90	80
	140	100	100	90	50	50	50	45	30
	350	100	80	60	30	30	20	10	10
DIQUAT									
Coho Salmon	75	100	100	100	100	100	100	100	100
	150	100	100	100	100	100	100	90	90
	350	80	80	60	50	50	50	50	50
	750	30	20	0	0	0	0	0	0
Bluegill	100	100	100	100	100	100	100	100	100
	500	100	100	90	60	60	60	60	60
	1000	0	0	0	0	0	0	0	0
TORDON 22K									
Coho Salmon	60	100	100	100	100	100	100	100	100
	150	100	100	100	100	100	90	80	70
	300	100	100	90	60	50	50	50	50
Bluegill	270	100	100	100	100	100	100	100	100

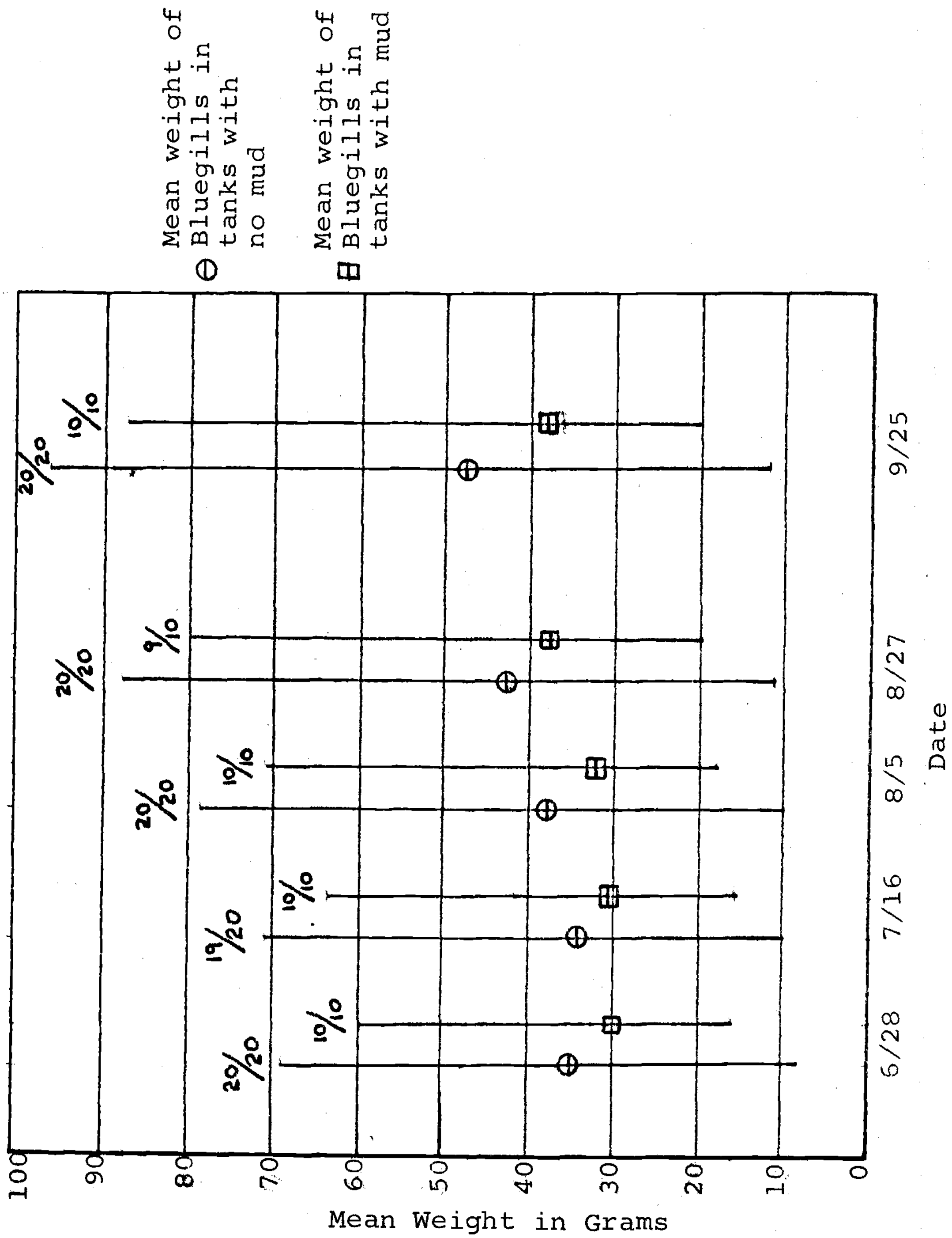

Figure 2. Weights of Bluegills held as controls for 90 days in 750-gallon tanks.

