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SENSITIVITY OF FINGERLING BROOK AND RAINBOW TROUT TO TOXAPHENE - R. E. REINERT 1962

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SENSITIVITY OF FINGERLING BROOK TROUT, SALVELINUS FONTINALIS, AND RAINBOW TROUT, SALMO GAIRDNERI ., TO TOXAPHENE WITH A BRIEF SURVEY OF THE TOXAPHENE LITERATURE

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INTRODUCTION

The main objective of this study was to determine the variation in tolerance to toxaphene between fingerling brook and rainbow trout and also to study the variation in tolerance between different weight classes of these two species.

It has been more than half a century since the first poison was used by fisheries biologists to remove undesirable fish from a body of water. Since that time many chemicals have been found to be toxic to fish. It was after the introduction of rotenone in 1934 that fisheries biologists became interested in the chemical control of fish populations. Rotenone has proven to be a useful tool in fisheries management, but it has certain limitations as a fish toxicant. It detoxifies rapidly, sometimes in 24 hours or less at higher temperatures (Clements and Martin, 1954). Very often detoxification occurs before the chemical has been distributed throughout the lake basin, and as a consequence incomplete kills frequently occur. It is somewhat impractical to use rotenone at temperatures between 40° and 60°F., because its toxicity is greatly reduced (Rose, 1957).

Most of the chlorinated hydrocarbons and organic phosphates marketed as insecticides since the end of World War II can be used as toxicants. The fish kills which followed the use of insecticides on crops in the southern states led to the discovery that these chemicals are extremely toxic to fish (Surber, 1948; Lawrence, 1950;

Young and Nicholson; 1951). Chlorinated hydrocarbons are usually more toxic to fish than the organic phosphates (Henderson, Pickering, and Tarzwell, 1959). Of the chlorinated hydrocarbons toxaphene (octachlorocamphene) has been most widely used as a fish poison. The effects of toxaphene upon fish and aquatic invertebrates have been investigated in the laboratory and in the field. The results of these studies have suggested considerable variation in tolerance to toxaphene among different species of fish and between different size groups of the same species. More precise data on differences between species and upon the effect of size will make this chemical a more useful toxicant.

LITERATURE SURVEY

Chemistry

Toxaphene is manufactured by the Hercules Powder Company of Wilmington, Delaware. It is made by chlorinating camphene, which in turn is made by isomerizing alpha pine, a major constituent of turpentine (Frear, 1955). The approximate empirical formula is $C_{10}H_{10}Cl_8$ and a possible structural formula has been proposed (Figure 1). The chlorine content is from 67 to 59 percent (Metcalf, 1955). The commercial product, technical toxaphene, is a yellow, waxy solid which has a mild and pleasant odor. Its melting point is between 65° and 90°C. and its density is 1.6 grams per milliliter. Toxaphene is insoluble in water, but it is soluble in organic

Figure 1. The approximate structural formula of toxaphene, also known as compound 3953, penphene, toxakil, alltox, geniphene, and camphene. The exact position of chloride ions on the toxaphene molecule is unknown.

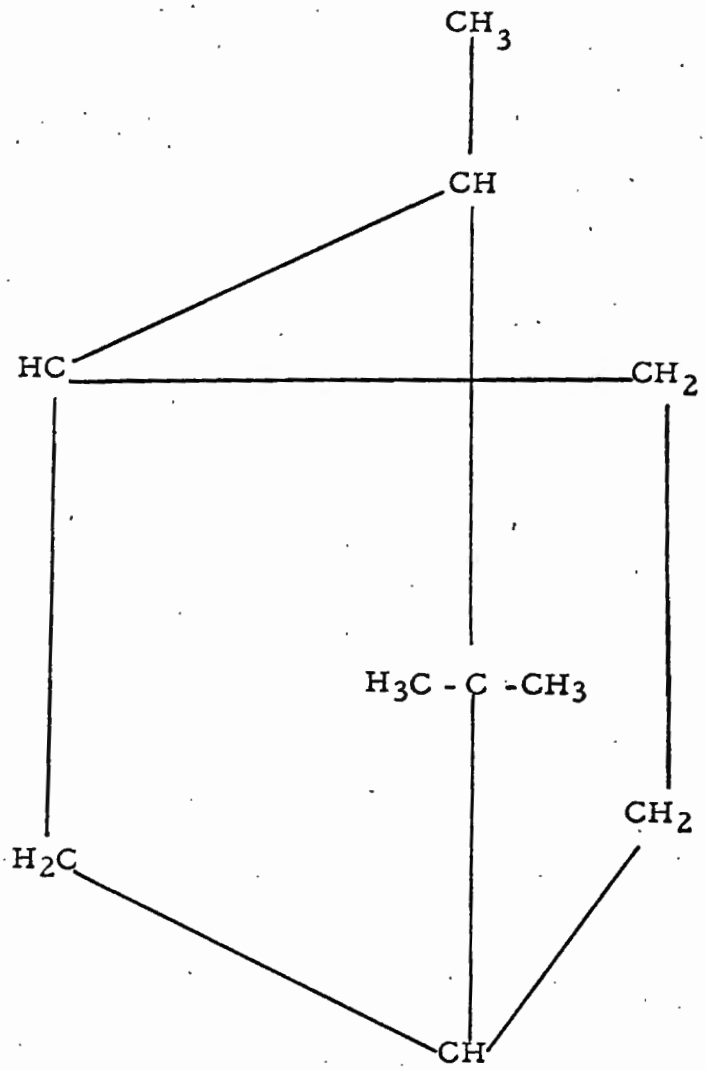

Fig. 1

solutes. It decomposes in the presence of heat, sunlight, or alkali yielding HCl(Frear, 1955). A temperature of 190°F. is required for the breakdown of toxaphene into non-toxic substances.

Agricultural Use

Toxaphene has been widely used as an insecticide since 1947. It is available for agricultural use in four forms; as dusts containing 10 or 20 percent toxaphene; as an emulsifiable concentrate containing 4, 6, or 8 pounds of toxaphene per gallon; as an oil solution; and as a wettable powder containing 40 percent toxaphene (Anon.[2], 1952).

Toxaphene is used on cotton, alfalfa, clover, tomatoes, and potatoes. Emulsifiable concentrates and wettable powders are used as sprays for control of the external parasites of beef cattle, sheep, wool-producing goats, and hogs. (Anon.[2], 1952).

Toxicity to Mammals

The acute toxicity of toxaphene varies widely among mammals. The acute oral median tolerance limit (TL_m) varies from 20 to 30 milligrams per kilogram for dogs to as high as 288 milligrams per kilogram for the guinea pigs (Anon.[4], 1953). The acute oral dosage for man has been estimated to be from 2 to 7 grams of technical toxaphene or 60 milligrams per kilogram of body weight. These figures indicate that a 150 pound man would have to drink approximately 9,000 gallons of water containing 100 p.p.b. to accumulate a lethal dose. Since toxaphene breaks down into non-toxic substances at

temperatures over 190°F, eating fish recovered from toxaphene treated lakes would not seem to be hazardous provided the fish are properly cooked.

In the form of dusts or wettable powders it is poorly absorbed through the skin. In the emulsified form there is danger of absorption through the skin and precautions should be taken in handling (Anon.[4], 1953). Stringer and McMynn (1958) recommend that goggles, rubber gloves, and boots be used when lakes are treated with the emulsifiable form of toxaphene. Accidental ingestion of lethal amounts of toxaphene emulsions has caused several deaths (Anon.[4], 1953). No deaths or illnesses have been reported which can be traced to ingestion of water treated with toxaphene.

