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THE ACUTE TOXICITY OF ZINC TO CUTTHROAT TROUT (Salmo clarki)

A Thesis

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CLAUDE WAYNE SAPPINGTON

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Determinations of the acute toxicity of zinc to Cutthroat trout (Salmo clarki) fingerlings were conducted using two bioassay systems. Methyl orange alkalinity of the test water was 23.9 ppm as calcium carbonate. PH values were near neutrality. In a standard, static bioassay, 24, 48 and 96 hr median tolerance limit (TLm) values of 0.62, 0.27 and 0.09 ppm zinc respectively were obtained. Results of a recirculating, flowing-water bioassay showed a 24 hr TLm value of 0.42 ppm zinc. No 48 or 96 hr values were found due to statistical non-significance of the data. The inconclusiveness of the results of the running system is due to changes in the concentration of zinc present in the test water as a result of sorption of the zinc ions to the bottom and sides of the test tank.

INTRODUCTION

The South Fork of the Coeur d'Alene River has received large volumes of domestic and industrial wastes for more than 50 years. As a result of extensive mining operations, large quantities of suspended matter (commonly called crushed rock "flour"), chemicals from ore concentrators and solutions containing heavy metals have been dumped into the river. Originally, the suspended matter imparted a "milky" appearance to the river. However, much of the contribution of suspended solids has been abated within the last year.

The South Fork joins the North Fork at Enaville to form the Coeur d' Alene River, which flows into Coeur d'Alene Lake at Harrison, Idaho. In their survey of Coeur d'Alene Lake, Kemmerer, Borard and Boorman (1923) noted that the muddy waters of the Coeur d'Alene River extended far out into the lake. Ellis (1940) made an extensive survey of the conditions of the Coeur d'Alene River and found the polluted portions of the river to be barren of organisms. He also noted that Coeur d'Alene Lake near the mouth of the river showed a large deficit in populations of phytoplankton, zooplankton, fish and bottom organisms as compared to the rest of the lake.

The North Fork of the Coeur d'Alene River has not been subjected to environmental changes brought about by the addition of domestic or industrial wastes and is presumedly in a condition similar to that of the South Fork prior to the addition of domestic and industrial wastes. North Fork waters then offer an excellent opportunity for the examination of the effects of specific heavy metals on the aquatic environment. It is almost impossible to work directly with South Fork waters because the synergistic and antagonistic effects of its many ions in solution preclude isolating the effects of individual ions. Any live-trap experiments of fish or other aquatic organisms in the South Fork could result in mortality with no insight to the cause of death.

In 1968, a proposal to assess the biological productivity of the Coeur d' Alene River was submitted to the Water Resources Research Institute. One phase of this proposal was designed to determined the toxic effects of zinc to Cutthroat trout (Salmo clarki Richardson). Experiments, consisting of static

bioassay tests, were conducted in a laboratory at the University of Idaho from December 1968 to July 1969. In addition, I evaluated the feasibility of using a recirculating, flowing-water system as a bioassay method.

Since zinc is an important constituent of the South Fork waters (R. E. Williams, 1968, personal communication), it was chosen as the parameter to be tested. Cutthroat trout were used as test organisms because (1) they are important sport fish of the area (2) they inhabit the unpolluted portions of the Coeur d'Alene River and (3) they are sensitive to environmental changes.

Over the past 40 years, numerous studies have been conducted on the toxicity of heavy metal ions to fish. Much of the earlier literature was reviewed by Douderoff and Katz (1953). More recently, Skidmore (1964) has compiled a review of the literature on the toxicity of zinc to aquatic organisms.

The use of bioassays is the generally accepted method of determining the effects of heavy metal ions and other toxicants on aquatic organisms. Two types of bioassays have been widely used. The static bioassay system is often used in tests to determine acute or short term effects. An acceptable method is described by Douderoff, et al. (1951) and Standard Methods (1965). For long term tests, a flow-through, running water bioassay is used to determine chronic effects of a toxicant. Various authors (Henderson and Pickering 1963; Mount and Brungs 1967; and Brungs and Mount 1967) have described several types of running systems. Burdick (1967) discussed situations to which continuous flow or static bioassay systems are applicable.

MATERIALS AND METHODS

A. Static System

Five liter glass aquaria were used as test containers for the static bioassay system. The aquaria were placed in an 84 x 18 inch tank containing water at a depth of 4 inches (Fig. 1). This arrangement was used to reduce fluctuations in the temperature of the test water in the aquaria. No thermostatic controls were used in this system, so the water equilibrated with the air temperature in the laboratory. Air temperature in the lab was maintained at about 15 C with a refrigerated air conditioner. Dissolved oxygen was supplied to the test water through air stones. I made no attempt to control the p^H of the test water but monitored this parameter during the test run. After each test, the aquaria were cleaned with a dichromate acid solution and throughly rinsed with distilled water.

In the static system, the water was changed every 24 hrs while a test was in progress. Twenty-four, 48 and 96 hr median tolerance limit (TLm) values were calculated by the Maximum Likelihood Method of Probit Analysis as described by Finney (1962). The TLm is the concentration of a toxicant necessary to kill 50 percent of the test organisms in a specified time period.

B. Running System

The second bioassay system consisted of a recirculating, flowing-water apparatus (Fig. 2). Overall length of the tank was 9 feet 6 inches with a width of 3 feet 2 inches and a depth of 1 foot. It was constructed of 3/4 inch plywood on the bottom and sides. One-quarter inch plexiglass was used on the front side and to divide the tank in half. The plywood was covered with several coats of fiberglass resin for waterproofing. The bottom of the intake side of the tank was built with a gradient of 8 degrees. The outlet side was horizontal. The tank was divided into a test channel and control channel by a

