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Acute Toxicity of Zinc to Some Fishes in High Alkalinity Water

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ABSTRACT

This study documents the acute toxicity effects of varying concentrations of zinc on certain fishes native to Illinois. Fourteen-day bioassays were performed with bluegill fry, channel catfish fingerlings, and largemouth bass fingerlings in waters relatively high in alkalinity and the salts of calcium and magnesium.

The 14-day median tolerance limit at 20 C was 11.0 mg/l soluble zinc for the bluegill, 8.2 mg/l soluble zinc for the channel catfish, and 8.0 mg/l soluble zinc for the largemouth bass. For protection of the fishes investigated, and in compliance with Illinois water pollution regulations, soluble zinc concentrations in Illinois streams having high alkalinity and hardness should not exceed 0.8 mg/1.

INTRODUCTION

The acute toxicity of heavy metals to fishes appears to be a function of the soluble fraction. And the solubility of heavy metals, in turn, appears to be dependent upon the alkalinity of water. The alkalinity of lakes and streams in Illinois are variable with concentrations, as $CaCO_3$, generally exceeding 200 mg/l in the northern and central part of the state and often less than 100 mg/l in the southern part of the state. Because this study deals with the acute toxicity of zinc to certain fishes in waters of relatively high alkalinity, the results are more applicable to the northern and central regions of the state than the southern region.

The Water Pollution Regulations of Illinois set forth maximum permissible concentrations for zinc as follows:

Potable water	5.0 mg/l
All surface waters	1.0 mg/l
Waste effluents	1.0 mg/l (total)

These limits are applicable statewide without consideration for the differing concentrations of alkalinity that occur regionally in the surface waters of the state. The regulations also stipulate:

> **Any substance toxic to aquatic life shall not exceed one-tenth of the 48-hour median tolerance limit (48-hr TLm) for native fish or essential fish food organisms.**

The median tolerance limit (TLm) is the concentration at which 50 percent of the test specimens survive. It is also referred to as TL50 which is the designation used in this report. A 48-hour bioassay is of little use except in examining very toxic substances such as chlorine. A 96-hour bioassay is the desirable minimum length. However, for assessing the acute toxicity of heavy metals on fish, a 96-hour time period of fish exposure to a selected toxicant is too short. During this study, an exposure time of 14 days (336 hours) was used.

The levels of total zinc in natural water bodies in Illinois generally do not exceed 0.5 mg/1. Soluble zinc concentrations are usually less than 0.05 mg/l except in those surface waters that are significantly influenced by urban centers. Elevated concentrations of zinc occur at the mud-water interface, during anaerobic conditions, in Illinois lakes. The concentrations thus far observed are generally two to three times higher than those detected in the overlying waters.

This study is part of a continuing effort to develop information that will be useful to persons and agencies whose activities are related to the enhancement of water quality in the streams and lakes of Illinois.

Scope of Study

This study was concerned with documenting the acute toxicity effects that varying concentrations of zinc have on certain fishes native to Illinois lakes and streams. The fishes observed were largemouth bass fingerlings, bluegill fry, and channel catfish fingerlings. Concentrations of zinc were quantified in terms of its fraction most toxic to fish, i.e., soluble zinc.

The bioassays were of 14 days' duration and they were performed with various fish sizes and water temperatures. The results were derived from water high in the salts of calcium and magnesium with correspondingly high alkalinity.

Plan of Report

The report is simple in structure. It consists of a literature review, description of methodology, report on fish reactions, analysis of results, and a summary. All data developed from the bioassays are included in the appendices. A description of the chemistry of zinc is offered to emphasize the complexity of dealing with certain heavy metals in this type of research and is not considered by any means authoritative. All information is presented in a manner that may be useful to persons or agencies involved in the day-to-day business of maintaining adequate fisheries and reasonable water quality in Illinois.

Acknowledgments

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LITERATURE REVIEW

Zinc Chemistry

Metal ions in natural waters may be classified as soluble or insoluble. The soluble ions are free ions or metal complexes with inorganic or organic ligands such as amino, fulvic, and humic acids (Guy and Chakrabarti, 1975). Insoluble ions are colloidal particulates of metal complexes or aggregates of hydrous metal oxides, i.e., $(OH)^{2+}$, $Fe(OH)^{+}$, and $Fe(OH)^{-}_{4}$, and metal complexes absorbed on suspended particulates such as $CuCO_3$ (Stumm and Morgan, 1970; Stiff, 1971; Mancy and Allen, 1977).

It is generally reasoned that in order for a metal to exert its toxicity it must be in soluble form as opposed to suspended or insoluble. In an investigation on the toxicity of zinc sulfate to bluegill, Cairns et al. (1971) found soluble zinc toxic and insoluble zinc nontoxic. The toxicity exhibited by a substance can be influenced by conditions in the solution. Modifying conditions for toxicity bioassays included effects of temperature, water hardness, alkalinity, pH, dissolved oxygen, and other physical and chemical factors present during the test. To differentiate between soluble and insoluble zinc for analytical purposes, that portion that will pass through a 0.45 μm pore size membrane filter is the soluble zinc. The combination of soluble and insoluble zinc is termed total zinc.

Numerous investigators have studied the effect of hardness by measuring the toxicity of metal solutions with increasing hardness. Several theories have been advanced to account for their results. Early workers attributed the decreased toxicity of zinc solutions at high hardness to complexation and precipitation of the metal ions from the solution. Lloyd (1960) considered hardness to be the most important single factor modifying the toxicity of zinc ions. He stated that the effect of hardness increases with an increase in the periods of survival, until there is almost a tenfold difference in the concentrations of zinc toxic in 2.5 days in a water having a total hardness of 320 mg/l and a water having a total hardness of 12 mg/1. In 96-hour experiments, Cairns and Scheier (1957) found zinc to be four times as toxic to the common bluegill in a soft water (total hardness approximately 50 mg/1) as in a hard water (total hardness 320 mg/1). Hence, it is important to know the total hardness of the dilution water when comparing data on the toxicity of zinc to fish.

However, with the advent of continuous-flow bioassay techniques, it has been found that some complexation may actually increase the toxicity of metal solutions. Mount (1966) theorized that the suspended zinc is at least partially converted to dissolved zinc because of lower pH values near the surface of the gills due to CO_2 excretion. The toxicity of suspended zinc will obviously depend on the physical-chemical nature of zinc; thus, a fine hydroxide precipitate will probably have a different toxicity than zinc silicate or zinc bound to organic matter.

One of the major difficulties in interpreting the effect of hardness on heavy metal toxicity stems from the inability to determine whether the observed effects are a result of calcium-heavy metals competitive interactions or physiological effects that resulted from the presence of Ca^{2+} or Mg^{2+}, or both. Mancy and Allen (1977) have attempted to explain these hypotheses. With regard to the former condition, the effect of hardness could be interpreted in terms of purely aqueous chemical interactions. Under a given set of circumstances, certain heavy metals can exist as soluble organic complexes. At relatively high Ca^{2} concentrations, as a result of metal-ligand exchange reactions, these heavy metals may be transformed into inorganic metal complexes that may precipitate as hydroxy compounds at a high pH. This effect, more pronounced at higher levels of hardness, is largely dependent on pH.

Consequently, if the toxicity effects were primarily due to the organic-metal complex, the presence of high levels of calcium will result in a decrease of its toxicity.

On the other hand, the presence of high levels of Ca^{2+} and Mg^{2+} has been thought to cause certain physiological changes in fishes that may result in a decrease in the toxic effects of heavy metals. Lloyd (1975) found that rainbow trout reared in hard water and acclimated to softer water for no longer than 3 days had similar zinc toxicity results when tested in both hard and soft water. Further experiments revealed that rainbow trout reared in hard water had to be acclimated to soft water for a minimum of 5 days before they became as sensitive as rainbow trout maintained in soft water. Apparently, there is an internal protective action of calcium, and some calcium must be lost by the hard water-reared fish before they attain soft water sensitivity. Preconditioning of fish in hard water will significantly decrease their susceptibility to toxic metals.

The effect of temperature upon the toxicity of water soluble toxins is difficult to predict. The published data for heavy metal ions tend to be conflicting. A decrease in temperature usually increases the survival time of fish in toxic solutions. Experiments with rainbow trout in solutions of zinc showed that the survival times increased 2.35 times when the temperature was reduced from 22 to 12 C (Lloyd, 1960). In addition, Lloyd concluded that if temperature affected only survival time, then the lethal threshold concentration would be unaffected.

