EFFECTS OF SELECTED AQUATIC HERBICIDES ON FISHES

рy

ROBERT HILL LEWIS

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

June 1959

APPROVED:

Redacted for privacy

Associate Professor of Fish and Game Management

In Charge of Major

Redacted for privacy

Head of Department of Fish and Game Management

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

Date thesis is presented may 13 1959

Typed by Lenora Bond

ACKNOWLEDGMENT

Sincere gratitude is offered to Associate Professor Carl Bond for his encouragement and assistance throughout this work.

Dr. Max Katz of the U. S. Public Health Service offered many helpful suggestions pertaining to the experimental techniques and assisted in obtaining test animals.

Assistant Professor Charles Warren offered timely suggestions concerning test animals and experimental technique.

My appreciation is extended to John L. Fryer for his time and effort in describing the constant flow apparatus and other aspects of the experimental procedures.

Mr. Virgil H. Freed, Associate Chemist, Agricultural Experiment Station, devoted a great deal of time discussing the herbicides used in this work.

Dr. William R. Furtick, Assistant Professor, Farm Crops Department, contributed a number of the herbicide samples used in the experimental work.

TABLE OF CONTENTS

																								Page
INTRO	שענ	TI	ON	•	Ø.		٠	*			•	٠	•	٠	*	•	*		•	•	•	•	•	1
REVIE	W O	I^{n}	LI	TE)	RA	TUI	æ	•		•	•	•			•	Þ	•	٠	•	0	•	*	•	3
	End ACF Bar Kur Sim Sod FB. Oma Acr	on on az iiu 2	in me	69 FC.	· · · · · · · · · · · · · · · · · · ·	*	* * * * *	* * * * *		* * * *	* * * *	* * * *		•		* * * * * * * * * * * * * * * * * * * *	•				•	•		3 4 5 5 6 7 7 7 8
METHO	BU	AN	D I	MA!	r E	RI	AL	S	٠		•		•	₽	•	۹.	•	•	٠	*	•	٠	•	8
	Exp Jar Con Sho Bic Jar	st rt	xp an -t sa	er: t : er: ys	ind Fl m	eni ow Exi	ts E 90:	wi xpe sus Ple	ther:	n I Lme e v	Pi: eni wi: P:	she ts th	es W: F': sei	itl isl	n P	is	she	es	•	•	•	•	•	8 11 16 18 20 20
EXPER	CIME	NT	AL	R	ES	UL/	ľS	•	٠	٠	•	8	٠	•		۰	•	٠	•	•	•	•	•	23
	Con	ıst	ENCABUICO AND POST AND POST POST POST POST POST POST POST POST	do P-I romandi 2	then in the second of the seco	al 560 (Ex. ne TO in owal ane ne XX XX XX	Po Ros	xpe	res	im	en · · · · · · · · · · · · · · · · · · ·	ts · · · · · · · · · · · · · · · · · · ·	D	isl	* * * * * * * * * * * * * * * * * * * *	* · · · · · · · · · · · · · · · · · · ·	• • • • • • • • • • • • • • • • • • • •							23 26 26 29 29 32 34 40 40 46 48 51 51 53
					X		7	Emi							•									55 55

TABLE OF CONTENTS (continued)

	Pag e
Bioassays with Plants Present	59
FB.2	5 9
Baron	61
ACP-M-569	61
Jar Experiments with Plants	61
FB.2	61
Endothal	66
DISCUSSION AND CONCLUSIONS	67
Endothal	67
ACP-M-569	69
Baron	69
Kuron	70
Simazine	71
Sodium TCA	72
FB.2	72
Omazene	74
Acrolein	74
Dalapon	75
Penite	76
Delrad	76
Phygon XL	77
Copper Sulfate	77
TCB with 5 per cent Emulsifier	77
BTBLTOGRAPHY	79

LIST OF TABLES

Table		Page
1	Names and Some Properties of Herbicides Used in Jar Bioassays	12
2	Survival of Chinook Salmon in Endothal Bioassays	24
3	Survival of Largemouth Black Bass in Endothal Bioassays	25
4	Survival of Chinook Salmon in ACP Bicassays	27
5	Survival of Chinook Salmon in Baron Bioassays	28
6	Survival of Chinook Salmon in Kuron Bioassays	30
7	Survival of Chinock Salmon in Simazine Bicassays	31
8	Survival of Chinock Salmon in FB.2 Bioassays	33
9	Survival of Chinook Salmon in Omazene Bioassays	35
10	Survival of Chinook Salmon in Acrolein Bioassays at 20° C	36
11	Survival of Chinook Salmon in Acrolein Bioassays at 13° C	3 8
12	Survival of Rainbow Trout in Acrolein Bioassays at 13° C	39
13	Survival of Largemouth Black Bass after Four Days Exposure to Endothal	41
14	Survival of Chinook Salmon after Pour Days Exposure to Simazine	42
1.5	Survival of Largemouth Black Bass after Seven Days Exposure to Dalapon	44
1.6	Survival of Chinock Salmon after Short- period Exposure to Penite	47
17	Survival of Chinook Salmon after Short-	10

LIST OF TABLES (continued)

Table		Page
18	Survival of Chinook Salmon after Short- period Exposure to Kuron	50
19	Survival of Chinook Salmon after Short- period Exposure to Delrad	52
20	Survival of Chinook Salmon after Short- period Exposure to Phygon XL	54
SI	Survival of Chinook Salmon after Short- period Exposure to Copper Sulfate	56
22	Survival of Chinook Salmon after Short- period Exposure to Trichlorobenzene plus 5 per cent Colloidal X-77 Emulsifier	57
23	Survival of Chinook Salmon after Short- period Exposure to Acrolein	5 8
24	The Effect of FB.2 on Chinook Salmon in the Presence of Ceratophyllum demersum	60
25	The Effect of Baron on Chinook Salmon in the Presence of Ceratophyllum demersum	62
26	The Effect of ACP on Chinock Salmon in the Presence of Ceratophyllum demersum	63
27	The Effect of FB.2 on Ceratophyllum demersum with controlled Light and Temperature	64
28	The Effect of FB.2 on <u>Flodea densa</u> with Uncontrolled Light and Temperature	66

LIST OF FIGURES

Figure		Pag e
1	Bioassay jars with chinook salmon and Ceratophyllum demersum	21
2	Short-term exposure of chincok salmon	 21

INTRODUCTION

This study was conducted to determine the toxicity of selected herbicides to fingerling chinook salmon, Oncorhynchus tshawytscha (Walbaum), rainbow trout, Salmo gairdnerii Richardson, and juvenile largemouth black bass, Micropterus salmoides (Lacepede). In conjunction it was desired to learn the susceptibility of the submergent plants, Ceratophyllum demersum L. and Elodea densa Planchon, to herbicides that demonstrated a low toxicity towards fish species used in this investigation. The majority of the compounds tested were recently developed herbicides that may prove useful in aquatic situations. This work was done during the summer and fall of 1958 at the Fisheries Laboratory of Oregon State College and was a continuation of the Farm Pond research project which was initiated in June of 1956 to determine the fish species and management practices best suited to Oregon farm ponds.

Toxicity to fishes of herbicides used in this work was studied by four approaches. The short term bicassay method as described by Doudoroff et al (8, p. 1380-1397) was used in most of the experiments. In this technique test animals are exposed to progressive concentrations of a toxicant for a specified period of time. The index of relative toxicity is expressed as the median tolerance

limit (TL_m) or the concentration at which 50 per cent of the introduced test animals survive for a given period of exposure. Exposure periods in the short term (standing water) bloassays of this work were 24 and 48 hours.

In the standing water tests a number of herbicides appeared to lose toxicity upon storage and aeration (11, p. 46). In order to get more accurate knowledge of the toxicity of these compounds, running water bicassays were performed in a constant flow apparatus. This apparatus, devised and constructed by Fryer (11, p. 15), allowed test animals to be subjected to a constant concentration of herbicide for a prolonged period of time. In this work experiments were run from four to seven days.

In some aquatic plant control situations it is feasible to treat the problem area on different dates by sections. In this type of treatment, or in running water, fishes might be in contact with herbicides for only a short period, as an avoidance reaction might enable them to evade the treated area. Exposure experiments were performed with herbicides in which test animals were introduced into concentrations comparable to those used in the field. These exposures were for 15 and 30 minute periods.

Another approach to determine toxicity to fishes involved the presence of aquatic plants in containers with

the test animals when herbicides were applied. This procedure was followed in order to ascertain whether aquatic plants might reduce the susceptibility of fishes by absorbing or otherwise detoxifying the herbicides.

As the objective of this study was to reveal herbicides that had a low toxicity towards fishes, the more promising compounds in this respect were tested against two submergent plant species. This part of the investigation followed the fish tolerance tests and was conducted inside the laboratory under controlled light and temperature and outside of the laboratory in naturally fluctuating physical conditions.

REVIEW OF THE LITERATURE

There is a paucity of published material concerning the tolerance of fishes to the herbicides that were used in the standing and flowing water bioassays of this work. The bulk of the information cited below has been obtained through personal correspondence with workers in various organizations. See table 1 for data on herbicides.

Endothal

In personal correspondence Dr. Robert C. Hiltibran,
Associate Biochemist with the Illinois State Natural
History Survey Division, Urbana, Illinois, has stated that

bluegill sunfish could survive for 21 days at 100 parts per million (p.p.m.) Endothal. At greater concentrations the survival decreased (14).

Mr. Charles R. Walker, Biochemist with the Missouri Conservation Commission, has reported in personal correspondence that 96 hour exposure of 100 p.p.m. sodium Endothal was non-toxic to Lepomis macrochirus, Lepomis humilis, Notropis umbratilis, Notropis lutrensis, Pimephales notatus and Ictalurus melas (24).

AGP -M-569

No literature on the toxicity of ACP to fish could be found; however, toxicity data is available on amino triazole, the major active ingredient of ACP. Fryer reports the 24 hour TL_m of coho salmon to amino triazole (Weedazol) to be 335 p.p.m. and the 96 hour survival of largemouth black bass exposed to 1000 p.p.m. amino triazole to be 100 per cent (11, p. 31).

Workers at the Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania, report the 24 hour TL_m of bluegill sunfish to amino triazole to be 14,300 p.p.m. and the biologically safe concentration to be 1,470.57 p.p.m. (1, p. 7).

Baron

Unpublished data received from Mr. J. R. Fisher of the Dow Chemical Company list the toxicity of Baron to lake emerald shiners (Notropis atherinoides) as follows: upper safe limit, 6 p.p.m.; fatal to approximately 50 per cent of the fish, 8 p.p.m.; fatal to 100 per cent of the fish, 15 p.p.m. (10).

Workers associated with the U. S. D. A. Soil Conservation Service, Durham, New Hampshire, reported a fish kill when 1 p.p.m. Baron was tested on rooted, emergent plants (19, p. 59).

