1 ppm; chromium, 96-hour TL_m, 110 ppm as dichromate, 170 ppm as chromate, 0.2 ppm as hexavalent chromium under conditions of continuous exposure; ammonia, 2.5 ppm, with toxicity dependent upon pH and extent of ionization.

The 96-hour TL_m's of bluegills in soft water to some of the organic insecticides most widely used in the south are as follows: DDT, 0.016 ppm; BHC, 0.790 ppm; toxaphene, 0.0035 ppm; parathion, 0.700 ppm.

TOLERANCE OF THE FRY OF COMMON WARM-WATER FISHES TO SOME CHEMICALS EMPLOYED IN FISH CULTURE

By R. O. JONES
Bureau of Sport Fisheries and Wildlife
Marion, Alabama

ABSTRACT

The toxicity of fifteen chemicals used in vegetation control or disease treatment was studied. The fry of largemouth black bass, *Micropterus salmoides*, bluegill, *Lepomis macrochirus*, and channel catfish, *Ictalurus punctatus* were used as test animals. Chemicals included were three forms of silvex, three formulations of endothal, simazine, atrazine, diquat, two forms of benzenehexachloride, roccal, acriflavine, malachite green and methylene blue. Results of toxicity tests were used to arrive at a safe concentration of the various chemicals for the species of fry used as test animals.

Several of the chemicals showed evidence of species specificity and one herbicide was sufficiently toxic to these species of fishes to warrant consideration as a fish toxicant.

INTRODUCTION

The purpose of this series of tests was to obtain information concerning the effects of some of the newer herbicides upon young fry; such information to serve as a guide for estimating safe concentrations of untried chemicals for the treatment of ponds containing fry. Other chemicals occasionally used in fish production techniques were included. The tolerance of three species of fry to fifteen chemicals was tested. All of the tests were conducted at the National Fish Hatchery at Marion, Alabama.

MATERIALS AND METHODS

TEST ANIMALS

The black bass (*Micropterus salmoides*) fry used throughout this series of tests ranged in size from 9 to 21 millimeters in total length and had an overall mean total length of 18 millimeters. They were removed from a brood pond on the day before the tests and were held for one hour in a two parts per million solution of pyridylmercuric acetate. They were then kept overnight in a holding house tank.

The bluegill (*Lepomis macrochirus*) fry had maximum and minimum total lengths of 25 and 15 millimeters, respectively, with a mean total length of 17.8 millimeters. They were handled in the same manner as the bass fry as to treatment and acclimatization period.

Channel catfish (*Ictalurus punctatus*) fry ranged in size from 10 to 16 mm and were in the yolk-sac or post absorptive stage.

The acclimatization period varied from one group to another, but was never less than 16 hours. As the water flowing through the holding house and used as diluent for the test solutions was also used to hatch the channel catfish eggs, no acclimatization period was required. These fry were transferred directly from hatching troughs to test solutions. The good survival of those placed in control units demonstrated that the prophylactic treatment wih P.M.A. was not needed.

CHEMICALS

The chemicals used in the tests are listed in Table Number I.

TABLE NUMBER I. CHEMICALS

Common Name	*Trade Name*	*Chemical Name*	*Form*	*Amount of Active Ingredients*
silvex*	Kuron	Propylene glycol butyl ether ester of silvex	Liquid	4 lbs. of silvex acid equivalent per gallon
silvex*	Kurosal S. L.	Potassium salt of silvex	Liquid	6 lbs. of silvex acid equivalent per gallon
silvex*	Kurosal G	Potassium salt of silvex	Granules	20% by weight of silvex acid
disodium endothal	Aquathol	Disodium endoxohexaehydrophthalate	Liquid	19.2% aqueous sol. (1.8#/gal.)
disodium endothal	Aquathol G	Disodium endoxohexaehydrophthalate	Granules	5% by weight of disodium endothal on clay carrier
endothal cocoamine	T. D. 47 Algicide	Di N, N dimethylcocoamine salt of 3, 6 endoxo-hexahydrophthalic acid	Liquid	Not known—used as 100% in test solutions
simazine	Simazine 80W	2-chloro-4,6 bis-(othylaminol)-s-triazine	Wettable powder	80% by weight
atrazine	Atrazine 80W	2-chloro-4 ethyl-amino-6-isoprophyl-amino-s-triazine	Wettable powder	80% by weight
ortho diquat	Ortho Diquat 4 spray	1:1′-ethylene-2:2′-dipyridylium dibromide	Liquid	Used as 100% in test solutions
benzene-hexachloride	BHC Emulsion	1,2,3,4,5,6 hexachloro-cyclohexane	Liquid	11% (gamma isomer)
benzene-hexachloride	BHC-Wettable Powder "Protecto"	1,2,3,4,5,6 hexachloro-cyclohexane	Wettable powder	12% (gamma isomer)
benzalkonium	Roccal	Alkyl dimethylbenzyl ammonium chloride	Liquid	10% (by vol. in test solutions)
acriflavine	Acriflavine (Neutral) N. F.	A mixture of 2ɣ8 diamino-10-methylacridinum chloride and 2ɣ8 diaminoacridine	Powder	Used as 100% in test solutions
malachite green	Malachite Green Crystals Z.F. (zinc free)	di-P-dimethylamino-triphenylcarbinol hydrachloride	Powder	Used as 100% in test solutions
methylene blue	Methylene Blue	Methylthionine chloride	Small crystals	Used as 100% in test solutions