A similar conclusion was reached by Cairns and Scheier (1957) on the toxicity of zinc to bluegills. There was little difference in the 96-hour TL50 values obtained at 18 and 30 C in a hard or soft dilution water. Burton et al. (1972) and Morgan et al. (1971) demonstrated that an increase in temperature could reduce survival time of bluegills exposed to both lethal and sublethal concentrations of zinc. The actual reduction in survival time was a function of the rate at which the temperature was increased. For example, bluegills acclimated to 20 and 30 C and then exposed to a lethal concentration of zinc (32 mg/l) at each temperature died 2.6 times faster at 30 than at 20° C. Fish acclimated to 20° C and exposed to a temperature increase of 1.5 C every 10 minutes died 4.8 times faster than fish maintained at 20 C. A sublethal zinc concentration of 5.6 mg/l produced no mortalities in 20 C acclimated bluegills in 96 hours but was toxic to fish when the temperature was increased from 20 to 30 C at a rate of 1.5° C every 10 minutes. From these data, it is clear that a changing thermal environment can amplify the effects of trace metal toxicity for fish far more than a constant high temperature.

The effect of temperature on zinc toxicity becomes somewhat less clear, however, when the information from additional studies is considered. Pickering and Henderson (1966) confirmed the findings of Cairns and Scheier (1957) for bluegill in that a temperature change had little influence on the 96-hour TL50 for zinc, but the same authors reported that raising the temperature from 15 to 25 C reduced the 96-hour TL50 in fathead minnows from 2.55 to 0.77 mg/l (i.e., the toxicity was increased by a factor of approximately 3). Rehwoldt et al. (1972) investigated the toxicity of six metal ions to several Hudson River fish species at 28 and 15° C. They claimed that the 24-, 48-, and 96-hour TL50s for copper, zinc, nickel, cadmium, and chromium are uninfluenced by the test temperature used. The mercury data, however, revealed TL50s which were significantly different as the test temperatures were varied. Clearly the influence of temperature on the toxicity of low concentrations of zinc is very species-dependent both qualitatively and quantitatively.

The low dissolved oxygen concentrations, which are characteristic of many polluted rivers have been shown by several authors to increase the toxicity of poisons to fish. Since most toxicity tests are made in well-aerated water, it is important to know what factor to apply to the results when predicting the effect of a reduced dissolved oxygen concentration on toxicity. Lloyd (1960) exposed rainbow, trout to five lethal concentrations of zinc sulfate at three nonlethal levels of dissolved oxygen, 8.9, 6.2, and 3.8 mg/l O_2 at 17.5 C in hard water (320 mg/l hardness) with a pH of 7.8. The trout were acclimated for 18 hours to the temperature and pH value, but not to the oxygen tension. Lloyd calculated that, over an exposure period of 1000 minutes, the concentration of zinc necessary to kill 50 percent of the fish was 1.4 times greater at an oxygen concentration of 8.9 mg/1 than it was at 3.8 mg/l. Another experiment was made with rainbow trout taken from the same stock but with the fish acclimated for 18 hours to the experimental dissolved oxygen levels, the temperature, and the pH of the previous test. Lloyd's results indicated that the toxicity of zinc to acclimated fish was less affected by the dissolved oxygen concentrations than in previous experiments.

Pickering (1968) evaluated the effects of three dissolved oxygen levels on the toxicity of zinc to bluegill in hard spring water under conditions of continuous flow exposure. The bluegill test results showed an increased mortality to zinc as a result of low dissolved oxygen concentrations. The difference in the average TL50 value between low and high test concentrations of dissolved oxygen was a factor of 1.5. He cited the report of Cairns and Scheier on the effects of periodic low oxygen on the toxicity of zinc in which they reported obtaining a similar factor of 1.6 in their experiment. The factors derived for the effects of dissolved oxygen on acute zinc toxicity are similar in short static tests and in longer flow-through tests. Pickering (1968) also concluded that dissolved oxygen concentrations have a significant effect on bluegill growth. Growth was best at the high and least at the low dissolved oxygen concentration.

Lloyd (1961) proposed the idea that an increase in toxicity resulting from a reduction in the concentration of dissolved oxygen is a result of a physiological reaction by the fish to such a change in the environment and is independent of the nature of the poison. The most apparent reaction of fish to a lowered oxygen content of the water is to increase the volume of water passed over the gills in order to obtain sufficient oxygen. If accompanied by an increase in temperature, it produces a higher metabolic rate with a greater demand for the uptake of oxygen from the water and the loss of CO_2 and metabolites from the blood. In both cases, the amount of water passed over the gills increases and the proportion of blood shunted into the thin gill lamellae increases. In effect, this increases the opportunity for the exchange of materials between the blood and the water, and accelerates the uptake of heavy metals from the water.

Lloyd (1961) also had another hypothesis related to the effect of low oxygen concentrations on the toxicity of poisons to fish. It assumes that a given toxic effect is produced by a specified concentration of poison at the gill surface, and suggests that this concentration is governed not only by the concentration of poison in the bulk of the solution but also by the velocity of respiratory flow. It also implies that the relation between the increase in toxicity of poisons to fish and a reduced dissolved oxygen concentration of the water will be the same for all poisons except those whose toxicities are affected by the pH of the water.

Although a considerable amount of information exists on zinc toxicity to fish, little can be found in the literature to explain the environmental impact a varying pH might have on zinc toxicity. Perhaps this variable was not measured or at least was not reported in the publications on zinc toxicity. Cairns and Scheier (1957) who investigated the effects of temperature and hardness on bluegill did not evaluate the effect of pH in the course of their study; however, tables in their published work indicate that the pH decreased from the beginning to the end of each test. Also, the pH was approximately 1.0 unit higher in the hard water than in the soft water. On the basis of 4 day (96 hour) static tests, Pickering and Henderson (1966) reported zinc to be noticeably more toxic to bluegills in soft water (hardness 20 mg/l and pH 7.5) than in hard water (hardness 360 mg/l and pH 8.2). Their results were based on concentrations added, and they state that settling of zinc occurred and that changes in pH resulted from the addition of a metal salt.

Mount (1966) commented that most studies dealing with environmental variables were made by varying the hardness and accepting the resulting pH. He stated that although this procedure is valid under most natural conditions, in pollutional situations the pH of a receiving body of water may be altered significantly. He further stressed the importance of assessing the individual effect of each factor in various pollutional combinations. Therefore, Mount conducted a series of continuous flow-through bioassays to determine the toxicity of zinc to fathead minnows at pH levels of 6, 7, and 8 and total hardness levels of 50, 100, and 200 mg/1. Zinc was most toxic at a pH of 8 and hardness of 50 mg/l and least toxic at a pH of 6 and hardness of 200 mg/1. Mount's study showed that at any given hardness level, as pH increased, the solutions became more toxic, and he attributed this phenomenon to the zinc complexes formed at higher pH levels.

Cairns et al. (1971) cited the work of Lloyd and Herbert who postulated that the increase in carbon dioxide content of the water at the surface of fish gills causes a local reduction in pH. Such a decrease in pH could cause insoluble particulate zinc which was trapped in the gill to become soluble. The solubility of zinc decreases markedly as the pH increases. At pH values of 8 or higher, zinc solubility is reduced a hundredfold compared with water of pH 7. If the pH underwent a local decrease near the fish gills and insoluble zinc compounds were converted to soluble forms, this might explain why zinc could be toxic to fish at pH values of 8 or higher.

Cairns et al. (1971) exposed bluegill to zinc sulfate for 96 hours under static test conditions at two temperature ranges and two pH ranges. Bluegill mortalities in concentrations of soluble zinc ranging from 10 to 32 mg/l were 0 to 10 percent at the high pH and 100 percent at the low pH. They surmised that bluegills exposed to zinc sulfate at a pH of 8 or higher were not killed in 96 hours at the same zinc concentrations which produced mortalities at pH 6.0 and 7.3. Their tables revealed that the measured zinc concentration was much less at pH 8 or 9 than at the lower pH. The dissociation of ionic zinc from a zinc compound appears to be the principal factor in cause of death. At pH 6.0 and 7.3, the zinc ion readily dissociated from $ZnSO_4$ and produced mortalities. At pH 8 or above, the dissociation of ionic zinc was much less and did not cause any mortalities under the conditions of their experiments.