A summarization by the Agricultural Experiment
Station of the Alabama Polytechnic Institute, Auburn,
Alabama, lists a concentration of 5 p.p.m. Baron as being
"safe" to fishes (17, p. 3).

Kendle has noted in unpublished data from Oregon State College, Corvallis, Oregon, that the estimated 24 hour TL_m of largemouth black bass to Baron was 4 p.p.m. (16).

Kuron

Unpublished data from Mr. J. R. Fisher of the Dow Chemical Company list the toxicity of Kuron to lake emerald shiners as follows: upper safe limit, 5 p.p.m.; fatal to approximately 50 per cent of the fish, 7 p.p.m.;

fatal to 100 per cent of the fish, 9 p.p.m. (10).

In personal correspondence Dr. Robert C. Hiltibran of the Illinois State Natural History Survey Division stated that bluegill withstood 5 p.p.m. Kuron without mortality. Above this concentration there was no survival (14).

Kendle has noted in unpublished data from Oregon State College that the estimated 24 hour TL_m of largemouth black bass to Kuron was 3.4 p.p.m. (16).

Simazine

Dr. Robert C. Hiltibran has stated in personal correspondence that bluegill sunfish are susceptible to 3.5 p.p.m. Simazine in 21-day bioassays. One group of 15 fish was dead after an exposure of seven days to this concentration. In a second experiment eight of the 15 fish were dead after 12 days, while the remaining fish survived for 21 days (14).

The toxicity results of Simazine to Lepomis

macrochirus, L. humilis, Notropis umbratilis, N. lutrensis,

Pimephales notatus and Ictalurus melas have been received

through personal correspondence with Mr. Charles R. Walker,

Missouri Conservation Commission. Bioassay results on

these fish species were favorable at 50 p.p.m. Simazine

over a 96 hour exposure (24).

Mr. Bernard R. Jones of the Bureau of Research and Planning, Department of Conservation, St. Paul, Minnesota, has reported to the Geigy Agricultural Chemicals Corporation that 300 p.p.m. Simazine killed 50 per cent of the test animals (chub minnow) within 24 hours. The probable safe concentration for this species under these conditions was considered to be about 30 p.p.m. (15).

Sodium TCA

Hilliard reports the 24 hour TL_{m} of white crappie to trichloroacetic acid, the parent acid of Sodium TCA, to be 116 p.p.m. and the 48 hour TL_{m} to be 88 p.p.m. (13, p. 92).

A summarization by the Agricultural Experiment Station of the Alabama Polytechnic Institute lists the concentration of trichloroacetic acid that is "safe" to fish as less than 50 p.p.m. (17, p. 3).

FB.2

No data were found on the toxicity of FB.2 to fishes.

Omazene

Dr. W. W. Dalquest of Midwestern University reported to the Olin Mathieson Chemical Corporation that Omazene had no effect on sunfish and silvery plains minnows at concentrations of 1 and 2 p.p.m. The median tolerance

limits of these species were estimated as 2.75 p.p.m. (7).

In an experiment reported in personal correspondence by Mr. R. D. Burrows and Mr. B. D. Combs, Bureau of Sport Fisheries and Wildlife, Entiat, Washington, one half of the fingerling chinook salmon exposed to 1.4 p.p.m. Omazene were dead after a 90 minute exposure (6).

Acrolein

Dr. Rene Blondeau of the Agricultural Research
Division of the Shell Development Company reported in
personal correspondence that during field work with
Acrolein fish showed severe agitation when they were
exposed to concentrations of from 10 to 15 p.p.m. Below
4 or 5 p.p.m. many fish seemed to escape after exposures
of several hours. Carp and thread fin shad appeared quite
sensitive to Acrolein, whereas largemouth black bass
seemed more resistant (4).

Applegate reports that no deaths occurred when rainbow trout, bluegill sunfish and sea lamprey were exposed to 5 p.p.m. Acrolein for 24 hours (3, p. 16).

METHODS AND MATERIALS

Experimental Animals and Plants

Chinook salmon, rainbow trout and largemouth black

bass were used as test animals in this work because of their availability and because of their wide distribution in the Northwest.

Chinook salmon were obtained from the McKenzie River salmon hatchery near Leaburg, Oregon. These fish were seined from the same pond throughout the investigation which allowed the use of fish from a homogeneous population as regarded size and condition.

Rainbow trout were obtained from the Alsea trout hatchery near Alsea, Oregon.

Largemouth black bass were seined from the Jefferson Junction barrow pit immediately west of the junction on U.S. Highway 99. Juvenile bass seined from this area were of a uniform size and in good condition.

All fish were transported to the laboratory in 10-gallon milk cans and care was taken to avoid crowding the animals in these containers. Ice was placed in the cans with the chinook salmon, which were carried at the rate of one pound per 10 gallons of water. At the laboratory, test animals were held in five-foot circular tanks with constant water supply for one week. At the end of this time the fish were placed into 50-gallon aquaria with aeration inside the laboratory and acclimated to the laboratory temperature for at least 48 hours prior to an experiment.

With the exception of those used in constant flow experiments, test animals were not fed after they were brought into the laboratory. While being held in the circular tanks chinook salmon and rainbow trout were fed ground liver, crustaceans and mosquito larvae; bass were fed mosquito larvae and earthworms. In constant flow tests bass were fed every two days.

During the acclimation period in the aquaria fish of abnormal appearance were removed and released. The largest fish selected for any experiment was no more than one-and-one-half times the size of the smallest fish. During the run fish that appeared dead were removed from the test jars and weighed and measured after observation and mechanical stimulation had failed to produce movement. In instances of a total or partial mortality in a test jar, each fish was weighed and measured. If all fish survived in a test jar, the aggregate was weighed in water, and a sample of three fish was taken to determine an average fork length for fish in that jar. Test animals were used for one complete experiment and then discarded.

The test plants, Ceratophyllum demersum and Elodea

densa were selected because of their wide range and

appearance in many Oregon waters. E. densa is particularly

troublesome in some western Oregon lakes and has proven

resistant to most herbicides that are low in cost or

relatively non-toxic to animals.

E. dense was obtained from the Oregon State College Botany Department and was used at rates of 20 and 40 grams per 15 liters of water in herbicide susceptibility tests.

Geratophyllum was grown in aquaria at the laboratory where this work was done and was used at the same rates as E. densa in susceptibility tests. In combination with fish bloassays it was used at the rate of 42 grams per 15 liters of water.

Plants were kept in the laboratory in 50-gallon aquaria for two weeks before tests were conducted. E. densa susceptibility was tested in both filtered and unfiltered water.

Jar Experiments with Fishes

Nine herbicides with possible use as aquatic weed control agents were selected for toxicity studies. The commercial and chemical names of these compounds in addition to the composition of active ingredient and water solubility are listed in table 1. All concentrations listed in this work are expressed in the amount of active ingredient, and experimental dilutions were computed on this basis. Hereafter all compounds will be referred to by their commercial or trade name.

Endothal, the disodium salt of endoxy hexahydro

Table 1
Names and Some Properties of Herbicides Used in Jar Bioassays

Commercial or Trade Name	Chemical Name	Empirical Formula	Active Ing redie nt (%)	Water Solubility (20° C.)	Form
F-98	Acrolein	C ₃ H ₄ O	100	22%	liquid
ACP-W-569	3 amino 1,2,4 triazole	C2N4H4 *	25		liquid
Simazine	2-chloro-4,6-bis- (ethylamino)-s-triazine	C7N5H12C1	20	5 p.p.m.	powder
Sodium TCA	Sodium trichloroacetate	C2 ^{C1} 3 ^O 2*Na	90	infinitely soluble	granular
Oma ze ne	Copper dihydrazinium sulfate	Cu(NH2•NH2)2SO4	50		powder
FB.2	1:1' ethylene-2:2' dipyridylium dibromide	$^{ ext{C}}$ 12 $^{ ext{H}}$ 12 $^{ ext{N}}$ 2 $^{ ext{B}}$ 2	25	70%	liquid
Baron	dalapon ester of 2,4,5 trichlorophenoxy ethanol	^C 11 ^H 9 ^O 3 ^C 15	50		liquid
Kuron	<pre>∮(2,4,5 trichlorophenoxy) propionic acid</pre>	C9H6C13O3	50	50 p.p.m.	liquid
Endotha1	Disodium 3,6 endoxy hexahydro phthalate	C8H8O3·Na2	19.2	infinitely soluble	liguid

^{*} formula of amino triazole only
The above information was taken from chemical company literature.

phthalic acid, was tested against largemouth black bass and chinook salmon.

ACP, a liquid formulation of amino triazole and an undisclosed additive, was tested on chinook salmon. This chemical has been tested on emergent aquatic vegetation.

Baron (Erbon) is the dalapon ester of 2,4,5 trichlorophenoxy ethanol. It was tested on chinook salmon.

Kuron is composed of low volatile esters of (2,4,5">(2,4,5">trichlorophenoxy) propionic acid. Kuron bioassays were run with chinook salmon.

Simazine is 2-chloro-4,6-bis(ethylamino)-s-triazine. It is primarily used as a soil sterilant and may offer possibilities for control of rooted aquatic plants. This compound was tested on chinook salmon.

Sodium TCA is generally used as a grass eradicator and has been tested on emergent aquatics. The compound is a sodium salt of trichloroacetic acid. In this work Sodium TCA was tested on chincok salmon.

FB.2 is a quaternary ammonium compound, 1:1'-ethylene-2:2'-dipyridylium dibromide, recently developed by Imperial Chemical Industries Ltd. This herbicide has proven effective as a desiccant and non-selective weedkiller in terrestrial usage. Unless the cost is prohibitive this compound may be used as a submergent aquatic plant eradicator. FB.2 was tested on chinook salmon in this

work.

Omazene, copper dihydrazinium sulfate, is primarily used as a fungicide. It was tested on chinook salmon.

In preparing test dilutions with the solid herbicides that included Simazine, Sodium TCA and Omazene, the proper amount of material was weighed on an analytical balance and introduced directly into the test jars. The proper concentrations were made up from the liquid herbicides volumetrically by either pipetting the toxicant directly into the test jars or by preparing a stock solution. This latter procedure was necessary with the more toxic compounds such as Acrolein, Baron and Kuron. Endothal, ACP and FB.2 had such low toxicity to test animals that the proper amounts needed for each test jar could be accurately removed from the sample container.

All standing water bioassays except for Acrolein were performed in a constant temperature room with the temperature thermostatically controlled at 20° C. Acrolein was tested in the 20° C. constant temperature room and in a 13° C. constant temperature room. Test aquaria consisted of 19-liter (five-gallon) cylindrical wide-mouth jars. The shape of these jars inhibited test animal injury when the organisms became excited or hyper-sensitive. All standing water tests were conducted with water pumped from Mary's River. Preliminary investigation of each toxicant

to determine the approximate range of toxicity was made by placing two fish in a one gallon wide-mouth jar that contained two liters of solution. All experimental concentrations were selected by using the method described by Doudoroff et al. (8, p. 1395).