The symptoms of toxaphene poisoning appear to be similar in all mammals. It causes a diffuse stimulation of the cerebrospinal axis and brings about convulsions which may lead to respiratory failure (Metcalf, 1955). Degenerative changes in the renal tubules and liver paraenchyma have been noted in mammals which have died from chronic poisoning (Anon.[4], 1953). Continued ingestion of this toxicant over a period of time may result in a build-up of it in the fatty tissue (Anon.[1], 1952). However, if the amount ingested over a given period is small, detoxification by the liver will keep pace with the intake and toxaphene will not be deposited in the tissues. In mammals, this toxicant is excreted in the urine and will disappear from the fat when ingestion is terminated (Metcalf, 1955; Anon.[1], 1952).

The concentrations of this toxicant used in fish eradication do not seem to have had a cumulative effect upon livestock. Livestock were restricted for 60 days to an area in which their only drinking water was lake water treated with 100 p.p.b. toxaphene. These animals showed no adverse symptoms (Hemphill, 1954).

Toxicity to Fishes

A survey of the literature shows that the amount of toxaphene used for fish eradication varies from 5 p.p.b. to 610 p.p.b. Complete fish kills in deep relatively sterile lakes have been achieved with treatments as low as 7.5 p.p.b. by Stringer and McMynn (1960). Stringer and McMynn (1960) found that a concentration of 20 p.p.b. produced only partial kills in three shallow turbid lakes. On the basis of laboratory tests Rose (1958) suggested that concentrations in excess of 25 p.p.b. would be needed to kill such tolerant species as carp and bullheads, while in warmer turbid waters he suggested that concentrations in excess of 200 p.p.b. might be necessary.

Among the warm-water fishes tested bluegills appear to be the most sensitive with a 96 hour TL_m of 3.5 p.p.b. (Henderson, Pickering, and Tarzwell, 1959). Salmonids also appear to be very sensitive. Katz (1961) reports a 96 hour TL_m of 8.4 p.p.b. for fingerling rainbow trout. For Chinook and Coho salmon he gives values of 2.5 and 9.4 p.p.b., respectively.

Low concentrations will often kill small fishes and not injure the larger ones. Selective poisoning experiments on six hard-water lakes in Michigan suggests that a concentration of 5 p.p.b. will selectively poison small, warm-water fishes (Fukano and Hooper, 1958).

Toxaphene appears to attack the central nervous system of fishes as well as mammals. Laboratory observations show a loss of equilibrium followed by convulsive swimming and respiratory motions. The affected fishes eventually sink to the bottom of the aquarium and die (Mayhew, 1955). In treated lakes fish will often swim on shore and strand themselves (Hooper, 1959).

Chlorinated hydrocarbons tend to increase the level of acetylcholine in the nerve tissue of insects (Winteringham and Lewis, 1960). They may also damage or affect the nerve cell or axon wall in such a way as to cause an uncontrolled loss of ions and enzymes (Metcalf, 1955). These effects noted in insects have not been demonstrated in mammals or fishes.

Temperature appears to influence the time required for fishes to be affected by toxaphene. At temperatures below 50°F. and at concentrations near the toxicity threshold it may take a week or more for a fish to show any visible effects of poisoning and fishes may continue to die for three weeks or more. However, if the water temperature is 70°F. the fishes will die within a ten-day period (Hooper, 1959).

Toxicity to Aquatic Invertebrates

Aquatic invertebrates appear to be less sensitive to toxaphene than fishes. Tanner and Hayes (1955) reported that bottom fauna and plankton reappeared in a reservoir before it had detoxified sufficiently to be restocked with fish. Rose (1958) found that there was a marked increase in the number of midge larvae one month after treating a shallow Iowa lake with a concentration of 100 p.p.b. He presumed this was due to the eradication of a large bullhead population which had acted as a predator on the chironomid larvae. Mollusks and oligochaetes appeared to be the only bottom fauna not affected in two Michigan lakes treated with a concentration of 100 p.p.b. (Hooper and Grzenda, 1957). This dosage eliminated the midge larvae from a Colorado reservoir (Cushing and Olive, 1957). This dosage reduced the number of Chironomidae, Ephemeroptera, and Odonata in several British Columbian lakes even though oligochaetes, rotifers, flagellates, and diatoms did not appear to be affected (Stringer and McMynn, 1958).

Low concentrations of toxaphene apparently causes little damage to aquatic invertebrate populations. In two Michigan lakes treated with 10 p.p.b. there was no marked depletion of the bottom fauna (Hooper,1959). Stringer and McMynn (1958) also found no appreciable mortality at this concentration.

The above data indicate that if toxaphene is used in concentrations below 10 p.p.b. there will be little damage to bottom fauna but when used in the 25-50 p.p.b. range, which is necessary in many lakes, certain groups of invertebrates

will be eliminated. Even if many kinds are killed, certain species will generally repopulate the lake before it can be restocked with fish.

Detoxification

One of the major difficulties encountered with the use of toxaphene is the length of time it takes treated waters to detoxify. Periods of from several months to more than three years have been reported in the literature (Rose, 1958; Stringer and McMynn, 1960). Shallow turbid bodies of water appear to detoxify quicker than deep, clear lakes. This may be due to the high ratio of substrate surface to water found in turbid lakes (Hooper and Grzenda, 1957). It has been shown that soil microorganisms can break down toxaphene into non-toxic substances (Smith and Wenzel, 1947). Microorganisms on the lake bottom and on the suspended soil particles of turbid lakes may perform a similar action. Laboratory tests have shown that periodic sterilization of the bottom substrate in aquaria greatly inhibits detoxification (Hooper and Grzenda, 1957).

Some of the other factors which may influence the rate of detoxification are: alkalinity, pH, sunlight, water stratification, flushing rate, oxygen content and temperature (Hooper and Grzenda, 1957; Mayhew, 1959; Hemphill, 1954; and Heneger, 1958). No single chemical or physical characteristic has so far been isolated as wholly responsible for the variation from lake to lake in the rate of detoxification.

Quantitative Methods of Analysis

In the past there has not been a satisfactory quantitative method for the determination of toxaphene in the small concentrations used for fish eradication (Hornstein,[1] June 1957; Hornstein,[2] 1957; and Anon.,[3] 1953). Recently the U. S. Fish and Wildlife Service in Denver, Colorado developed a method which is reported to detect amounts of toxaphene as low as 1 p.p.b. (Kallman, Cope and Navarre, 1962). In this method the pesticide is extracted with organic solvents and the interfering substances are removed. Chlorine from the toxaphene is separated as silver chloride by paper chromatography. The size and intensity of spots formed by known and unknown concentrations are compared visually.

This method was used to study the bathymetric distribution of toxaphene and its rate of detoxification in a lake in New Mexico. Results showed that at no time did the concentrations in the lake approach the theoretical level added to the lake. A much higher level of toxaphene was detected on the lee side of the lake than on the windward side. Both the trout and bullheads that were analyzed for toxaphene showed a high concentration of toxicant within their bodies. The bullhead, a species which is highly resistant to toxaphene, was able to concentrate almost three times as much as the trout (Kallman, Cope, and Navarre, 1962).

MATERIALS AND METHODS

Water

Water used in these experiments was obtained from the Wolf Lake Hatchery. Preliminary tests showed that both rainbow and brook trout from the Wolf Lake Hatchery remained in better condition when kept in hatchery water than when they were kept in either lake water or tap water. The pH of this water ranged from 7.2 to 7.9. Methyl Orange alkalinity ranged from 139 to 158. The phenophthalein alkalinity was zero. Dissolved oxygen ranged from 8.2 to 9.8 p.p.m. in the aerated containers. At the conclusion of test runs the dissolved oxygen content varied from 6.4 to 8.5 p.p.m.