Solbe (1973) explained that in fresh natural waters the solubility of zinc, Zn^{2+}, is essentially controlled by the solubility of zinc carbonate. This, in turn, is a function of the concentration of carbonate ion, and is dependent upon the pH value and concentration of bicarbonate ion in the solution. Total hardness is related to bicarbonate ion concentration. Table 1 shows the solubility of zinc carbonate under various conditions. In computing these values, Solbe assumed that organic complexing of zinc did not occur, that less soluble zinc hydroxocarbonates were not formed and precipitated out of solution, and that equilibrium had been reached within the system. The concentration of dissolved zinc would be reduced tenfold by either a ten unit increase in hardness or a one unit increase in pH.

Table 1. Zinc Carbonate Solubility for Given pH and Hardness Values*
(Zinc carbonate values in mg/l)

Hardness (mg/l)	pH values 6	7	8
50	131	13.1	1.31
100	65.3	6.53	0.65
200	32.6	3.26	0.33
500	13.1	1.31	0.13

**from Solbe'(1973)*

Zinc Toxicity

The water quality of a body of water (particularly hardness, temperature, dissolved oxygen, and pH) has an important effect on the toxicity of poisons to fish. Table 2 lists short-term toxicity data from other sources. When referring to this table, the reader should consider that various water quality criteria are omitted, and that fundamental testing data (i.e., toxic compound used, type of exposure) have not been given. The influence of pH, dissolved oxygen, hardness, and other factors on the solubility and form of zinc are critical to an understanding of the toxic values obtained. Many early publications on zinc toxicity ignored the importance of water characteristics and their subsequent effect on zinc complexation. Recently, more emphasis has been placed on reporting all pertinent physico-chemical data measured during an investigation to determine an acute toxicity value. Knowledge of these facts might shed some light on the reasons for variability in toxicity results.

Partial and complete life cycle toxicity tests on fish, involving all developmental stages, have been used extensively in the establishment of water quality criteria for aquatic life. During extended chronic exposures of fish to selected toxicants, certain developmental stages have frequently shown a greater sensitivity than others. McKim (1977)"states that the embryo-larval and early juvenile life stages were the most, or among the most, sensitive. He based his conclusions on 56 life cycle toxicity tests completed during the last decade with 34 organic and inorganic chemicals and four species of fish.

Other investigators also show that fish eggs and fry are extremely sensitive to metallic poisoning. Affleck (1952) indicated a high sensitivity of eggs and alevins of brown and rainbow trout to zinc poisoning, where concentrations as low as 0.01 mg/l were found to be lethal or toxic. Pickering and Vigor (1965) have shown an acute toxicity of zinc to eggs and fry of the fathead minnow. They found median tolerance limits of 3.92 to 3.98 mg/l for 1 day old eggs. The TL50 values continued to drop until on the 12th day the level had fallen to 1.57 to 1.69 mg/l. Newly hatched fry exposed for 2 days to zinc sulfate had a TL50 of 0.95 mg/l indicating an even greater sensitivity to zinc at this stage of the life cycle. Skidmore (1966, 1967) studied the effects of zinc sulfate on zebra fish eggs and found that they also increase in sensitivity during the course of embryonic development. Later embryonic stages of 2 to 4 days and newly hatched fry 4 to 13 days old were highly susceptible to the toxic effects of zinc.

Birge and Just (1975) conducted bioassays on heavy metals utilizing avian, amphibian, and piscine embryos as bioindicator organisms. In light of their findings, they too noted that zinc appeared to be more toxic to late embryonic stages and newly hatched fry while mercury was definitely more harmful to early embryonic stages. Although zinc has been shown to be toxic to certain species at various levels, it has been demonstrated to be an essential trace element for normal embryonic development, and the deficiency of zinc may lead to serious embryonic abnormalities. Therefore, they stressed that the pattern of zinc toxicity to embryonic systems may vary significantly from that of nonessential elements such as mercury.

Sabodash (1974) studied the effect of zinc sulfate on the development and viability of grass carp larvae under varying water hardnesses. The addition of zinc sulfate was shown to have a beneficial effect on the size, weight, and length of carp embryos and larvae. Further investigation revealed that zinc ions have a more stimulatory effect in water of increasing hardness due to the high calcium content. Because zinc promotes the inclusion of calcium into the metabolic processes, it is essential in development, especially in the early stages.

Crandall and Goodnight (1962) observed a reduction in growth and delay of sexual maturity in the common guppy when subjected to a 90-day exposure of 1.15 mg/l zinc in water containing 80 mg/l total hardness. Bengtsson (1974a, 1974b, 1974c, and 1974d) studied the effects of zinc on the growth, reproduction, behavior, and histology of the minnow *Phoxinus pboxinus L.* Reduced growth occurred in yearlings at 0.13 mg/l zinc in fresh water over a 150-day

Table 2. Summary of Zinc Toxicity Data from Other Sources

Species	Size	Temperature (°C)	Alkalinity (mg/l)	pH range	TL50 (mg/l)	Reference source
Bluegill	adult	24	low	7.5	5.2 (48 hr)	Cairns et al. (1965)
Bluegill	1.5 g	25	18		6.75 (24 hr)	Pickering and Henderson (1966)
Bluegill	1.5 g	25	18		5.46 (48 hr)	Pickering and Henderson (1966)
Bluegill	1.5 g	25	18		5.46 (96 hr)	Pickering and Henderson (1966)
Bluegill	1.5 g	25	300	8.1	40.9 (96 hr)	Pickering and Henderson (1966)
Bluegill	1.5 g	15	18		7.95 (24 hr)	Pickering and Henderson (1966)
Bluegill	1.5 g	15	18		6.14 (48 hr)	Pickering and Henderson (1966)
Bluegill	1.5 g	15	18		6.44 (96 hr)	Pickering and Henderson (1966)
Bluegill	0.64 g	5	low	5.8-7.8	23 (24 hr)	Cairns et al. (1978)
Bluegill	0.64 g	15	low	5.8-7.8	19.1 (24 hr)	Cairns et al. (1978)
Bluegill	0.64 g	30	low	5.8-7.8	8.85 (24 hr)	Cairns et al. (1978)
Bluegill	3.7 g	18	low		3.32 (96 hr)*	Cairns and Scheier (1968)
Bluegill	3.2 g	30	low	6.8-8.2	2.78 (96 hr)*	Cairns and Scheier (1957)
Bluegill	3.2 g	18	high	8.1-8.8	11.3 (96 hr)*	Cairns and Scheier (1957)
Bluegill	3.2 g	30	high	8.3-8.8	11.2 (96 hr)*	Cairns and Scheier (1957)
Bluegill	2.5 g	25	300	7.7-8.0	11.4 (20 da)*	Pickering (1968)
Bluegill	18.7 g	20	44	7.3-8.3	32 (12 hr)	Burton et al. (1972b)
Bluegill	18.7 g	30	44	7.3-8.3	32 (4.7 hr)	Burton et al. (1972b)
Bluegill	37 g	7	36	7.0-7.8	11.5 (96 hr)*	Cairns et al. (1971)
Rainbow trout	16 cm	15.5	high	7.6-7.9	4.76 (48 hr)	Solbe (1973)
Rainbow trout	fingerling	17.5	240	7.8	3.86 (48 hr)	Herbert and Shurben (1964)
Rainbow trout	fingerling	17.7	41.5	6.9	0.91 (48 hr)	Herbert and Shurben (1964)
Rainbow trout	yearling	16.9*	high	7.3-7.5	4.0 (48 hr)	Brown and Dalton (1970)
Rainbow trout	4.42 g	15	low	5.8-7.8	1.56 (24 hr)	Cairns et al. (1978)
Rainbow trout	4.42 g	30	low	5.8-7.8	2.1 (24 hr)	Cairns et al. (1978)
Rainbow trout	73.4 g	15	high	7.3-7.9	4.6 (5 da)	Ball (1967)
Rainbow trout	3-15 months	17.5	240	7.8	4 (7 da)	Lloyd (1960)
Striped bass	<20 cm	17	low	7.8	10 (48 hr)	Rehwoldt et al. (1971)
Striped bass	<20 cm	17	low	7.8	6.7 (96 hr)	Rehwoldt et al. (1971)
Fathead minnows	1-2 g	25	50	7	10 (96 hr)*	Mount (1966)
Fathead minnows	1-2 g	25	102	8	9.0 (96 hr)*	Mount (1966)
Perch	34.5 g	12.1	high	7.2-7.8	16 (5 da)	Ball (1967)

** Average of results*

test period. The under-yearling minnows were the most sensitive of the three age groups tested and were least able to compensate for rotating water current. Adult minnows exposed to different concentrations of zinc nitrate developed hemorrhages and lesions. Also vertebral damage occurred between 0.2 and 2.4 mg/l zinc which is below the 96-hour TL50 dosage of 3.2 mg/1. Lastly, Bengtsson researched the long-term effects of zinc nitrate on the reproduction and mortality of different developmental stages of the minnow. The most sensitive parameter appeared to be the mortality of newly hatched fry. Zinc did not inhibit spawning behavior but did reduce egg production. Similarly, Brungs (1969) conducted a 10 month continuous flow bioassay on the chronic toxicity of zinc to fathead minnows. Chronic exposure produced reduced growth rates through 2.8 mg/1, reduced egg hatching through 1.2 mg/1, and reduced spawning performance (i.e., egg production) through 0.18 mg/1.