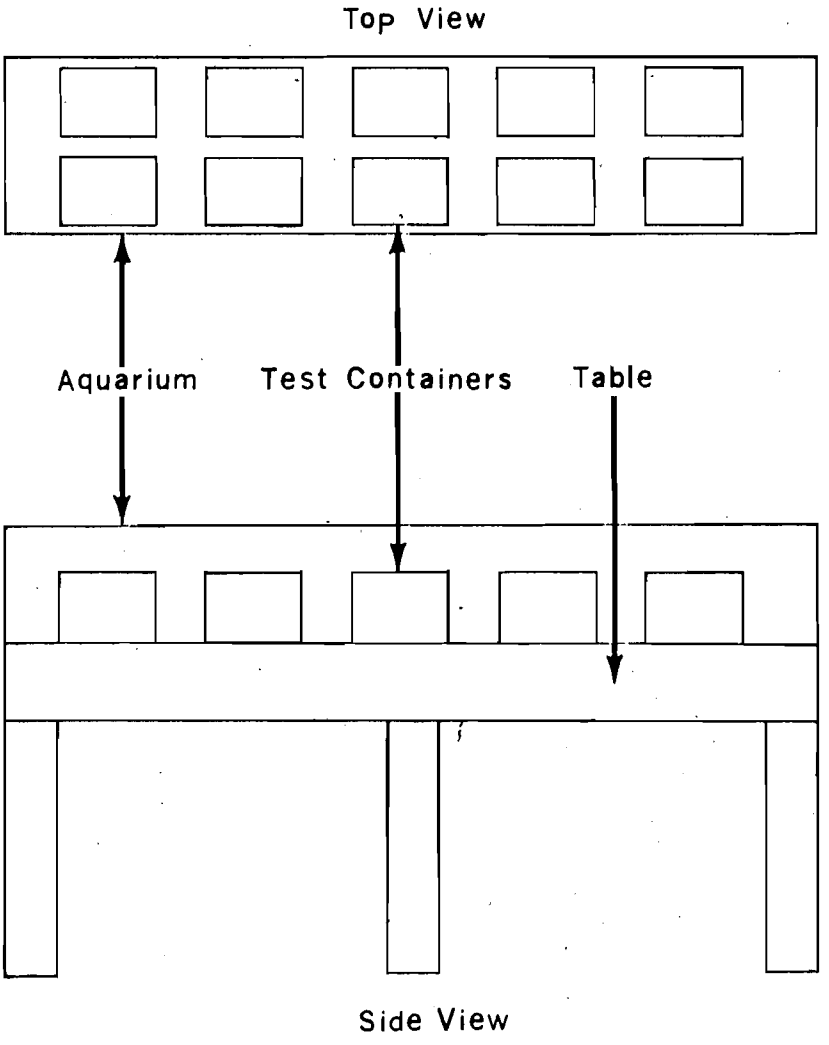

Figure 1. Diagram of apparatus used for static bioassay tests (about 1/20 scale).

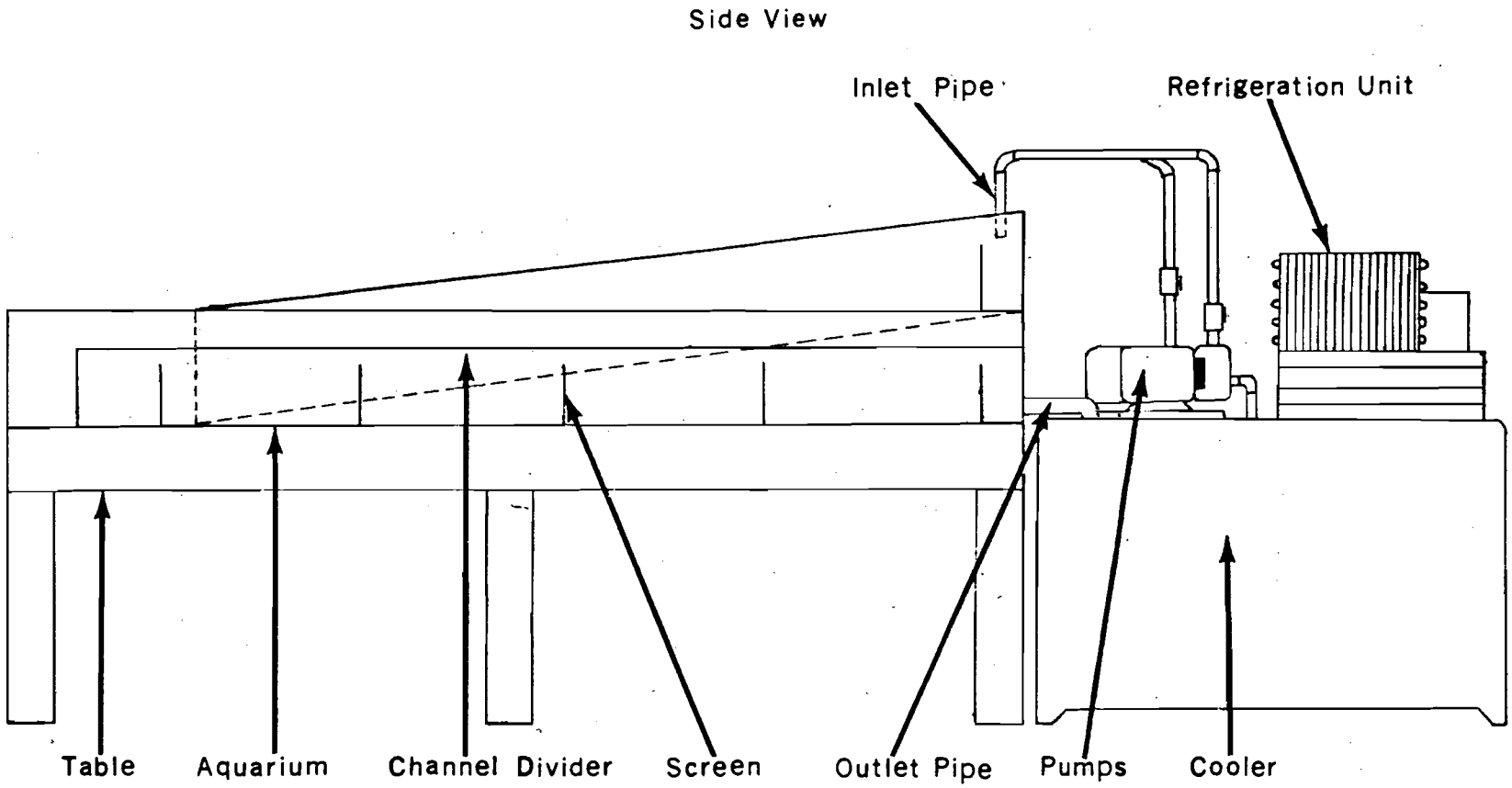

Figure 2. Recirculating, flowing-water bioassay system (about 1/20 scale).