All test solutions except those containing Acrolein were aerated by the use of aeration stones that were connected to an air compression unit. Solutions of Acrolein, a volatile compound, were aerated by bubbling pure oxygen into the test jars at the approximate rate of one bubble per second.

In preparing for the standing water bicassays, jars were filled to the 15 liter mark and allowed to remain at least 12 hours under aeration until the test water was tempered and was saturated with dissolved oxygen. The proper amount of the test herbicide was then added and thoroughly mixed with the diluent. With all bicassays except those involving Acrolein, ten test animals were selected from the 50 gallon aquaria and introduced into each jar. Acrolein bicassays were conducted using both five and 10 fish per jar. The exposure period for each herbicide was 48 hours. Close observation of the test animals was made during this time and survival was noted at the end of the 24 hour and 48 hour periods. The median tolerance limit was estimated by plotting the survival

points of these periods on semilogarithmic paper, with test concentrations ruled off on the logarithmic scale and survival percentages on the arithmetic scale (8, p. 1397). A curve was fitted to these points by inspection, and the concentration which corresponded to the 50 per cent survival point on the graph was then observed.

After each run was terminated, test jars were washed thoroughly with detergent soap, rinsed well and refilled with water to be held for use in subsequent tests.

Prior to and at the end of all experiments the dissolved oxygen, hydrogen ion concentration and alkalinity content of the water were measured. During the experiments dissolved oxygen content in the test dilutions was measured when mortality first appeared. No tests were instigated unless the diluent water contained at least 6.0 p.p.m. dissolved oxygen, and results were not accepted as reliable in a bicassay where the dissolved oxygen fell below 4.5 p.p.m. Dissolved oxygen determinations were made by the Winkler method (23, p. 38).

Constant Flow Experiments with Fishes

Results obtained in the standing water bloassays indicated that a number of the herbicides were somewhat volatile. To eliminate the possibility of toxicant being "blown off" by test jar aeration and to keep test animals

in contact with a constant amount of toxicant, constant flow bicassays were conducted with Simazine, Endothal and Dalapon. Dalapon, the sodium salt of dichloropropionic acid, is a systemic, translocated grass killer produced by the Dow Chemical Company. This company reports that lake emerald shiners were exposed to 3,000 p.p.m. Dalapon for three days without any adverse effect; however, 5,000 p.p.m. was found to be lethal (22, p. 4). The toxicity of this compound to coho salmon and largemouth black bass was studied by Fryer (11, p. 29-31). Dalapon and Endothal were tested on largemouth bass and Simazine was tested on chinock salmon in this work.

The constant flow apparatus operated by a gravity water flow through five-gallon pyrex bottles that were immersed in a water bath thermostatically controlled for temperature stability at 20° C. (11, p. 15). Intake water passed through a head jar that also contained a thermostatically controlled heater which adjusted the incoming diluent to 20° C. before it entered the test bottles. Outflow lines from each of the five test bottles could be manipulated vertically thus controlling the amount of water that passed through each bottle. Uniform addition of toxicant was accomplished with a Brittingham laboratory pump (21, p. 798-801). The toxicant was pumped into the system from four 15-gallon pyrex bottles. As the amount

of toxicant delivered per time period could be measured, the water outflow could be adjusted to obtain the desired concentrations in the test bottles. Outfall delivery from each dilution was calibrated using a stop watch and 500 milliliter graduate every 24 hours during an experiment in order to maintain a constant concentration. Chemical pump delivery was measured before each experiment was started.

After the proper flows had been established, 10 test animals were introduced into each test bottle and acclimated for 48 hours. At the end of this time the proper amount of herbicide was placed in the toxicant bottles and mixed into a stock solution. The chemical pump was engaged and fish condition was noted at least six times daily throughout the run. Dead fish were removed, weighed and measured when observed. Dissolved oxygen concentrations at the outfalls were measured when the experiment started and periodically thereafter until the run was terminated.

Short-Term Exposures with Fishes

In aquatic weed treatment where the preservation of fishes is desired, it is sometimes necessary to divide the area and treat the sections concurrently. This is essential because of the high biological oxygen demand of

decomposing plants. Furthermore it is possible that fish in a treated area might be able to detect the presence of the herbicide and avoid prolonged contact by moving to an untreated section. Because of this possibility exposures were devised to ascertain whether fish could withstand, for short periods, concentrations of herbicides comparable to those used in the field. Herbicides selected for exposures were Acrolein, Delrad, Trichlorobenzene with 5% emulsifier, Penite (sodium arsenite on vermiculite), copper sulfate, Baron, Kuron and Phygon XL. The test animal was the chincok salmon.

In preparing for the exposures, five-gallon jars were placed in pairs and brought up to the 15 liter mark with water in the 20° C. constant temperature room. Five fish were placed in one jar of each pair and allowed to acclimate for 24 hours. At the end of this time a measured amount of herbicide was introduced into the other jar of the pair. Fish were taken from the acclimation jars and placed in cylindrical wire baskets with a diameter of five inches and a length of ten inches (figure 1). The baskets were quickly immersed in the solution and allowed to remain for 15 and 30 minutes per concentration. After this exposure the fish were transferred back to the acclimation jars where observation was made for 24 hours. The acclimation or fresh water jars

were aerated throughout the test.

Bioassays with Plants Present

In order to simulate field conditions more closely bioassays with herbicides and fishes were performed with aquatic plants present in the test jars (figure 2). The herbicide concentrations selected for this work coincided with the approximate median tolerance limits estimated by the standing water bioassay section of this work. Fish survival that differed widely from the previous data might be attributed to the presence of plants. Herbicides tested against chinook salmon and <u>Ceratophyllum</u> were Baron, FB.2 and ACP.

These experiments were conducted in the 20° C. constant temperature room. The only difference in technique between these experiments and the preceding standing water bicassays was the addition of 42 grams of Ceratophyllum to the 15 liter dilution that contained five test animals. Survival of fish and plants was noted at the end of 24 and 48 hours.

Jar Experiments with Plants

Test animals were found to have a relatively high tolerance to FB.2 and Endothal. In order to determine the effect of these herbicides on aquatic plants, Elodea densa



Figure 1. Bioassay jars with chinook salmon and Ceratophyllum demersum.



Figure 2. Short-term exposure of chinook salmon.

and <u>Ceratophyllum demersum</u> were treated in five-gallon standing water jars both in the constant temperature room and outside the building. Concentrations were selected that would indicate the approximate minimum lethal dose necessary for complete necrosis. Both filtered and unfiltered water was used with FB.2 as particles in the water occlude this compound.

Plants were acclimated in 50 gallon containers for two weeks either in the 20° C. constant temperature room or outside of the building depending upon the area in which experimentation was to be carried out. Five-gallon jars were used as test containers, and plants were introduced into 15 liters of water at the rates of 20 and 40 grams wet weight. Herbicides were placed into the jars with the same technique that was employed with the fish bioassays. After treatment, those jars that were tested outside of the laboratory were placed so that they were accessible to sunlight. The extremes of temperature in this situation were recorded by means of a maximum-minimum thermometer. Tests ran from four to 16 days, and condition of the plants was noted daily until death occurred or until the concentration was judged ineffective. Both the starting and terminating pH and alkalinity of the test concentrations were noted.

EXPERIMENTAL RESULTS

Jar Experiments with Fishes

Endothal

Results of bioassays conducted to determine the toxicity of Endothal to chinook salmon resulted in the estimation of the following median tolerance limits:

24 hour, 149 p.p.m. and 48 hour, 132 p.p.m. (table 2).

At the end of one hour all test animals were dead in 180 p.p.m. and higher concentrations. Fish in concentrations lower than 115 p.p.m. appeared normal throughout the experiment. Chinook that remained alive after 48 hours in concentrations where deaths occurred were noted to have protruding eyeballs and were much more sluggish than control fish.

Experiments conducted to determine the toxicity of Endothal to largemouth black bass resulted in the following estimated median tolerance limits: 24 hour and 48 hour, 202 p.p.m. (table 3). Total mortality occurred within two hours in the 320 p.p.m. solution and within 12 hours in 240 p.p.m. Fish appeared normal during the run in concentrations lower than 135 p.p.m. Test animals that remained alive in concentrations where partial mortality occurred were sluggish and partially narcotized.

Table 2
Survival of Chinook Salmon in Endothal Bioassays

		No. Test Survivin	Animals After		est Animal s .ng After	Length mm 278 200 60.3 200 59.8 200 57.2 200 59.3 200 61.2 20 61.2 20 65.1	Avg. Weight
Conc. p.p.m.	No. Test Animals	24 Hours	48 Hours	24 Hours	48 Hours		Grams
32	10	10	10	100	100	60.3	2.91
56	1.0	10	10	100	100	59.8	2.87
100	10	10	10	100	100	57.2	2.43
115	10	10	8	100	80	59.3	2.55
135	20	19	10	95	50	61.2	3.11
155	20	7	3	35	15	71.8	3.92
180	10	0	0	O	O	60.0	2.59
210	10	0	0	0	0	64.8	3.35
240	10	O	0	O	0	59.2	2.93
320	10	Ô	0	0	0	65.1	3.36
0	10	10	10	100	100	58.0	2.56
Starting Ending p						Alkalinity lkalinity	- 51 - 167

Table 3
Survival of Largemouth Black Bass in Endothal Bioassays

		No. Test Survivin	Animals g After		est Animals ng After	Avg. Length mm 64.4 64.5 62.6 50.9 39.6 39.5 63.0 Alkalinity lkalinity	Avg.
Conc. p.p.m.	No. Test Animals	24 Hours	48 Hours	24 Hours	48 Hours		Length
135	10	10	10	100	100	64.4	2.93
155	5	5	5	100	100	64.5	3.14
180	10	8	8	8 0	80	62.6	2.76
210	20	8	8	40	40	50.9	1.85
240	10	0	0	0	0	39.6	0.87
320	10	O	0	O	0	39.5	0.85
O	5	5	5	100	100	63.0	2.84
	pH - 7.78 H - 7.85						- 41 - 122

ACP-M-569

Toxicity tests with ACP against chinook salmon yielded the following estimated median tolerance limits: 24 hour, 185 p.p.m. and 48 hours, 152 p.p.m. (table 4). At the end of three hours only one fish remained alive in 370 p.p.m. The three largest fishes in the jar died first in this concentration. The lowest concentration that caused mortalities was 135 p.p.m., and test animals in weaker dilutions never appeared to be in distress.