Test Containers

The containers used were wide-mouthed one-gallon jars. After each test the jars were cleaned with chromic acid cleaning solution and rinsed several times with distilled water. Each glass air tube was treated in the same manner. During the tests the top of each jar was covered by a 4" x 4" glass plate.

Toxicant

The toxicant used in these experiments was an emulsified form of toxaphene sold under the trade name of Cooper - Tox Number 6. This product is manufactured by William Cooper and Nephews, Chicago, Illinois. It contained six pounds of toxaphene per gallon of solution. A stock solution was made by diluting 1 ml. of Cooper-Tox Number 6 to 1 liter and then

diluting 1.39 ml. of this solution to 1 liter. The stock solution contained 1000 p.p.b. It may detoxify when stored in glass containers for long periods (Hooper and Grzenda, 1957). For this reason new stock solutions were made for each test.

Fish

The fingerling brook and rainbow trout used in these experiments were obtained from the Wolf Lake Fish Hatchery which is located near Kalamazoo, Michigan. The fish were transported from the hatchery to the Institute for Fisheries Research in cans of 15 gallon capacity. A temperature of approximately 45°F. was maintained during the trip by transporting the cans in an ice-water bath.

Milk cans containing fish were held at a constant temperature for 24 hours before the fish were used in the tests. This was done in order to acclimate the fish to the experimental temperature (40°F.) and to eliminate fish which had been injured. Fish which showed visible injuries were removed and discarded. Prior to the beginning of each test the experimental fish were weighed in a beaker of water and the weight of each fish was recorded.

Aeration

Preliminary studies showed that trout survived better in aerated water. For this reason test containers were aerated for 24 hours before each test and during the ninety-six-hour test period. Containers were aerated from the laboratory air supply. The aerators used were glass tubes which had been

drawn down to a fine point. One areator was placed in each test container and the air was regulated so that a steady stream of bubbles was produced. Air hoses and clamps were adjusted each day in order to keep the areation of containers as uniform as possible. However, maintenance of the same rate of areation in all containers was difficult because pressure in the air line fluctuated.

Temperature

Water in test containers was maintained at a temperature of 40°F. (± 2°F.) by placing the containers in a water bath which was regulated by a thermostatically controlled cooling unit. The temperature of the bath was recorded by a Taylor recording thermometer.

PROCEDURE

Forty one-gallon jars were used in each experimental trial. A single fish was introduced into each jar. Ten jars held fish which served as controls. The control fish were given the same treatment as experimental fish except that no toxicant was added to the water. The thirty experimental fish were divided into three groups of ten fish. Each group was used to test a different concentration. The concentrations of toxaphene used were: 1,3,6,9,15, and 18 p.p.b. Thus, two trials were necessary to test the entire range of concentration. Fish were conditioned to the test water for a 24-hour period prior to each experiment. From 0.3 to 1.07 grams of fish were used for each liter of water.

The fish were observed each morning and evening following the addition of toxaphene. The dead fish were removed, measured, and an estimate of the time of death recorded. Tests were terminated after 96 hours and the remaining fish were measured. If more than 10 percent of the control fish died, results of the test were discarded. Termination of tests after 96 hours does not mean that no mortality occurred after this period. For instance, in one group of ten fish, three (30 percent) died after 96 hours; leaving them for an additional 96 hours (4 days) another five (50 percent) died. A 96-hour period was used in these experiments because it was a convenient test period and one which is widely used in bio-assay studies. The 96-hour median tolerance limit (TL_m) was used to compare the effects of the various concentrations of toxaphene upon the different weight classes of brook and rainbow trout. To avoid extensive handling trout were not measured until the end of each test.

Because only a small number of trout could be stored in the laboratory for any length of time, it was necessary to make frequent trips to the Wolf Lake Hatchery for fresh trout. Both species of trout were divided into three groups according to their weight (Table 1). One weight class was tested during each trial run.

Median tolerance limits and their confidence limits were computed by plotting the percentage of fish affected by various dosages against dosage on logarithmic probability paper (Codex 3128). (Figures 2-7). The method used is

TABLE 1

Maximum, minimum and average weight of the three groups of brook and rainbow trout used in experiments.

	Weight (grams)		
Group	Minimum	Maximum	Average
	Rainbow Trout		
1	0.9	1.2	1.1
2	1.4	1.7	1.5
3	2.4	3.1	2.7
	Brook Trout		
1	1.2	1.6	1.4
2	1.6	2.2	2.0
3	2.7	3.4	3.2

Figure 2. Percentage mortality of Group 1 rainbow trout (average weight 1.1 grams, average length 32 mm.) exposed to different concentrations of toxaphene. The 96-hour median tolerance limit (TL_m) is shown.

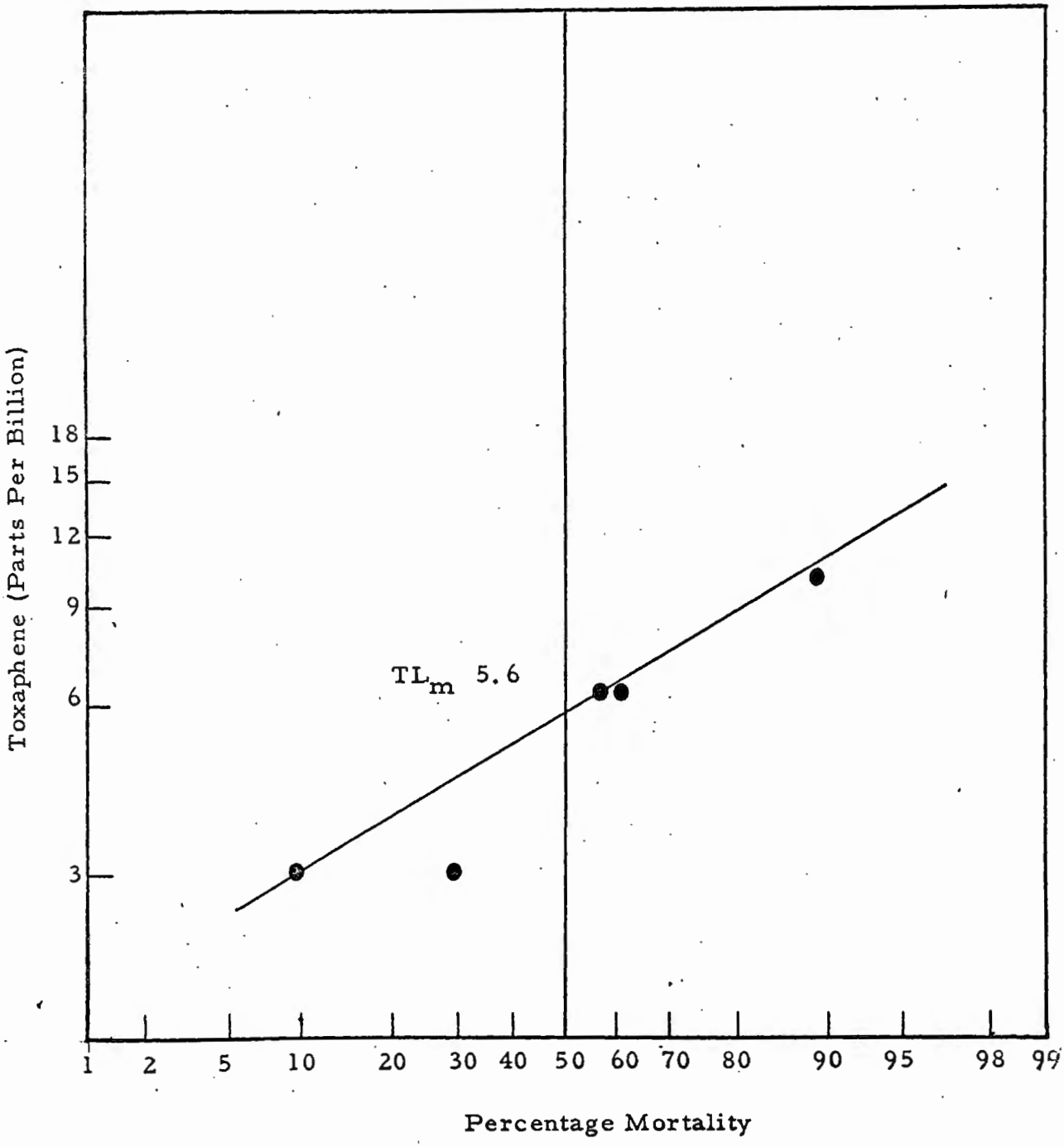
Toxaphene (Parts Per Billion)
18
15
12
9
6
3
TL$_m$ 5.6
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99
Percentage Mortality

Fig. 2

Figure 3. Percentage mortality of Group 1 brook trout (average weight 1.4 grams, average length 44 mm.) exposed to different concentrations of toxaphene. The 96-hour median tolerance limit (TL_m) is shown.