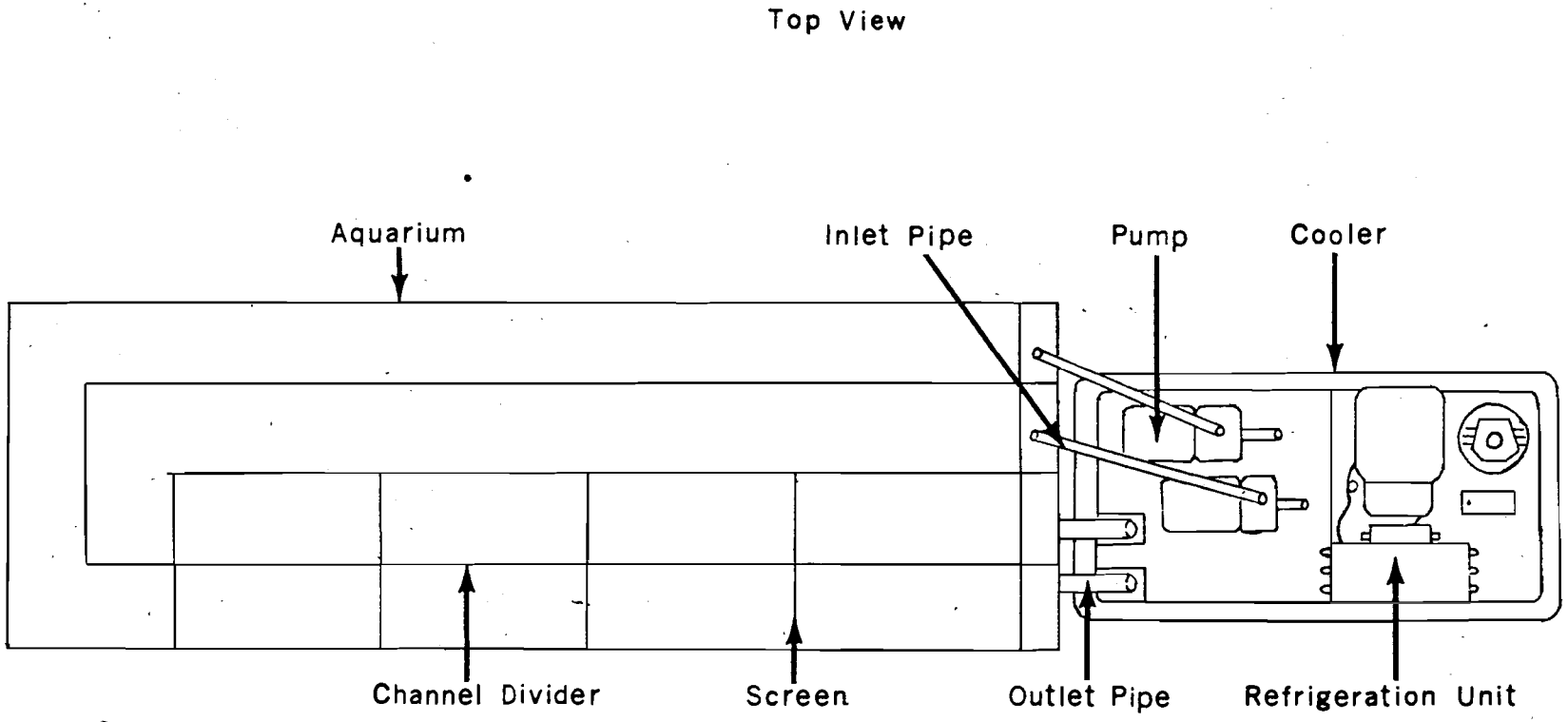

Figure 3. Recirculating, flowing-water bioassay system (about 1/20 scale).

plexiglass divider (Fig. 3). There was an inlet and outlet pipe for each channel.

Circulating water was supplied by two model 3SPC Oberdorfer self-priming centrifugal pumps. A 20 foot section of 1 inch (I.D.) tygon tubing was attached to the outlet pipe of each channel. The tubing was then coiled and placed in the bottom of a cooling tank. The other end of the tubing was connected to the intake of one of the pumps which returned the water to the upper portion of the tank for recycling.

Distilled water in the cooling tank was maintained at a constant temperature by a submerged refrigeration unit. However, due to an inefficient heat exchange through the walls of the tygon tubing, 18 to 24 hrs were required for the test water to equilibrate to the temperature of the cooling water. After several preliminary runs, I decided not to use the refrigeration unit. I obtained more comparable results by keeping the same temperature in the holding tank, static system and running system.

C. Test Water

The source of the test water was the North Fork of the Coeur d'Alene River, about 5 miles above the confluence of the North and South Forks of the Coeur d'Alene River. The water was transported back to the laboratory in 5 gal plastic containers and used as needed for testing. A 30 gal aquarium containing water from the North Fork served as a holding tank for fish prior to testing. Acclimation time prior to testing was at least 7 days.

D. Fish

At each concentration of zinc tested, a minimum of two aquaria containing five fish each were placed in the static system. Controls were run simultaneously. The trout averaged 56.5 mm in total length with a range of 0.37 to 99.0 mm. The mean wet weight was 1.59 gm, with a range of 0.37 to 7.82 gm. The fish were not fed for 1 day prior to testing, nor were they fed while a test was in progress. The Cutthroat trout were supplied by the Idaho State Fish Hatchery near Sandpoint, Idaho. About 200 to 300 fish were obtained at any one

time. They were transported to the laboratory in a 5 gal plastic bag placed in a cooler and super-saturated with oxygen. Transport time averaged about 3 hrs.

In the running system, the fish were confined to the horizontal portion of the tank which was divided into four sections by using plastic screens. Five trout were placed in each section with a total of 20 fish in each channel. Shelters for the fish were made from 3 inch poly-vinyl chloride (PVC) pipe cut into 2 inch lengths. Each piece was then cut in half lengthwise and placed concave side down.

E. Water Chemistry

The zinc used in the tests was added as $ZnSO4 \cdot 7H_2O$. Zinc sulphate was added to distilled water in the proper amount to obtain a stock solution of 1 gm/liter zinc. At intervals of 24 hrs, samples of the test water were collected and analyzed for zinc by using a Jarrell-Ash atomic absorption spectrophotometer.

Temperature, p^H, alkalinity and electrical conductivity were determined at the beginning of each test and at the termination of each 24 hr period that the test was in progress. Dissolved oxygen was determined occasionally by the Winkler Method (unmodified).

RESULTS

A. Physical and Chemical Factors

During the period of testing, the mean water temperature was 14.3 C ($\pm$ 1 degree), but on occasion it ranged from 11.5 to 16.0 C. The p^H of the water varied from 6.6 to 7.6. No phenopthalein alkalinity was present. The mean methyl orange alkalinity was 23.9 ppm as $CaCO_3$ with a range of 17.0 to 32.0. The mean value for dissolved oxygen was 8.0, ranging from 6.2 to 10.0 ppm (Table 1).

B. Static System

Twenty-four, 48 and 96 hr TLm values of 0.62, 0.27 and 0.09 ppm of zinc were obtained from the static system (Table 2). The graphs calculated from analysis of the data represent regression of percent mortality expressed as probits against log concentration in ppm of zinc (Figs. 4, 5 and 6). The TLm value is obtained by plotting the point where the regression line intersects the probit value of 5 which corresponds to 50 percent survival. The value on the abscissa at this point estimates the dosage required to produce 50 percent mortality. Statistical data were based on a 95 percent confidence level with 8 degrees of freedom.

C. Running System

In the running system, a 24 hr TLm value of 0.40 ppm of zinc was obtained (Fig. 7). No TLm values were estimated for 48 and 96 hr tests (Figs. 8 and 9) due to statistically non-significant regression lines (Table 2). Statistical information was based upon a 95 percent confidence interval with 9 degrees of freedom.

Table 1. Chemical and physical parameters of the test water during bioassays in the static and running systems.

	Temperature C	pH	Alkalinity	Dissolved Oxygen
STATIC SYSTEM				
Mean	14.3		23.9	8.0
Range	11.5 - 15.5	6.6 - 7.3	20.0 - 29.0	6.2 - 10.0
RUNNING SYSTEM				
Mean	14.6		25.2	8.4
Range	12.5 - 16.0	6.8 - 7.6	17.0 - 32.0	7.1 - 10.0

Table 2. TLm values, confidence limits and statistical analyses for static and running systems.

