State Water Survey Division
WATER QUALITY SECTION
AT
PEORIA, ILLINOIS

Illinois Department of
Energy and Natural Resources

SWS Contract Report 283

ACUTE TOXICITY OF CHLORIDES, SULFATES, AND TOTAL DISSOLVED SOLIDS TO SOME FISHES IN ILLINOIS

by

Paula Reed and Ralph Evans

Prepared for and funded by the
Illinois Environmental Protection Agency,
Division of Water Pollution Control

September 1981

CONTENTS

ACUTE TOXICITY OF CHLORIDES, SULFATES, AND TOTAL DISSOLVED SOLIDS TO SOME FISHES IN ILLINOIS

by Paula Reed and Ralph Evans

INTRODUCTION

This report presents the results of a study undertaken to assess the acute toxicity to certain fishes of various concentrations of chloride, sulfate, and resultant total dissolved solids. A review of the results of the water quality monitoring program developed by the Illinois State Water Survey in cooperation with the U.S. Geological Survey during the period 1945-1971 and reported on by Larson and Larson (1957), Harmeson and Larson (1969), and Harmeson et al. (1973) suggests that chlorides, sulfates, and total dissolved solids are not significant sources of pollution. After an evaluation of the Water Survey's water quality data, Nienkerk and Flemal (1976) concluded that the statewide discharge-weighted mean concentrations for these constituents are as follows:

Chloride:	25 mg/1
Sulfate:	70 mg/1
Total dissolved solids:	303 mg/1

In light of the rules governing maximum permissible concentrations of these substances in the waters of Illinois these *mean* concentrations are minimal. However Nienkerk and Flemal (1976) suggest that sulfate and chloride are among those mineral constituents most influenced by anthropogenic processes. Although they speculate that a major source of sulfate in the waters of northeastern Illinois may be atmospheric fallout and a major source of chloride in the waters of southeastern Illinois may be the excessive seepage of saline groundwater, they nevertheless conclude that the principal causes of sulfate and. chloride concentrations exceeding background levels are such activities as: the use of street de-icing salt, waste disposal, coal mining, and oil production.

The work of Butts et al. (1976) confirmed that high chloride content in Illinois streams can be related to oil production and groundwater seepage. They found for some streams of the Saline River basins that the chloride content exceeded 500 mg/1 about 10 to 45 percent of the time. At the same stream locations the total dissolved solids exceeded 1000 mg/1 about 30 to 60 percent of the time.

More recently Toler (1980) reported that a reconnaissance of 50 stream sampling sites on much of the surface-mined area in Illinois revealed sulfate concentrations ranging from 25 to 4100 mg/1. Indeed, sulfate was the major mineral constituent in the samples from all sites. On the basis of comparisons with streams having little or no upstream mining activities he concluded that concentrations of sulfate in excess of 100 mg/1 in base stream flow are probably attributable to drainage from mine spoils.

The Illinois Pollution Control Board (1977, with amendments through 1979) recognized the likelihood that excess mineral contributions from human activities are superimposed upon the background concentrations of certain minerals in the state's surface waters. The limitations promulgated by the Board for the three constituents (in milligrams per liter) are:

	Chloride	*Sulfate*	*Total dissolved solids*
General stream quality	500	500	1000
Public water supplies	250	250	500

In addition to the general stream standards and the public water supply limitations the Board established the following rule regulating the total dissolved solids concentrations in effluent discharges:

> Total dissolved solids shall not be increased more than 750 mg/1 above background concentration levels unless caused by recycling or other pollution abatement practices, and in no event shall exceed 3500 mg/1 at any time; provided, however, this Rule shall not apply to any effluent discharging to the Mississippi River, which, after mixing as set forth in Rule 201, meets the applicable water quality standard for total dissolved solids.

In this case the background concentration is that of the production water. And although an effluent can contain up to 3500 mg/1 of total dissolved solids (more where discharge is to the Mississippi River) the rule does not permit a violation of the general stream quality standard of 1000 mg/1.

The Board's regulations also stipulate, in part:

> Any substance toxic to aquatic life shall not exceed 1/10 of the 96-hour median tolerance limit (96-hr.-TL) for native fish or essential fish food organisms.

The median tolerance limit (TL) 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 96-hour bioassay is a desirable minimum length. During this study, an exposure time of 14 days (336 hours) was used.

Of pertinent interest to this study is the validity of the maximum permissible concentrations of chloride (500 mg/1), sulfate (500 mg/1), and total dissolved solids (1000 mg/1) permitted in Illinois water in accordance with the general stream quality rule. The intent of the rule, among others, is to protect the state's waters for aquatic life. This study is also part of a continuing effort to develop information useful to persons and agencies whose activities relate to the enhancement of water quality in the streams and lakes of Illinois.