Jones (1947) conducted early studies on the reactions of fish to toxic solutions. Sticklebacks were able to detect dissolved zinc down to 10 mg/l and avoided it if possible. Waller and Cairns (1971) devised an apparatus for the continuous monitoring of fish to zinc through the use of light beams. Their system detected premortal aberrations in fish movement caused by zinc. The detection of stress occurs in sufficient time to permit survival of the fish if stress conditions are reversed at the time of detection. The lowest concentration of zinc detected by golden shiners and goldfish during a 96-hour exposure was between 3.64 and 2.94 mg/1. Bengtsson (1974e) conducted a similar experiment with minnows. Initially the minnows developed hyperactivity as a response to a gradually increased zinc concentration. This behavior was followed by a period when the fish displayed hypoactivity. In addition there were changes between the diurnal and nocturnal activity. Sparks et al. (1972) observed

that zinc concentrations as low as 2.55 mg/l could be detected in bluegills by an increase in breathing rate or a change in breathing rate variance. They also found that approximately one-tenth of this physiologically detectable zinc level (0.235 mg/l) was sufficient to inhibit spawning in ripe bluegills and to kill newly hatched larvae.

Toxic concentrations of zinc compounds cause adverse changes to the morphology and physiology of fish. Jones (1938) reported gill damage and heavy secretions of mucus in sticklebacks killed by high concentrations of zinc. The mucus was believed to be the major cause of death through mechanical obstruction of thegills. Lloyd (1960) determined that the cause of death of fish in solutions of zinc sulfate was not from the precipitation of mucus on the gills but probably from zinc-induced damage to the gill epithelium. Guppies reared in zinc solutions experienced stunted growth, had a higher mortality rate, and showed less sexual dimorphism (Crandall and Goodnight, 1962). Histological examination revealed many abnormalities among the internal organs. The liver had degenerated, the pancreas was undersized, the kidneys were distorted and hemorrhaged, and the skeletal muscles were underdeveloped and vacuolated. Crandall and Goodnight exposed guppies to chronic concentrations of zinc. Under these conditions, guppies were less active than the controls. Appetites decreased, swimming was abnormal, and they experienced a loss of equilibrium. Acutely toxic concentrations of zinc cause severe cytological damage to the gills and some coagulation of mucus over large areas of the body. No other morphological changes have been noted. In contrast, chronically toxic concentrations of zinc compounds cause general enfeeblement and widespread histological changes to many organs, but not to gills.

Fish exposed to either a toxic or nontoxic solution of zinc absorb zinc by an unknown route, possibly through the gills while respiring or through the mouth with swallowing (Skidmore, 1964). Once absorbed internally, the zinc may be concentrated especially in the gills, gut, and liver. If the exposed fish are returned to zinc-free water, most of the absorbed zinc is subsequently lost.

The toxic effects of zinc salts on the gills of experimental fishes have been documented by several authors. Data from Skidmore (1970) suggest that epithelial damage decreased the permeability of the gills to oxygen and did not increase their permeability to cations. Zinc did not act as a rapid internal poison. Furthermore, he attributed death to tissue hypoxia when maximum gill ventilation was no longer sufficient to supply the oxygen needs of the fish. Skidmore and Tovell (1972) demonstrated that acute exposure of rainbow trout to 40 mg/l zinc sulfate caused an acute inflammatory reaction in the gill with a separation of the epithelium outward from the pillar cells. This was followed by circulatory breakdown, tissue destruction, respiratory collapse, and death.

The effects of dissolved zinc on the gills of the stickleback were studied by Matthiessen and Brafield (1973). They, too, noted the detachment of the epithelial cells upon exposure to zinc. Many acutely poisoned fish recovered when transferred to zinc-free hard water. The epithelial cells regenerated but were accompanied by the appearance of chloride cells on the secondary lamellae. They postulated that the chloride cells were produced as a direct or indirect response to the influx of zinc ions. Through biochemical analyses, Burton et al. (1972a) confirmed an earlier hypothesis that the major physiological change preceding death in acute toxicity studies with zinc was tissue hypoxia. The hypoxia was directly related to gill tissue damage which disrupts normal gas exchange at the gill surface.

Hiller and Perlmutter (1971) reported the effects of the metal zinc in virus-host interactions in the rainbow trout cell line, RTG-2. Titers of infectious pancreatic necrosis virus showed a significant increase when cultured in the presence of 10 mg/l zinc. It is suggested that zinc cations reduce the net negative charge on either the cellular or viral surfaces or both. This reduces the electrostatic barrier between virus and cell, thereby enhancing the adsorption of virus to cell surface. They proposed that investigations for the purpose of determining biologically safe concentrations of these metal cations should also include studies on their interactions with virus-host systems.

EQUIPMENT AND METHODS

A modification of a proportional dilutor by Mount and Brungs (1967) was used. Water flow was provided through 12 glass test chambers. Each chamber had a volume of 22 liters, and the flow rate, 250 milliliters per minute (ml/min), produced a 95 percent volume displacement every 6 hours. The apparatus permitted flow of five different concentrations of toxicant into duplicative test chambers with two chambers available for control purposes. All tests were performed for at least 14 days.

Equipment Modifications and Appurtenances

The major modification in the dilutor apparatus was a syringe style pipettor with a two-way check valve from

Manostat, which was fed from a container of toxicant. A normally open four-way Skinner air solenoid valve was placed into the circuit of the electrical switch which operated the water solenoid valve in the standard Mount and Brungs dilutor. The system worked in the following manner.

During cycling of the dilutor, the water bucket arm descends to engage the switch and breaks the electrical circuit. This shuts off the water solenoid valve and opens the air solenoid valve causing the arm of the air cylinder to be extended. The extended arm depresses the plunger ot the pipettor to inject an exact amount of toxicant from the syringe into the mixing bowl. When the bucket arm rises to complete the electrical circuit again, the water solenoid valve opens and the air solenoid valve causes the air cylinder arm to retract. Two external springs return the plunger of the syringe to the locked position of the pipettor necessary for the intake of desired syringe volume through the two-way check valve. The original internal spring was replaced by external springs to ensure the reliability necessary for the very frequent and long-term cycling in bioassays. The advantages of this system are an easily adjustable volume of toxicant, a fail-safe design directly timed by dilutor function, an ability to dispense solutions with suspended particles, and a relatively low price for a system comprising an air solenoid valve, air cylinder, and pipettor.

A well on the laboratory site, in the same aquifer as the municipal wells, was the source of water for the zinc study.

Two header boxes were used. The first one, consisting of a steel barrel lined with fiber glass, housed a thermoregulator which could be set at a desired temperature. Significant cooling from the preset water temperature energized a relay which activated a solenoid-controlled valve on a hot water line. Water flowed from the steel barrel to a polyethylene plastic header box where air agitation kept the contents mixed and provided a sustained dissolved oxygen level.

The following characterize the dilution water used in the zinc bioassay:

	mg/l		*mg/l**
Chemical oxygen demand	ND	Fluoride	0.79
Ammonia-N	0.06	Magnesium	25.3
Nitrate-N	3.6	Iron	0.22
Phosphate-P	0.20	Zinc	0.07
Sulfate	147	pH	8.52
Chloride	65.2	Hardness	313
Copper	0.005	Alkalinity	268

Note: ND = not detected
** Except pH*

Stock Solutions and Chemical Analyses

The zinc stock solutions were prepared by dissolving American Chemical Society analytical reagent grade hydrous zinc sulfate in deionized water. Because of the high alkalinity (268 mg/1) of the dilution water, precipitation occurred as the stock solution was added to the test chambers. As previously discussed, insoluble zinc is not as toxic to fish as soluble zinc. To analyze for the soluble zinc, the particulate had to be removed. At least four times during the first 24 hours of each bioassay, and at least daily thereafter, a 50-ml sample was taken from the middle of each test chamber. Next, 25 ml of that sample was forced through a 0.45 μm pore size membrane filter, resulting in a filtrate to be analyzed for soluble zinc. The other 25 ml of the sample was analyzed for total zinc. All zinc analyses were determined by a Perkin-Elmer atomic absorption spectrophotometer, model 305 A.