Time (hrs)	Upper Limit	TLm	Lower Limit	F (05)	F (cal)	X^2 (05)	X^2 (cal)
STATIC SYSTEM							
24	0.97	0.62	0.48	5.32	9.12	15.50	14.91
48	0.38	0.27	0.16	5.32	22.11	15.50	11.06
96	0.11	0.09	0.06	5.32	14.31	15.50	15.22
RUNNING SYSTEM							
24	34.75	0.40	0.26	5.12	5.18	16.90	63.70
48	-----	-----	-----	5.12	3.16	16.90	118.40
96	-----	-----	-----	5.12	2.05	16.90	113.92

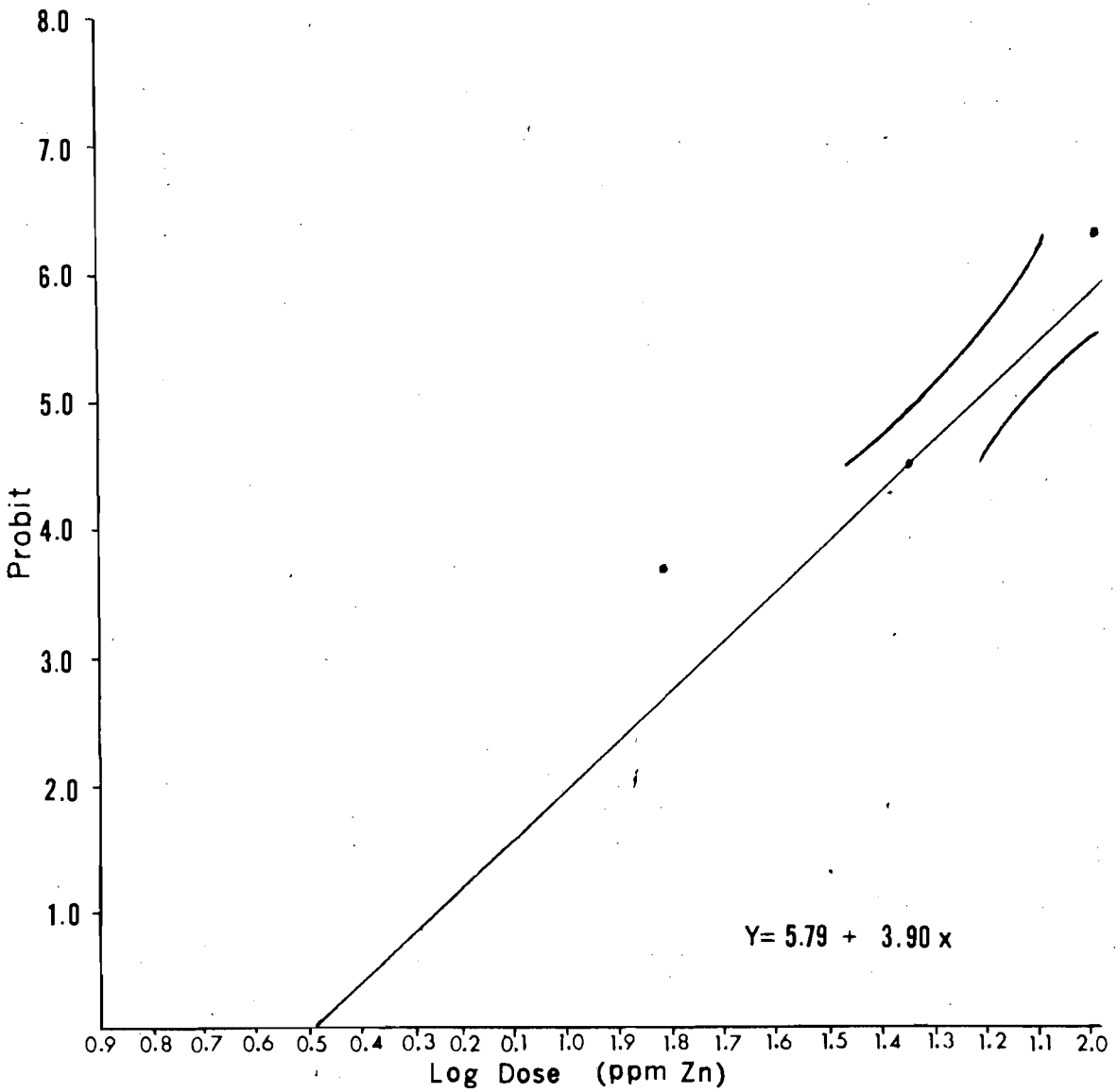

Figure 4. 24 hr TLm for static system. The straight line represents the estimated mortality. The points are actual mortality values. The curved lines are the 95 percent confidence limits of the estimate.

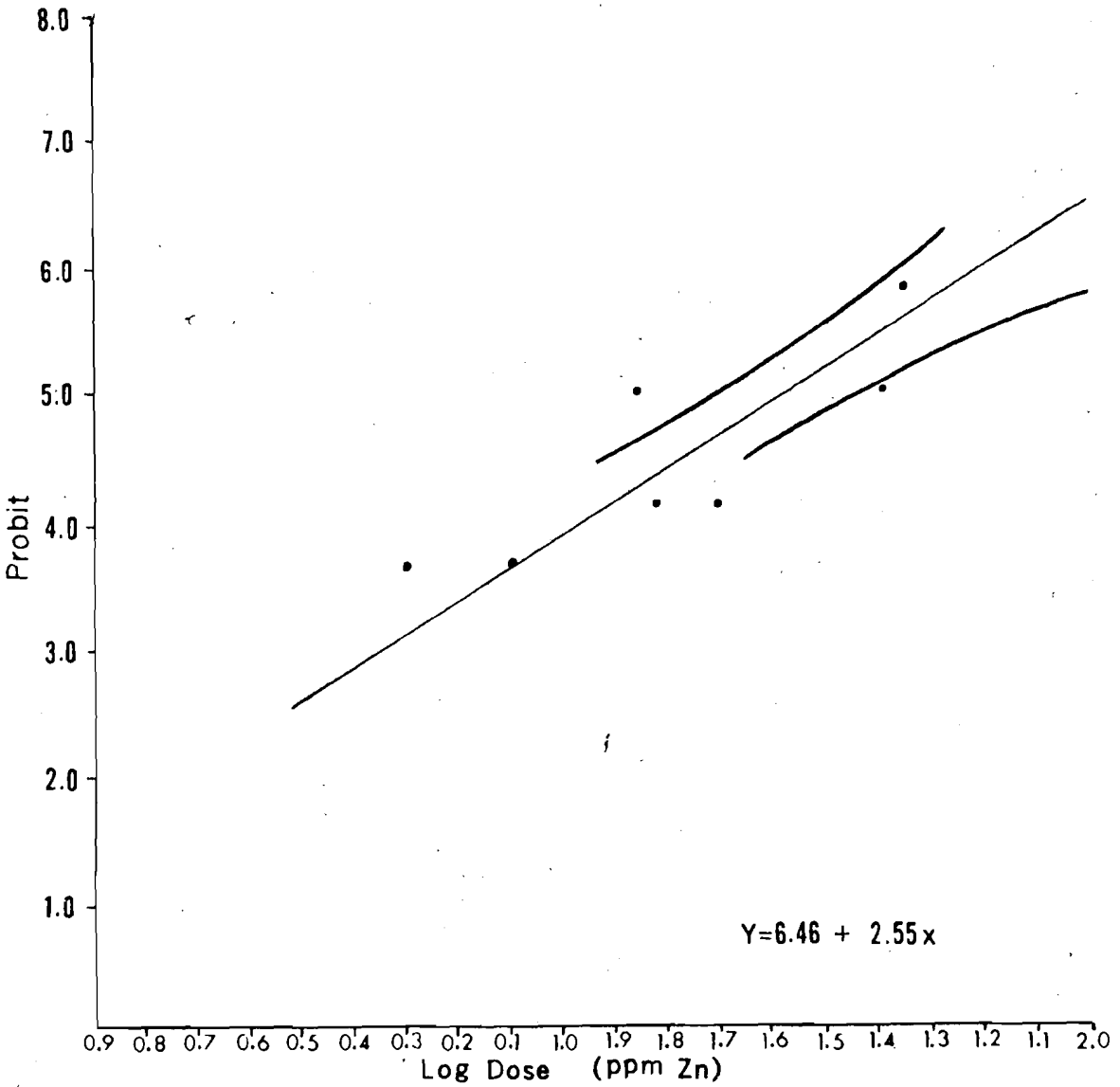

Figure 5. 48 hr TLm for static system. The straight line represents the estimated mortality. The points are actual mortality values. The curved lines are the 95 percent confidence limits of the estimate.