Scope of Study - -

As part of this investigation certain fishes native to Illinois lakes and streams were exposed to varying concentrations of chloride, sulfate, and resultant total dissolved solids in an effort to ascertain acute toxicity effects. The fishes used as test specimens were largemouth bass fingerlings, bluegill fry, and channel catfish fingerlings. Thirty-three bioassays were performed requiring the use of 3360 test specimens.

The bioassays were of 14-day durations and were performed with various fish sizes and water temperatures. The dilution water was high in the salts of calcium and magnesium with correspondingly high alkalinity.

Plan of Report

The report contains a description of the equipment and methods used for all bioassays; a two-part description of the observed reactions of fishes to chloride and sulfate; and a three-part discussion of the results concerning chlorides, sulfates, and total dissolved solids. All data developed from the bioassays are included in the appendices.

Acknowledgments

This study was conducted under the general supervision of Stanley A. Changnon, Jr., Chief, Illinois State Water Survey, and Dr. William C. Ackermann, Chief Emeritus, Illinois State Water Survey. Many persons of the Water Quality Section assisted in the study. Dave Hullinger and Dana Shackleford provided guidance and assistance in the analysis of chloride, sulfate, and total dissolved solids. Laurie Hebel, Lew Hoffman, and Rick Twait performed analyses, lent direction to the operation of the dilution apparatus, and occasionally maintained continuous 24-hour observations of aquaria. Mr. Maurice Whitacre of the Department of Conservation offered advice on the maintenance of test specimens and supplied many of them. Linda Johnson typed the original manuscript, and Gail Taylor edited it. Illustrations were prepared under the supervision of John W. Brother, Jr.

EQUIPMENT AND METHODS

A modification of a proportional dilutor developed 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, 113 milliliters per minute (ml/min), produced a 95 percent volume displacement every 10 hours. The apparatus permitted the 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 Modificationg and Appurtenances

Previous work by the Water Survey, involving studies of the acute toxicity to fishes of residual chlorine and ammonia (Roseboom and Richey, 1977), copper (Richey and Roseboom, 1978), and zinc (Reed et al., 1980), relied on a syringe style pipettor to inject an exact amount of toxicant from the container of a stock solution to the mixing bowl of the dilutor apparatus. This toxicant feed system is satisfactory when dealing with toxicant concentrations of small magnitude. Since this study involved the use of toxicants generally exceeding 10,000 mg/1 in test tanks, another method of delivery had to be devised. The dilution apparatus used consisted of a chemical metering pump supplied by Fluid Metering, Inc., which derives its feed of stock solution from a 200-liter container. The system operates in the following manner.

During the cycling of the dilutor, the timer activates the water solenoid valve to open and begin filling the dilution water chambers as it simultaneously engages the chemical metering pump to start pumping toxicant from the stock solution container into the toxicant bowl. As water from the dilution water chambers overflows into the water bucket, the bucket fills and descends, thereby engaging the switch and breaking the electrical current. This shuts off the water solenoid valve and the chemical metering pump. As dilution water and toxicant combine in the mixing chambers, the water bucket arm rises to complete the electrical circuit. Then the cycle repeats itself. The advantages of this system are an easily adjustable volume and rate of feed at the pump, a fail-safe design directly timed by dilutor function, an ability to maintain high concentrations of toxicant in a flow-through unit, and a relatively low price for a system comprising a timer, a chemical metering pump, and a water solenoid.

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

Two header boxes were used. The first one is a polyethylene plastic barrel equipped with a thermoregulator which can be set at a desired temperature. Significant cooling from the pre-set water temperature energizes a relay which activates a solenoid-controlled valve on a hot water line. Water flows from the first polyethylene plastic barrel to a second polyethylene plastic header box, where air agitation keeps the contents mixed and provides a sustained dissolved oxygen level.

The following characterize the dilution water used in the bioassays (all values except pH are in milligrams per liter):

Chemical oxygen demand	Not detected	Magnesium	25.3
Ammonia-N	0.09	Iron	0.11
Nitrate-N	3.6	Zinc	.07
Phosphate-P	0.20	pH	8.33
Sulfate	183	Hardness	412
Chloride	87	Alkalinity	291
Copper	.008	Cadmium	.004
Fluoride	0.79	Lead	<.08

Stock Solutions and Chemical Analyses

The sodium chloride stock solutions were prepared by dissolving technical grade sodium chloride in dilution water. Due to the rather low toxicity of sodium chloride to fish, large quantities of toxicant were used daily in the dilutor. To accommodate the preparation of the toxicant and to assure its thorough mixing, a circulating pump was used.