Baron (Erbon)

The median tolerance limits of chinock salmon exposed to Baron were as follows: 24 hour, 2.62 p.p.m. and 48 hour, 2.27 p.p.m. (table 5). No mortalities occurred in concentrations of 1.8 p.p.m. or less during the experiment; however, fish that were confined in solutions of from 0.75 p.p.m. to 2.4 p.p.m. were observed to swim in a vertical position with their heads up by the second hour of the test. By the fourth hour the majority of the fish that remained slive were lying on their dorsal surface on the bottom of the jars with only visible pectoral fin and opercle movements. Those fish capable of swimming did so on their backs or twirled as they progressed. By the twenty-fourth hour many of these fish appeared in better condition, but normal swimming was still not possible.

Table 4
Survival of Chinook Salmon in ACP Bioassays

		No. Test Survivin	Animals ag After		est Animals ng After	Avg.	Avg.
Conc. p.p.m.	No. Test Animals	24 Hours	48 Hours	24 Hours	48 Hours	Length mm	Weight Grams
56	10	10	10	100	100	81.0	5.5
75	10	10	10	100	100	83.0	5.87
100	10	10	10	100	100	74.0	4.92
135	30	27	20	90	67	65.5	3.3
180	20	10	4	50	20	69. 9	3.95
210	40	21	4	52	10	70.2	4.12
240	30	5	1	1 6	3	75.0	5.08
280	10	0	0	0	0	77.5	5.81
320	10	0	0	0	Ö	82.8	7.55
370	10	0	O	0	0	78.1	5.51
Ö	20	20	20	100	100	77.2	5.65
Starting	g pH - 7.58				Starti	ng Alkalini	ty - 62
Ending g						Alkalinity	- 109

Table 5
Survival of Chinook Salmon in Baron Bioassays

		Survivin	Animals R After		est Animals ng After	Avg.	Avg.
Conc. p.p.m.	No. Test Animals	24 Hours	48 Hours	24 Hours	48 Hours	Length mm	Weight Grams
0.32	1.0	10	10	100	100	73.0	4.26
0.56	20	20	20	100	100	70.7	3.64
0.75	10	10	10	100	100	72.1	3.52
1.0	80	20	20	100	100	71.8	3.73
1.8	10	10	10	100	100	71.5	3.53
2.4	20	14	8	70	40	78.0	4.66
3.2	20	1	0	5	0	72.6	4.78
4.2	10	0	0	0	0	73.8	4.91
5.6	10	0	0	0	O	68.2	4.04
Ō	10	10	10	100	100	74.0	3.8ે
Starting Ending p	pH - 7.43 H - 7.36					ng Alkalini Alkalinity	

A number of the test animals remained on the bottom as described above throughout the test.

Kuron

Toxicity tests with Kuron against chinook salmon resulted in the following estimated median tolerance limits: 24 hour, 1.36 p.p.m. and 48 hour, 1.23 p.p.m. Mortalities occurred after four hours in concentrations of 3.2 p.p.m. and higher concentrations. There was 100 per cent survival of test animals in concentrations of 0.32 p.p.m. and 0.56 p.p.m. Kuron (table 6).

Simazine

The median tolerance limits of chinook salmon to Simazine were estimated to be as follows: 24 hour, 6.74 p.p.m. and 48 hour, 6.63 p.p.m. (table 7). Test animals appeared in distress after $2\frac{1}{8}$ hours in concentrations of 10 p.p.m. and over, and total mortality was noted in 13.5, 18 and 24 p.p.m. Simazine after 24 hours. Only one fish succumbed after the initial 24 hour period of the experiment. Fish in distress swam erratically near the surface of the solution. After 6 hours had elapsed, foam appeared on the surface of the test dilutions. At the end of 48 hours the dissolved oxygen content of the Simazine jars had dropped to approximately 4 p.p.m.

Table 6
Survival of Chinook Salmon in Kuron Bioassays

		No. Test Survivin	Animals g After	Survivi	est Animals ng After	Avg. Length mm	Avg. Weight Grams
Conc. p.p.m.	No. Test Animals	24 Hours	48 Hours	24 Hours	48 Hours		
0.32	10	10	10	100	100	81.5	6.1
0.56	10	10	10	100	100	79.2	5.56
0.75	30	40	31	100	77	74.7	4.99
1.0	40	31	28	77	70	77.6	4.88
1.8	20	5	5	25	25	74.6	5.46
3.2	30	O	O	0	O	72.9	4.94
4.2	10	0	O	O	0	67 • ଞ	4.02
5.6	10	O	Q	O	O	6 8.5	4.47
Startin:	g pH - 7.61				Starti	ng Alkalini	ty - 67
Ending p						Alkalinity	- 93

Table 7
Survival of Chinook Salmon in Simazine Bioassays

		No. Test Animals Surviving After			est Animals ng After	Avg.	Avg.
Conc. p.p.m.	No. Test Animals	24 Hours	48 Hours	24 Hours	48 Hours	Length ma	Weight Grams
3.2	10	10	10	100	100	63.1	2.85
5.6	10	10	10	100	100	64.6	2.92
7.5	20	4	3	20	15	64.4	3.25
10.0	20	1	1	5	5	64.1	3.07
13.5	20	O	0	0	Q	61.5	3.56
18.0	10	O	O	O	0	64.1	3.07
24.0	10	0	0	0	O	63.2	2.97
0	10	10	10	100	100	61.8	2.87
Starting Ending p	pH - 7.5 H - 7.65					ng Alkalinit Alkalinity	ty - 47 - 67

Sodium TCA

Determination of the approximate median tolerance limits of chinook salmon to Sodium TCA was not attempted because of the low toxicity demonstrated by this compound. No test animal mortality was sustained in 16 concentrations that ranged from 24 p.p.m. to 870 p.p.m. Test fish in all concentrations retained normal actions throughout the 48 hour test period.

FB.2

Tests to determine the toxicity of FB.2 to chincok salmon resulted in the following estimated median tolerance limits: 24 hour, 29.4 p.p.m. and 48 hour, 28.0 p.p.m. (table 8). By the second hour of the test complete mortality was noted in the 100 p.p.m. dilution. At the end of eight hours partial mortality had occurred in concentrations of 32, 42, 56 and 75 p.p.m. Fish in distress swam violently in a circular manner for a short period of time and ultimately sank to the bottom of the test jar where they remained in a narcotized condition for approximately one hour before death occurred. Test jars in which partial mortality occurred took on a dark-green color after 40 hours of the experiment had elapsed.

Table 8
Survival of Chinook Salmon in FB.2 Bioassays

		No. Test Survivin	Animals	Per Cent To Survivi	est Animals ng After	Avg.	Avg. Weight Grams
Conc. p.p.m.	No. Test Animals	24 Hours	48 Hours	24 Hours	48 Rours	Length	
5.0	10	10	10	100	100	57.0	1.98
10	10	10	3	100	30	61.0	2.87
13.5	20	20	20	100	100	56.5	2.28
18	10	10	10	100	100	62.5	2.94
24	10	10	9	100	90	57.0	1.93
28	10	5	5	50	50	64.0	3.35
32	40	17	9	42	22	67.7	3.46
37	10	3	2	30	20		
42	10	0	0	0	. 0	65.4	3.07
56	20	0	O	0	0	64.3	3.23
75	10	O	O	0	O	63.0	3. 05
100	10	0	. 0	0	0	62.7	3.1
0	20	50	50	100	100	5 8.8	2.24
Starting						ng Alkalini	
Ending ph	- 7.63		, A		Ending	Alkalinity	- 84

Omazene

Bioassays to determine the approximate median tolerance limits of chinook salmon to Omazene resulted in the following data: 24 hour and 48 hour, 0.83 p.p.m. (table 9). Chinook tested at 0.56 p.p.m. Omazene appeared normal throughout the 48 hours of experimentation, whereas those confined to 0.75 p.p.m. and 1.0 p.p.m. were in distress and sustained a partial mortality. Concentrations of 1.8 p.p.m. and above produced a total mortality. There were no additional mortalities after the 24 hour period of the experiment. Apparently fish that were able to survive the first half of the experiment were not affected by the compound during the second half of the test.

Acrolein

The estimated median tolerance limits of chinook salmon exposed to Acrolein in the 20°C. constant temperature room were as follows: 24 hour and 48 hour, 0.081 p.p.m. The validity of this TL_m is questionable as it was based on total survival in 0.075 p.p.m. Acrolein and total mortality in 0.087 p.p.m., which was the next highest concentration (table 10). After two hours had elapsed, there was no survival in 1.35 and 2.4 p.p.m. as well as 4.2 p.p.m. Acrolein. Only 20 per cent of the test animals remained alive in 0.42 p.p.m. at this time.

Table 9
Survival of Chinock Salmon in Omazene Bioassays

	No. Test Animals	No. Test Survivin	Animala A After	Survivi	est Animals ng After	Avg.	Avg.
Conc. p.p.m.		24 Hours	48 Hours	24 Hours	48 Hours	Length mm	Weight Grams
0.1	10	10	10	100	100	62.4	2.94
0.18	īŏ	10	10	100	100	57.5	2.11
0.32	10	10	10	100	100	63.0	3.1
0.56	20	20	20	100	100	58.∙8	2.45
0.75	10	7	7	70	70	62.1	2.57
1.0	20	3	3	1.5	15	60.9	2.4
1.8	10	0	0	O	O	60.0	3.05
3.2	īo	O	O	O	O	62.5	2.94
5.6	īo	Ō	0	O	0	59 . 0	2.8
10.0	ĩo	Ö	O	0	O	61.0	2.97
0	īŏ	10	10	100	100	62.3	3.1
€ 4	· · · · · · · · · · · · · · · · · · ·				Starti	ng Alkalini	ty - 50
Ending	g pH - 7.45 oH - 7.6				Ending	Alkalinity	- 57

Table 10
Survival of Chinook Salmon in Acrolein Bioassays at 20° C.

		No. Test Survivin	Animals After		est Animals ng After	Å v g.∙	Avg.
Conc. p.p.m.	No. Test Animals	24 Hours	48 Hours	24 Hours	48 Hours	Gength mm	Weight Grams
0.075	10	10	10	100	100	70.0	3.88
0.087	īŏ	Ō	Ō	O	O	68.4	3.6
0.135	īō	O	O	0	O	70.4	3.98
0.42	10	Ō	O	O	0	70.5	3.89
0.75	10	0	O	O	O	67.2	3∙5
0	ĩo	10	10	100	100	67.7	3.6 8
Starting Ending p	pH - 7.12 oH - 7.25					ng Alkalinit Alkalinity	

Results of experiments conducted at 13° C. to estimate the median tolerance limit of chinook salmon to Acrolein were as follows: 24 hour and 48 hour, 0.075 p.p.m. (table 11). Test animal survival corresponded in these experiments to that in the 20° C. environment in that there was no partial mortality in any test jar. At the end of the 24 hour and 48 hour periods there was 100 per cent survival in one jar containing a concentration of 0.075 p.p.m. Acrolein and complete mortality in a duplicate jar having the same concentration.