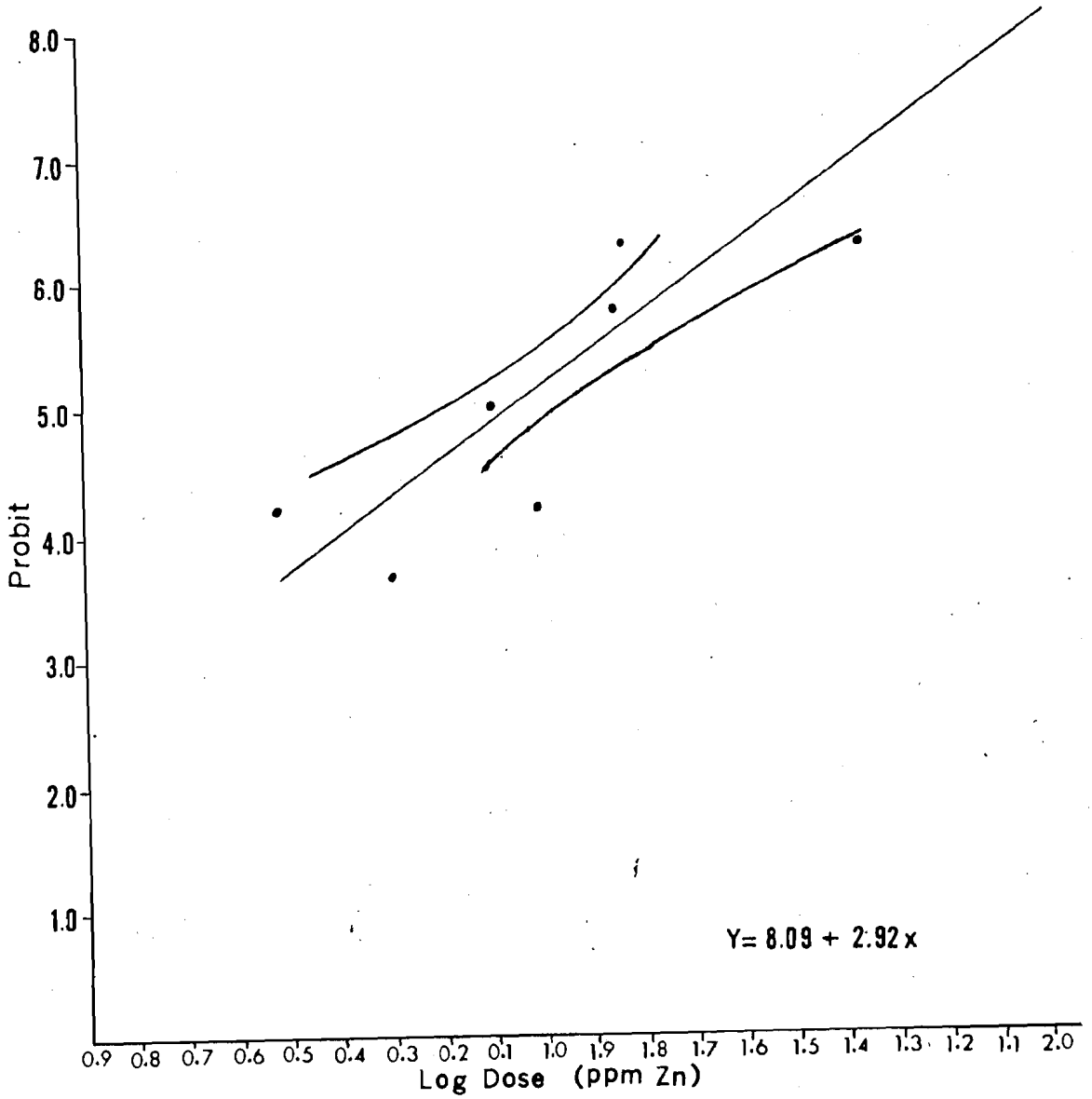

Figure 6. 96 hr TLm for static system. The straight line represents the estimated mortality. The points are actual mortality values. The curved lines are the 95 percent confidence limits of the estimate.