At least once during the first 24 hours of each bioassay, and generally daily thereafter, chloride analyses were made by removing a sample from the middle of each test chamber. All chloride determinations were performed in accordance with the argentometric method. Results are expressed in mg/1 chloride (Cl^-) .

The sodium sulfate stock solutions were prepared by dissolving technical grade sodium sulfate in dilution water. Due to the low solubility of sodium sulfate in 20 C dilution water, it became necessary to use dilution water heated to 30-35 C to achieve the desired stock concentration. Since sodium sulfate is relatively low in toxicity, large volumes of toxicant were also used daily in the proportional dilutor. A circulating pump was utilized to facilitate the preparation of the toxicant and to assure thorough mixing of the sodium sulfate and dilution water. During the winter months it became necessary to use a submersible thermostat heater and to supply aeration by means of air stones in the stock solution container because the sulfate stock solution had a tendency to stratify.

At least once during the first 24 hours of each bioassay, and generally daily thereafter, sulfate determinations were made by removing a sample from the middle of each test chamber. All sulfate analyses were performed in accordance with the turbidimetric method. A Bausch and Lomb Spectronic 20 was used for all absorbance readings. All results are expressed as mg/1 sulfate ($SO_4^=$).

All analyses were performed as outlined in *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, 1975).

Hardness and alkalinity were determined in one control chamber and two other test chambers on three occasions during each bioassay. Analyses for pH were conducted on the same three occasions, but samples were taken from six test chambers rather than three. Dissolved oxygen levels, measured by a Yellow Springs Instrument Model 57 oxygen meter, were recorded daily from all test chambers. Water temperature also was measured daily by a standard graduated centigrade thermometer. Hardness determinations were by the EDTA titrimetric method with Eriochrome Black T as an indicator. Alkalinity and pH were determined by a Metrohm Herisau pH meter, Model 588, with 0.02 N H_2SO_4 as a titrant for alkalinity.

Salinity and conductivity measurements of all test chambers were recorded generally on a daily basis with a Yellow Springs Instrument S-C-T meter, model 33. Analyses for total dissolved solids (TDS) were generally determined daily

Table 1. Test Conditions for Chloride Bioassays

	Average fish weight (grains)	*Average fish length (cm)*	*Range chloride (mg/1)*	*Range total diss. solids (mg/1)*	*Range pH (units)*	*Average alkalinity (mg/1)*
Bass						
8-6-79	--	4.1	6460-9718	--	8.40-8.57	184
8-8-79	--	2.8	9665-9713	--	8.45-8.46	184
8-9-79	--	3.1	9587	--	8.43-8.43	--
8-13-79	--	3.1	10199-10947	--	8.52-8.54	201
8-27-79	--	3.9	6119-9493	--	8.45-8.59	210
10-22-79	--	--	10490-14075	92-21313	--	--
11-5-79	2.11	5.1	5898-15308	9951-24529	8.30-8.48	271
11-12-79	2.01	5.2	9647-9847	16111-16178	8.22-8.38	298
1-21-80	3.75	6.6	5358-11067	9741-19158	8.18-8.40	291
2-4-80	4.38	6.8	6247-11371	10520-18869	8.19-8.39	293
11-10-80	1.92	5.6	5968-14126	10617-23289	8.20-8.70	286
12-2-80	2.26	5.6	6237-14432	10981-23437	8.40-8.61	322
Bluegill						
7-10-79	2.64	5.6	6825-10690	--	8.42-8.70	211
7-16-79	4.51	6.6	6775-10704	--	8.28-8.62	221
7-24-79	7.24	7.3	5971-9161	--	8.08-8.54	186
11-15-79	2.24	5.3	11446-11646	19036-19161	8.21	298
11-26-79	2.31	5.3	5277-11546	9434-18549	8.20-8.40	298
12-10-79	0.33	2.8	5105-11231	9378-19143	8.22-8.41	294
Catfish						
8-18-80	1.54	5.6	5175-13783	8899-21265	7.13-8.42	253
9-2-80	2.37	6.4	5185-13151	8951-20618	8.32-8.48	257
9-9-80	3.51	7.1	13340-13592	21287-21303	8.32-8.33	267