Vertical lines denote range of weights. Small numbers at top of vertical line show number of fish sampled over total population.

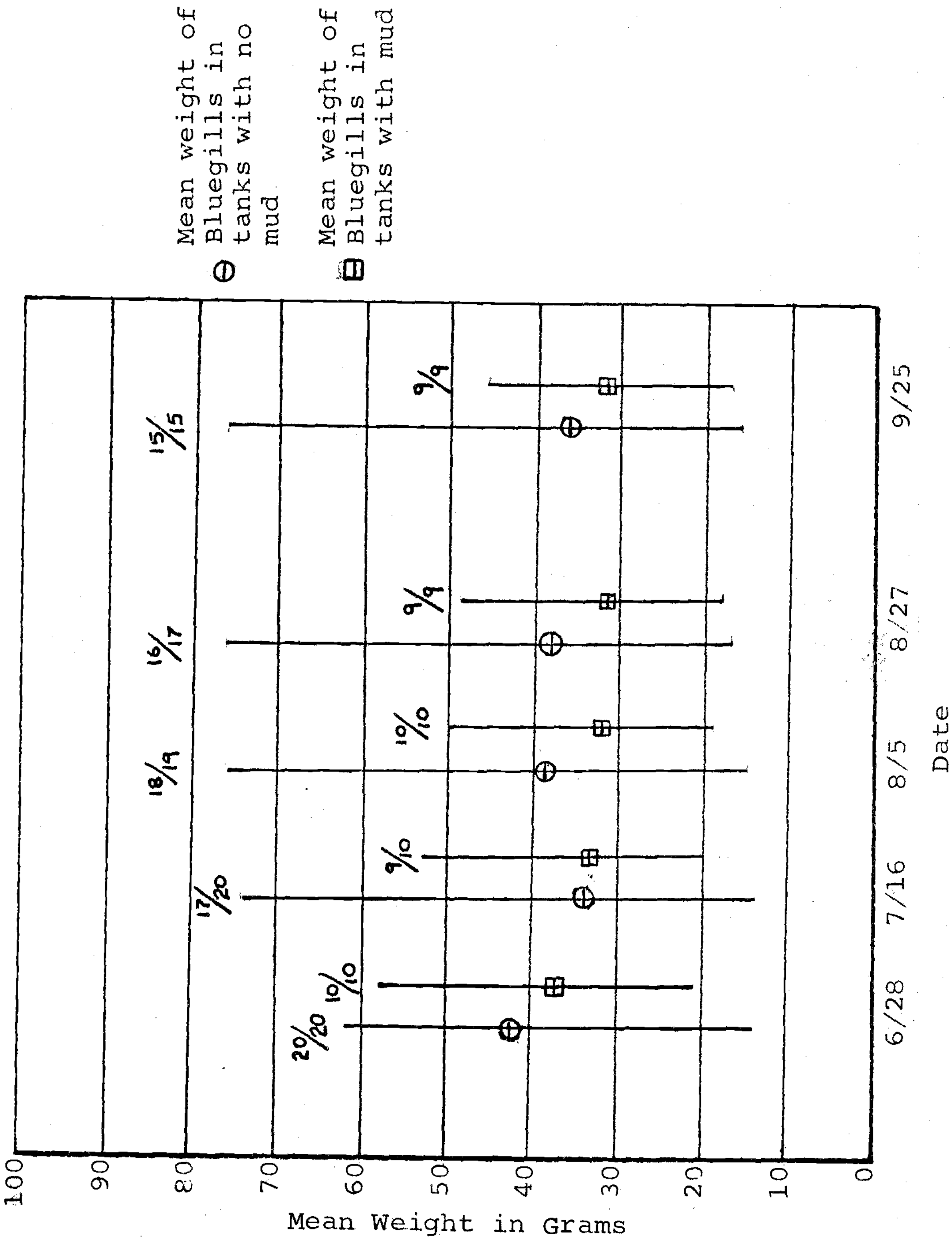

Figure 3. Weights of Bluegills exposed to Tordon for 90 days in 750-gallon tanks.

Vertical lines denote range of weights. Small numbers at top of vertical line show number of fish sampled over total population.