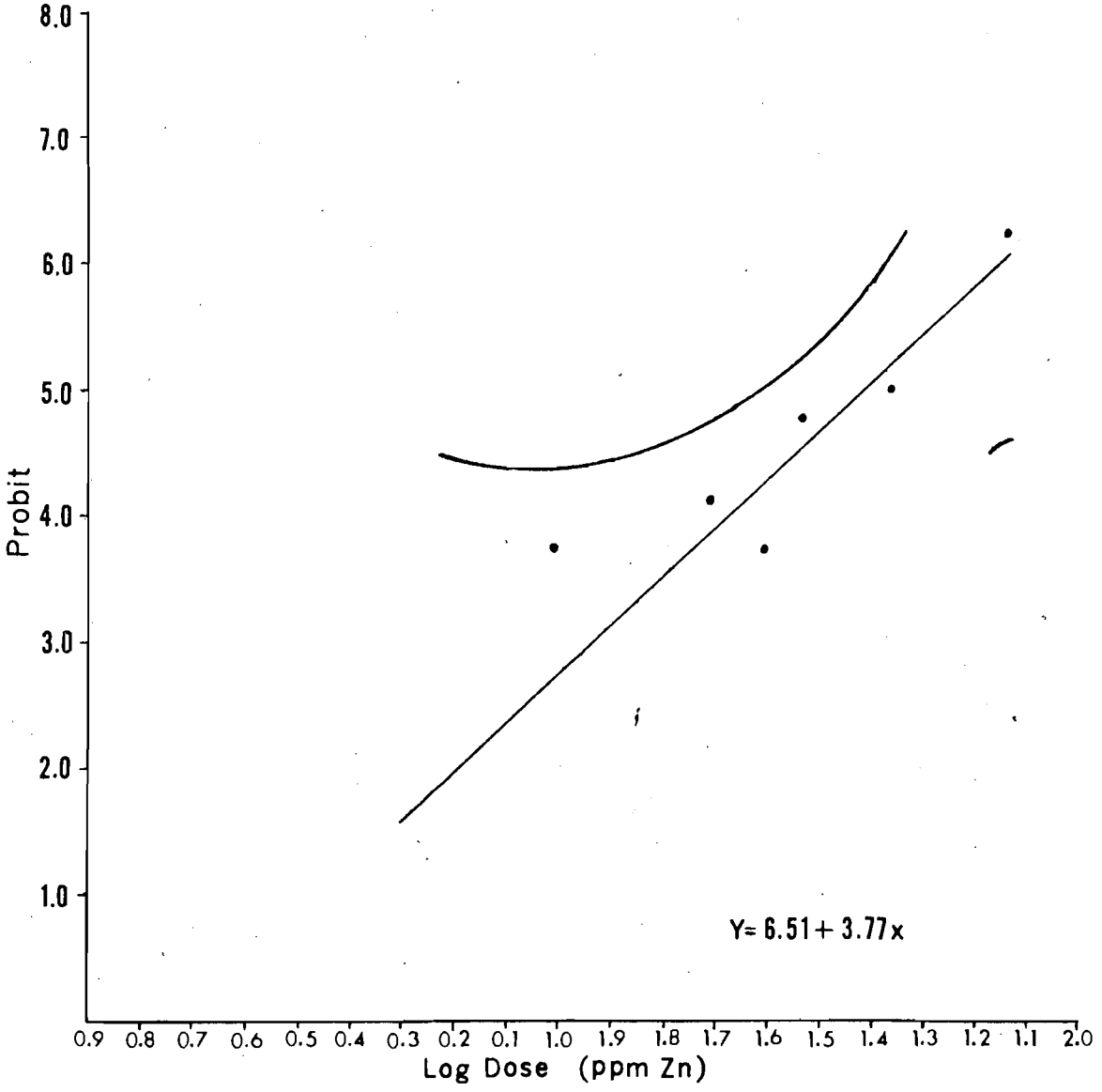

Figure 7. 24 hr TLm for running system. The straight line represents the estimated mortality. The points are actual mortality values. The curved lines are the 95 percent confidence limits of the estimate.

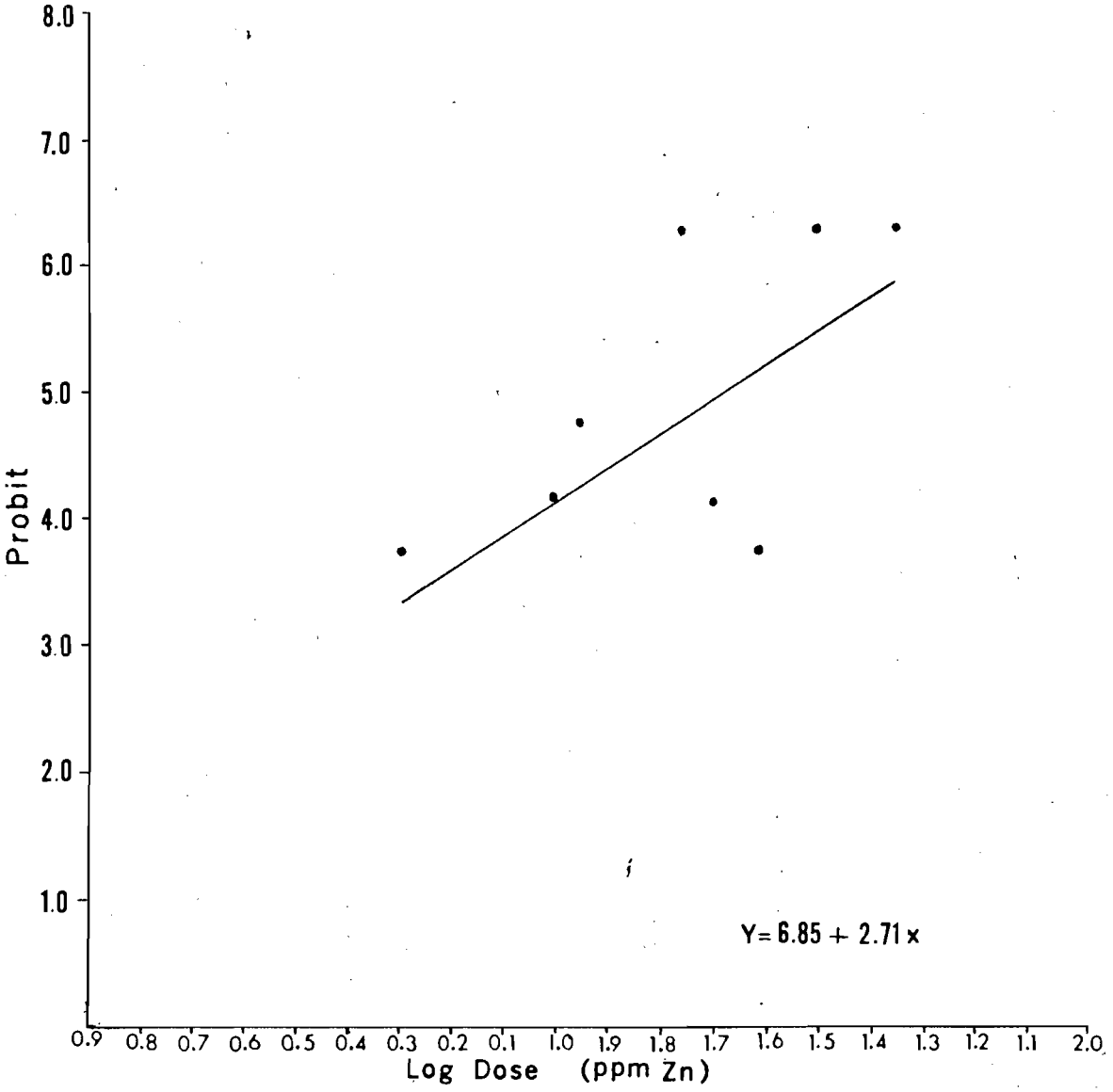

Figure 8. 48 hr TLm for running system. The straight line represents the estimated mortality. The points are actual mortality values. This line is statistically non-significant.