Table 1. Concluded

	Average hardness (mg/1)	Percent dissolved oxygen saturation	Average temperature (°C)	Range salinity	Range species conductivity (micro-MHOS)
Bass					
8-6-79	493	92	21	--	--
8-8-79	480	90	21	--	--
8-9-79	--	92	21.7	--	--
8-13-79	480	97	20.8	--	--
8-27-79	514	93	21.4	--	--
10-22-79	--	--	20.6	--	--
11-5-79	407	92	20.5	--	--
11-12-79	416	93	20.3	--	--
1-21-80	415	85	20.3	9.2-20.8	14000-29800
2-4-80	424	81	20.1	9.2-19.0	13800-27200
11-10-80	515	83	19.4	10.7-23.1	15800-31800
12-2-80	527	81	18.6	10.8-24.3	15500-33200
Bluegill					
7-10-79	365	86	22.4	--	--
7-16-79	393	77	22.7	--	--
7-24-79	429	80	21.4	--	--
11-15-79 416	--	--	--	--	--
11-26-79	428	94	20.6	8.8-19.5	13200-27800
12-10-79	416	96	20.4	9.0-19.9	13800-28200
Catfish					
8-18-80	383	86	17.8	8.8-21.9	12700-30300
9-2-80	387	81	19.9	8.7-21.6	13000-30500
9-9-80	405	87	20.1	21.0-21.2	30500

from all test aquaria using filtration and residue on evaporation at 103 to 105 C. Some ranges and averages of these analyses along with other pertinent data representing test conditions during each bioassay are included in tables 1 and 2. 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 specimens for the chloride and sulfate bioassays. They were largemouth bass *(Micropterus salmoides)*, bluegill *(Lepomis macrochirus)*, and channel catfish *(Ictalurus punctatus)*. Table 3 lists the type and number of fishes used, average weight of the fishes, and sources of the fishes for each of the bioassays.

All 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 metabolic waste.

At the beginning of each bioassay, the temperature, salinity, conductivity, 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, and the exact time of each mortality was recorded. Appendices A, B, C, D, E, and F 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

It is customary to record the behavior of fishes exposed to toxicants during the performance of bioassay work at the Water Survey. This is done for several reasons. A principal one is the desire to develop information useful to personnel in Illinois who have the responsibility for investigating fish kills and determining the likely causes of fish mortality. Observations under controlled conditions of such factors as behavior during stress, sites of hemorrhaging, changes in pigmentation, and body configuration may make it possible to interpret similar observations under field conditions.

A control group of fish was maintained with each bioassay at the ratio 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 toxicant. There was never any occurrence of a mortality in the control tanks at any time during the bioassays. All fish behaved normally and eagerly accepted food.

Table 2. Test Conditions for Sulfate Bioassays

	Average fish weight (grams)	Average fish length (cm)	Range sulfate (mg/l)	Range total diss. solids (mg/l)	Range pH (units)	Average alkalinity (mg/l)
Bass						
9-22-80	1.24	4.8	7556-17484	13321-25469	8.39-8.53	267
9-30-80	1.26	4.7	8627-18868	12001-27277	8.39-8.55	265
10-6-80	1.31	4.8	9953-14567	16104-23666	8.44-8.52	291
10-22-80	1.45	5.1	11201-18989	16306-29573	8.60-8.64	305
10-27-80	1.77	5.4	10323-14907	17183-25986	8.41-8.57	316
Bluegill						
5-19-80	0.67	3.5	9801-17483	15400-26024	8.50-8.60	302
6-2-80	0.59	3.5	9418-18009	15460-26611	8.50-8.65	299
6-9-80	1.09	4.1	13483-13844	21467-21456	8.55-8.59	
Catfish						
6-16-80	1.01	4.7	8845-18205	—	8.48-8.60	
6-23-80	1.27	4.9	9032-19245	13877-25968	8.49-8.63	296
7-7-80	1.55	5.2	6769-14564	10722-20440	8.41-8.60	262
7-28-80	1.85.	5.6	7019-15584	11052-22954	8.46-8.51	257

	Average hardness (mg/l)	Percent dissolved oxygen saturation	Average temperature (°C)	Range salinity	Range species conductivity (micro-MHOS)
Bass					
9-22-80	416	86	20.2	7.0-19.9	10500-28100
9-30-80	420	83	20.3	6.9-16.8	10300-24300
10-6-80	461	84	20.0	9.3-18.3	13700-26500
10-22-80	461	83	20.2	9.0-19.0	13300-26500
10-27-80	493	83	19.9	9.0-19.2	13600-28100
Bluegill					
5-19-80	501	89	21.0	9.5-17.2	15000-26000
6-2-80	.512	84	21.7	8.6-19.1	13500-28100
6-9-80	—	81	20.8	12.8-15.5	19200-23100
Catfish					
6-16-80	507	80	20.9	7.9-17.0	12000-25000
6-23-80	486	84	20.8	8.8-16.2	13500-23700
7-7-80	440	82	21.0	6.3-15.6	9800-23500
7-28-80	397	87	19.4	7.3-14.1	10900-21100