Because of the large range of values between the soluble and total zinc, the absorption curve was not always linear. Therefore, two curves were used, one for the soluble zinc and one for the total zinc.

Table 3 shows a comparison between the total and soluble zinc values obtained from July 31, 1978, to January 23, 1979. Much of the zinc in the test chambers formed insoluble compounds, especially at the higher concentrations. At least four duplicate standards were plotted for each daily curve. Correlation coefficients for the daily standards ranged from 0.9946 to 1.0000.

Hardness and alkalinity were determined in one control chamber and two other test chambers every day. Dissolved oxygen levels, measured by a Yellow Springs Instrument Model 51B oxygen meter, and pH were measured daily in all test chambers. The water temperature also was measured daily by a standard calibrated Centigrade thermometer. Hardness determinations were by EDTA titrametric method with Eriochrome Black T as an indicator. Alkalinity and pH were determined by an Orion digital pH meter, model 801A, with 0.02N H_2SO_4 as a titrant for alkalinity. The averaged results of these analyses are shown in table 4. Illumination for the 16-hour photoperiod was furnished by a combination of Duro-Test and Wide Spectrum Gro-lux fluorescent lighting in circuit with a timer.

Test Specimens

Three native Illinois fishes were selected as test species. The largemouth bass *(Micropterus salmoides)* used in this investigation were obtained from the fish hatchery maintained by the Illinois Department of Conservation at Spring Grove. In all, 526 largemouth bass at an average weight of 2.27 grams were tested. The 357 bluegill *(Lepomis macrochirus),* obtained from the Illinois Department of Conservation's fish hatchery at Carbondale, had two distinct weight groups of 2.3 and 0.81 grams per fish. The 470 channel catfish *(Ictalurus punctatus),* also from the hatchery at Carbondale, had a representative weight of 2.88 grams. All 1353

Table 3. Comparison of Total and Soluble Zinc

Tanks	Total zinc (mg/l)	SD	Soluble zinc (mg/l)	SD	Total zinc (mg/l)	SD	Soluble zinc (mg/l)	SD
	July 31, 1978				*November 14, 1978*			
6, 9	20.2	2.0	15.3	0.49	*		42.0	4.51
1, 11	16.1	1.09	10.6	0.23	35.0	2.71	31.7	0.95
5, 7	12.9	0.62	8.4	0.33	25.4	2.78	21.7	1.71
4, 8	9.8	0.45	5.6	0.50	22.6	3.17	19.2	1.37
2, 12	5.8	0.41	3.0	0.40	11.0	1.79	8.2	0.67
	August 21, 1978				*November 28, 1978*			
6, 9	15.2	0.61	11.2	1.32	30.3	4.95	19.3	1.26
1, 11	13.2	0.45	7.6	1.86	21.7	1.49	13.4	1.01
5, 7	6.4	1.26	4.7	1.88	16.4	0.85	8.8	1.49
4, 8	7.3	0.68	4.4	1.16	15.9	1.56	8.3	1.24
2, 12	3.2	0.68	1.7	0.47	8.6	0.21	3.9	0.64
	October 4, 1978				*December 5, 1978*			
6, 9	18.4	0.00	14.2	0.70	26.9	0.49	17.4	1.65
1, 11	15.1	0.00	9.8	1.08	26.5	8.06	11.6	2.22
5, 7	12.6	0.14	8.1	0.57	16.4	3.25	6.9	1.24
4, 8	9.6	0.14	6.0	0.67	13.8	0.07	6.2	1.25
2, 12	5.1	0.00	2.9	0.48	6.4	0.21	2.9	0.76
	October 18, 1978				*January 9, 1979*			
6, 9	24.5	0.71	20.2	1.25	33.5	1.16	29.8	1.49
1, 11	20.0	0.00	14.6	1.14	29.3	2.50	22.9	1.53
5, 7	18.2	0.85	11.0	0.96	22.6	3.38	17.4	1.35
4, 8	15.2	0.00	8.4	1.13	19.1	2.89	13.5	0.78
2, 12	7.6	0.57	4.2	0.61	8.7	0.34	5.9	1.03
	October 31, 1978				*January 23, 1979*			
6, 9	32.8	1.27	28.6	1.55	17.3	1.79	11.7	1.25
1, 11	26.0	3.90	21.3	3.32				
5, 7	16.1	2.74	11.9	3.18				
4, 8	14.9	2.41	10.2	2.44				
2, 12	7.9	1.42	4.9	1.39	13.9	1.06	8.1	0.80

Note: SD = Standard deviation

** At high levels, this analysis was inhibited by zinc precipitate interference*

test specimens were acclimated to the 20 C dilution water for a minimum of 10 days. When necessary, the temperature was increased 1 C per day and maintained at the desired temperature for 10 days. Holding tanks were continually flushed with dilution water to eliminate any metabolical waste.

At the beginning of each bioassay, the temperature and toxicant concentration for each test chamber were determined. One fish at a time was randomly placed in the different aquaria until each of the 12 chambers held 10 fish. Because of rapid mortality at high concentrations, each test chamber was continuously monitored the first 32 hours. The exact time of each mortality was recorded. Appendices A, B, and C provide the exact mortality times for largemouth bass, bluegill, and channel catfish. After death, the fish were thoroughly blotted to remove excess moisture, and their lengths and weights were determined.

Reactions of Fishes

Largemouth bass exhibited numerous symptoms of stress in the higher toxicant concentrations. Initially, fish would hover near the water surface in respiratory distress, often with streamers of zinc precipitate trailing from their bodies. As time progressed, however, the bass assumed a variety of positions in the aquaria. Some appeared to be resting on the bottom of the tank. Breathing was labored and prodding produced a sluggish movement forward. Others became rigid, maintaining a position perpendicular to the bottom of the tank. And still others near the bottom attempted to regain their equilibrium by swimming upward in a short, straight diagonal line, but nearly always sank to the bottom.

Certain bass individuals reacted to the zinc toxicant by jerking and twitching since it apparently irritated the muscle and nerve tissues of the fishes. In the lower concentrations

Table 4. Test Conditions for Largemouth Bass, Bluegill, and Channel Catfish Bioassays

	Soluble zinc (mg/l)	Average temperature (°C)	Average fish weight (grams)	pH range	Average alkalinity (mg/l)	Average hardness (mg/l)	Percent dissolved oxygen concentration
Largemouth bass							
7/31/78	15.3-2.8	20.0	1.67	7.69-8.33	199	244	87.0
8/21/78	10.8-1.6	20.0	2.41	8.02-8.48	217	238	88.6
10/4/78	14.0-2.7	19.7	2.28	7.92-8.36	264	308	91.0
10/18/78	20.9-4.1	20.3	2.47	7.74-8.23	285	316	92.0
10/24/78	18.6-4.3	20.2	2.50	7.81-8.15	275	322	92.4
Bluegill							
8/7/78	10.7-3.0	20.0	2.62	7.72-8.32	210	253	90.2
8/21/78	11.3	20.0	1.98	7.68-8.85	216	247	92.5
10/31/78	28.9-6.0	20.1	0.92	7.46-8.22	297	366	94.2
11/14/78	42.9-8.5	20.1	0.69	7.55-8.04	311	396	94.8
Channel catfish							
11/28/78	19.3-3.9	20.2	2.48	7.78-8.24	318	370	90.3
12/5/78	17.9-3.4	20.2	2.29	7.87-8.42	327	378	90.6
1/9/79	31.0-5.9	19.9	3.02	7.72-8.20	335	397	93.1
1/16/79	12.5-5.2	20.1	3.01	7.95-8.18	331	402	94.4
1/23/79	12.4-8.0	20.0	3.58	7.92-8.05	324		99.3

this irritation would often subside, as many fishes underwent an acclimation to the zinc. But, for other bass, the twitching so irritated them that they would break the water surface on their 'death run.' This involved a sudden dash forward, a quick jump out of the water, and then a rapid swimming around the tank in circles. At the end of this death run the bass would be ventral-side up with greatly flared, nonmoving gills and a gaping mouth. Shortly thereafter the muscle twitching ceased and the fish died. It should be noted that this death run reaction was not limited to any particular zinc concentration but was observed in each toxicant chamber, i.e., with 100, 75, 60, 45, and 25 percent zinc concentration. Approximately 3 percent of all bass mortalities resulted from this reaction.