Exposure of rainbow trout to Acrolein at 13° C. resulted in the following estimated median tolerance limit: 24 hour and 48 hour, 0.062 p.p.m. (table 12). These results were similar to the chinook toxicity tests at 13° C. and 20° C. in that there was no partial mortality in any test jar after 24 hours and 48 hours had elapsed.

Although tables 11 and 12 denote concentrations that produced a partial mortality, these data were averaged from more than one test jar. Survival variation between jars of the same concentration is thought to be caused by either a very narrow tolerance range of the test animals or by hypersensitivity of the test fishes. All fishes introduced into Acrolein became extremely excitable. The possibility exists that an active individual in a test jar could have a stimulating effect on the other individuals,

Table 11 Survival of Chincok Salmon in Acrolein Bioassays at 13° G.

	No. Test Animals		t Animals ng After		est Animals ng After	Avg.	Avg. Weight Grams
Conc. p.p.m.		24 Hours	48 Hours	24 Hours	48 Hours	Length ma	
0.0135	5	5	5	100	100	85.1	7.51
0.024	5	5	5	100	100	82.0	7.5
0.042	10	10	10	100	100	ଌ ୍. 7	ଃ • 3
0.056	10	10	10	100	100	87.9	8.04
0.075	10	5	5	50	50	84.0	6.21
0.135	5	0	0	0	0	89.0	7.6
0.24	5	0	0	0	0	87.0	7.8
0.42	5	0	0	0	0	89.0	8.1
0.75	: 5	O	Ö	0	0	81.5	7.2
O	5	5	Š	100	100	91.8	9.02

Table 12 Survival of Rainbow Trout in Acrolein Bioassays at 13° C.

		No. Test Animals Surviving After			est Animals ng After	Avg.	Avg.
Conc. p.p.M.	No. Test Animals	24 Hours	48 Rours	24 Hours	48 Hours	Length ma	Weight Grams
0.056	15	10	10	66	66	ଃ5∙5	7.4
0.065	īo	5	5	50	50	84.0	7.4
0.075	15	Ó	0	0	0	85.5	7.56
0.087	5	0	0	0	0	88 .0	7.96
0.1	5	O	0	O	O	85.0	6.2
0.18	10	0	O	0	0	S9 ∙ 5	7.0
0.32	5	0	0	O	O	0.88	8.2
O	15	15	15	100	100	83.0	7.03
	pH - 7.4 H - 7.4					ng Alkalini Alkalinity	

hence causing death more rapidly because of over-exertion in the presence of the toxicant.

Constant Flow Experiments

Endothal

The effect on largemouth black bass exposed to four concentrations of Endothal for four days is presented in table 13.

No mortalities occurred during this experiment in any solution, but feeding reactions of bass in 135 p.p.m. Endothal implied that these fish were under stress. On the second day, bass in this concentration took earthworms in a sporadic manner. On occasion if a worm was taken, it was rejected immediately. This feeding action continued throughout the remainder of the test.

Bass in 10, 56 and 100 p.p.m. Endothal fed normally during the experiment and these fish were more aware of the experimenter than those held in 135 p.p.m.

Simazine

The results of Simazine's effect on chinook salmon held in flowing water are summarized in table 14.

The first indication of test animal distress occurred on the second day when there were nine mortalities in the 10 p.p.m. solution. The remaining fish would not accept

Table 13

Survival of Largemouth Black Bass after Four Days Exposure to Endothal

		9	· • · · · ·	est Anima ng After	2ls	Average	Average	Average Dissolved	
Conc. p.p.m.	No. Test Animals	l	2 Cays	3 days	4 days	Length (mm)	Weight (Grams)	(p.p.m.)	
10	10	100	100	100	100	61.0	2.6	6.1	
5€	10	100	100	100	100	62.5	3.05	5.87	
100	10	100	100	100	100	60.4	2.9	6.05	
135	10	100	100	100	100	63.0	3.61	5.8	
0	10	100	100	100	100	60.0	2.84	5.92	

Table 14
Survival of Chinook Salmon after Four Days Exposure to Simazine

		P.		'est An i ma .ng A fter	31 3	Average	Average	Average Dissolved	
Conc. p.p.m.	No. Test Animals	dey	2 days	3 dey s	4 deys	Length (mm)	Weight (Grams)	Охудеп (р.р.ш.)	
1	10	100	100	100	100	73.2	3.83	6.1	
ã•2	10	100	100	90	90	74.0	4.05	5.8	
5.6	10	100	100	80	60	71.9	3.2	6.05	
10	10	100	10	0	O	71.5	3.16	5.9	
О	10	100	100	100	100	70.5	3.03	6.35	

food. During the third day total mortality was recorded in this concentration.

Test animal distress was noted in 5.6 p.p.m. on the second day. The fish swam in a vertical position near the top of the container. By the end of the third day there was 80 per cent survival in the solution. Chinook salmon in this concentration would not accept worms during the fourth day and at the end of this period, when the experiment was terminated, there was 60 per cent survival in the bottle.

Fish held in 3.2 p.p.m. Simazine demonstrated a 90 per cent survival by the end of the third day and those remaining in the bottle accepted food. At the end of the run on the fourth day, there was still a 90 per cent survival in this solution.

Chincok confined to 1 p.p.m. Simazine and those in the control bottle sustained no mortalities during the experiment. These fish took worms readily and were quick to become excitable when the experimenter approached.

Dalapon

The seven-day survival of largemouth black bass exposed to four concentrations of Dalapon in flowing water is expressed in table 15.

All bass held at 1000 p.p.m. Dalapon were in distress

Table 15
Survival of Largemouth Black Bass after Seven Days Exposure to Dalapon

		or of the state of				Average	Average	Average Dissolved		
No. Test Animals	lay	2 day	3 day	4 day	5 day	6 day	7 day	Length (mm)	Weight (Grama)	oxygen (p.p.m.)
10	100	100	100	100	100	100	100	60.6	2.96	5.8
10	100	100	100	100	100	100	100	60.0	2.51	8.8
10	100	50	40	30	10	0	0	62.7	3.63	5.75
10	30	O	0	0	0	0	0	61.6	5.24	5.7
10	100	100	100	100	3.00	100	100	60.0	2.84	5.8
	10 10 10 10	Animals day 10 100 10 100 10 100 10 30	No. Test Animals 1 2 day 10 100 10 100 10 100 10 100 10 30	No. Test Animals 1 2 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	No. Test Animals 1 2 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	No. Test 1	No. Test Animals 1 2 3 4 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	No. Test 1 2 3 4 5 6 7 Animals day day day day day day day 10 100 100 100 100 100 100 100 10 100 50 40 30 10 0 0 10 30 0 0 0 0 0 0	No. Test 1 2 3 4 5 6 7 Length Animals day (.mm) 10	No. Test 1 2 3 4 5 6 7 Length Weight Animals day day day day day day day day day (mm) (Grams)

by the end of the first day, and there was only a 30 per cent survival at this point. Distress was characterized by a loss of equilibrium, and the fish swam in a vertical position near the top of the bottle. Total mortality was noted in this solution on the morning of the second day.

Test animals introduced into 500 p.p.m. Dalapon appeared normal until the second day of the test. During this period there was a 50 per cent mortality and fish that remained alive swam on their dorsal surface at the top of the container. By the end of the third day there was a 60 per cent mortality in the solution and the fish that had not succumbed were lying on their dorsal surface at the bottom of the test container. By the fifth day there was only 10 per cent survival in this bottle and this fish died early on the sixth day.

There was no mortality in 125 p.p.m., 250 p.p.m. Dalapon or in the control jar at the end of seven days, but two bass in 250 p.p.m. appeared in some distress on the fifth day of the run. By the sixth day these two fish had apparently regained their equilibrium. Active feeding took place in these jars except during the third day when bass in 250 p.p.m. appeared uninterested in worms.

Bass survival for 48 hours in a standing water solution of 1000 p.p.m. Dalapon concurrent with this flowing water experiment confirmed results obtained by

Fryer (11, p. 31). These fish were on their backs on the bottom of the test jar in a narcotized condition at the end of 24 hours, but at the end of the test the fish had practically recuperated and 100 per cent survival was noted in the solution.

Short-term Exposures with Fishes

Penite

Sodium arsenite is commonly used in the field as a submergent plant herbicide at concentrations that range from 3 p.p.m. to 8 p.p.m. of the active ingredient, arsenic trioxide. Canadian workers who performed tolerance tests with chum salmon report that the 48 hour median tolerance limit of this species to Penite 6X is approximately 11 p.p.m. The calculated time to 50 per cent sample mortality with 84.5 p.p.m. As 203 was reported as 426 minutes (2, p. 28). A 100 per cent survival, after observation for 24 hours, was noted from exposure of chinook salmon to this compound at 5 p.p.m. and 10 p.p.m. (table 16). Fish did not appear in distress while held in the dilutions, but a number of the test animals held in 10 p.p.m. attempted to jump out of the immersion basket.

Table 16
Survival of Chinock Salmon after Short-period Exposure to Penite

Conc. p.p.m.	No. Test Animals	Winutes Exposure	Per Cent Survival of Test Animals at End of Exposure	1	in F	resh 3	Water 4	Jars 8	st Ani After 12 hour	<u>r</u> 24	Avg. Length (mm)	Avg. Weight (Grams)
5	10	15	100	100	100	100	100	100	100	100	79.5	4.87
5	10	30	100	100	100	100	100	100	100	100	85.1	5.32
10	10	15	100	100	100	100	100	100	100	100	74.7	4.18
10	10	30	100	100	100	100	100	100	100	100	86.0	6.02
0	5	15	100	100	100	100	100	100	100	100	82.0	5.14
0	5	30	100	100	100	100	100	100	100	100	74.6	4.1

Baron

This compound may receive extensive usage as a general vegetation control agent on ditch banks and has been tested as a submergent plant killer at concentrations of from 1 to 3.91 p.p.m. Exposure results of chinook salmon to 5 and 10 p.p.m. Baron are listed in table 17. Fish, when removed from 15 and 30 minute exposures to 10 p.p.m., had difficulty swimming for a few minutes. A number of these rested on their backs on the bottom of the fresh water container in a narcotized condition, but after 15 minutes all of these test animals but one had gradually returned to normal.

Kuron

Kuron is marketed as a brush killer and has been tested in aquatic areas at concentrations of from 1 to 2 p.p.m. One hundred per cent survival was noted with chincok salmon that were exposed to 5 and 10 p.p.m. (table 18). Two fish held in 10 p.p.m. Kuron were in distress at the end of 30 minutes exposure, but these individuals revived after they were replaced into the fresh water observation jar. This compound appeared to

¹ Applied as a spray.