Table 3. Types, Numbers, Weights, and Sources of Fish Used in Bioassays

Bioassay	*Type of fish*	*No. of fish*	*Average wt. of fish (grams)**	*Sources of fish*
Chloride	Bass	1200	2.08 4.08	IDOC, Spring Grove; Opel's Fish Hatchery, Worden, IL
	Bluegill	560	0.33 2.40 5.9	IDOC, Spring Grove; Opel's Fish Hatchery, Worden, IL
	Catfish	260	2.47	Seven Springs Fish Farm, Evansville, IL
Sulfate	Bass	600	1.41	IDOC, Spring Grove; National Fish Hatchery, Hebron, Ohio
	Bluegill	260	0.78	Fender's Fish Hatchery, Baltic, Ohio
	Catfish	480	1.42	Seven Springs Fish Farm, Evansville, IL

* Bass and bluegill used in chloride bioassays fell into several distinct weight groups, as indicated
Note: IDOC = Illinois Department of Conservation

Chloride

At high chloride concentrations, channel catfish exhibited numerous symptoms of stress. At the beginning of each bioassay the fish experienced a definite loss of equilibrium. This was accompanied by respiratory difficulty; opercular movement was rapid and shallow. Many individuals swam frantically at the water surface. As time progressed, the eyes appeared glazed and respiration became increasingly labored. In addition, the catfish assumed a variety of positions in the water column. Some performed short bursts of swimming in a zigzag fashion at the surface of the water. Others lay on their sides on the bottom of the tank. Some of the fishes underwent a stiffening of their bodies and maintained a position perpendicular to the bottom of the tank. Certain individuals hung at the surface in this rigid position while others stood on their tails.

A few channel catfish experienced muscle spasms and twitching along with tail chasing. Afterwards their bodies became rigid, and death soon followed. Certain physical characteristics that accompanied the catfish mortalities were produced by chloride. They included hemorrhaging in the gills, in the brain, and at the base of the pectoral fins. Curvature of the body was a common reaction to the toxicant. Death was determined by lack of reaction to prodding and the cessation of gill movement.

The appetite of the channel catfish during the bioassay was a function of the concentration of the chloride. In concentrations above 10,000 mg/1 chloride, the fishes completely ignored food. In the moderate range of approximately 7500-9000 mg/1 chloride, their appetites fluctuated. Initially, the chloride produced a suppression of the appetite. Later, after perhaps some acclimation to the chloride, there was a slight improvement in appetite. Concentrations at or below 5000 mg/1 chloride slightly decreased the appetite of the catfish initially, but after awhile all fish eagerly accepted food.

The stress patterns of the bluegill exposed to chloride concentrations in excess of 10,000 mg/1 were similar to those of the channel catfish. Initially respiration was sluggish and there was a general darkening of body color. The fish experienced a loss of equilibrium, lying on their sides at the surface and floating sideways. Others attempted short dives downward in the water column and later floated back to the top. Coughing and regurgitation were experienced by some bluegill in distress. .

As time progressed, some, of the fishes underwent a frenzied, convulsive type of activity. Other bluegill became rigid and maintained a vertical position in the water. The eyes appeared glazed. Death usually occurred within nine hours and produced certain distinctive features, including flared gills, severe curvature of the spine, and hemorrhaging in the gills and at the pectoral fins.

At concentrations less than 10,000 mg/1 chloride, the same stress patterns occurred as noted before, but with less severity. Deaths seemed to occur more quietly. There was apparent hemorrhaging at the gills as well as the tail, at the base of the dorsal fin, and in the head.

The appetites of the bluegill exposed to chloride varied inversely with the concentrations. In the higher concentrations, the fishes ignored food completely. In lesser chloride concentrations, the bluegill initially would refuse to eat, but as time continued there was a gradual improvement in appetite from a poor to fair status. At concentrations of less than 5000 mg/1 chloride, all bluegill ate normally.

The largemouth bass exposed to chloride concentrations in excess of 9000 mg/1 revealed stress behavior patterns similar to those of the bluegill and channel catfish. At the beginning of each bioassay, the fish would hover at the water surface with respiratory problems. They exhibited a loss of equilibrium by lying on their sides in the water column. Some bass attempted to right themselves by diving down towards the bottom of the aquarium, but they nearly always rose back to the surface. Certain individuals reacted to the chloride through spinal curvature; in a couple of severe cases, the body was almost L-shaped and there was evidence of internal hemorrhaging.