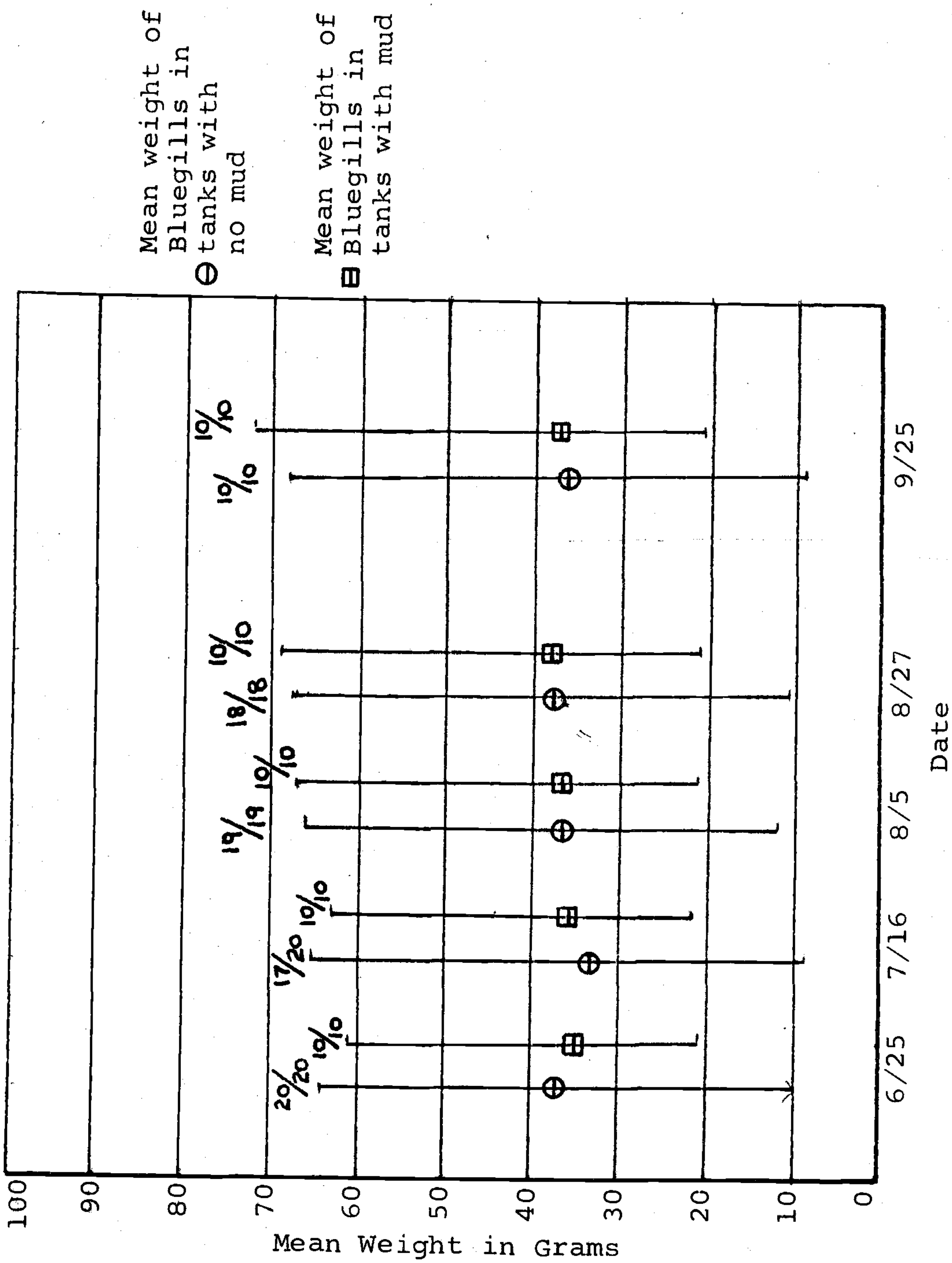

Figure 4. Weights of Bluegills exposed to Diquat for 90 days in 750-gallon tanks.

Vertical lines denote range of weights. Small numbers at top of vertical line show number of fish sampled over total population.

Relationship Between Species

There appears to be no consistent relationship between guppy TLm values and those of the game species. Guppies were the least tolerant of the species tested in some of the chemicals, while in other herbicides they were more tolerant than any of the game fish.

DISCUSSION

AmChem 64-296B

Of the 13 chemicals tested AmChem 64-296B was found to be most toxic. All of the 96-hour TLm values for the species tested were less than one ppm (Table III). Additional exercise tests showed that a concentration equal to the 96-hour TLm for coho salmon (.49 ppm) killed 65 percent of the test fish in 96 hours. In the past only a little work on the toxicity of AmChem 64-296B to fish has been reported. McKinley (1966) reported that concentrations of .1 ppm failed to create any mortality to goldfish (*Carassius auratus*).

CIPC

The data for CIPC exhibited in Table III showed that there was little difference in the TLm values for any of the species tested. The 24-hour and 96-hour TLm values for guppies were found to be 9.1 ppm and 8.7 ppm, respectively. The small difference between 24-hour and 96-hour TLm values suggests that CIPC is either fairly fast-acting or that the test solutions are detoxified. In other words, if a fish survives the initial concentration for 24 hours, in many tests it has a good chance of surviving through the 96-hour period.