* Silvex is the common name of 2(2,4,5 trichlorophenoxy) propionic acid.

DILUENT

The water used in preparation of the test solutions was taken from a continuously flowing tap in the fish holding house. This water came from a 700-foot flowing well and passed by gravity flow through an 0.8 acre pond. Before use it was held in immersed containers to allow stabilization of temperature and approach to equilibrium with atmospheric gases. Analyses of the well water and that taken from a tap in the holding house are shown in Table Number II. All oxygen concentration readings were above 5 parts per million and a great majority exceeded 8 parts per million.

CONTAINERS

Ten-quart plastic pails were used to hold the test solutions. The pails were thoroughly cleaned before each series of tests and those which had held the strongest lethal concentrations in the preceding tests were used to contain control fry in the series following. This method plus additional testing of the pails provided adequate demonstration that there was no toxic carry-over from prior use of the pails.

TABLE NUMBER II. CHEMICAL CHARACTERISTIC OF DILUENT WATER

	Sample Taken at Well	*Sample from Holding House after Passing through 0.8 Acre Pond*
Temperature °C	21.2	28.0
pH	7.9	8.4
Dissolved Oxygen (ppm)	0.5	9.2
Free Carbon Dioxide	3.0	0.0
CO_3	0.0	3.0
HCO_3	131.	92.
Total Alkalinity * (ppm)	107	75
Total Hardness * (ppm)	108.	78.
Calcium * (ppm)	73	48

* Equivalent of $CaCO_3$.

PROCEDURE

Four liters of diluent water were poured into the containers. These were then immersed to the level of the contained water in holding house tanks through which the supply water was flowing. They were kept thus until the following morning. The minimum interval of this stabilizing period was about 16 hours. Test solutions were prepared just prior to adding the fry.

Exact quantities of chemicals were weighed or measured into the containers and quickly stirred in with a glass rod. No special effort was made to bring dry chemicals into solution. When liquid chemicals were added in volumes of 25 milliliters or more an equal volume of water was first withdrawn from the container.

Fry were put into the solutions very soon after they were prepared. Ten fry were put into each unit and tests were repeated when results were not clearcut. Temperatures of the solutions were routinely taken and dissolved oxygen determinations made occasionally. Observations were made and the number of dead fry recorded at intervals from 1 to 96 hours. In a few instances the surviving fry were observed up to 140 hours, but because of the small size of the fry, the lack of food, and the relatively high water temperatures, starvation was not overlooked as a factor which might contribute to mortality in periods beyond 96 hours. Control units which were identical to test units with the exception of the added chemicals were set up with each series of dilutions. The number of fry used as controls was in excess of 14 percent of the total number.

RESULTS

The effect of each of the chemicals on the three species of fry is discussed separately in the following paragraphs. All concentrations are expressed as parts per million of active ingredient. For acriflavine, malachite green, methylene blue and T.D. 47 this was taken as 100 percent of the substance as obtained.

Kuron

The tolerance of black bass fry was tried at eight concentration levels *e.g.* 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 5.0 and 10.0 parts per million. The fry used ranged in size from 16 to 21 millimeters with a mean total length of 19 millimeters. Ten fry were placed in each of ten containers. Each held four liters of solution. Two of the units were at zero concentration for controls. In the controls and all concentrations less than 1 part per million no death occurred in 120 hours. All fry survived in the 1 part per million solution for more than 72 hours but 2 fry died during the interval between 72 and 96 hours. Two parts per million killed 80% of the fry in 12 hours. The 5 and 10 parts per million concentrations killed 100 percent in 4 and 2 hours, respectively. During these observations the temperature range was from 19.2 to 20.8 degrees centigrade.