Table 17
Survival of Chinook Salmon after Short-period Exposure to Baron

Conc.	No. Test Animals	Minutes Exposure	Per Cent Survival of Test Animals at End of Exposure	1	in F:	Surv resh \ 3 hour	Water 4	Jars 8	After 12	24	Avg. Length (mm)	Avg. Weight (Grams)
5	10	15	100	100	100	100	100	100	100	100	86.6	6.13
5	10	30	100	100	100	100	100	100	100	100	84.2	5.58
10	10	15	100	100	100	100	100	100	100	100	81.0	5.12
10	10	30	100	90	90	90	90	90	90	90	ଥ5 . 7	5.81
0	5	15	100	100	100	100	100	100	100	100	87.0	5.52
0	5	30	100	100	100	100	100	100	100	100	83.5	5.26

Table 18
Survival of Chinook Salmon after Short-period Exposure to Kuron

Conc.	No. Test Animals	Minutes Exposure	Per Cent Survival of Test Animals at end of Exposure	1	in F	resh V	Water 4	of Tes Jars 8 hour	Afte:	<u>r</u> 24	Avg. Length (mm)	Avg. Weight (Grams)
5	10	15	100	100	100	100	100	100	100	100	71.1	3.34
5	10	<i>3</i> 0	100	100	100	100	100	100	100	100	69.4	3.06
10	10	15	100	100	100	100	100	100	100	100	63.7	2.57
10	10	30	100	100	100	100	100	100	100	100	70.7	3.2
0	5	15	100	100	100	100	100	100	100	100	71.0	3.18
0	5	30	100	100	100	100	100	100	100	100	62.5	2.44

settle out on the bottom of the test jar, although the solution was agitated when the material was introduced. It is possible that the material that fell out of solution was merely the carrier of the active ingredient.

Delrad (Rosin amine D acetate)

This compound is used as an algicide at concentrations that range from 0.3 to 1.0 p.p.m. A toxicity summarization by Alabama workers reports that Delrad is "safe" to fishes at a concentration of 0.5 p.p.m. (17, p. 3). Experiments conducted at the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, indicated that the 24 hour TLm of the fathead minnow to Delrad was 0.23 p.p.m. (18, p. 513). Chinook salmon sustained no mortalities when exposed to 0.5 p.p.m. and 1 p.p.m. Delrad for 15 and 30 minutes (table 19). One fish was in distress at the end of 30 minutes in 1 p.p.m., but this individual recovered after being placed into the fresh water jar.

Phygon XL (Dichlone)

This material has recently been introduced as an algicide effective at concentrations that range from 0.01 to 0.15 p.p.m. In tests conducted in the Fisheries Research Laboratory at Oregon State College, Fryer reports the 24 hour TL_m of largemouth black bass exposed to

Table 19
Survival of Chinook Salmon after Short-period Exposure to Delrad

Conc.	No. Test	Minutes	Per Cent Survival of Test Animals at End of	1	<u>in F</u>	Survi resh V	Nater 4	Jars 8	After 12	24		Avg. Weight
p.p.in.	Animals	Exposure	Exposure	hour	hour	hour	hour	hour	hour	hour	(mm.)	(Grams)
0.5	10	15	100	100	100	100	100	100	100	100	72.5	3.17
0.5	10	30	100	100	100	100	100	100	100	100	70.1	2.98
1.0	10	15	100	100	100	100	100	100	100	100	68.7	2.78
1.0	10	30	100	100	100	100	100	100	100	100	69.0	3.03
0	5	15	100	100	100	100	100	100	100	100	7 3.8	3.34
O T	5	30	100	100	100	100	100	100	100	100	67.7	2.74

Phygon XL to be approximately 0.08 p.p.m. and the concentration at which silver salmon showed no distress for 96 hours to be 0.042 p.p.m. (11, p. 25). In this work chinook salmon were exposed to 0.1 and 0.5 p.p.m.

Phygon XL (table 20). While immersed in the test solutions, chinook moved sporadically as though in severe distress, and two fish were dead in 0.5 p.p.m. at the end of the 30 minute exposure. The remaining fish held in this concentration for 30 minutes were dead after two hours in the fresh water observation jar. Fish exposed to 0.5 p.p.m. for 15 minutes demonstrated a 40 per cent survival at the end of 24 hours of observation.

Copper Sulfate

Copper sulfate is one of the most widely used herbicides to control aquatic vegetation. The concentrations utilized in treatment vary from 0.1 to 10 p.p.m. Lawrence reports that copper sulfate is "safe" to fishes at concentrations of from 0.5 to 2.0 p.p.m. (17, p. 3). Research conducted at the Robert A. Taft Sanitary Engineering Center indicates that the 24 hour TL_m of the fathead minnow to copper sulfate is approximately 0.19 p.p.m. (18, p. 513). There was no visible distress revealed by any test animal during immersion in 1 and 2 p.p.m. solutions of copper sulfate (Cu₂SO₄·5H₂O) and the

Table 20
Survival of Chincok Salmon after Short-period Exposure to Phygon XL

Conc.	No. Test Animals	Minutes Exposure	Per Cent Survival of Test Animals at End of Exposure	1	in F	resh V	Vater 4	Jars	st Ani After 12 hour	24	Avg. Length (mm)	Avg. Weight (Grams)
0.1	10	15	100	100	100	100	100	100	100	100	80.1	5.83
0.1	10	30	100	100	100	100	100	100	100	100	83.3	6.25
0.5	10	15	100	80	50	50	50	40	40	40	79.6	4.77
0.5	10	30	©	20	10	0	0	0	0	0	82.2	5 .65
0	5	15	100	100	100	100	100	100	100	100	79.5	5.02
0	5	30	100	100	100	100	100	100	100	100	SO.7	€.2

salmon appeared normal through the 24 hour observation period (table 21).

Trichlorobenzene plus 5 per cent Colloidal X-77 Emulsifier

TCB, generally combined with an emulsifier, has been employed to control submergent aquatic vegetation. Eicher reports that TCB is deadly to largemouth black bass and bluegill sunfish at 5 p.p.m. (9, p. 179).

Results summarized in table 22 point out that chinook salmon withstood short exposures of 50 p.p.m. TOB plus emulsifier without mortality. After 12 hours in the fresh water jars, two fish died that had been exposed to 100 p.p.m. TOB for 30 minutes. There was no observable change in the test animals during the exposure period, and all the fish appeared active when they were returned to the fresh water jars.

Acrolein

This compound has been described as having an intense phytotoxic effect on submergent plants at concentrations as low as 0.5 p.p.m. (20, p. 335). Exposure results of chinook salmon to 2 and 4 p.p.m. Acrolein indicate that the compound is extremely toxic even for short periods (table 23). Fish exposed to 4 p.p.m. showed immediate discomfort and were narcotized, with only opercular

Table 21
Survival of Chinook Salmon after Short-period Exposure to Copper Sulfate

Conc. p.p.m.	No. Test Animals	Minutes Exposure	Per Cent Survival of Test Animals at End of Exposure	1	<u>in F</u>	resh 1	ater 4	Jara 8	st An After 12 hour	r 24		Avg. Weight (Grams)
	10	15	100	100	100	100	100	100	100	100	71.7	3.32
1	10	30	100	100	100	100	100	100	100	100	71.9	3.1 8
2	10	15	100	100	100	100	100	100	100	100	67.3	2.77
2	10	30	100	100	100	100	100	100	100	100	75.2	3.5
0	5	15	100	100	100	100	100	100	100	100	69.2	2.78
0	5	30	100	100	100	100	100	100	100	100	72.0	3.08

Table 22

Survival of Chinook Salmon after Short-period Exposure to Trichlorobenzene plus 5 per cent Colloidal X-77 Emulsifier

Conc.	No. Test	Minutes	Per Cent Survival of Test Animals at End of	1	in P	resh) 3	Water 4	Jars 8	st And	24	Avg. Length	
p.p.m.	Animals	Exposure	Exposure	nour	nour	nour	nour	nour	hour	nour	(mm)	(Grams)
50	10	15	100	100	100	100	100	100	100	100	∂4.3	5.93
50	10	30	1.00	100	100	100	LOO	100	100	100	36 .4	6.2
100	10	15	100	100	100	100	100	100	100	100	მ3∙0	5.63
100	10	30	100	100	100	100	100	100	80	80	95 .3	6.17
0	5	15	100	100	100	100	100	100	100	100	86.9	6.31
o	5	30	100	100	100	100	100	100	100	100	79.3	5.48

Table 23
Survival of Chinook Salmon after Short-period Exposure to Acrolein

Conc.	No. Test Animals	Minutes Exposure	Per Cent Survival of Test Animals at End of Exposure	1	in Fr	resh V	ater 4	Jars 8	st And After 12 hour	24	Avs. Length (mm)	Avg. Weight (Grams)
2	10	15	100	0	0	0	0	0	0	0	96.5	10.4
2	10	3 0	100	0	0	0	0	0	0	0	93.5	10.18
4	10	1 5	90	0	0	0	0	0	O	0	90.0	8.24
4	10	30	50	0	0	0	9	0	0	0	87.4	8.02
0	5	15	100	100	100	100	100	100	100	100	୫ ୨.2	9.2
0	5	30	100	100	100	100	100	100	100	100	92.0	9.51

movement visible, at the end of 20 minutes. At the end of the 30 minute exposure there was 50 per cent survival in this concentration. Chinook held in 2 p.p.m. Acrolein appeared normal at the end of 15 and 30 minute exposures. After one hour in the fresh water jars, there was 100 per cent mortality of fish exposed to both 2 and 4 p.p.m.

Bicassays with Plants Present

FB.2

The effect of FB.2 on the combination of chincok salmon and Ceratophyllum is denoted in table 24. Mortalities occurred in each of the three concentrations, but no uniform difference in test animal survival was evident between jars that contained plant and fish and jars that held fish only. In the solutions that contained 28 p.p.m. and 37 p.p.m. FB.2 fish survival was higher when the plant was not present. This result was reversed in 32 p.p.m. where test animal survival was higher in solutions that contained both plant and fish. A number of fish were in evident distress after six hours of the test, and the first mortalities occurred at this time. After 48 hours Ceratophyllum was dead in all concentrations of FB.2. All test solutions except the control jar solutions took on a cloudy appearance as the bioassay progressed.

Table 24

The Effect of FB.2 on Chinook Salmon in the Presence of Ceratophyllum demersum

		Grams				Survi imals			Condition of Plant	Average Length of Test	Weight
Conc.	No. Test	of	1	2	6	18	24	48	after	Animals (mm)	Animals
p.p.m.	Animals	Plant	hour	hou r	hou r	hour	hour	hour	48 Hours		(Grams)
2 8	10	84	100	100	100	4 0	30	10	dead	90.0	9.75
2 8	10	0	100	100	100	80	50	50		86.0	8.35
32	10	8 4	100	100	8 0	60	60	10	d e ad	94.0	9.45
32	10	0	100	100	8 0	20	10	0		89.5	8.4
37	10	8 4	100	100	10	0	0	0	dead	95.0	10.0
37	10	0	100	100	100	30	3 0	20		96.0	9.55
0	5	42	100	100	100	100	100	100	normal	84.0	7.9
0	5	0	100	100	100	100	100	100		90.0	8.5

Baron

Ceratophyllum apparently had no uniform effect on the survival of chinook salmon when they were exposed to Baron (table 25). Fish tested in 3.7 p.p.m. Baron demonstrated a higher survival where Ceratophyllum was present, but in 2.8 p.p.m. higher survival occurred in dilutions where the plant was absent.