These test results (Table III) showed slight differences from those reported by other workers. Applegate *et al*. (1957) found a CIPC concentration of five ppm non-toxic to bluegill fingerlings. Further, Davis and Hardcastle (1959) estimated the 48-hour TLm for bluegill to be 12 ppm. The discrepancy in results between the experiments reported here and those conducted by Davis and Hardcastle might be explained by differences in water chemistry and variances between stocks of fish tested.

Prometone

Prometone was found to be nearly as toxic to fish as was CIPC. Coho salmon were the most resistant of the species tested with a 96-hour TLm of 9.6 ppm. Bluegills with a 96-hour TLm of 7.0 ppm were the least resistant of the species tested. These results compare closely to those reported by Walker (1964), who found that for a combination of several species of centrarchids the 96-hour TLm was seven ppm.

By the end of 24 to 30 hours the suspension of Prometone broke down, and the chemical appeared much like droplets of oil in the water. This could be one apparent explanation for the low mortality after 48 hours. If fish were placed in the water 24 hours following addition of the herbicide, no mortality was noted during the next 96 hours.

Diquat

Toxicity of Diquat to test fish was found to vary from results obtained by other workers. The 24-hour estimated TLm for bluegills was 100 ppm. Surber (1962) stated that the 24-hour TLm for bluegills varies from 91 ppm in soft water to 410 ppm in hard water. In these tests the 48-hour TLm for bluegills was 36.5 ppm (Table III) which is considerably lower than Meyer's (1964) report of McKee and Wolf's (1963) findings of 80 ppm. These differences can possibly be explained by the difference in size of test organism, and differences in alkalinity of test waters.

The 24-hour and 48-hour estimated TLm values for coho salmon exposed to Diquat were 66 ppm and 48 ppm, respectively. Bond *et al*. (1960) found the 48-hour TLm for juvenile chinook salmon (*Oncorhynchus tshawytscha*) to be 29 ppm which is lower than the coho salmon results of this study.

When coho salmon were exercised while being exposed to a concentration equal to the 96-hour static water TLm, 55 percent died in 48 hours.

A long-term exposure of bluegills to concentrations of Diquat shown in Table I not only retarded growth, but also created a 50 percent mortality. The test was begun on June 28 and two fish, one weighing nine grams and a second fish weighing 16 grams, died before the end of

August. When the tanks were drained on the 25th of September, eight additional fish were found dead. Oxygen concentration, pH, and temperature of the water were all measured weekly, and there was little variation between any of the test ponds. At the relatively low concentration in the tank to which mud was added (Table I), there were no deaths among test animals. These data indicate that in the absence of soil bottoms Diquat has a tendency to have a cumulative effect on the fish and concentrations that were determined to be safe through bioassays may be lethal over a long exposure to fish.

Tordon

Of the three species of fish tested, bluegills were found to be the least resistant to Tordon (Table 3). The TLm values for guppies, the most resistant of the three species tested, ranged from 43 ppm for 24 hours to 28.5 for 96 hours. The 96-hour TLm for the bluegills was only 4.5 ppm lower than the 24-hour TLm. These results suggest that Tordon solutions detoxify at a fairly rapid rate.

The 96-hour TLm values for Tordon reported by Lynn (1965) were as follows: brook trout (*Salvelinus fontinalis*), 420 ppm; rainbow trout, 230 ppm; fathead minnow (*Pimephales promelas*), 135 ppm; brown trout

(*Salmo trutta*), 240 ppm; green sunfish (*Lepomis cyanellus*), 420 ppm; and black bullhead (*Ictalurus melas*), 420 ppm.

The TLm values for guppies, coho salmon, and bluegill were found to be considerably lower than those reported by Lynn (1965) (Table III). The differences between these results and Lynn's are not readily explainable but could possibly be attributed to difference in water quality or difference in size of test animals.

The exercise tests indicate that the safe limit might be somewhat lower than indicated by the TLm (Table VI). When coho salmon were exercised in a concentration equal to 96-hour TLm (21 ppm), by the end of 48 hours there was a 45 percent mortality (Table VI). In addition to restricting the rate of growth of bluegills, prolonged exposure to the concentrations of Tordon ranging from 3.0 to 12.5 ppm caused a 25 percent mortality during the 90-day growth experiments (Figure 3).