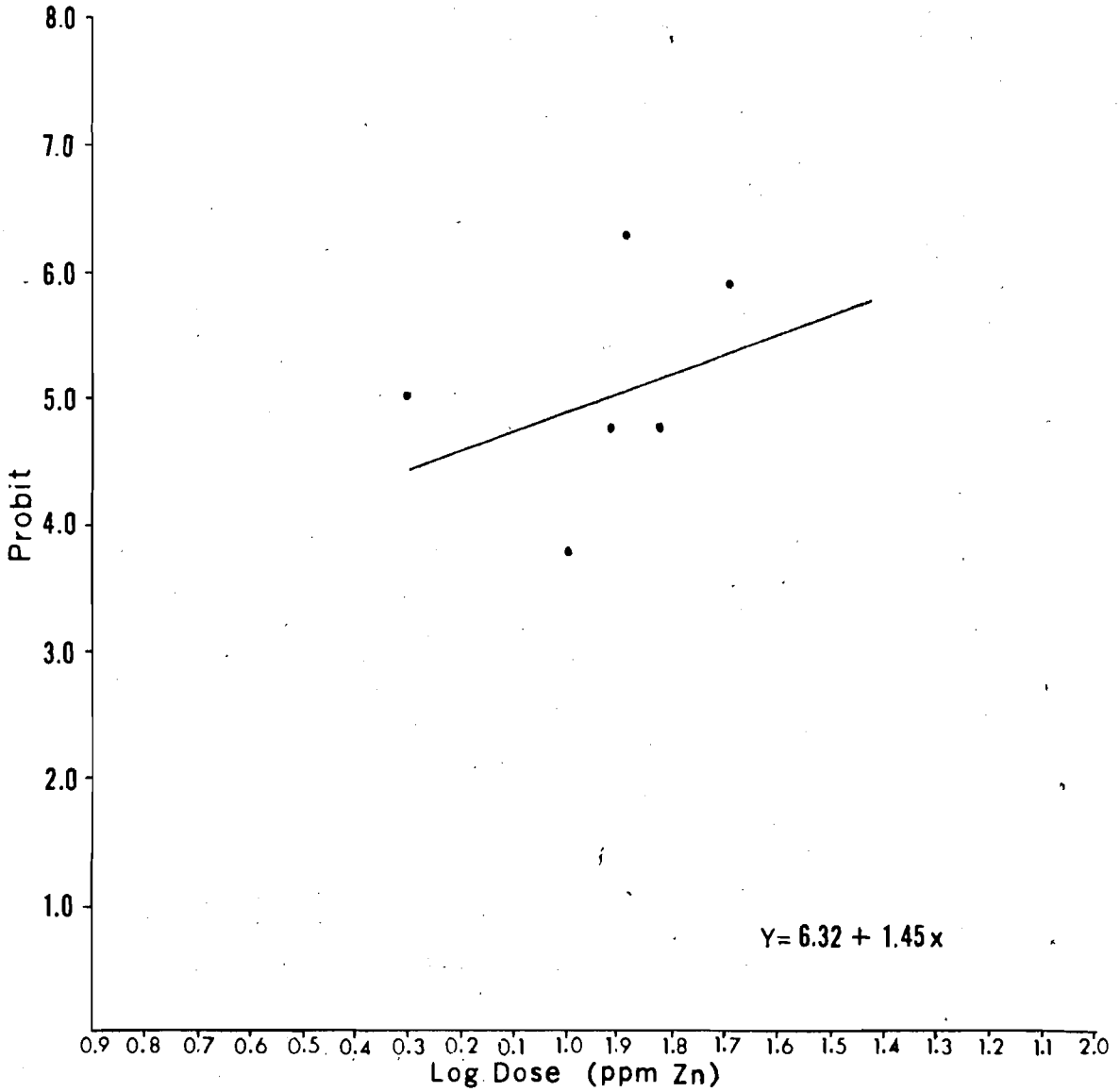

Figure 9. 96 hr TLm for running system. The straight line represents the estimated mortality. The points are actual mortality values. This line is statistically non-significant.

DISCUSSION

Examination of the dose response curves indicates that Cutthroat trout are extremely sensitive to the toxic action of zinc. I found a lower tolerance to zinc by Cutthroat trout than that found by other authors for different species of trout (Table 3). In general, Cutthroat trout seem to be very sensitive to changes in their environmental conditions (D.W. Chapman, 1970, personal communication). I believe that this environmental sensitivity is partially reflected in the low tolerance these fish show to the effects of zinc. Also, the low alkalinity of the test water (23.9 ppm $CaCO_3$) probably had very little antagonistic effect on the toxicity of the zinc.

Hardness and other environmental conditions are known to influence the toxic action of heavy metal ions. Lloyd (1960) has shown that Rainbow trout are less sensitive to zinc sulphate in hard water than in soft water. He demonstrated a seven fold increase in sensitivity between trout in hard and soft water (Table 3). Less conclusive results of other investigators show that hardness moderates the toxic effect of heavy metal ions. Cairns and Scheier (1957) reported a 96 hr TLm of 11.3 ppm zinc for bluegill sunfish (Lepomis macrochinus) in water with a hardness of 30,000 ppm calcium carbonate. A 96 hr TLm value of 2.8 ppm zinc was obtained in water of 40 ppm calcium carbonate. Jones (1938), working with sticklebacks (Gasterosteus aculeatus), found that all fish survived 10 days in water containing 2 ppm zinc and a hardness of 50 ppm calcium carbonate. No fish survived 2ppm zinc in water that contained no calcium hardness.

Dissolved oxygen levels and temperature can also have effects upon the susceptibility of fish to a toxicant. Lloyd (1960, 1961) demonstrated that for Rainbow trout a reduction of dissolved oxygen (8.9 to 3.8 ppm) present in water will cause an increase of 40 percent in the toxicity of zinc to the fish. It should be noted that lowering the dissolved oxygen content of the water will cause an increase in the ventilation rate of the fish. As a result, more of the toxicant is brought into contact with the gill surfaces. Low dissolved oxygen levels exert an added stress separate from the stress produced by a toxicant. The added stress factor probably enhances the effect of a toxicant.

Table 3. TLm values for three species of trout.

Test Animal	TLm	Zn conc. (ppm)	Temperature C	Alkalinity*	Author
Rainbow trout (Salmo gairdneri)	48 hr	4.0	17.5	320	Lloyd (1960)
Rainbow trout	48 hr	2.0	17.5	50	Lloyd (1960)
Rainbow trout	48 hr	0.6	17.5	12	Lloyd (1960)
Rainbow trout	24 hr	0.13	10.5	27	Affleck (1952)
Rainbow trout	24 hr	4.0	----	---	Goodman (1951)
Rainbow trout	48 hr	3.5	----	---	Goodman (1951)
Rainbow trout	5 day	4.6	----	290	Ball (1967)
Brown trout (Salmo trutta)	20 day	0.13	10.5	27	Affleck (1952)
Atlantic salmon (Salmo salar)	21 day	0.70	----	78	Grande (1967)

*Expressed as ppm of $CaCO_3$.

In another series of tests, Lloyd (1960) calculated that increased temperature (l 2.0 to 22.0 C) generally reduced the tolerance of trout to zinc by at least one-half. A temperature increase has much the same effect as low dissolved oxygen. There is an increase in metabolic and ventilation rates and a concurrent decrease in the concentration of oxygen present in the water.

The recommended maximum temperature and minimum dissolved oxygen level suggested for salmonid fishes by the Committee on Water Quality Criteria (1968) are 20 C and 6.0 ppm respectively. The mean temperature and dissolved oxygen levels for my tests were 14.3 C and 8.0 ppm respectively. These values were within safe limits for Cutthroat trout, i.e. neither temperature nor dissolved oxygen was limiting.

Upon removal of the dead fish, I observed a moderate amount of mucus covering the bodies of the dead fish. Examination of the gills did not show mucus present in large enough quantities to cause mechanical blockage of the gills, however, no histological examinations were performed. Carpenter (1927) working with minnows (Phoxinus phoxinus) and Jones (1938) working with sticklebacks (Gasterosteus aculeatus) reported that high concentrations of zinc caused death by mechanical obstruction of the gills. Some histological examinations of Rainbow trout by Lloyd (1960) showed no appreciable amount of mucus, but he observed some cellular breakdown of the gill epithelium at a concentration of 20 ppm zinc. Other fish in the test water containing 4 ppm zinc showed swelling of the gill lamella before death.