Death from the zinc toxicant produced certain distinctive features among the largemouth bass, i.e., hemorrhaged fins (especially the pectoral fin), usually an open mouth and flared gills, and hemorrhaging about the mouth and facial area. In one instance a spine became sigmoid shaped. Death was determined by lack of reaction to prodding and the cessation of gill movement.

The stress patterns of the bluegill exposed to the zinc were somewhat different from those of the largemouth bass. At the onset of the bioassays and particularly in the higher concentrations, the fish would rise to the water surface. There was a rapid beating of the pectoral fins as breathing became hampered. Most of the fishes remained in a stationary position but several were swimming with open mouths. As stress continued, the bluegill would lose their equilibrium in the water column and come to rest on their sides at the bottom of the tank. Respiration was extremely sluggish, and there was a general darkening of the body color. Depending upon the toxicant concentration, these stress symptions would last from 2 to 24 hours before death occurred.

At soluble zinc concentrations less than 12 mg/l some fish would temporarily experience stress symptoms such as hovering at the water surface, beating of the pectoral fins, and maintaining a stationary position in the aquarium. After *96* hours, these fish usually did not appear stressed. Apparently, the fish that were able to survive the periods of stress could acclimate to the presence of zinc in solution and return to normal behavior. Some bluegill attempted to eat but could not while others resumed normal eating patterns. Table 5 shows the eating patterns of the bluegill exposed to soluble zinc concentrations. At soluble zinc concentrations greater than 12 mg/1, the fish ignored food.

The majority of bluegill mortalities occurred in the first 5000 minutes of each bioassay. Hemorrhaging was evident on the fins, around the mouth and facial area, and in the gill region. One incident of a bluegill racing around the tank in circles, flaring the gills, and settling on the bottom upside down happened in a 75 percent zinc solution. This period of stress lasted for approximately 12 hours before death occurred.

The channel catfish in soluble zinc concentrations of 8.0 mg/1 or greater experienced distress symptoms similar to those of the bluegill and largemouth bass. At first they hovered at the surface with respiratory problems. Some had

Table 5. Eating Patterns of Fish Exposed to Soluble Zinc of Given Concentrations (mg/l)

Time (hours)	*16.3*	*10.3*	*7.5*	*5.7*	*2.9*
Largemouth bass (2.27 grams)					
0-72	ignored food	ignored food	ignored food	poor appetite	good appetite
73-140		ignored food	poor appetite	fair appetite	good appetite
141-183			fair appetite	good appetite	eating as well as controls
184-336			fair appetite	good appetite	eating as well as controls
	24.4	*21.0*	*14.1*	*12.4*	*5.9*
Bluegill (1.55 grams)					
0-72	ignored food	ignored food	ignored food	poor appetite	fair appetite
73-140			poor appetite	appetite slightly improved	appetite slightly decreased
141-183			poor appetite	appetite slightly improved	fair appetite
184-336				fair appetite	fair appetite
	18.2	*14.0*	*10.4*	*8.4*	*4.9*
Channel catfish (2.88 grams)					
0-72	ignored food	ignored food	ignored food	poor appetite	fair appetite
73-140			ignored food	fair appetite	good appetite
141-183				appetite slightly improved	eating as well as controls
184-336					eating as well as controls

zinc precipitate trailing from their fins. Later they exhibited reduced swimming activity as some would swim slowly through the water and others would rest on the bottom, not moving at all. All had very slow ventilation rates. Schooling behavior was absent and prodding produced slow reactions.

After 6 to 8 hours of distress in the higher concentrations, behavior was more erratic among the catfish. Those attempting to swim lost their equilibrium and made a weak effort to right themselves by swimming toward the water surface in a short diagonal line. Some of the catfish became rigid, maintaining a position perpendicular to the bottom of the tank and gulping air. Often death would occur in this position. Others who appeared to be resting on the bottom and were thought to be dead would, when removed from the water, exhibit muscle spasms and begin respiring seconds later. Death followed shortly thereafter. As with the bass and bluegill, hemorrhaging was evident on the gills, around the mouth, and on one or all of the fins.

At soluble zinc concentrations less than 8.0 mg/l, channel catfish experienced stress symptoms for approximately the first 72 hours. Usually by the end of the bioassay, these fish did not appear stressed. Eating patterns had improved and, in fact, some were eating as well as the controls. This might indicate that some of the catfish were acclimating to the zinc.

A control group of fish was maintained with each bioassay at the rate of 20 control fish to 100 test fish. The control fish were kept under exactly the same conditions as the test fish in all respects except for the addition of the zinc toxicant. At no time during the course of these experiments were there any mortalities in the control tanks. The fish behaved normally, showing none of the symptoms observed for the fishes exposed to zinc. All control fish eagerly accepted food.

RESULTS AND DISCUSSION

To estimate the median lethal time, i.e., that time at which 50 percent mortality will occur in a particular test chamber, the percent mortality for that chamber and its duplicate was plotted against the time of mortality. Figure 1 demonstrates the procedure, showing that 50 percent mortality occurred in 638 minutes at the soluble zinc concentration of about 30 mg/1. The acute toxicity curve was developed by plotting the median lethal times against the corresponding zinc concentrations on log-log paper. The arrow in figure 2 indicates the point that represents the condensation or the median lethal time of figure 1. If less than 50 percent mortality occurred in a particular test chamber, that median lethal time was plotted on the acute toxicity curve at the 14-day line.

The acute toxicity curves determine the TL50, that concentration at which the toxicity curve becomes asymptotic to the time axis. Figures 2, 3, and 4 give the TL50s for this investigation.

When applicable, the U. S. graphical method was also used to determine the 14-day TL50. After 14 days, a mortality less than 100 percent but greater than 50 percent, and a second mortality less than 50 percent but greater than 0 percent, were plotted against the soluble zinc concentration. The line was drawn, and the concentration that would be lethal to 50 percent of the fish was determined. Figure 5 demonstrates the procedure. The TL50 shown, however, is slightly higher than the 8.2 mg/l determined by the acute toxicity curve for channel catfish depicted in figure 2.

By comparing figures 2, 3, and 4, one can see that at 20 C largemouth bass were the most sensitive species tested having a TL5 Oof 8.0 mg/l soluble zinc. Channel catfish experienced a similar sensitivity to the zinc toxicant as they had a TL50 of 8.2 mg/l soluble zinc. The least sensitive species studied were the bluegill. Figure 4 reveals their TL50 of 11.0 mg/l soluble zinc.

Illinois water pollution regulations require a factor of one-tenth to be applied to the TL50s when the maximum permissible concentrations are being established. Therefore, to protect largemouth bass, channel catfish fingerlings, and bluegill fry and fingerlings in water with high alkalintiy and hardness, the soluble zinc should not exceed 0.80, 0.82, and 1.1 mg/1, respectively.

Summary

- Bluegill fry, channel catfish fingerlings, and largemouth bass fingerlings were subjected to varying concentrations of soluble zinc in waters relatively high in alkalinity and the salts of calcium and magnesium.
- Because of the high alkalinity and pH of the dilution water, much of the zinc precipitated. In addition, zinc complexes also occurred in the soluble zinc, reducing the zinc ion concentration.
- Acute toxicity curves were developed for each species permitting assessment for 14-day TL50s.
- The 14-day TL50 at 20° C was 11.0 mg/l soluble zinc for the bluegill.
- Channel catfish with a mean weight of 2.88 grams experienced a 14-day TL50 of 8.2 mg/l soluble zinc at 20° C.
- In the case of the largemouth bass, apparently the most sensitive of the species studied, the 14-day TL50 at 20 C was 8.0 mg/l soluble zinc.
- For the protection of the fishes investigated and in compliance with the Water Pollution Regulations of Illinois, the soluble zinc concentrations in Illinois streams having water characteristics of high alkalinity and hardness should not exceed 0.8 mg/1.