Kurosal SL (Silvex, Potassium Salt)

The 48-hour TLm value for Kurosal SL to bluegills was found to be 20 ppm. However, Hughes and Davis (1963a) found the 48-hour TLm for bluegills to be 95 ppm. The same chemical showed a 48-hour TLm of 240 ppm for coho salmon (Bond *et al.*, 1965). Surber and Pickering (1962) report a 48-hour TLm for bluegills exposed to

Kurosal SL to be 15 ppm, while Hughes and Davis (1963b) report a 48-hour TLm in terms of the acid equivalent to be 100 ppm.

The wide variation in results obtained by various workers is difficult to explain. One plausible explanation might be that slight differences in formulation can greatly affect the results of bioassays using Silvex or its derivatives. The differences between species of fish may have contributed to the variations between the results reported here and those reported by Bond et al. (1965). Difference in water supply may have contributed to the difference between my data and those of Suber and Pickering and Hughes and Davis.

Paraquat

Rainbow trout, coho salmon, bluegills, and guppies were all fairly resistant to the toxicity of Paraquat. Of the four species tested, coho salmon showed least tolerance to Paraquat. The 48-hour TLm values of Paraquat shown in Table III ranged from 28.5 ppm for coho salmon to 87 ppm for guppies.

The 48-hour turnover TLm for bluegill was ten ppm lower than the static water TLm (Table IV). This was one of the greatest differences noted between turnover and static water TLm values. Only bluegills exposed to Hyvar showed a larger difference between the static water TLm and the turnover TLm.

When rainbow trout were exercised in a concentration equal to the 96-hour static water TLm (22.0 ppm) the results were similar to the results obtained when coho salmon were exercised in other herbicides. Approximately 50 percent of the fish died in 48 hours (Table VI).

Matthews (1964) reported good control of most aquatic weeds in a stream with five ppm of Paraquat, and he observed no immediate effect on the fish present in the stream.

Hyvar X

Only two species of fish were tested in Hyvar X. Rainbow trout were far less resistant to this chemical than were bluegills. However, as can be seen in Table IV, Hyvar X was rapidly detoxified. It is interesting to note that for both species the 96-hour turnover TLm is actually higher than the 24-hour turnover TLm because some of the fish which were turned over at the 24-hour observation period regained their equilibrium.

Seventy-five percent of the fish which were exercised at a concentration equal to the 96-hour TLm died in 24 hours, and 100 percent died in 48 hours. With the exception of the mortalities observed using coho salmon in CIPC, this was the highest percent mortality observed in the exercise tests.

Simazine

None of the four species tested in Simazine showed any effects through 96 hours when exposed to 32 ppm active ingredient. Walker (1964) reported the 96-hour TLm for bluegill to be approximately 40 ppm. The 48-hour TLm for chinook salmon has been estimated by Lewis (1959) to be 6.63 ppm. Meyer (1964) states that McKee and Wolf (1963) estimated the 48-hour TLm for chinook salmon to be 7.7 ppm. The differences in toxicity of Simazine to bluegills from Walker's work appear to be slight and possibly explained by the difference in water supply and difference in size of test animals.

The differences from the results obtained by Lewis for chinook salmon are somewhat more difficult to explain. Lewis does not state which formulation of Simazine he tested, but as it contained 20 percent active ingredient (80 percent "inert" ingredient) it probably was Simazine 20W. The Simazine used in this study was Simazine 80W and was 80 percent active ingredient (20 percent "inert" ingredient). The "inert" ingredients in the chemical used by Lewis were four times as great as the chemical we tested. As the chemical he tested was approximately four times as toxic as the Simazine 80W reported here, one plausible explanation for the differences is that the additives in the formulation are more toxic than the Simazine. A second factor which probably led to the divergence in results was the difference in susceptibility between coho and chinook salmon. It is difficult to postulate reasons for differences noted between this work and that of McKee and Wolf (1963). Meyer (1964) did not report what formulation of Simazine was used by McKee and Wolf.

IPC

The estimated TLm values for IPC were about four times as great as those estimated for CIPC (Table III). Neither coho salmon nor guppies showed any ill effects at concentrations up to 28 ppm. The 48-hour estimated TLm of 58 ppm for white crappie (*Pomoxis annularis*) reported by Hilliard (1952) is slightly higher than those values reported here for coho salmon and guppies. Surber (1948) concluded that there is "no immediate danger to fish from the use of such new herbicides as ... o-iso-propyl N-phenyl carbamate ... at ten ppm or less."

SD11831

The results show that SD11831, a new experimental herbicide developed by Shell Development Company, is fairly non-toxic to fish. Of the two species of fish tested coho salmon were slightly less resistant than were guppies (Table III). When coho salmon were exercised at 35 ppm, 50 percent died in 48 hours. With the exception of those observed for Tordon this was the lowest mortality rate noted when fish were exercised in concentrations equal to the 96-hour TLm (Table VI). Information received from Shell Development Company indicated that gambusia and goldfish were not harmed when exposed to 20 ppm.