One hundred thirty channel catfish fry were used in tests with Kuron. These fry had just reached the stage of complete yolk-sac absorption. They survived a concentration of 0.5 parts per million for 96 hours. One part per million killed 50 percent in 75 hours and 1.5 parts per million killed one hundred percent in less than 14 hours. The temperature range of the solutions for this series was 23.5 to 25.0 degrees Centigrade.

One hundred twenty bluegill fry were used in tests with Kuron. Their mean total length was 18.3 millimeters. Two series of tests were made with bluegill fry. In the first trial, during which the solution temperatures ranged from 21

to 27 degrees Centigrade, all fry exposed to 0.25 parts per million survived for 96 hours. One half part per million killed 50 percent in 48 hours. In the second trial concentrations of 0.3, 0.4, 0.5 and 0.6 parts per million were used. Fry in the 0.3 concentration all survived 72 hours but one died between 72 and 96 hours. At 0.4 ppm 30 percent were dead in 65 hours. At 0.5 and 0.6 ppm, 90 percent of the fry were dead in 65 hours. During this latter series the temperature range was from 23.3 to 25 degrees Centigrade.

Kurosal S.L.

This chemical was not received in time to try its effect on black bass fry or channel catfish fry, but data were obtained using bluegill fry.

One hundred twenty bluegill fry were employed in this series. Their mean total length was 17.4 millimeters. The temperature range during these tests was from 24.4 to 28.8 degrees Centigrade.

No deaths occurred in concentrations up to and including 100 parts per million of active ingredient. A concentration of 150 parts per million caused 60 percent mortality at 96 hours, and 100 percent at 120 hours. All bluegill fry were killed in less than 24 hours by a concentration of 400 parts per million.

Kurosal G.

This material was not received in time to obtain toxicity data from black bass fry or channel catfish fry. Bluegill fry were exposed to eleven different concentrations ranging from 1.25 to 400 parts per million. A total of 145 fry were used in this series. The mean total length of the fry was 17.3 millimeters. Temperatures of solutions during these tests ran from 24.1 to 26.0 degrees Centigrade. No loss of bluegill fry was experienced at concentrations of 150 parts per million or less. At 200 parts per million 30 percent died in 48 hours and at 400 parts per million all fry were dead in 80 hours.

Aquathol (endothal liquid) (Extreme caution should be used in handling this product)

The black bass fry exposed to solutions of endothal had a mean total length of 18.7 millimeters. The temperature range was from 19.2 to 20.2 degrees Centigrade. Three concentrations of active ingredient were tried, *i. e.* 2.5, 5.0 and 10 parts per million. None of these caused mortality in 96 hours. Since the use of concentrations stronger than 2 parts per million was not anticipated, no further tests were made.

Channel catfish fry were exposed to concentrations of 2.5, 10, 15, 20, 25, 50 and 100 parts per million. No catfish fry were lost at any of these concentrations. Further tests were deemed unnecessary. Temperature range of solutions in these units was 24 to 26 degrees Centigrade. The fry used were very small, some still in yolk sac stage.

Bluegill fry proved to be a little more sensitive to the liquid formulation of disodium endothal than were channel catfish fry. One hundred forty bluegill fry having a mean total length of about 17 millimeters were used in this series. With bluegill fry, as with catfish fry, 100 parts per million of disodium endothal was the greatest concentration tried. Forty percent of the fry died in 96 hours at this concentration. Fifty parts per million resulted in no loss in 96 hours under the same conditions.

Endothal (granular)

Black bass fry were exposed to solutions containing 2, 5 and 10 parts per million of the active ingredient. Their average total length was 18.7 millimeters. The temperature range of the solutions during exposure was 19 to 21 degrees Centigrade. No loss occurred in the 2 ppm solution in 96 hours. In 5 parts per million 40 percent died in 40 hours and in 70 parts per million 80 percent died in less than 48 hours.

Fifty channel catfish fry were used in testing granular endothal. These fry were still in the yolk sac stage. During the tests the temperatures of the solutions ranged from 24 to 26 degrees Centigrade. Concentrations from two to 100 parts per million were tried. After an exposure of 96 hours no deaths had occurred at any concentration up to and including 50 parts per million. Only one higher concentration was tried and that was 100 parts per million. At this latter strength 50 percent of the fry were dead at 96 hours.