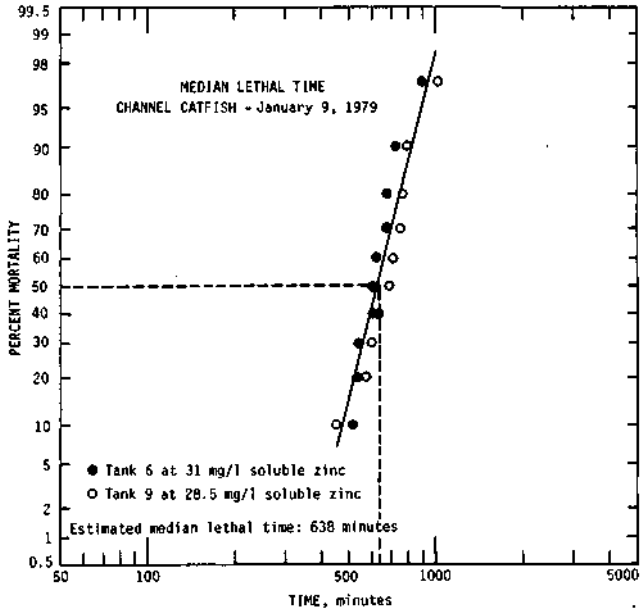

Figure 1. Percent mortality for channel catfish

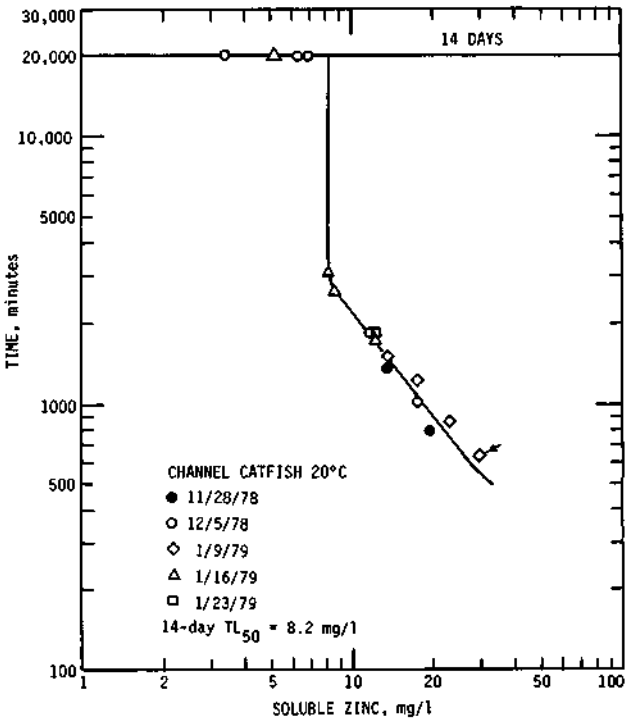

Figure 2. Acute toxicity curve for channel catfish

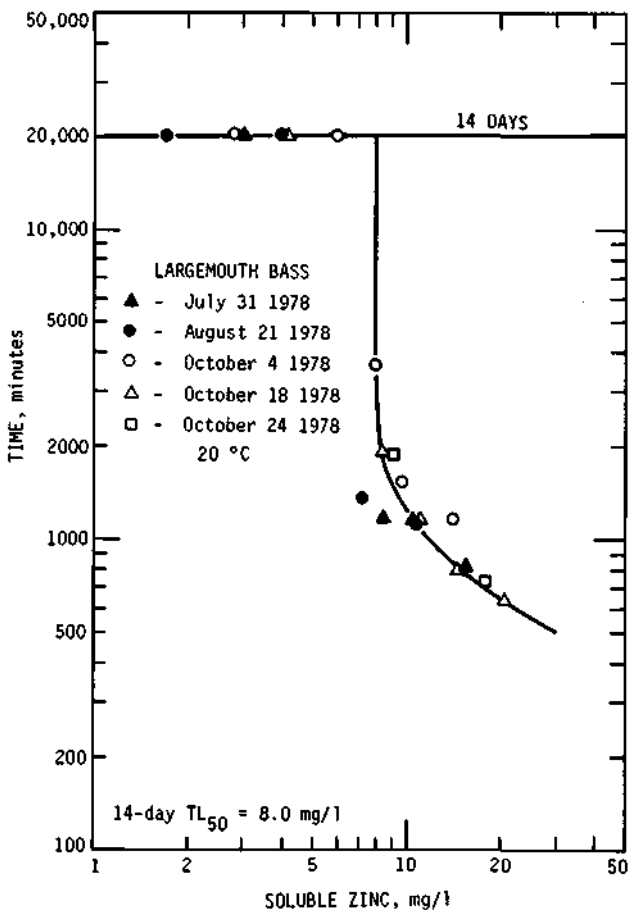

Figure 3. Acute toxicity curve for largemouth bass

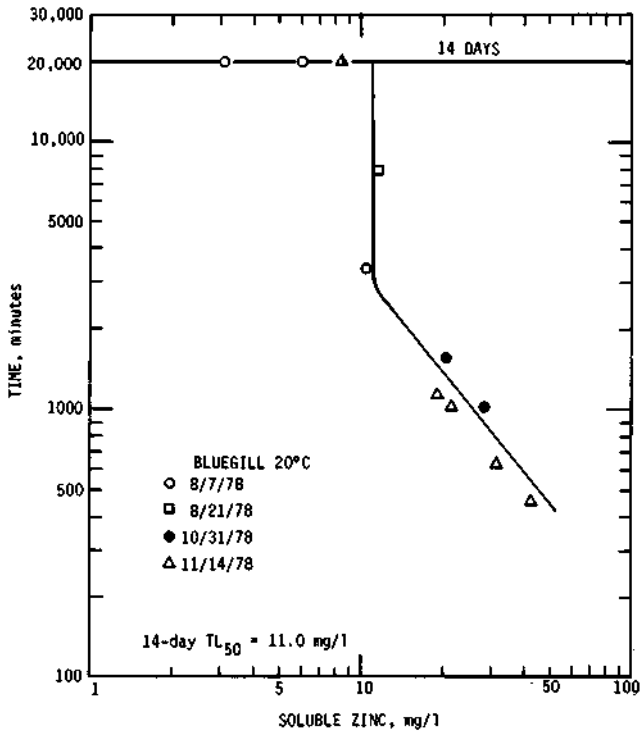

Figure 4. Acute toxicity curve for bluegill

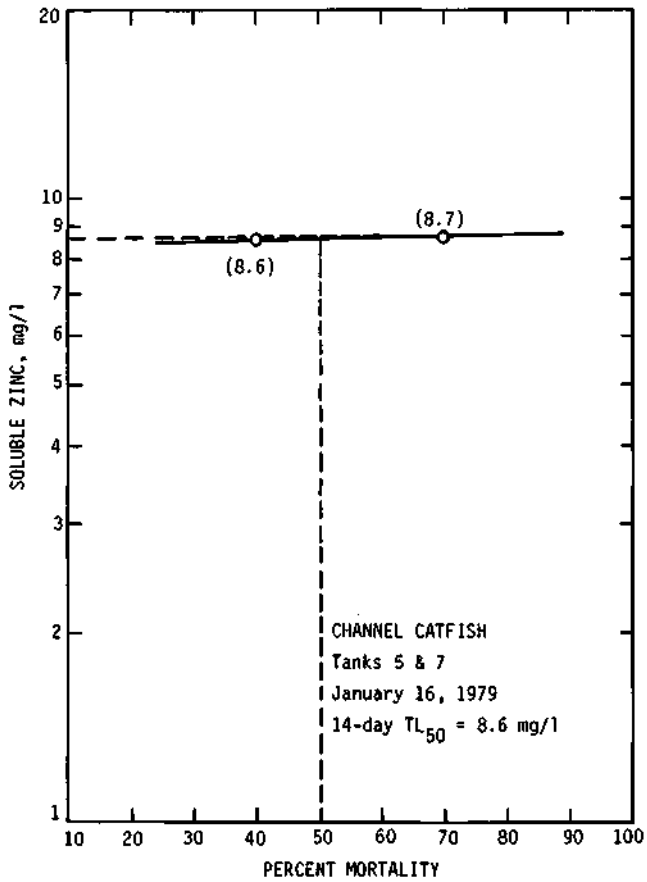

Figure 5. The 14 day TL50 for channel catfish by U. S. graphical method, January 16, 1979

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Appendix A. Observations of Percent Largemouth Bass Mortality