Other signs of distress included coughing, regurgitation, and gulping of water. As the fish neared death, respiration became more labored. Some experienced tremors or muscle spasms resulting in rapid bends or flips.

Upon expiration the largemouth bass exhibited certain distinctive characteristics as a result of their exposure to chloride. These included flared gills, gaping mouth, loss of pigmentation, and hemorrhaging in the gills, mouth, head, and at the base of the pectoral and caudal fins.

At chloride concentrations less than 9000 mg/1 the stress symptoms were the same as those at the higher concentrations, but they generally took longer to occur and were less severe. The appetites of the largemouth bass also were inversely correlated to the concentration of chloride. At the high concentrations, the chloride suppressed all appetites, but as the percent of toxicant present decreased, there was an initial absence of eating and then a gradual improvement in their eating habits. Lower concentrations of chloride did not adversely affect the appetites of the largemouth bass. Most ate well from the beginning to the end of the bioassay. In fact some were eating as well as the controls. This might indicate an acclimation to chloride.

Sulfate

Bluegill exhibited numerous symptoms of stress when exposed to sulfate concentrations in excess of 15,000 mg/1. Typically there was an immediate loss of equilibrium and general body control. Some fishes were observed lying on their sides at the surface, others were doing "barrel rolls," and still others were seen diving to the bottom of the tank and floating back to the top. All were experiencing respiratory difficulty as they rapidly beat their pectoral fins. As the bioassay continued, many bluegill preferred to stay near the bottom of the aquarium and exhibited very little movement. Breathing became more labored and sluggish.

Some noticeable symptoms of distress from the sulfate toxicant included spinal curvature, tremors, flared gills, gaping mouth, and hemorrhaging in the gills and head. Most bluegill underwent a change in pigmentation. Some experienced a darkening of body color, while others were pale in color upon death. In one instance, a fish displayed dark vertical bands above the lateral line and light ones below. Spiny rayed fins were erect. In sulfate concentrations greater than 10,000 mg/1 but less than 15,000 mg/1, the stress behavior was similar to that in the higher sulfate concentrations. Upon introduction to the toxicant, many exhibited disorientation and visited the surface briefly. Some were seen swimming sideways. Respiration was sluggish and was accompanied by a rapid beating of the pectoral fins. There was a change in pigmentation, with some becoming darker and others becoming lighter in color. Apparently the sulfate solution irritated the muscle and nerve tissues of certain bluegill to such an extent that they reacted by twitching and trembling. As they neared death and were severely distressed, the fishes stayed on the bottom of the tank.

At concentrations less than 10,000 mg/1 sulfate there was a drastic decrease in mortalities. Apparently after the initial shock was over, the bluegill gradually acclimated to the toxicant. All mortalities involved distress characteristics exactly like those which occurred at the higher concentrations.

Channel catfish appeared to react to the sulfate toxicant in a manner similar to the bluegill. At the onset of each bioassay, there was a loss of equilibrium. Some were seen stiffening their bodies and hanging vertically in the water column at the surface. Opercular movement was rapid and shallow as the catfish tried to compensate for the shock and introduction into a different fluid medium. Some fishes were so distressed by the sulfate toxicant that they vomited. As time progressed respiration became increasingly difficult and many rested on the bottom of the tank. Schooling behavior was somewhat erratic at this point.

Certain distressed individuals underwent a tail chasing phenomenon and death tremors. Upon their expiration, many catfish displayed an open or gaping mouth, flared gills, erect spiny-rayed fins, curvature of the body, and hemorrhaging at the base of the pectoral, dorsal, and caudal fins and in the head.

In sulfate concentrations in excess of 15,000 mg/1, the largemouth bass exhibited stress symptoms similar to those of the bluegill and channel catfish. Initially they hovered at the water surface with breathing difficulties. There was a rapid fluttering of the pectoral fins as they tried to adjust to the toxicant. All experienced a loss of balance as they entered the sulfate solution. Many rolled back and forth in a barrel roll fashion or simply lay on their sides at the surface. Later it was noted that some fish had spinal curvature. Muscle twitching was also displayed by a few individuals. Generally, most mortalities occurred within 12 hours at the higher concentrations. Many bass revealed gaping mouths, flared gills, and hemorrhaging at the head and operculum.

At sulfate concentrations in the moderate range, between 10,000 mg/1 and 15,000 mg/1, the same stress symptoms were observed but appeared to be less severe. As usual, the fishes experienced breathing difficulty at the beginning of each bioassay. A loss of equilibrium followed/with some individuals lying on their sides. Several were observed swimming upside down. Many bass appeared darker in color as the bioassay continued. Death in the moderate sulfate range was accompanied by distress characteristics similar to those in the higher concentrations. These included curvature of the body, an erect dorsal fin, open mouth, flared gills, and hemorrhaging at the operculum and in the gills. Most mortalities occurred within 24-48 hours. The appetites of the largemouth bass exposed to these sulfate concentrations were non-existent or very poor. Many completely ignored food or consumed a little food now and then.