The tests using bluegills involved 100 fry having a mean total length of 17.8 millimeters. Temperatures of the solution during the exposure ranged from 22.7 to 27 degrees Centigrade. At concentrations of one and two parts per million no deaths had occurred at 96 hours. In fact both of these concentrations and the control were held for 160 hours without the loss of a single fry. At three parts per million 90 percent of the fry were dead in 24 hours. At concentrations of 4, 5 and 10 parts per million all fry died in less than 16 hours. Three stronger concentrations were tried: 25, 50 and 100 parts per million. Twenty-five parts per million killed 70 percent of the fry in 96 hours. Fifty parts per million killed 80 percent of the fry in twenty hours and 100 parts per million killed 90 percent in six hours. Differences of toxicity in lower and higher concentrations might be caused by solubility of active ingredient or its unequal distribution in the product.

Ten pounds of this chemical per acre foot of water has been suggested for vegetation control. As the formulation contains 4.5 percent of active ingredient this will amount to only about one-sixth of a part per million.

It is noteworthy that although channel catfish fry readily tolerated 50 parts per million of disodium endothal, concentrations stronger than 2 parts per million appear to be unsafe for black bass and bluegill fry.

Simazine 80W

The crystalline substance is soluble in water to only about 5 parts per million. It is recommended by the Geigy Company to control aquatic vegetation and algae. The suggested concentration is 3 to 4 parts per million in ponds not exceeding one acre. A company technical bulletin mentions three reports on simazine which indicate that it is safe to a variety of minnows; to bluegills, largemouth bass and catfish at concentrations of 10 to 37 parts per million. Mr. Charles Walker (Missouri) reports safe levels up to 50 parts per million, but does not state kind or size of fish.

In these tests largemouth black bass fry having a total length of 10 to 15 millimeters were used and solution temperatures remained very close to 20 degrees Centigrade. Twenty-five fry were used in each four liter unit. Concentrations from 2 to 25 parts per million were tried. These very small bass fry survived the 25 parts per million concentration for 45 hours. Further observations were made at 69 and 93 hours and at these intervals serious losses had occurred at all concentrations including the control units. These fry had not been held in the diluent water for acclimatization as were the later groups of fry used.

The channel catfish fry used were taken at about the end of the yolk absorption phase (15 to 18 millimeters in total length). The temperature of the solutions ranged from 23.3 to 25.8 degrees Centigrade. Concentrations from 2.5 to 100 parts per million were tried. All groups, including those exposed to 100 parts per million survived a 96-hour period of exposure.

The bluegill fry tolerance test utilized 70 fry having a mean total length of 19.7 millimeters. Temperatures of the solutions ranged from 25 to 27 degrees Centigrade. All concentrations tried including 100 parts per million were tolerated for 96 hours. Higher concentrations were not tested.

Atrazine 80W

One hundred seventy-five bass fry ranging from 10 to 15 millimeters in total length were used. The temperature of the solutions remained near 20° C. Disease of fry masked results of exposure beyond 48 hours. In 48 hours of exposure concentrations of 2, 3, 4 and 5 parts per million were sub-lethal. Ten parts per million killed 28% and 25 parts per million killed 96% in the same period.

Concentrations of 2.5, 5, 10, 25, 50 and 100 parts per million were tried using 70 yolk sac fry of channel catfish. Temperatures of the solutions ranged from 23.3 to 25.8 degrees Centigrade. Concentrations up to and including 10 parts per million were sub-lethal to these fry. Twenty-five parts per million killed 100% in 17 hours, fifty parts per million killed 90% in 8 hours, and one hundred parts per million killed 90% in 5 hours.

Bluegill fry having a mean total length near 19 millimeters were exposed to solutions of Atrazine 80W. One hundred seventy fry were used in these tests. Concentrations up to and including 10 parts per million were sub-lethal in 112 hours. Twenty-five parts per million killed 70 percent, fifty and one

hundred parts per million killed 100 percent in 87 hours. The temperature range of these solutions was 25 to 27 degrees Centigrade.

The highest concentration suggested for vegetation control in Marion Hatchery ponds was 4 parts per million. The fact that Atrazine is much more soluble in water than simazine may explain its greater toxicity.