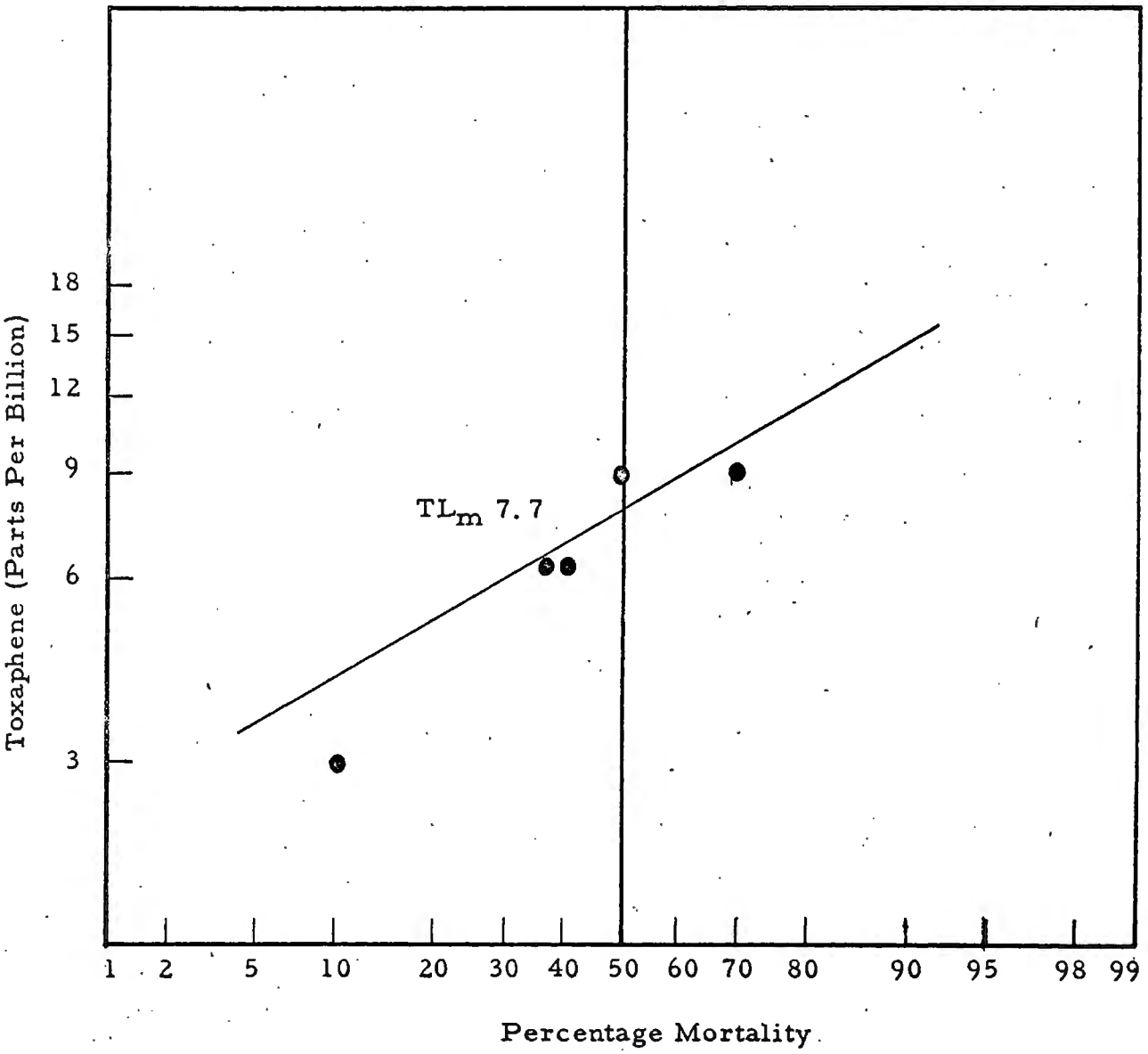
Toxaphene (Parts Per Billion)
18
15
12
9
6
3
TL$_m$ 7.7
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99
Percentage Mortality

Fig. 3

Figure 4. Percentage mortality of Group 2 rainbow trout (average weight 1.5 grams, average length 46 mm.) exposed to different concentrations of toxaphene. The 96-hour median tolerance limit (TL_m) is shown.

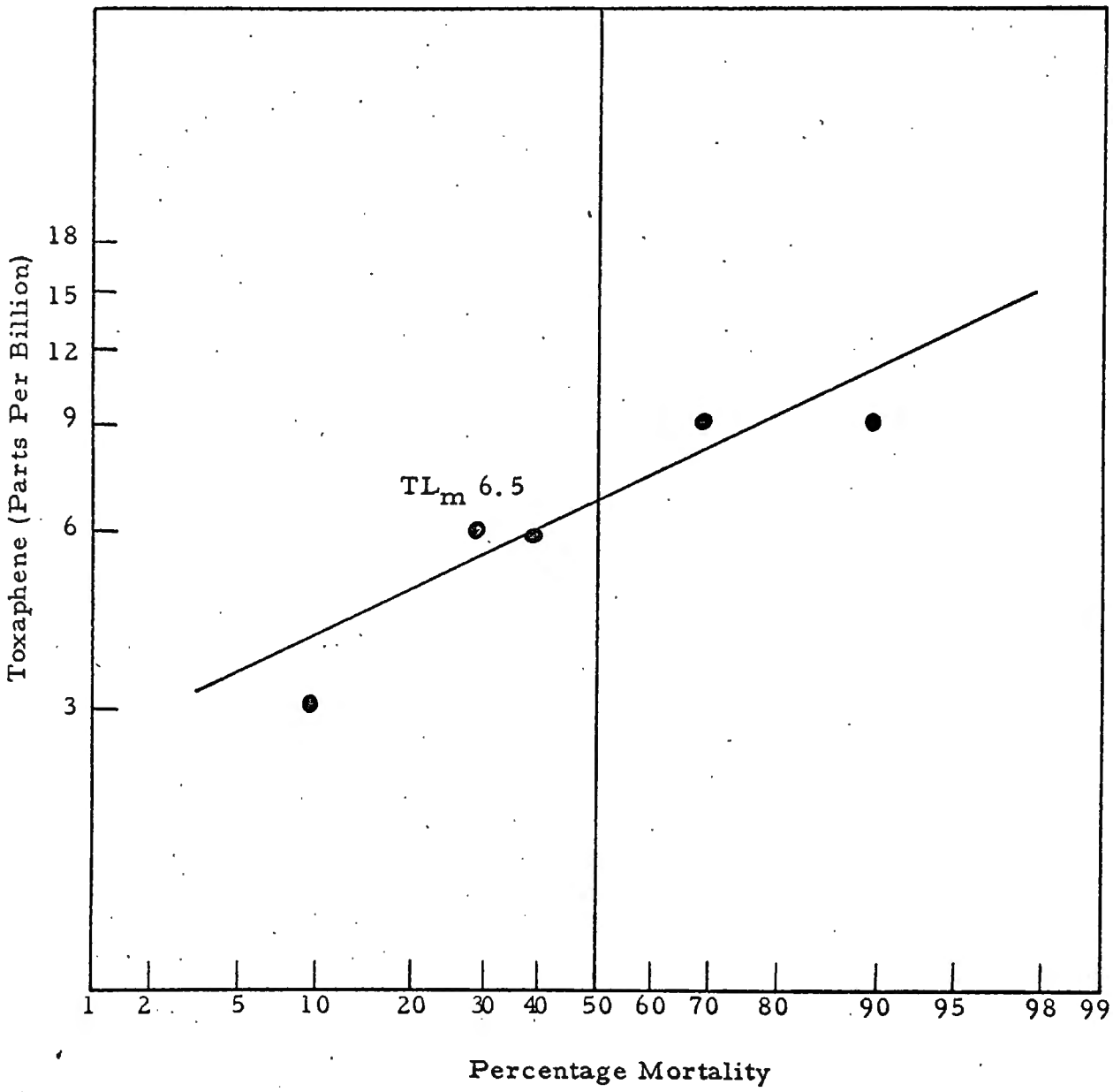
Toxaphene (Parts Per Billion)
18
15
12
9
6
3
TL$_m$ 6.5
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99
Percentage Mortality