Fenac

Bluegills and guppies were found to be relatively resistant to Fenac (Table III). The guppies were slightly more resistant than were bluegills. In the past there has been a wide range of reports concerning the safe limits for the use of this chemical. Redear sunfish (Lepomis microlophus) weighing three grams showed no mortality when exposed to 12,000 ppm (George, 1963). However, the TLm for rainbow trout was 7.5 ppm (McKee and Wolf, 1963, as reported by Meyer, 1964). The 48-hour TLm for "bluegill, trout, river and lake shiners, muskellunge and walleyed pike" are above 20 ppm (Gallagher and Collins, 1963). The values obtained in this study were somewhat higher than the values obtained by Gallagher and Collins, but were much lower than the values obtained by George. The differences in results found by many workers are difficult to explain, but may involve inert ingredients and water quality.

Atrazine

The results obtained in the Atrazine tests reported here (Table III) differ widely from those reported by Walker (1964). He found that the 96-hour TLm for groups of mixed species of fish was about 12 ppm. We observed only a 40 percent mortality in bluegills at 56 ppm. The differences might be explained by the fact that Walker's report was for combined species, *Lepomis macrochirus*, *Lepomis gibbosus*, and *Micropterus salmoides* and not solely for the bluegill. The differences in alkalinities might have added to the divergence, as might a slight difference in formulation.

Exercise Tests

To date little work has been performed on the swimming ability of fish in toxic solutions. Mount (1962) relates that Endrin did not affect the ability of bluntnose minnows (*Pimephales notatus*) to maintain a sustained swimming speed. Cairnes and Scheier (1963) found that by increasing the concentration of sodium alkyl benzene sulfonate the sustained swimming speed of the pumpkinseed (*Lepomis gibbosus*) was lowered. Lemke and Mount (1963) exposed bluegills to various concentrations of alkyl benzene sulfonate for 30 days, and then swam them in fresh water. They found no difference in endurance between controls and fish exposed to various concentrations of the chemical.

The experiments of this investigation were not designed to test the effects of herbicide concentrations on the sustained swimming speed of fish, but rather to determine the effects of an additional stress (i.e., forced swimming at 0.5 feet per second) on the tolerance of fish to certain herbicides.

The combination of moderate exercise and subjection to herbicides was found to have a deleterious effect on fish. In nearly every instance the 48-hour static water TLm killed over 50 percent of the fish in 24 hours (Table VI). Further, with the exception of Tordon (45 percent mortality) the 96-hour static water TLm resulted in a 50 percent mortality of fish in 48 hours. From these data it would appear that when a moderate stress in the form of activity is applied to a fish which is exposed to a toxicant, it can become an accessory factor which enhances the toxic effect. For the chemicals tested, moderate exercise in effect cut the lethal time in half.

Turnover Times

The observance of turnover times shows that with the exception of fish exposed to Hyvar X, a turned-over fish eventually died. Many times the turnover TLm closely approximated the static water TLm (Table IV).

Since in nature loss of equilibrium causes a fish to become easy prey, the actual death of a fish exposed to a toxicant may be somewhat sooner than suggested by either static water or turnover TLm values. Therefore the median turnover time is important (Table V).

Short-Term Exposures

The short-term tests demonstrated that fish can withstand high concentrations of the herbicides if the exposure is not prolonged. In nearly all cases, concentrations at least five times the 96-hour static water TLm were necessary to create any mortality. Much higher concentrations were necessary to create a 50 percent or greater mortality. These tests show that if only a section of a pond is treated, and if the fish can swim to another section, then relatively higher concentrations than previously thought might be safe to use.

Long-Term Exposures

Selected herbicides can have both good and bad effects on benthos of ponds as has been reported by many workers. Since benthos provides food for fish, and thus influences the growth of fishes, their results are reviewed here. Harp and Campbell (1964) working on ponds in Missouri found that there was an increase in tendipedids and oligochaetes when Silvex was used in concentrations up to 4.6 ppm active ingredient. They further report that Chrysops was the only genus to decrease in the treated enclosures. Walker (1959) applied Simazine to two similar ponds, A and B, at the following rate: Pond A, 100 pounds per acre, ten percent active ingredient; Pond B, 44 pounds per acre, 20 percent active ingredient. He found in Pond A that the benthic organisms numbered 259.6 per square foot. Pond B had 153.3 per square foot; whereas Pond C, the control, had only 72.3 per square foot. Surber and Everhart (1950) found that a similar increase in benthic organisms occurred after hatchery ponds had been treated with Nigrosine.