Diquat

Diquat (ortho-diquat) is a quaternary ammonia compound with herbicidal and desiccant properties. It is inactivated upon contact with soil. It has use as a crop desiccant, a potato foliage killer, and has possibilities for non-selective control of aquatic weeds. Chemically it is 1:1'-ethylene-2:2'-dipyridylium dibromide. The commercial product Ortho Diquat 4 Spray is an aqueous solution containing 4 pounds of diquat per gallon. It is a clear, mobile liquid, red in color, with a specific gravity at 20 degrees Centigrade of 1.2, and a pH of 7. It will not crystallize at 0 degrees Centigrade. It is stable in neutral or acid solution. If diluted with alkaline water it should be used within a few hours. It is non-volatile, but caution should be used in handling. Rubber gloves are recommended for handling the concentrate and splashes on the skin should be washed off immediately. It is incompatible with anionic wetting agents. It has been successfully mixed with 2,4-D, substituted ureas, dalapon, amitrol and simazine. It should not be used in muddy water. For use as an algicide in fish ponds, concentrations stronger than one part per million should not be required.

Eighty yolk-sac channel catfish fry were used to test their tolerance to Diquat. Temperatures of the solutions ranged from 23.3 to 25.8 degrees Centigrade. The highest concentration tried was 10 parts per million. All fry survived in the solutions for 111 hours. Bluegill fry averaging 19.5 millimeters were tested in solutions of Diquat. Concentrations used were 1.3, 5, 10 and 25 parts per million of active ingredient. No mortality occurred in the 1, 3 and 5 parts per million solutions in 96 hours. There was 20 percent mortality in 24 hours in the 10 parts per million solution. In the 25 parts per million solution 40 percent of the fry had died in 15 hours and 50 percent of the fry died in 30 hours. During the remainder of a 96-hour exposure no further deaths occurred. Further tests show that 4 parts per million was safe under conditions of the test but 5 parts per million was doubtful.

Black bass fry were more sensitive to Diquat than either of the above species. One half part per million was the highest safe concentration found when the solution temperature was 26 degrees Centigrade, one part per million when the temperature was from 22 to 23 degrees Centigrade. In one instance good survival was obtained in the 3 parts per million solution, but black bass fry appear to be sensitive to diquat and great caution should be exercised when it is used in ponds containing these fry.

BHC Emulsion

A working stock solution of this chemical was prepared on the basis of an estimated 116 milligrams per milliliter of gamma isomer in the commercial product used.

Black bass fry ranging in total length from 13 to 19 millimeters were exposed to concentrations of the gamma isomer of 0.025, 0.05, 0.1, 0.2 and 0.5 parts per million. During the exposures the temperatures of the solution varied from 19.0 to 20.3 degrees Centigrade. Observations were made at intervals for 128 hours. No losses occurred in the 0.025 and 0.05 parts per million solutions. Thirty percent mortality occurred in the 0.1 part per million solution in eight hours and eighty percent in the 0.2 part per million solution in four hours. One-half part per million killed all bass fry in less than two hours.

Channel catfish fry were exposed to 0.025, 0.05, 0.1 and 0.2 parts per million concentrations of gamma isomer. No loss had occurred in any of these solutions at 96 hours. Data on higher concentrations were not obtained.

Bluegill fry were exposed to concentrations of the gamma isomer of 0.025, 0.05, 0.1, 0.15, 0.25, 0.40 and 0.5 parts per million. No mortality had occurred at 96 hours at concentrations of 0.1 part per million and lower. A concentration of 0.2 part per million resulted in death of 80 percent of the fry in 15 hours and 0.4 part per million killed all fry in the same exposure time. In a repetition of this test no mortality occurred in a 0.1 part per million solution in 96 hours, but at 0.15 fifty percent died in this time and seventy percent at

a 0.25 part per million concentration. The temperature during this series was 26 to 28 degrees Centigrade. Unaccountably in this same series only 20 percent died in the 0.2 part per million solution in 96 hours. Under the conditions of these tests 0.1 part per million of gamma isomer appears to be the highest concentration that can be safely used in the presence of bluegill fry.

BHC Wettable

This formulation contains the same insecticidal ingredient as the liquid described above. The product used bore the trade name of "Protecto" and from information on the label contained 12 percent of active ingredient. Solutions were prepared on the basis of 12 percent by weight of the gamma isomer of BHC in the wettable powder. The stock solution was prepared by accurately weighing 0.8333 grams of the dry powder and diluting it to one liter with deionized water. An insoluble fraction remained and very slowly settled out. The stock bottle was shaken each time a measured sample was withdrawn.

The tolerance of black bass fry to wettable BHC powder was tested using fry ranging in total length from 13 to 19 millimeters. The temperature varied from 19.0 to 20.3 degrees Centigrade. Concentrations of 0.025, 0.05, 0.1, 0.2 and 0.5 parts per million of gamma isomer were tried. No mortality had occurred in the solutions up to and including 0.2 parts per million at 128 hours. The 0.5 part per million unit had a mortality of 30 percent during the 128-hour exposure.