Fig. 4

Figure 5. Percentage mortality of Group 2 brook trout (average weight 2.0 grams, average length 54 mm.) exposed to different concentrations of toxaphene. The 96-hour median tolerance limit (TL_m) is shown.

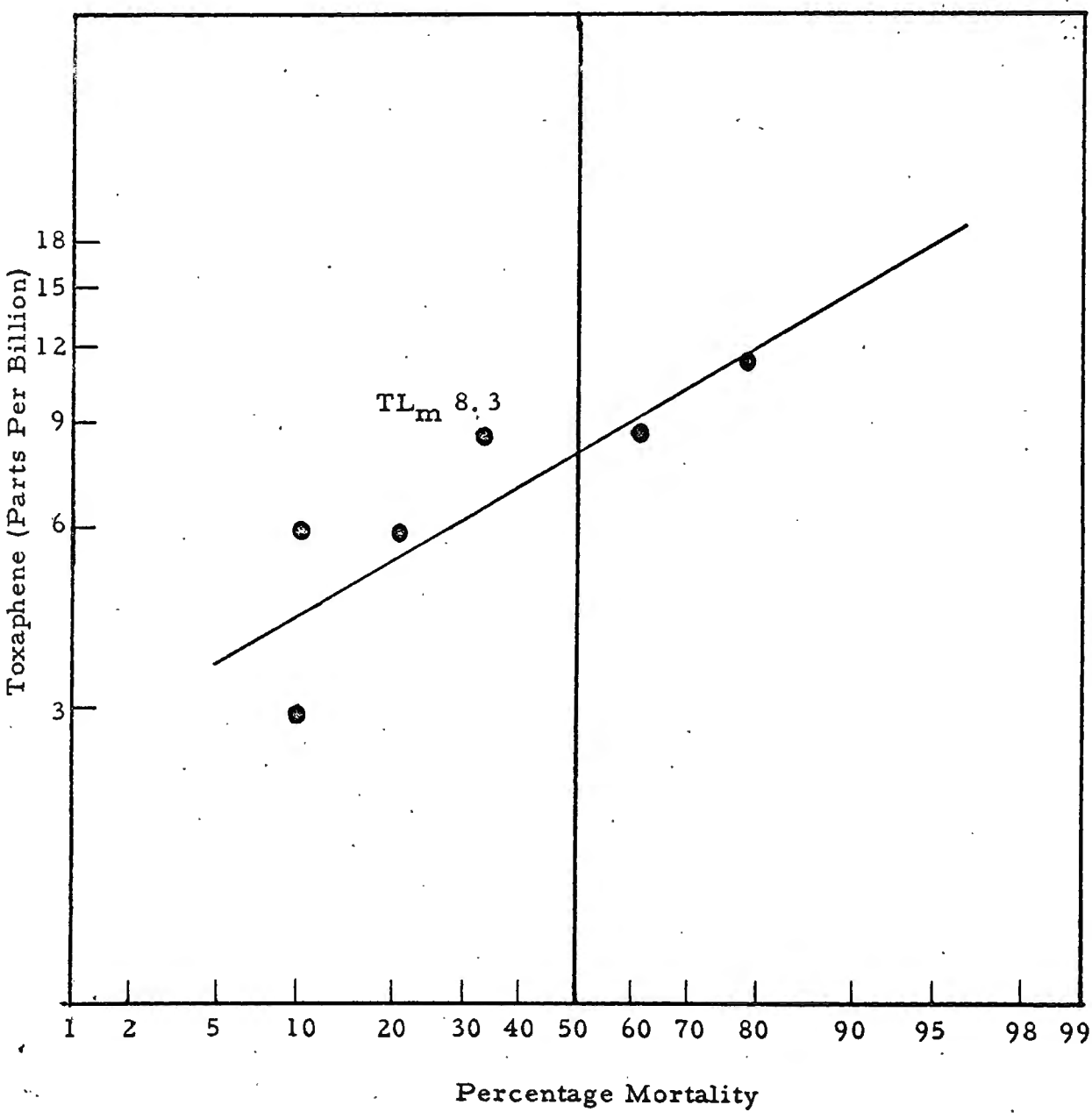
Toxaphene (Parts Per Billion)
18
15
12
9
6
3
TL_m 8.3
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99
Percentage Mortality

Fig. 5

Figure 6. Percentage mortality of Group 3 rainbow trout (average weight 2.7 grams, average length 64 mm.) exposed to different concentrations of toxaphene. The 96-hour median tolerance limit (TL_m) is shown.