Cowell (1965) shows that neither sodium arsenite at four ppm nor Silvex at two ppm had any effect on phytoplankton populations in farm ponds in New York. He further states that Silvex had no effect on zooplankton, but that four ppm sodium arsenite caused a marked reduction in zooplankton populations.

Lemke and Mount (1963) exposed bluegills to various concentrations of alkyl benzene sulfonate for 30 days. The results of three tests were variable depending on when they were run. The tests run in the spring gave the most consistent results. They found that generally the mean increase in weight varied inversely with the concentration of the test solution.

The 90-day exposures demonstrated that low concentrations of Diquat and Tordon can reduce growth rates in fish (Figures 2-4). Diquat can be lethal over a prolonged period in concentrations up to 14 ppm. The mud contained in the tank which had concentrations of Diquat absorbed much of the herbicide, thus detoxifying the test solution (Table I). However, the mud in the Tordon tank apparently did not absorb the chemical appreciably.

RECOMMENDATIONS

Neururer and Slanina (1960) developed a security quotient (SQ) for comparing herbicides. It is SQ = fd/pd, where SQ = security quotient, fd = dose tolerated by fish, and pd = dose necessary to kill weeds. The security quotient as used here is not intended to show safe limits, but rather it will relate herbicides to each other. For the purposes of the following recommendations, the dose tolerated by fish (fd) will be defined as the maximum observed concentration which had no effect for 96 hours on the least tolerant species tested. When available the manufacturer's suggested dosage for control of aquatic weeds was used in place of dose necessary to kill weeds. When the manufacturer did not make recommendations of adequate concentrations, the figure used was that which has been shown by other workers to be necessary for control of aquatic weeds. The SQ, fd, and pd for the aquatic herbicides tested are shown in Table IX. Some of the herbicides tested are still in the experimental stage as aquatic herbicides so that the necessary concentration for control of aquatic weeds is still unknown. Other herbicides are strictly terrestrial herbicides, so no security quotient is figured.

Table IX. Security Quotient, Doses Tolerated By Fish, Doses Necessary to Kill Plants, and the Authority Making the Recommendations of Five Aquatic Herbicides.

Herbicide	SQ	fd (ppm)	pd (ppm)	Authority
Diquat	5.4	13.5	2.5	Manufacturer
Kurosal SL	5.4	13.5	2.5	Surber (1961) and Lawrence (1962)
Paraquat	4.0	10.0	2.5	Manufacturer
Simazine	3.2	32.0	10.0	Surber (1961)
Fenac	6.0	18.0	3.0	Gallagher and Collins (1963)

Diquat

Diquat with an SQ of 5.4 appears to be one of the safest herbicides tested (Table IX). However, the long-term tests indicate that prolonged exposure can be lethal to bluegills, suggesting a cumulative effect. From these tests it appears that if the manufacturer's recommendations are followed, there is little chance for mortality to occur in fish. However, prolonged exposure to this herbicide should be avoided since even a relatively low concentration of up to 4.5 ppm retarded growth.

Kurosal SL

The potassium salt formulation of Silvex with an SQ of 5.4 (Table IX) appears to be a safe chemical to use for control of aquatic weeds. However, only bluegills were exposed to this herbicide. Because of the wide range of results reported by various workers a safe procedure would be to run bioassays using water and fish from waters to which the Kurosal will be added prior to the use of the chemical.

Paraquat

An SQ of 4.0 was obtained for Paraquat. In the acute toxicity bioassays of the four species of fish tested, the most tolerant was the guppy, and the least tolerant was the rainbow trout with coho salmon and bluegills being intermediate. From the standpoint of acute toxicity, this herbicide would probably be safe for use in aquatic habitats.

Simazine

The acute toxicity of Simazine to fish in this study was somewhat less than what has been reported by other workers. We observed no ill effects to any of the four species of fish at the highest tested concentration of 32 ppm. This would, therefore, give an SQ of greater than 3.2 (Table IX). Other research would indicate this chemical might be somewhat more toxic. As pointed out earlier, this chemical apparently has no ill effects on benthic organisms of concentrations up to ten ppm. The biggest disadvantage to the use of this chemical, as pointed out by Surber (1961), is that it costs $3.00 per pound. This means that treating an acre of water five feet deep would cost about $240. If the expense can be decreased, this chemical would be very promising for control of algae.

Fenac

Fenac with an SQ of 6.0 would indicate that it is the safest of the herbicides tested. However, two basic considerations must be taken into account. (1) Recommendations by Gallagher and Collins (1963) indicate that this chemical should be used in combination with other herbicides. Therefore, the combination of herbicides to be used should be tested on fish prior to introduction of the chemical into the water. (2) This herbicide remains in the soil for a prolonged period of time and may affect benthic organisms. If these two considerations become inconsequential, then from our tests Fenac would appear to be a desirable aquatic herbicide.