Channel catfish fry were exposed to concentrations of 0.25, 0.05, 0.1, 0.2, 0.3 and 0.4 parts per million for 96 hours. No deaths occurred at any of these concentrations. Greater concentrations were not tried. Temperatures of the solutions in this series were 26-27 degrees Centigrade.

Because of minor inconsistencies and the encroachment of disease in one or possibly two lots of fry five separate series of tolerance tests were made with bluegill fry.

In the first series exposed in June when the solution temperatures ranged from 23.3 to 25° C. a concentration of 0.5 part per million showed only a 10 percent loss in 96 hours. These fry had a mean total length of 17.8 millimeters. In late July and early September when solution temperatures ran from 25.5 degrees Centigrade in the early morning to 28 degrees in mid-afternoon, 200 more bluegill fry were exposed to various concentrations of wettable BHC. Seventeen different concentrations were tried. These ranged from 0.2 to 2.5 parts per million. No fry were killed at 0.7 part per million and less. In two separate units only 1 fry of 10 died during 96 hours of exposure to 1 part per million. Fifty percent were lost in the 1.2 parts per million solution and the percent mortality increased to 90 in the 2.5 parts per million concentration.

From the above data it appears that a concentration of 0.25 part per million might be safely used in the presence of the three kinds of fry tested in the wettable form but not as the emulsion, with the possible exception of channel catfish fry in the case of the latter.

T.D. 47 Algicide

Chemically T.D. 47 is the di N. N dimethylcocoamine salt of 3,6 endoxohexahydrophthalic acid. The manufacturer (Penco Agricultural Chemicals, Products Development Bulletin 8B issued December 12, 1960) claims it to be a highly effective algicide at rates ranging from 0.05 to 5.0 parts per million.

A lack of clarity in the information furnished made it advisable to prepare solutions and report concentrations in terms of volume of the stock liquid rather than weights of acid or disodium equivalents. The granular form of T.D 47 was not available for testing.

Black bass fry were exposed to various concentrations of T.D. 47. One hundred thirty fry ranging in total length from 9 to 14 millimeters were used. The temperatures of the solutions tested varied from 23.5 to 25.5 degrees Centigrade. Fry were exposed to concentrations from 0.025 to 0.4 parts per million. The first series consisted of 0.1, 0.2 and 0.4 parts per million. In the latter two concentrations, all fry were dead at 16 hours. In 0.1 part per million 20 percent were dead in 48 hours and 30 percent in 96 hours. In a second trial, fry were exposed to concentrations of 0.025, 0.05, 0.075, 0.1, 0.125 and 0.15 parts per million. Those up to and including 0.075 gave no evidence of a toxic effect. Concentrations of 0.1 and 0.125 appeared to be on the borderline. In the former, two fish out of ten were dead after 46 hours, but only one in the latter. In

the unit containing the 0.15 parts per million solution 67 percent were dead in 22 hours. A third series was run using concentrations of 0.15, 0.175 and 0.2 parts per million all of which were lethal. All fry were dead in the 0.2 unit in two hours and in the 0.175 unit in three hours. Sixty percent were dead in the 0.15 parts per million unit in four hours. In the light of the above findings it appears that, under the conditions of these tests a concentration of T.D. 47, 0.075 parts per million is the highest which may be considered safe for largemouth black bass fry.

Channel catfish fry were exposed to T.D. 47 at concentrations of 0.025, 0.05, 0.1 and 0.2 parts per million. The fry used were still in the yolk sac stage. The temperatures of the solutions during the tests ranged from 24 to 26 degrees Centigrade. No evidence of a toxic effect appeared at any of these concentrations. Higher concentrations were not tried.

Bluegill fry having a mean total length of 17.9 millimeters were exposed to T.D. 47 in solutions whose temperatures during the tests ran from 22.7 to 27 degrees Centigrade. Five concentrations, 0.5, 0.75, 1.0, 1.5 and 2.0 were tried. In the control unit and in a 0.5 part per million concentration none of the fry died in 100 hours and only one of ten in 0.75 parts per million in 100 hours. Twenty percent were dead in 44 hours at 1.0 part per million. A concentration of 1.5 parts per million killed all fry in eight hours and 2.0 parts per million in six hours. Under the conditions in which these tests were made, 0.75 part per million is the greatest concentration which may be safely used in the presence of bluegill fry.