In the running system, accurate TLm values were not obtained due to decreases in the concentration of zinc while the tests were in progress. To eliminate the effect of uptake by the fish, a test run lasting 8 days was made with no trout in the tank. There was an initial concentration of 1.95 ppm zinc in one stream channel which dropped to one-third of its original value over the 8 day period (Fig. 10). Results from the other stream channel, run simultaneously, showed a drop in concentration from 0.7 to 0 ppm within 5 days. At the conclusion of an earlier test run, a sample of the rinse water contained 0.2 ppm zinc.

The change in concentration of zinc in the system without fish and the residual zinc present in the rinse water indicate that the zinc ions present in solution were being adsorbed or complexed onto the sides and bottom of

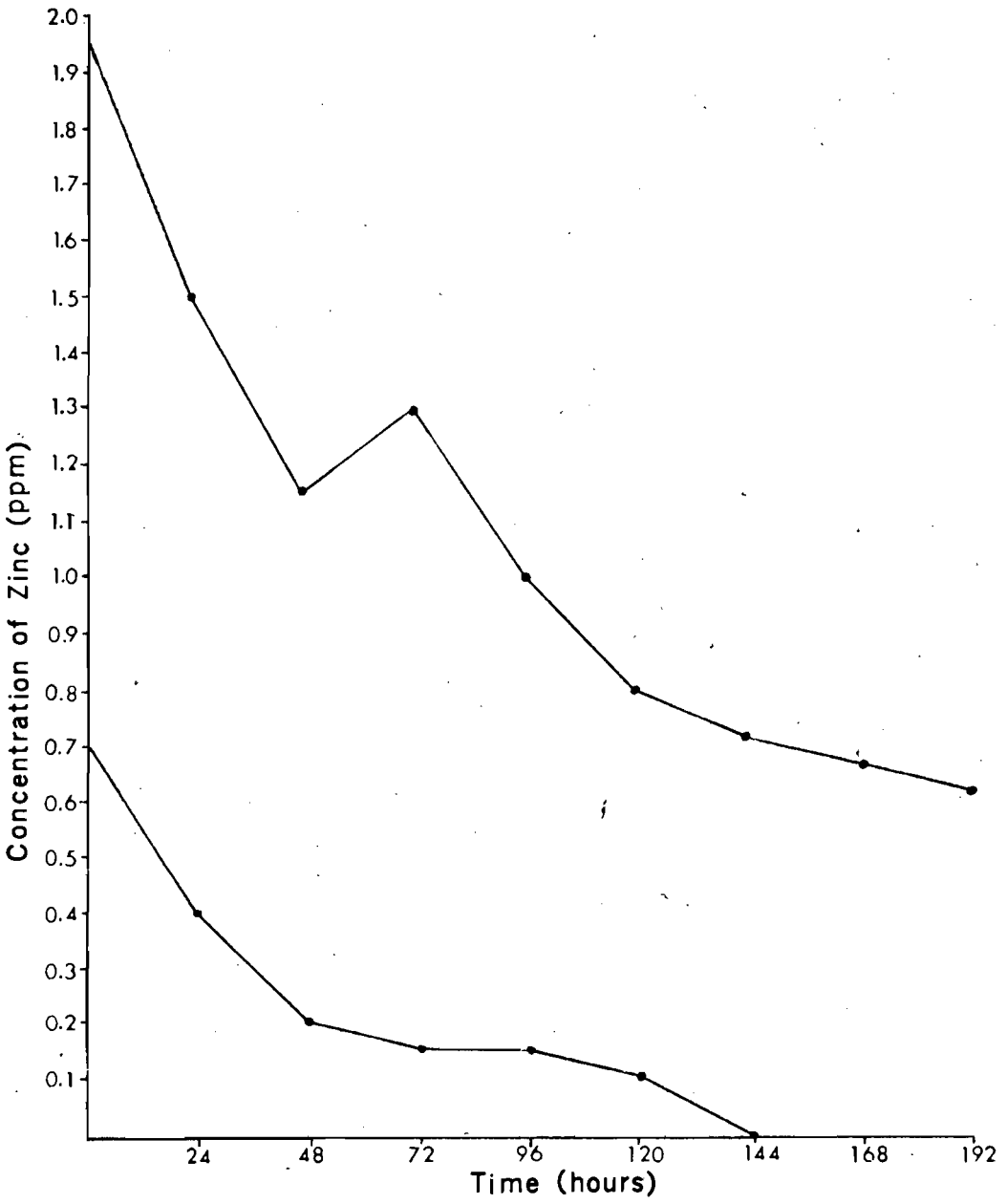

Figure 10. Decrease of zinc concentration in test water during an 8 day period (no fish present).

the tank. Funk (1969, personal communication) found polyethylene, polypropylene and glass containers to be capable of adsorbing considerable amounts of heavy metal ions. In their study of acute toxicity of heavy metals to aquatic insects, Warnick and Bell (1969) mentioned that the metal concentration decreased considerably over a two-week period. They attributed the decrease to adsorption, precipitation, complexing and other factors. Sprague (1969) states that sorption on the walls of test containers is apparently the cause of decreases in the concentration of many toxicants.

The zinc concentration of 0.40 ppm which occured at the 24 hr TLm value does not show close agreement with the 24 hr TLm value of 0.60 ppm for the static system. It must be understood however that these values were terminal concentrations. The lower concentration of zinc at which the 24 hr TLm value occured for the running system is undoubtedly a consequence of adsorption that had taken place on container walls in the running system (see Fig. 10). If the zinc concentration to which the fish were exposed throughout a specified time period had been used, better agreement might have been observed between the zinc concentrations at the 24 hr TLm values for the two systems.

Herbert and Shurben (1963) stated that current speeds up to 55 percent of the maximum sustainable velocity produced no noticeable increase in the toxicity of zinc sulphate to Rainbow trout. At all times, the current in the running system measured less than 0.25 cubic feet per second, and the fish had no trouble maintaining their positions. In addition, the shelters that had been placed in the stream channels provided the fish with resting areas that allowed them to move out of the current.

Based on my experiments, I concluded that a recirculating, flowing-water bioassay system has limited use for bioassay procedures. This system might possibly be employed in experiments in which TLm values of 24 hrs or less are desired. However, as a result of the changes in concentration, it is not satisfactory for most acute or long term tests.

SUMMARY

1. Determinations of the acute toxicity of zinc to Cutthroat trout fingerlings were performed in a laboratory at the University of Idaho from December 1968 to July 1969.
2. The dilution water used in the tests was from the North Fork of the Coeur d'Alene River. It had a low alkalinity (23.9 ppm as calcium carbonate) and a pH close to neutrality. This water had very little antagonistic effect upon the toxic action of the zinc as compared to other studies where higher alkalinity had a more pronounced effect upon trout survival.
3. One system employed was a standard, static bioassay in which 24, 48 and 96 hr median tolerance limit (TLm) values of 0.62, 0.27 and 0.09 ppm zinc were obtained. Results indicate that Cutthroat trout are very sensitive to the toxic action of zinc as compared to other species of trout.
4. The other bioassay consisted of a recirculating, flowing-water apparatus. A 24 hr TLm of 0.40 ppm zinc was obtained. No 48 or 96 hr values were estimated due to abrupt changes in the zinc concentration of the test water.
5. Changes in the concentration of zinc in the test water were due to adsorption of the ions onto the bottom and sides of the tank. I conclude that a recirculating, flowing-water bioassay system is unsatisfactory for short-term bioassay procedures.

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