ACP

Chincok salmon showed practically the same survival when tested with ACP regardless of the presence of Ceratophyllum (table 26). There appeared to be a higher survival with vegetation present in 135 p.p.m. and 180 p.p.m. ACP after 24 hours of the test, but by the 48th hour in these concentrations there were more fish remaining alive in dilutions free of Ceratophyllum.

Jar Experiments with Plants

FB.2

Results from the addition of <u>Ceratophyllum demersum</u> into four concentrations of FB.2 indicated that the plant was susceptible to the compound in a controlled light and temperature situation (table 27). Plants held in the higher concentrations were necrotic after 24 hours in the

Table 25

The Effect of Baron on Chinock Salmon in the Presence of Ceratophyllum demersum

		Grams			er Cent 'est Ar				Condition of Plant	Average Length of Test	Weight
Conc. p.p.m.	No. Test Animals	of Plant	hour	2 hour	6 hour	18 hour	24 hour	48 hour	after 48 Hours	Animals (mm)	Animals (Grams)
2.1	10 10	84	100 100	100	100 100	100 100	100 100	100 90	necrotic	79.5 86.5	6.09 7.0
2.8 2.8	10 10	8 4 0	100 100	100 100	100 100	100 100	100 100	70 100	necrotic	92.7 92.0	7.72 7.42
3.7 3.7	10 10	8 4 0	100 100	100 100	90 100	90 30	90 10	80 0	dead	92.0 93.5	7.73 8.95
0	5 5	42 0	100 100	100 100	100 100	100 100	100 100	100 100	normal	85.0 86.0	7.4 6.12

Table 26

The Effect of ACP on Chinock Salmon in the Presence of Ceratophyllum demersum

		Urams				Survi			Condition of Plant	Average Length of Test	Average Weight of Test
Conc. p.p.m.	No. Test Animals	of Plant	i hour	2 hour	6 hou r	18 hour	24 hour	48 hour	after 48 hours	Animals (mm)	Animals (Grams)
135	10	84	90	90	90	60	50	30	necrotic	97.7	9.32
135	îŏ	Ō	100	1.00	100	100	90	20		93.0	8.73
180	10	8 4	100	100	100	20	20	0	necrotic	91.5	7.49
180	ĩo	Ō	100	100	100	100	90	10		85.2	7.05
240	10	84	100	100	100	70	70	0	necrotic	86.2	7.1
240	10	ō	100	100	100	08	50	O		91.5	8.15
0	5	42	100	100	100	100	100	100	normal	90.0	7.46
Ö	5 5	Õ	100	100	100	100	100	100		100.0	9.72

Table 27

The Effect of FB.2 on Ceratophyllum demersum with Controlled Light and Temperature

Concentration p.p.m. active	Grams of	Cond	ition of	Plant P	fter*
ingredient	Plant	l day	2 days	3 days	4 days
1	40	1	1	3	3
1	20	ī	3	4	5
5	40	1	1	3	5
5	20	1	4	4	5
10	40	1	3	4	5
10	20	2	4	4	5
20	40	1	3	4	5
20	20	2	4	4	5
0	40	1	1	1	1
0	20	1	1	1	1

^{*} Condition key:

1 - normal

4 - severe necrosis

2 - chlorosis 5 - dead

3 - partial necrosis

solutions and dead after 96 hours. Apparently the amount of plant present affects its susceptibility in a given amount of dilution water. When 20 grams of plant were tested in 1 p.p.m. and 5 p.p.m. FB.2, there was complete necrosis by the end of 96 hours. When 40 grams of plant were tested in these concentrations with the same amount of dilution water, there was only partial necrosis after 96 hours.

The exposure of <u>Elodea densa</u> in constant light and temperature and unfiltered water indicated that the plant was susceptible to FB.2 under these conditions, although

a longer period of time was needed for the toxic action to take place than when the plant was treated under fluctuating physical conditions outside of the laboratory.

Severe chlorosis had occurred in 1, 5, 10 and 20 p.p.m.

FB.2 after five days of exposure, and the plant was considered dead in the majority of the jars after 12 days. Exceptions were noted in one dilution of 5 p.p.m. and another of 10 p.p.m. Although these plants broke apart easily, they were not considered dead.

The exposure results of E. densa to FB.2 tested outside of the laboratory in fluctuating physical conditions are indicated in table 28. Filtered diluent water was used in this experiment to determine if a loss of toxicity occurred because of occlusion of the herbicide by suspended material in unfiltered water. Within two days chlorosis of E. densa was evident in 20 p.p.m. FB.2, and after three days chlorosis of plants had occurred in all of the test solutions except the control. At the end of four days the plant broke apart when agitated in the test solutions, and death of all tissue was evident on the sixth day. Plants in the control jar appeared normal at this time. The average dilution temperature during the experiment was 20.7° C.

An experiment that differed from the above only by the concentrations chosen indicated that lower water

Table 28

The Effect of FB.2 on Elodea densa with Uncontrolled Light and Temperature

Concentration	Grams	Condition of Plant After*					
p.p.m. active	of	1	2	3	4	5	6
ingredient	Plant	day	days	days	days	days	days
1	20	1	1	2	3	5	5
1	20	1	1	2	2	4	5
5 5	20	1	1.	2	3	5	5
5	20	1	1	2	3	5	5
10	20	1	1	2	4	5	5
10	20	1	1	2	4	5	5
20	20	1	1	2	4	5	5
20	20	1	2	2	4	5	5
0	20	1	1	1	1	1	1
Temperatures:							
minimum (F	P.)	57	55	54	55	51	50
	PI	82	86	83	89	86	86

*Condition key:

1 - normal

4 - severe necrosis

5 - dead

temperatures were not as conducive to FB.2 phytotoxicity as higher water temperatures. E. densa tested at 0.5, 1 and 2 p.p.m. FB.2 were necrotic, but not entirely dead, at the end of 10 days. The average test solution temperature during this experiment was 16.4° C.

Endothal

Elodea densa showed some chlorosis after being tested

^{2 -} chlorosis

^{3 -} partial necrosis

in 10, 25, 50 and 100 p.p.m. Endothal for 16 days outside of the laboratory in naturally fluctuating physical conditions, but apparently the plant had little susceptibility to this compound. The average test solution temperature during this experiment was 20.2° C.

DISCUSSION AND CONCLUSIONS

Endothal

Jar experiment results indicate that chinook salmon are more susceptible to Endothal than are largemouth black bass. The difference in the estimated median tolerance limits of these two species, however, is greater than the difference of the highest concentrations at which the bass and salmon appeared normal. Stated more simply bass were in distress over a wider range of concentrations than were chinook. The range for chinook was between 100 and 155 p.p.m., whereas the range for bass was between 135 and 210 p.p.m. Both species of test animal when in distress were characterized by protruding eyeballs and a narcotized condition.

Constant flow results indicate that largemouth black bass can withstand constant concentrations up to and including 135 p.p.m. Endothal for four days without apparent ill effects. Bass in contact with 135 p.p.m.

Endothal over an extended period of time might experience indirect adverse effects because of sporadic feeding methods. This may not be a factor in concentrations of 100 p.p.m. and lower.

According to test results <u>Flodea densa</u> has little susceptibility to Endothal in situations of fluctuating light and temperature. Concentrations up to 100 p.p.m. Endothal produced chlorosis of the plants, but no deaths were recorded after 16 days of exposure.

Endothal killed <u>Geratophyllum</u> <u>demersum</u> within four days at concentrations of 1, 3.2, 5.6 and 10 p.p.m. in greenhouse experiments where the temperature varied from 20° C. to 24° C.

Potamogeton pusillus and a broad-leaved Potamogeton which was not identified were completely killed by an application of 0.33 p.p.m. Endothal in a largemouth black bass-bluegill sunfish pond near Turner, Oregon, in July of 1958. This pond contained 1.32 acre feet of water and was treated with 25 pounds of 5 per cent Endothal on attaclay. Plant death was complete within one week. There was no fish mortality due to the chemical observed after treatment.

The relatively high tolerance displayed by chinook salmon and largemouth black bass to Endothal allows possible use of this compound as a submergent plant

herbicide in areas where the preservation of these species is desired. The 48 hour median tolerance limits of chinook salmon and largemouth black bass were 132 p.p.m. and 202 p.p.m. respectively. Field concentrations of more than 5 p.p.m. Endothal would probably not be acceptable economically.

ACP-M-569

ACP, a liquid formulation, appears to be more toxic to salmon than the powdered Amino Triazole from which it is derived (11, p. 31). Apparently the additives in the liquid formulation increase toxicity.

Ceratophyllum in bicassays with chinook salmon and ACP apparently had no uniform effect on fish survival in laboratory conditions. It is possible that in these test conditions the herbicides ACP, FB.2 and Baron had a more rapid mode of action on the salmon than on Ceratophyllum. Fishes may have taken up a lethal amount of the compound before any detoxification by the plant could occur.

Baron

Chinook salmon subjected to Baron in standing water bioassays appeared less tolerant of the compound than species tested by workers in other areas (17, p. 3).

Although no mortalities occurred in 48 hours at less than

1.8 p.p.m. Baron, the possibility is remote that the distressed fish in the solutions could survive in natural conditions. The inability to maintain themselves in any current and the incapacity to escape more tolerant predators might limit their survival.

Chinook appeared narcotized after a 30 minute exposure to 10 p.p.m. Baron. No deaths occurred during the 24 hour observation period that followed the exposure, but fish would probably be unable to evade aquatic areas treated at this concentration because of the narcosis.

Survival of chinook was not uniformly influenced by the inclusion of <u>Ceratophyllum</u> in bicassays with Baron. Fish tested in 3.7 p.p.m. Baron, where plants were present, had a larger survival than the test animals held in this concentration without plants, but a larger sample would have been necessary to show a significant difference. After three days in the laboratory conditions, <u>Ceratophyllum</u> appeared susceptible to 3.7 p.p.m. Baron.

Kuron

Less tolerance was shown by chinook salmon held in Kuron than when this species was tested in Baron. Under the conditions of these experiments, chinook can be exposed for 48 hours to concentrations up to 0.56 p.p.m. Kuron without apparent damage.

No mortality was observed after 24 hours when chinook were exposed to 5 and 10 p.p.m. Kuron for 30 minutes. Fish were in distress in 10 p.p.m. at the end of 30 minutes, and apparently a longer exposure would have proved fatal for these test animals.