Roccal

Roccal is available commercially as a ten percent aqueous solution of alkyldimethylbenzylammonium. It is a strong germicide and has many general uses. At a fish hatchery it may be used to disinfect tanks, nets and other tools and it may be used in the control of certain fish diseases. In reporting the tolerance of fry to this chemical all concentrations are expressed as milligrams per liter of active ingredient.

Black bass fry were exposed to solutions having temperatures from 23.0 to 25.5 degrees Centigrade. These fry were from 9 to 13 millimeters in total length. The fry remained for 57 hours in 0.25, 0.5 and 1.0 parts per million. Nine of the ten fry exposed to two parts per million died within 24 hours, and those exposed to four parts per million were all dead in less than two hours.

Channel catfish fry in the yolk sac stage were exposed to solutions of Roccal whose temperatures remained between 25.0 and 27.4 during the tests. Concentrations of 0.25, 0.5, 1.0, 2.0 and 4.0 parts per million. All fry survived for 96 hours in the 0.25 and 0.5 concentrations and only one died in the 1.0 solution in the same period.

Two parts per million killed all fry in less than 19 hours and four parts per million killed all fry in one hour.

On the basis of 60 bluegill fry exposed, this species appears to be more sensitive to Roccal than either bass fry or channel catfish fry. At solution temperatures of 25.5 to 28 degrees Centigrade, bluegill fry averaging 17.3 millimeters in total length were exposed to concentrations of Roccal of 0.5, 1.0, 1.5, 2.0 and 2.5 parts per million of active ingredient. After putting ten fry into each of the containers the first observation was made after a time interval of 16 hours. At that time one fry was dead in the 0.5 concentration. Only two were living in the one part per million solution and these died in the next three hours. In the 1.5, 2.0 and 2.5 parts per million concentrations all fry were found dead at the initial observation at 16 hours. If Roccal is to be used to control disease among fry, stronger doses for shorter periods should be tried.

Acriflavine

This chemical is a mixture of 2,8-diamino-10-methylacridinium chloride and 2,8-diaminoacridine. It is a bacteriostatic agent and is used when holding or transporting fish to retard the spread of infection. It was not tried with black bass fry. Channel catfish fry in the yolk sac stage were still alive after 67 hours in a 5 parts per million solution, but two of the ten died before the next observation at 91 hours. All of the fry at ten parts per million died between 19 and 31 hours. Those held in concentrations of 25 and 50 parts per million all died between 6 and 19 hours. Solution temperatures in this series of tests ranged from 25.0 to 26.0 degrees Centigrade.

The bluegill fry tested with acriflavine had a mean total length of 19.3 millimeters. Solution temperatures ranged between 25.5 and 28.0 degrees Centigrade. Concentrations of 2.5, 5.0, 7.5, 10.0 and 12.5 parts per million were used. No deaths occurred at 2.5 or 5.0 parts per million in 96 hours. At 96 hours 50 percent of those at 7.5 parts per million and 90 percent of those at 10 parts per million had died. The 12.5 parts per million solution caused a 90 percent mortality in 24 hours and 100 percent in 40 hours.

Malachite Green

Malachite Green goes by numerous commercial and popular names. It is one of the analine dye group, having the empirical formula $C_{23}H_{25}ClN_a$. Its dark green crystals are very soluble in water. For use as a dye it is prepared as a double salt with zinc chloride. For use at hatcheries the zinc free form should be purchased. It has limited use in laboratories as a chemical reagent and as a stain for plant tissue or bacterial spores. At many fish hatcheries it is used to control fungus in fish eggs.

The toxicity of this chemical to black bass fry was tested at six concentrations from 0.025 to 0.4 part per million. All of these concentrations proved to be toxic. Ninety percent mortality occurred in the 0.025 solution between 27 and 51 hours. One hundred percent mortality occurred in the 0.05 part per million concentration in less than 17 hours, at the 0.1 level in less than seven hours, at the 0.2 level in less than five hours, and at the 0.3 and 0.4 level in less than three hours. It should be noted that no deaths occurred during the first hour in any of these concentrations.

The tolerance of channel catfish fry to Malachite Green was not tested.

Bluegill fry proved to be considerably more sensitive to Malachite Green than did black bass fry. The bluegill fry used had a mean total length of 20 millimeters and temperatures of the test solutions varied between 25.5 and 28.0 degrees Centigrade. These fry were exposed in groups of ten to concentrations of 0.01, 0.02, 0.025, 0.03 and 0.05 parts per million of Malachite Green. These, like the bass fry were not observed until 16 hours had elapsed. At that time 20, 30, 40 and 80% mortality had occurred in the four stronger concentrations, respectively. Only the 0.01 part per million solution and the control unit had no dead fry. Observations were continued at intervals through 96 hours. At that time none of the fry in the control unit had died. Three of the ten were dead in the 0.01 solution. In the four successively increasing concentrations the percent mortality had changed to 80, 90, 90 and 100, respectively. From the above observations it is obvious that under the conditions in which these tests were conducted, black bass and bluegill fry are extremely sensitive to very low concentrations of Malachite Green.