Prometone

Preliminary investigations by Dennis Wilson (1966) conducted at Oregon State University indicate that this chemical might be toxic to *Elodea densa* at concentrations of about ten ppm. The toxic level to guppies was 4.9 ppm. In this concentration five percent of the fish died in 48 hours. Coho salmon were the most resistant of the species tested. Only ten percent of the test animals were killed in 8.7 ppm at the end of 96 hours. If the coho salmon did not die in the first 24 hours, they were able to withstand the concentration through 96 hours. As shown in Table III guppies reacted nearly the same. At the present time it is recommended that this herbicide not be used as an aquatic herbicide in the presence of desirable fishes. Also, when this chemical is being used as a soil sterilant near aquatic environments, caution should be taken not to spray it directly into the water.

Tordon

Tordon is one of the safest terrestrial herbicides tested. In the long-term exposures, the tanks containing Tordon were characterized by a lush plankton bloom which did not appear in the other tanks. This chemical shows relatively low acute toxicity to fish. The TLm values show that bluegills were least tolerant of the species tested. However, concentrations up to 12 ppm killed 25 percent of the test animals during the 90-day exposures and retarded growth rates. Mud in the tanks did not absorb the herbicide as it did with Diquat (Table I). This herbicide appears to be safe to use near aquatic environments if prolonged exposure is avoided.

CIPC

It is recommended that CIPC not be used as an aquatic herbicide. Lawrence (1961) reports that a concentration of 100 ppm is necessary for a 100 percent kill of *Elodea densa*, while a concentration of ten ppm CIPC showed no effect on *Elodea densa*. Rainbow trout were the most tolerant species tested. The 96-hour TLm was 9.0 ppm. None of the coho salmon which were exercised in the 72-hour static water TLm of 9.0 ppm survived for 24 hours.

IPC

Terrestrial use of IPC around aquatic environments appears to be safe. Lawrence (1962) states that IPC at ten ppm arrested growth of *Elodea canadensis* but did not kill the plants. The only tests executed with this chemical were static water bioassays. However, both coho salmon and guppy TLm values were about four times as great as they were in CIPC.

Hyvar X

If standard precautions are taken, terrestrial use of Hyvar X near aquatic environments seems to be safe. In preliminary screening at Oregon State University with Hyvar X, there is an indication of no effect on *Elodea densa* at 50 ppm. This concentration is greater than the 72- or 96-hour TLm for rainbow trout. Bluegills were much more resistant to the effect of the chemical than were rainbow trout. Many of the fish were able to recover after turning over.

Atrazine

From the data presented in this thesis, fish can tolerate relatively high concentrations of Atrazine. Only recently has this chemical been experimentally used in aquatic environments. If it proves to control plants at relatively low concentrations, then it has good possibilities of being used as an aquatic herbicide. We observed no ill effects to coho salmon or guppies exposed to 56 ppm. However, at this concentration 40 percent of bluegills were killed.

AmChem 64-296B

Because of the high acute toxicity of AmChem 64-296B to fish, it should not be used as an aquatic herbicide where fish are to be protected. Further, for any terrestrial use near aquatic environments precautions should be taken to prevent spray drift. Since even small amounts of this chemical introduced into fish environments can probably cause damage, the possibilities of its leaching out of the soil and being carried to a stream or lake in runoff should be considered. There is a need for additional fish toxicity work with this chemical.

SD11831

This chemical is an experimental herbicide developed by the Shell Chemical Company. Coho salmon showed a good tolerance of the chemical. In the exercise tests Tordon and SD11831 produced the best results. From these preliminary data it would appear that the possibilities for use of SD11831 as an aquatic herbicide should be examined.

Additional Recommendations

Besides the acute toxicity data reported for 13 herbicides, this thesis has pointed up two additional major considerations that should be taken into account when making recommendations as to use of chemicals in aquatic environments. The first is that even though fish that are exposed to low concentrations may show no observed ill effects for the standard 96-hour test period, over a prolonged period growth of fish may be retarded. This growth retardation was exhibited by bluegills when exposed to either Diquat or Tordon.

The second additional consideration of significance is that exercising the fish while they were exposed to the herbicide placed an additional stress on the fish, producing profound effects. Concentrations equal to the 96-hour static water TLm for all chemicals tested except Tordon (45 percent in 48 hours) killed at least 50 percent of the test animals in 48 hours. The exercise tests demonstrate that TLm values for fish which are under additional stress are probably somewhat lower than indicated by static water acute toxicity bioassays.

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