At less than 10,000 mg/1 sulfate, the bass appeared to be okay and acted normally after an initial adjustment period. Appetites were usually good, and in fact many were eating as well as the controls. This might indicate an acclimation to the sulfate toxicant at this level.

RESULTS AND DISCUSSION

To estimate the median lethal time — the time at which 50 percent mortality will occur in a particular test chamber — the percent mortality for that chamber and its duplicate is plotted against the observed time of mortality. Figure 1 illustrates the procedure, showing that 50 percent mortality occurred in duplicate chambers in 329 minutes (the median lethal time) at the chloride concentration of about 10,900 mg/1. In this manner median lethal times and corresponding chloride concentrations have been determined for each bioassay. An acute toxicity curve can then be developed by plotting the median lethal times against the corresponding chloride concentrations, as shown

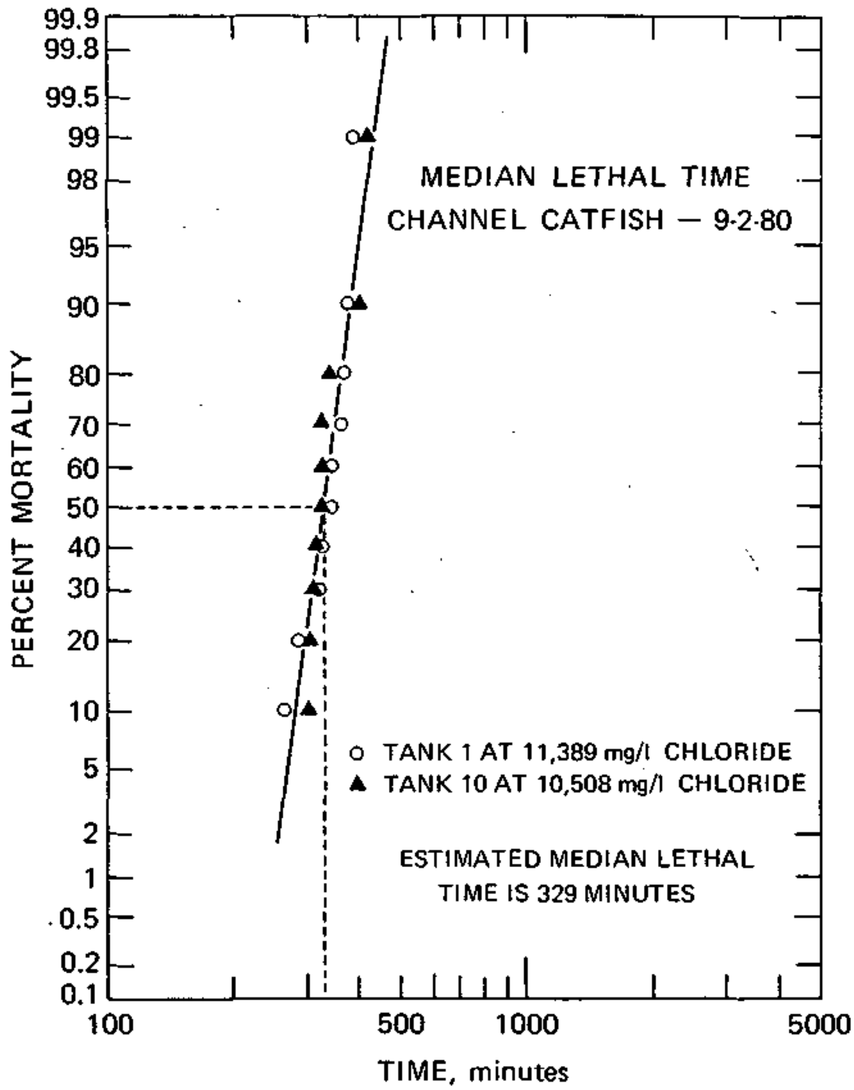

Figure 1. Percent mortality for channel catfish (Cl~)

in figure 2. The arrow in figure 2 represents the condition developed from figure 1. If less than 50 percent mortality occurred in a test chamber within 14 days, the time selected for representing the median lethal time is 14 days. For the purposes of this study, 24-hour and 96-hour designations are also included in addition to 14-day times.

From the acute toxicity curves the TL50 value is determined. The TL50 is that concentration at which the curve becomes asymptotic to the time axis.