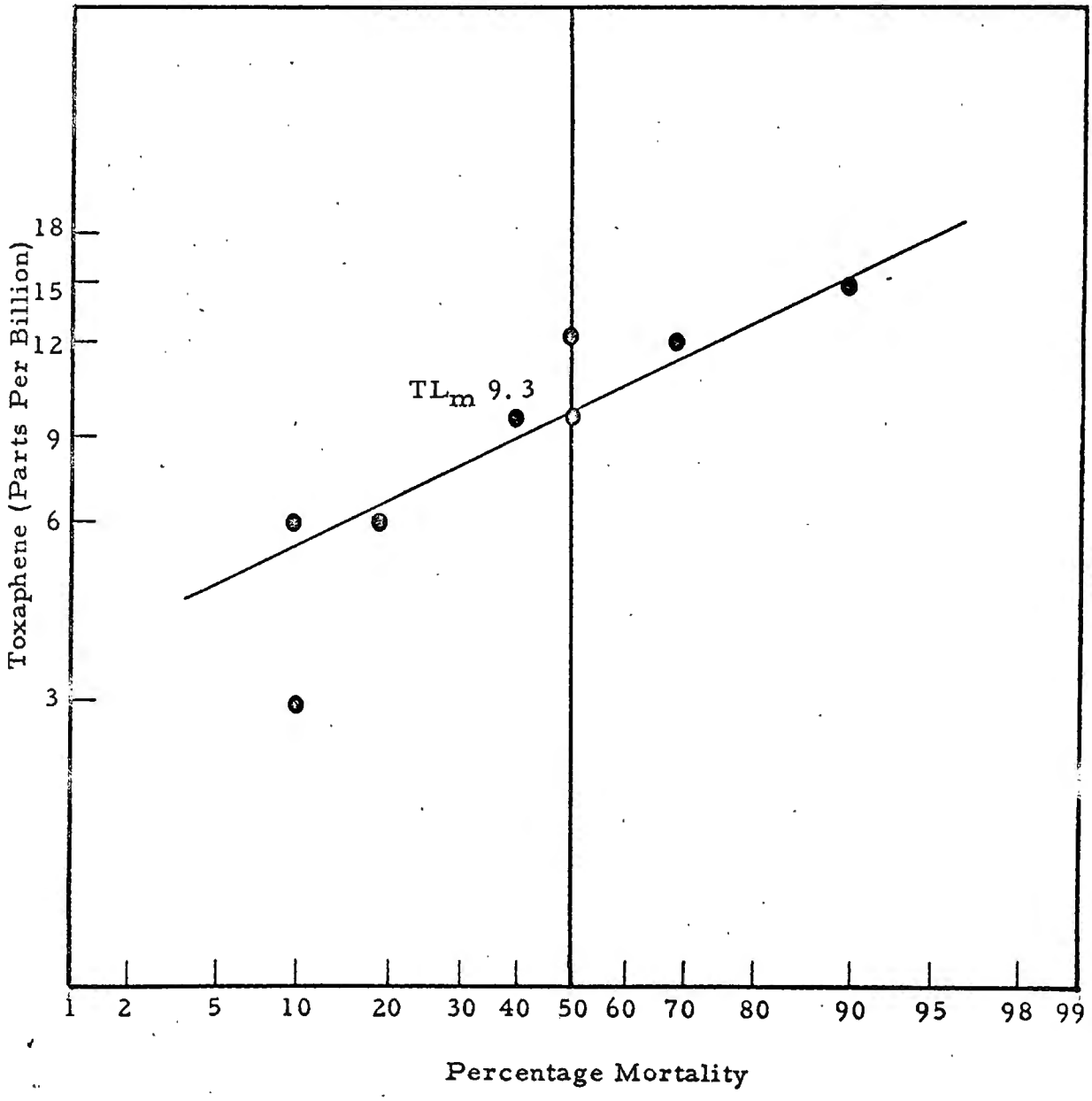
Toxaphene (Parts Per Billion)
18
15
12
9
6
3
TL$_m$ 9.3
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99
Percentage Mortality

Fig. 6

Figure 7. Percentage mortality of Group 3 brook trout (average weight 3.2 grams, average length 72 mm.) exposed to different concentrations of toxaphene. The 96-hour median tolerance limit (TL_m) is shown.

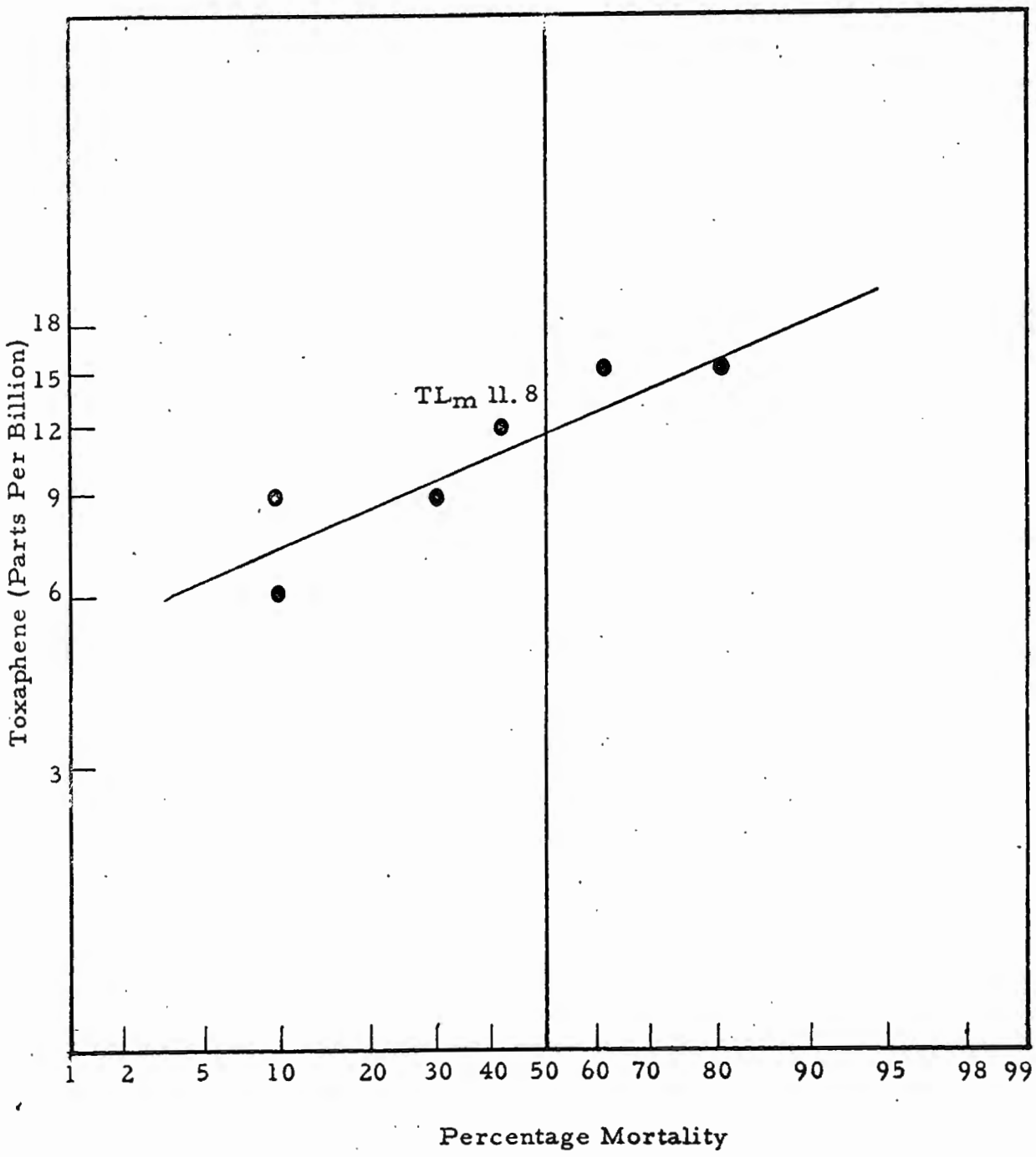
Toxaphene (Parts Per Billion)
18
15
12
9
6
3
TLm 11.8
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99
Percentage Mortality

Fig. 7

outlined by Litchfield and Wilcoxon (1949). For zero and 100 percent mortalities corrected values were obtained from a table of maximal and minimal corrected probits. A straight line was fitted by inspection to the plotted points. Particular weight was given to points in the region of 40 to 60 percent effect. The fit of the line was tested with a $(Chi)^2$ test (Litchfield and Wilcoxon, 1949).

RESULTS AND CONCLUSION

Both the brook and rainbow trout showed an increased tolerance to toxaphene with an increase in body weight (Figure 8). All three weight classes of brook trout showed a higher tolerance than the three corresponding weight classes of rainbow trout (Figure 8). Since the average weight of the brook trout in all three classes was slightly higher than that of the rainbow trout an analysis of covariance was performed to determine if the differences in tolerance between the two species were real or simply due to the greater weight of the brook trout. The regression lines for the two species were parallel and these lines differed in their elevation. An F test showed that this difference in elevation was significant at the 5 percent level. This demonstrated that there was a real difference in tolerance between the brook and rainbow trout tested, with the brook trout being the more tolerant species.