Percent mortality	*Time (minutes) for given concentrations of soluble zinc (mg/l)*									
Date, 7/31/78; Average weight, 1.67 grams; Water temperature, 20°C										
	15.3	*15.2*	*10.7*	*10.6*	*8.2*	*8.7*	*5.8*	*5.4*	*2.8*	*3.1*
10	456	447	799	725	665	783	786	945	1304	1317
20	525	745	903	890	895	865	970	971	1304	1328
30	543	755	925	1011	1010	925	1020	971	4152	3260
40	720	850	1000	1011	1045	970	1045	995	4154	4155
50	786	890	1010	1100	1070	980	1070	1210	4234	
60	800	970	1240	1160	1314	1334	1158	1512	5594	
70	940	1011	1369	1506	1404	1402	1210	1608	12,792	
80	1375	1160	1789	1513	1542	1423	1430	2843		
90	1451	1240	2277	2277	2770	1657	2842	14,235		
100	2277	1543	2277	2277	4153	1667		14,548		
Date, 8/21/78; Average weight, 2.41 grams; Water temperature, 20°C										
	10.8	*7.5*	*7.4*	*4.1*	*4.1*	*4.1*	*4.1*	*1.6*	*1.7*	
10	860	848	868		1699	2510	970	14,082		
20	860	970	1040		15,013	5125	4918			
30	1119	1097	1192		15,013					
40	1119	1428	1310							
50	1222	1434	1312							
60	1222	1595	1427							
70	1428	1605	1528							
80	1428	1770	1800							
90	1770	1895	2037							
100	1770	3484								
Date, 10/4/78, Average weight, 2.28 grams; Water temperature, 19.7°C										
	14	*14*	*9.7*	*9.5*	*8.1*	*8.0*	*6.1*	*5.9*	*2.7*	*2.8*
10	624	677	675	776	2872	1965	2426	2874		
20	722	713	865	880	2872	2876	4800	2874		
30	916	799	1000	916	2872	2876		3412		
40	960	1400	1138	1733	2872	2876				
50	1015	1635	1914	1733	4800	3412				
60	1066	2874	4800	1733	4800	3412				
70	1066	2874	4800	1733		6332				
80	1230	2874	4800	1733						
90	1340	2937	4800	2875						
100	1577	3413	4800	2913						

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Appendix A. (Concluded)

Percent mortality	*Time (minutes) for given concentrations of soluble zinc (mg/l)*									
Date, 10/18/78; Average weight, 2.47 grams; Water temperature, 20.3°C										
	20.9	*20*	*14.9*	*14.5*	*10.7*	*11.4*	*8.8*	*8.0*	*4.1*	*4.3*
10	380	481	475	628	548	627	1290	1280		2860
20	383	542	475	684	675	1060	1410	1461		3206
30	384	554	543	684	799	1281	1487	1711		
40	408	643	645	832	950	1281	1521	2860		
50	485	714	684	845	1071	1290	1521	2860		
60	626	736	798	1072	1071	1677	1677	2860		
70	696	761	1070	1072	1160	2860	2860	2860		
80	1071	1070	1160	1072	1280	2860	2929	2860		
90	1071	1380	1556	1160	1647	2860	2969	3186		
100	1460	1575	1914	1280	2860	2860	4600	3186		
Date, 10/24/78; Average weight, 2.50 grams; Water temperature, 20.2°C										
	18.6	*17.9*	*8.9*	*9.3*	*5.9*	*5.8*	*4.3*	*4.4*		
10	446	433	810	1223	2650	1370				
20	508	453	901	2240	2650	1556				
30	680	487	901	2240	2917	2992				
40	718	565	2240	2240	4098					
50	863	719	2240	2253						
60	957	864	2657	2444						
70	984	1296	3687	2444						
80	1296	1296		2645						
90	1296	1296		2648						
100	1296	1729								

Appendix B. Observations of Percent Bluegill Mortality*

Percent mortality	*Time (minutes) for given concentrations of soluble zinc (mg/l)*									
Date, 8/7/78; Average weight, 2.62 grams; Water temperature, 20°C										
	10.7	*10.0*	*8.3*	*8.5*	*6.3*	*5.7*	*3.0*	*3.3*		
10	3576	2169	2734	2569		3675				
20	3576	2230	3999	5000						
30	3576	2277	5001							
40	3576	2794	5001							
50	5000	3583	13,698							
60	5000	3583								
70	5000	3583								
80		4081								
90		4081								
100		5000								
Date, 8/21/78; Average weight, 1.98 grams; Water temperature, 20°C										
	11.3	*11.2*								
10	4917	3986								
20	5214	4964								
30	9002	9004								
40	9002	9004								
50	9002	9004								
60	12,125	9004								
70		9004								
80		10,679								
90										
100										
Date, 10/31/78; Average weight, 0.92 grams; Water temperature, 20.1°C										
	28.9	*28.3*	*20.7*	*20.6*	*12.0*	*12.4*	*12.4*	*12.0*	*6.0*	*6.1*
10	718	530	650	863	948	1730	1730	3371		
20	730	730	685	1142	2400	2400	2854			
30	821	746	840	1665	2400	2400	3372			
40	821	960	1131	1730	6038	2400	3372			
50	1055	1027	1257	1730	14,432	4300	6037			
60	1148	1138	1300	2400		4300				
70	1194	1257	2400	2400		8647				
80	2402	1531	3226	2400						
90	2402	1531	3845	2400						
100	2402	1900	4435	3845						
Date, 11/14/78; Average weight, 0.69 grams; Water temperature, 20.1°C										
	42.9	*41.6*	*32.0*	*31.8*	*20.5*	*23.0*	*19.7*	*18.2*	*8.4*	*8.5*
10	306	287	425	425	516	590	425	753		3204
20	416	373	460	426	820	654	750	852		
30	460	417	516	545	830	682	820	1120		
40	473	417	568	625	842	1000	915	1280		
50	473	473	640	654	852	1120	1120	1280		
60	516	517	701	700	1000	1280	1290	1526		
70	545	517	701	770	1280	1470	1400	1526		
80	547	565	784	857	1946	1543	1526	1565		
90	597	598	1120	898	2414	1619	1649	1661		
100	642	598	1528	1120	2414	1762	2414	3851		

Appendix C. Observations of Percent Channel Catfish Mortality*

Percent mortality	Time (minutes) for given concentrations of soluble zinc (mg/l)									
Date, 11/28/78;Average weight, 2.48 grams;Water temperature, 20.2°C										
	19.3	*19.3*	*13.7*	*13.1*	*8.2*	*8.8*	*8.3*	*8.6*	*4.0*	*3.9*
10	578	680	872	785	2408	2408		3271		
20	640	785	913	907						
30	660	785	1182	937						
40	710	788	1600	956						
50	710	806	1700	1206						
60	735	820	2408	1206						
70	760	917	2956	1349						
80	835	982		1374						
90	1058	1107		1796						
100	1830	1224								
Date, 12/5/78;Average weight, 2.29 grams;Water temperature, 20.2°C										
	17.9	*16.9*	*11.8*	*11.4*	*7.1*	*6.8*	*6.8*	*5.8*	*3.4*	*3.4*
10	725	420	898	1133		2419		3269		
20	802	613	1139	1242						
30	898	799	1544	1360						
40	898	801	1775	1467						
50	1104	955	1944	2419						
60	1104	1242	2419	2419						
70	1135	1628	2419	2419						
80	1585	1773	2419							
90	1658	1808	3087							
100	1830	1955	4673							
Date, 1/9/79;Average weight, 3.02 grams;Water temperature, 19.9°C										
	31	*28.5*	*23.7*	*22*	*17.1*	*17.8*	*13.4*	*13.6*	*5.9*	*6.3*
10	517	450	709	625	815	1102	960	726		2948
20	538	577	723	675	841	1102	1309	1202		
30	540	600	767	796	898	1124	1560	1202		
40	600	623	794	796	1003	1146	1622	1350		
50	600	691	794	915	1003	1202	1622	1397		
60	626	711	836	929	1003	1447	1642	1560		
70	675	750	874	960	1124	1560	1642	1700		
80	675	767	959	1046	1323	1622	2400	1781		
90	723	796	1124	1046	1560	1690	2400	1910		
100	897	1005	1314	1212	2400	2400	2400	2400		
Date, 1/16/79;Average weight, 3.01 grams;Water temperature, 20.1°C										
	12.5	*12.1*	*8.6*	*7.7*	*8.7*	*8.6*	*5.2*	*5.2*		
10	1100	1196	2214	2214	1100	2214	3662			
20	1249	1442	2214	2214	2214	2214				
30	1398	1523	2214	2214	2214	2756				
40	1398	1523	2214	2214	2214	3662				
50	1732	1633	3097	2891	2214					
60	2214	2214	8430	3195	2755					
70	2214	2214		4550	2998					
80	2214	2214								
90	2214	2687								
100	3662	3662								

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Appendix C. (Concluded)

Percent mortality	*Time (minutes) for given concentrations of soluble zinc (mg/l)*		
Date, 1/23/79;Average weight, 3.58 grams;Water temperature, 20.0°C			
	11.7	*12.4*	*8.0*
10	1380	1399	2098
20	1399	1418	2456
30	1437	1643	3421
40	1643	2098	3421
50	2098	2098	3900
60	2098	2098	
70	2098	2098	
80	2098	2456	
90	2098	2654	
100	3421	3421	