Methylene Blue

Methylene Blue is the common name for methylthionine chloride. It is used as a chemical reagent, as a stain in bacteriology, and to some extent as an antiseptic. It is sometimes used in the treatment of Ichthiophtheriasis in aquarium fishes.

The tolerance of black bass fry to this chemical was tested using fry having an average total length of 19 millimeters and a range of 16 to 23 millimeters in solutions at a temperature range of 19.2 to 20.5 degrees Centigrade. Concentrations of 2.5, 5, 10 and 25 parts per million were tried. Those exposed to 10 parts per million or less were alive after a 72-hour period of observation. The group in the 25 parts per million concentration all died in less than 23 hours. The fry in this group had gill covers widely distended and the gill chambers completely clogged with flocculated particles of methylene blue. Death apparently was caused by mechanical interference with respiration.

Channel catfish fry in the 15 to 18 millimeter size range were tested for their tolerance to methylene blue in solutions ranging in temperature from 25 to 28 degrees Centigrade. Concentrations of 5, 10, 15, 20 and 25 parts per million were used and observations made at 17, 72, 96 and 112 hours of exposure. At 17 hours no deaths had occurred at 5 and 10 parts per million. Forty percent mortality had occurred at 15 parts per million, fifty percent at 20 and forty percent at 25 parts per million. At 72 hours all fry were dead in the 15, 20 and 25 parts per million concentrations. Eighty percent were dead at 10 parts per million and none at five parts per million. After 96 hours of exposure the only survivors were in the five parts per million solution. All of these were

still living. At 112 hours there were no survivors excepting the controls. Three control units of ten fry each were used. All of these fry were alive and apparently in normal condition after the 112-hour period.

Bluegill fry with a mean total length of 18.5 millimeters and a range of 16 to 21 millimeters were exposed to concentrations of 2.5, 5.0, 7.5 and 10 parts per million of methylene blue. The solution temperatures ranged from 25.3 to 27.3 degrees Centigrade. All of the fry in these concentrations were living after 96 hours of exposure.

DISCUSSION

A total of 3,685 fry were utilized in the tests described in the preceding paragraphs. Five hundred twenty-five of these served as controls. The remainder comprised some 316 separate test units. At most concentration levels ten fry were sufficient to give the information desired. In some instances as many as 40 fry were used at a single concentration.

Data from the tests have been summarized in Table Number III. All of the concentrations listed as safe under the conditions of the tests were survived by at least 90 percent of the fry for a period of 72 hours unless otherwise indicated. The sub-lethal concentrations which have been tabulated are not invariably the maximum tolerated by fry. In instances in which the strength of the solution tolerated was far in excess of the maximum concentration which it would be practical or desirable to employ, greater concentrations were not tried. In these instances the graphical determination of TLM (Median tolerance limit) values was not possible. Such determinations were not made on the remaining data as they would not serve the purpose for which these tests were made.

TABLE NUMBER III

CONCENTRATIONS* OF CHEMICALS READILY TOLERATED BY FRY UNDER CONDITIONS OF THESE TESTS

Chemicals	*Channel Catfish*	*Largemouth Black Bass*	*Bluegills*
Kuron (silvex acid equivalent)	0.5	1.0	0.3
Kurosal S. L. (silvex acid equivalent)	..	..	100.
Kurosal G. (silvex acid equivalent)	..	..	150.
Endothal (Liquid)	100.	10.	50.
Endothal (Granular)	50.	2.	2.
Simazine	100.	25.	100.
Atrazine	10.	5.	10.
Diquat	10.	1.0 at 22.5°C. 0.5 at 26.0°C.	4.
BHC (Emulsion)	0.2	0.05	0.1
BHC (Wettable)	0.4	0.2	0.5
T. D. 47†	0.2	0.075	0.75
Roccal	1.0	1.0	0.25
Malachite Green†	–	0.025‡	0.01
Methylene Blue†	5.0	5.0	5.0
Acriflavine†	5.0	–	5.0

* Concentrations are given in parts per million of active ingredient.
† Concentration is in parts per million of entire product.
‡ Survival for 27 hours—90% mortality in 51 hours.