Both brook and rainbow trout showed an increased mortality rate with an increase in exposure time (Tables 2-7). Kallman et al. (1962) found that the longer the time

Figure 8. The 96-hour TL_m for the three weight classes of brook and rainbow trout. The 95 percent confidence limits for each group interval is indicated for each TL_m value. Confidence limits were calculated for each weight class from the pooled values of the two test runs at each concentration.

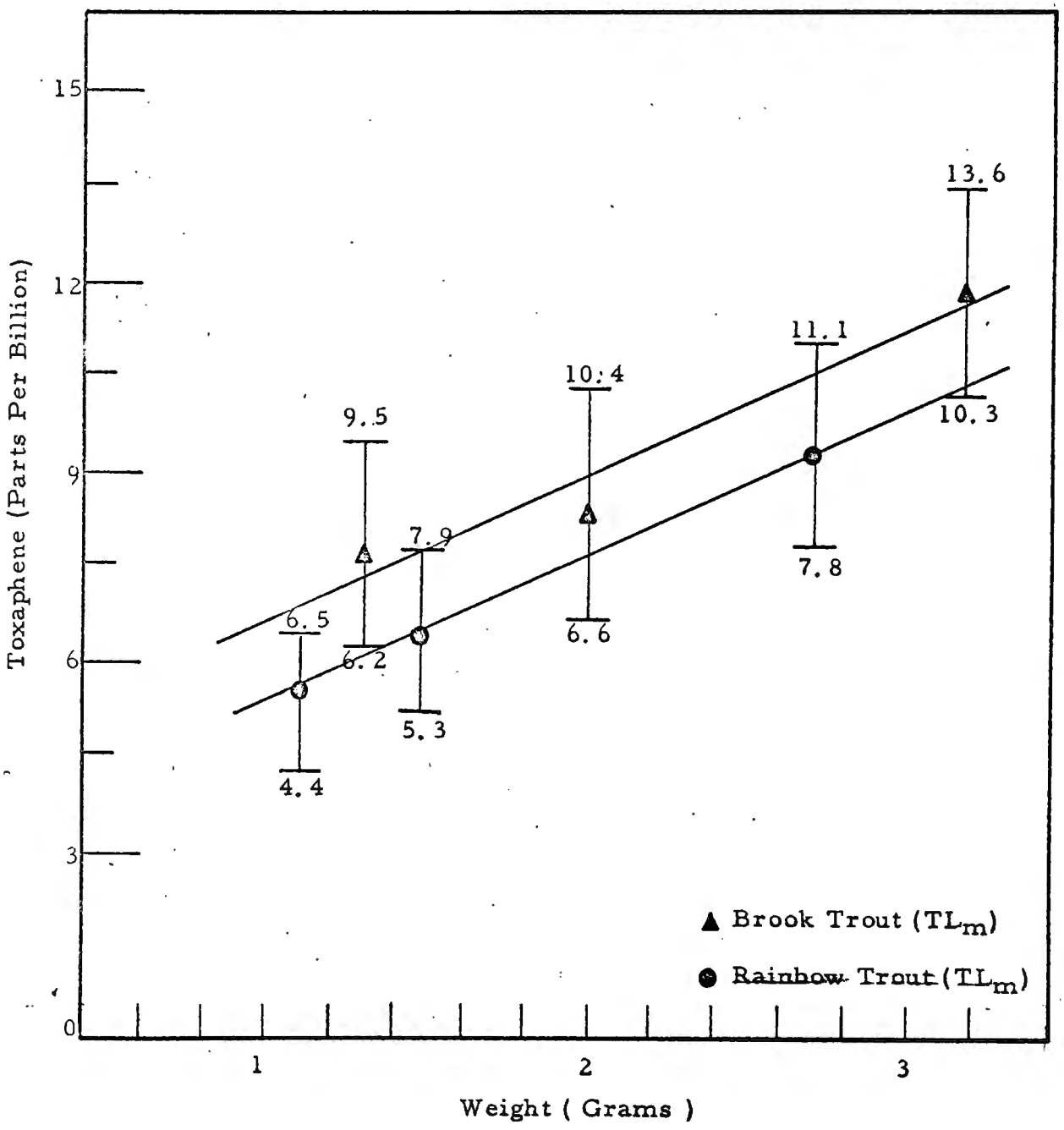
Toxaphene (Parts Per Billion)
15
12
9
6
3
0
1
2
3
Weight (Grams)
13.6
11.1
10.4
9.5
7.9
6.5
6.2
10.3
7.8
6.6
5.3
4.4
▲ Brook Trout (TL_m)
● Rainbow Trout (TL_m)

Fig. 8

Table 2. Percentage mortality of rainbow trout exposed to various concentrations of toxaphene. Ten fish were used in each test (Average weight 1.1 grams, average length 32 mm., Group 1 of Table 1).

Test	Concentration (p.p.b.)	Hours of Exposure 12	24	36	48	60	72	84	96
Test 1	1	0	0	0	0	0	0	0	0
	3	0	0	0	10	10	10	20	30
	6	0	0	0	0	10	30	50	60
	9	0	0	20	40	70	90	90	90
	12	0	10	70	100	-	-	-	-
Test 2	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	10	10
	6	0	0	10	10	30	50	50	60
	9	0	10	40	60	100	-	-	-
	12	20	50	80	80	80	100	-	-

Table 3. Percentage mortality of brook trout exposed to various concentrations of toxaphene. Ten fish were used in each test (Average weight 1.4 grams, average length 44 mm., Group 1 of Table 1).

Test	Concentration (p.p.b.)	Hours of Exposure 12	24	36	48	60	72	84	96
	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	10
	6	0	0	0	0	0	0	10	40
	9	0	0	10	10	10	30	30	50
	12	10	20	20	50	80	100	-	-
Test 2	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	6	0	10	10	10	10	10	40	40
	9	0	20	20	30	40	40	60	70
	12	0	10	40	90	100	-	-	-

Table 4. Percentage mortality of rainbow trout exposed to various concentrations of toxaphene. Ten fish were used in each test (Average weight 1.5 grams, average length 46 mm., Group 2 of Table 1).

Test	Concentration (p.p.b.)	Hours of Exposure 12	24	36	48	60	72	84	96
Test 1	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	10	10
	6	0	0	0	0	10	10	10	30
	9	0	10	10	10	20	40	60	70
	12	0	0	20	70	100	-	-	-
Test 2	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	20	30	40
	9	0	0	0	0	10	50	90	90
	12	0	10	10	50	70	90	90	100

Table 5. Percentage mortality of brook trout exposed to various concentrations of toxaphene. Ten fish were used in each test (Average weight 2.0 grams, average length 54 mm., Group 2 of Table 1).

Test	Concentration (p.p.b.)	Hours of Exposure 12	24	36	48	60	72	84	96
	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	6	0	0	0	10	10	10	20	20
	9	0	0	0	20	20	20	20	30
	12	0	0	10	40	50	70	100	-
Test 2	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	10	10	10
	6	0	0	0	0	0	0	0	10
	9	0	0	0	20	30	30	40	60
	12	0	10	10	20	20	50	70	80
	15	10	10	50	100	-	-	-	-

Table 6. Percentage mortality of brook trout exposed to various concentrations of toxaphene. Ten fish were used in each test (Average weight 2.7 grams, average length 64 mm., Group 3 of Table 1).