Simazine

Comparison of the survival results from various fish species tested in Simazine points out that chinook salmon are the most sensitive of the experimental fishes used with this herbicide. Although the standing water tests indicated that chinook were safe in 5.6 p.p.m. for 48 hours, these data are not supported by the flowing water part of this work. In the latter test only 60 per cent of the salmon survived 5.6 p.p.m. Simazine after 96 hours. The cause of this survival difference might be from loss of the toxic properties due to aeration in the standing water test and from the additional 48 hours of exposure to the herbicide in the flowing water experiment. In the constant flow experiment only the fish subjected to 1 and 3 p.p.m. Simazine fed actively at the end of the four-day run.

There was some evidence that chinook in the presence of Simazine exerted a greater dissolved oxygen demand than did the control fish.

Sodium TCA

Practical concentrations of Sodium TCA, that would be feasible for aquatic weed control, were found to have no apparent effect on chinook salmon. No mortalities occurred in 48 hours at the highest test concentration of 870 p.p.m.

FB.2

A 48 hour exposure of chinook salmon to FB.2 indicates that under the test conditions the compound is apparently not harmful at concentrations up to and including 18 p.p.m. The occurrence of 70 per cent mortality in 10 p.p.m. FB.2 at the end of 48 hours may be explained by the fact that test jar aeration failed during the last period of this experiment. The presence of the dark green color in test solutions after 40 hours may have been brought on by a chemical breakdown of the herbicide or by combination of the compound with alkaline wastes emitted by the test animals.

The presence of <u>Ceratophyllum demersum</u> in association with chinook salmon and FB.2 had no uniform effect on fish survival. Before and after <u>Ceratophyllum</u> became necrotic there appeared to be a larger oxygen demand in jars that contained the plant than in jars without vegetation. This increased demand appeared slight in the test solutions,

but in an unaerated area plants might assist in lowering the dissolved exygen to a critical level for chinock salmon.

Ceratophyllum demersum appears susceptible to FB.2 in conditions of controlled light and temperature. Concentrations of 1, 5, 10 and 20 p.p.m. FB.2 killed plant samples by the end of four days. The amount of plant present in a dilution apparently affects its survival. In the lower concentrations of this experiment 40 grams of plant per 15 liters of dilution did not become necrotic as rapidly as plants tested at 20 grams per 15 liters of dilution.

Elodea densa appeared more resistant to FB.2 in controlled physical conditions than in an environment of naturally fluctuating light and temperature. The plant was considered dead after 12 days of exposure to 1, 5, 10 and 20 p.p.m. FB.2 in the laboratory, while outside the building E. densa tested in filtered water was considered dead after six days in these concentrations. Filtering the diluent may have minimized detoxification by reducing the occlusion of FB.2 by suspended material (5, p. 446). Apparently the toxic effect of FB.2 on E. densa is augmented by higher water temperatures.

Omazene

Omazene appeared quite toxic to chinook salmon during the first 24 hours of exposure; concentrations of 0.75 p.p.m. and over produced mortalities. The absence of mortality during the second period of the test may be explained by the fact that under conditions of high pH and alkalinity, copper sulfate precipitates into insoluble copper compounds (18, p. 513). Also the limited dilution volume may have allowed the fish to remove and discard the available copper with sloughed off gill mucus.

Acrolein

Acrolein was the most toxic compound tested in this work. Chinook salmon appeared very sensitive to the herbicide at temperatures of 13° C. and 20° C. The fish were hypersensitive in all of the solutions; the slightest movement by the experimenter caused them to respond violently. Derivation of a safe concentration of Acrolein by the method proposed by Hart et al. (12, p. 130-131) resulted in a concentration of 0.024 p.p.m. for this species at 20° C.

The effects of Acrolein on rainbow trout were similar to those produced on chinook salmon. There was mortality at 0.056 p.p.m., the lowest concentration in the battery of test jars, and the reaction of startled test animals

indicated a hypersensitive condition.

Exposure results indicate that 2 and 4 p.p.m. Acrolein are lethal to chinook after 15 and 30 minute immersions. This compound showed a rapid mode of action on the test animals; deaths occurred after 20 to 30 minutes in the stronger concentration. The experimenter opines that chinook would not be able to escape areas treated with 4 p.p.m. Acrolein. Apparently physiological damage sustained in solutions of 2 p.p.m. asserted itself within one hour, as the fish in this concentration were dead in the observation jars at the end of this time.

Acrolein was found to be difficult to handle in the small amounts concerned either indoors or outdoors. The compound acted as a lachrymator and was irritating to the throat if inhaled. This attribute may not be a problem where precise measurements are not necessary. The compound appeared to be volatile in the test solutions, as its odor was always present in the constant temperature rooms.

Dalapon

Dalapon proved to be more toxic to largemouth black bass in flowing water than in standing water bloassays (11, p. 31). Apparently this compound breaks down under aeration. Bass in 1000 p.p.m. Dalapon succumbed during

the first two days of the constant flow experiment, and total mortality occurred in 500 p.p.m. at the end of five days. Concentrations of 125 p.p.m. and 250 p.p.m. Dalapon apparently were not harmful to the test animals for the seven days of the test. Except for one instance in 250 p.p.m., active feeding continued during the extent of the run in the two lower concentrations and the control. According to these results Dalapon is probably safe to use on marginal aquatic vegetation at the recommended concentrations.

Penite

Short-term exposures with Penite indicated that chinook salmon can remain in contact with 5 and 10 p.p.m. of the active ingredient, arsenic trioxide, for 30 minutes without apparent ill effects. The test animals demonstrated an avoidance reaction in 10 p.p.m. Penite. Cognizance of the herbicide's presence might allow the fish to escape to an untreated section of the water area.

Delrad

Test data indicate that Delrad is not lethal to chinook salmon after a 30 minute exposure to 0.5 and 1 p.p.m. of this compound. One fish appeared in distress after being held 30 minutes in 1 p.p.m.

Phygon XL

According to exposure results, chinook salmon are unable to withstand 0.5 p.p.m. Phygon XL for 30 minutes. Total mortality occurred during immersion in the test solution or within 24 hours after being replaced in the observation jars. Reaction of the test animals indicated that they would be unable to evade an area treated at this concentration. After exposure of 15 minutes to 0.5 p.p.m. Phygon XL, a partial mortality may be expected. Apparently an exposure of 0.1 p.p.m. for 30 minutes causes no ill effects on chinook salmon.

Copper Sulfate

In situations similar to the test conditions, chinook salmon can be exposed to 1 and 2 p.p.m. copper sulfate (Cu₂SO₄·5H₂O) for 30 minutes without apparent harm. Test animal activity in the experimental solutions gave no indication of the possibility of evading this compound.

TCB with 5 per cent Emulsifier

Trichlorobenzene with 5 per cent Colloidal X-77
emulsifier is somewhat toxic to chinook salmon after an
exposure of 100 p.p.m. for 30 minutes. In this work
20 per cent of the test animals succumbed that had been
exposed under the above conditions. A 30 minute exposure

of 50 p.p.m. TCB and emulsifier is apparently not harmful to this species.

BIBLIOGRAPHY

- 1. Academy of Natural Sciences of Philadelphia.

 Toxicity of 3 amino 1,2,4 triazole to Lepomis

 macrochirus. Philadelphia, 1956. 7 numb. leaves.

 (Mimeographed)
- 2. Alderdice, D. F. and J. R. Brett. Toxicity of sodium arsenite to young chum salmon. In. Fisheries Research Board of Canada's Pacific Progress Report no. 108. Nanaimo, B.C., Sept. 1957. p. 27-29.
- 3. Applegate, Vernon C. et al. The toxicity of 4,346 chemicals to larval lampreys and fishes. Washington, D.C., 1957. 157 p. (U.S. Dept. of Interior. Fish and Wildlife Service. Special Scientific Report Fisheries 207)
- 4. Blondeau, Rene, Technologist. Agricultural Research Division, Shell Development Company, Modesto, California, 1958. Letter.
- 5. Brian, R. C. et al. A new herbicide 1:1-ethylene-2: 2-dipyridylium dibromide. Nature 181:446-447. 1958.
- 6. Burrows, R. D. and B. D. Combs, Fishery Research Biologists. Bureau of Sport Fisheries and Wildlife, Entiat, Washington. 1958. Letter.
- 7. Dalquest, W. W. Midwestern University, Wichita Falls, Texas. 1958. Letter.
- 8. Doudoroff, P. et al. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage and Industrial Wastes 23:1380-1397. 1951.
- 9. Eicher, George J., Jr. Localized weed control in management of game fish. Transactions of the American Fisheries Society 76:177-182. 1946.
- 10. Fisher, J. R. The Dow Chemical Company, Seattle, Washington. 1958. Letter.
- 11. Fryer, John L. Toxicity of some new herbicides to fish. Master's thesis. Corvallis, Oregon State College, 1957. 50 numb. leaves.

- 12. Hart, W. B., P. Doudoroff and J. Greenbank. The evaluation of the toxicity of industrial wastes, chemicals and other substances to fresh-water fishes. Philadelphia, Atlantic Refining Company, 1945.
- 13. Hilliard, Douglas K. The effects of herbicides and related compounds on fish. Master's thesis. Corvallis, Oregon State College, 1952. 98 numb. leaves.
- 14. Hiltibran, Robert C., Associate Biochemist. Illinois State Natural History Survey Division, Urbana, Illinois. 1958. Letter.
- 15. Jones, Bernard R. Bureau of Research and Planning, Dept. of Conservation, St. Paul, Minnesota. 1958. Letter.
- 16. Kendle, Earl. Unpublished research, Fish and Game Department, Oregon State College. 1957.
- 17. Lawrence, J. M. Methods for controlling aquatic weeds in fish ponds with emphasis on use of chemicals.

 Auburn, 1958. 4 p. (Alabama. Agricultural Experiment Station. Progress Report ser. no. 69)
- 18. Maloney, Thomas E. and C. Mervin Palmer. Toxicity of six chemical compounds to thirty cultures of algae. Water and Sewage Works 103:509-513. 1956.
- 19. Northeastern Weed Control Conference. Aquatic Weeds. Reprint from the supplement to the Proceedings of the Twelfth Annual Meeting. New York, New York, Jan. 1958. 8 p. (Mimeographed)
- 20. Overbeek, J. van, W. H. Hughes and R. Blondeau. Acrolein for the control of water weeds and disease carrying snails. Science 129:335-336. 1959.
- 21. Setter, L. R., W. E. Brittingham and R. F. Wessels. The Brittingham laboratory pump. Sewage and Industrial Wastes 25:798-801. 1953.
- 22. The Dow Chemical Company. Dalapon bulletin no. 2. Midland, Michigan, Dec. 1953. 20 p.

- 23. Theroux, Frank R., Edward F. Eldridge and W. Le Roy Mallmann. Laboratory manual for chemical and bacterial analysis of water and sewage. 3d ed. New York, McGraw-Hill, 1943. 274 p.
- 24. Walker, Charles R., Biochemist, Missouri Conservation Commission, Columbia, Missouri. 1958. Letter.