Test	Concentration (p.p.b.)	Hours of Exposure 12	24	36	48	60	72	84	96
Test 1	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	20
	9	0	0	0	0	0	20	40	40
	12	0	10	10	20	30	30	50	50
	15	10	30	60	90	90	90	90	90
	18	30	50	100	-	-	-	-	-
Test 2	1	0	0	0	0	0	0	0	0
	3	0	0	0	10	10	10	10	10
	6	0	0	0	0	0	10	10	10
	9	0	0	0	0	0	40	50	50
	12	0	0	10	20	40	60	60	70
	15	20	50	80	100	-	-	-	-

Table 7. Percentage mortality of brook trout exposed to various concentrations of toxaphene. Ten fish were used in each test (Average weight 3.2 grams, average length 72 mm., Group 3 of Table 1).

Test	Concentration (p.p.b.)	Hours of Exposure 12	24	36	48	60	72	84	96
1	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	10
	9	0	0	0	0	0	0	0	10
	12	0	0	0	10	10	10	20	40
	15	0	10	20	29	40	70	80	80
	18	30	40	70	90	100	-	-	-
2	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	30	30
	12	0	0	0	0	0	30	30	40
	15	10	10	10	10	10	20	30	60
	18	10	40	70	70	100	-	-	-

rainbow trout and bullheads were in a treated lake the greater the amount of toxaphene concentrated within their bodies. From this and other evidence it appears that toxaphene has a cumulative effect. The fat content of the mammalian body seems to influence tolerance due to its ability to store this toxicant. The fatty tissue of fish may serve the same purpose, however, this has not been demonstrated. Another factor which may influence tolerance to toxaphene among different size classes of fish is the gill to body surface ratio which decreases as the fish grow. Thus, as fish grow the gill area available for absorption of toxaphene becomes proportionally smaller.

A comparison of the 96 hour TL_m estimates from this study with data obtained by Katz (1961) shows approximately the same results. For rainbow trout varying between 2 and 3 1/8 inches in length and averaging 3.2 grams Katz lists a 96-hour TL_m of 8.4 p.p.b. The rainbow trout in group 3 of this study which averaged 2.5 inches in length and had an average weight of 2.7 grams had a 96 hour TL_m of 9.3 p.p.b. Field data indicate that concentrations as low as 7.5 p.p.b. give complete kills of brook and rainbow trout (Stringer, 1959). This is considerably lower than the 18 p.p.b. which were recorded in this study (Tables 2-7). There are several conditions which may explain the difference; (1) The fish in this study were maintained at a temperature of 40°F. which is considerably lower than the water temperature in most field situations. (2) The 96-hour time limit

set for those experiments was insufficient time to kill fish at the lower concentrations. (3) Fish were contained in three liters of water and they may have been able to metabolize a significant portion of the toxicant in this volume of water thereby reducing the concentration. Fish in a lake would not be able to do this and the concentration would change very little. (4) The high ratio of water volume to container surface area and the rapid circulation of water may have caused adsorption of a significant fraction of the toxicant by the container, thus lowering the toxicity of the water.

The difference in tolerance levels between the brook and rainbow trout studied in this experiment as calculated by the difference in the regression lines for the two species is approximately 1.5 p.p.b. (Figure 8). Since the confidence levels for the regression lines for both species overlap it would appear that selective poisoning of rainbow trout from a lake that contained both rainbow and brook trout of approximately the same size would be very difficult. However, the evidence of this study does indicate that it would be possible to poison selectively small rainbow or brook trout without harming larger fish of either species.

SUMMARY

This study was made to determine the effect of toxaphene on fingerling brook and rainbow trout. The investigation was concerned with the variation in tolerance between the

two species and also the variation in tolerance between different weight classes of each species.

1. Both the brook and rainbow trout showed an increased tolerance to toxaphene with an increase in body weight.

2. All three weight classes of brook trout showed a higher tolerance to toxaphene than the three corresponding weight classes of rainbow trout.

3. An analysis of covariance demonstrated that there was a significant difference in tolerance between the two species with the brook trout being the more tolerant.

4. The difference in tolerance levels between the brook and rainbow trout as calculated by the difference in regression lines for the two species was approximately 1.5 p.p.b.

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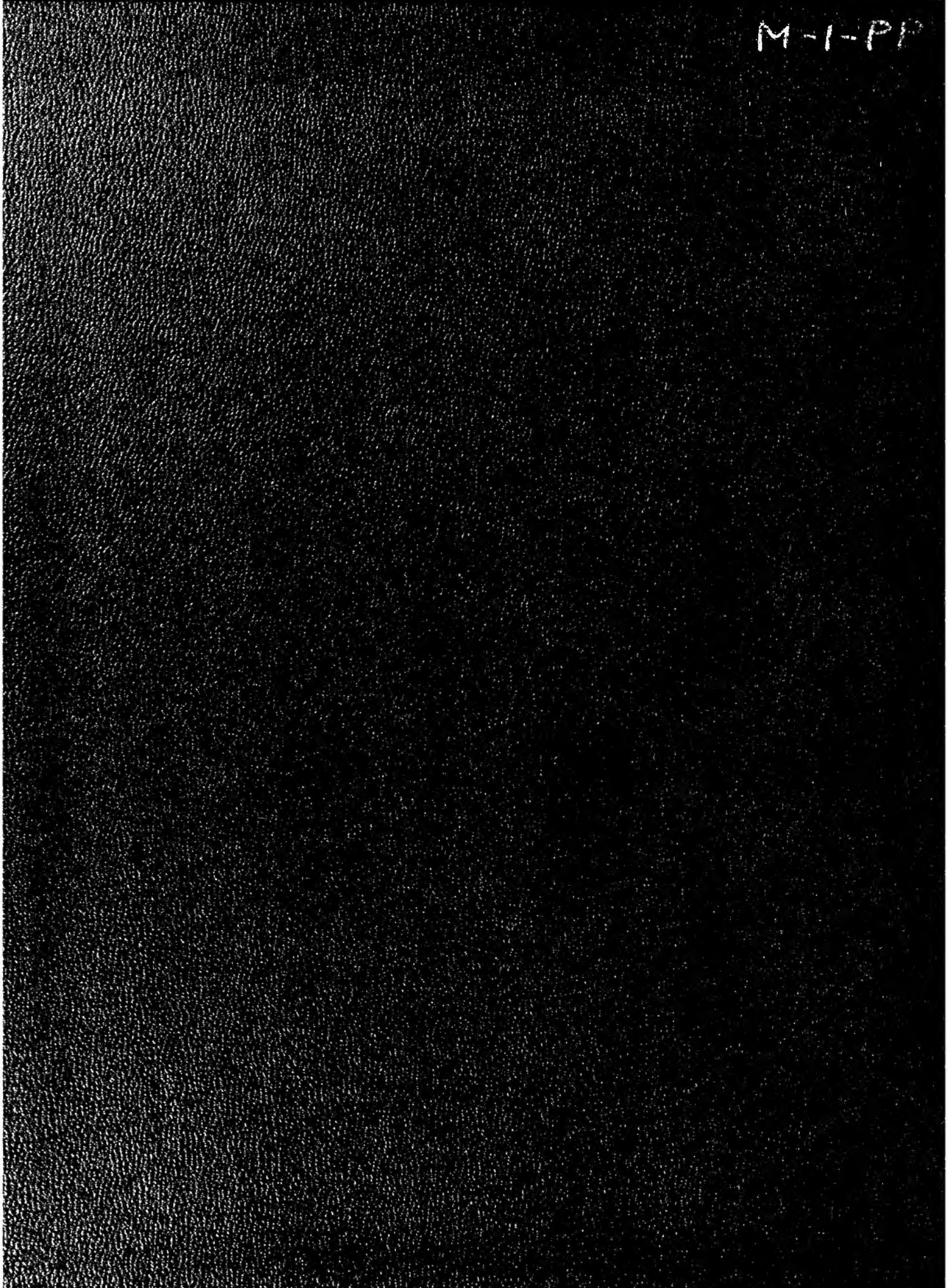
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