As mentioned previously, the water pollution regulations in Illinois require an application factor of 1/10 to the TL50 for determining the maximum permissible concentration of any substance toxic to aquatic life. Because the TL50 concentration is derived here from the acute effects of the substance on fishes it is assumed that an allowable concentration of 1/10 the TL50 concentration in Illinois waters will minimize chronic effects related to growth, reproduction, and genetic characteristics of aquatic organisms. Nevertheless the uniform application of the factor (1/10) for *all* toxic substances is a questionable practice without adequate substantiation for Illinois conditions. Under present conditions, however, the 1/10 factor is required and shall remain so until evidence has been developed to justify a reevaluation of its usefulness.

Chloride Bioassays

The reactions of catfish, bass, and bluegill to concentrations of chloride are shown in figures 2, 3, and 4, respectively. It is apparent from these figures that all three species of fish exhibit a similar sensitivity to chloride at water temperatures of about 20 C. The TL50 concentrations range from 8000 to 8500 mg/1 chloride with the bass appearing to be slightly more tolerant to chloride than the other two species.

The figures also suggest that there is not a perceptible difference between TL50 concentrations for bioassays with time lengths of 24 hours, 96 hours, or 14 days.

Sulfate Bioassays

The reactions of catfish, bass, and bluegill to concentrations of sulfate are shown in figures 5, 6, and 7, respectively. Here also it is apparent that all three species are similarly sensitive to sulfate at water temperatures of about 20 C. The TL50 concentrations at 14 days range from 10,000 to 11,000 mg/1. Of the three species, bass is the least sensitive to sulfate.

The figures also show that the TL50 concentrations will differ depending on the time length of the bioassay. Generally, the shorter the time length of the bioassay (24 hours versus 96 hours versus 14 days), the higher the resultant TL50, as shown in the figures. A summary of TL50s for figures 5, 6, and 7 appears on page 22.

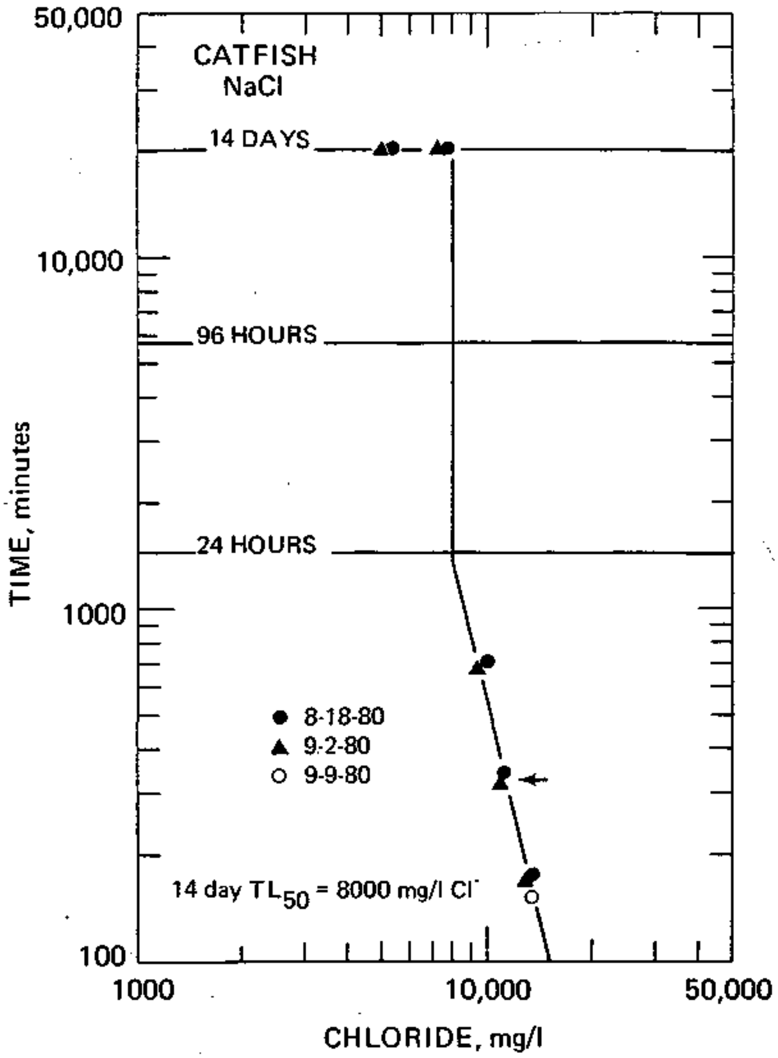

Figure 2. Acute toxicity curve for channel catfish (Cl^-)

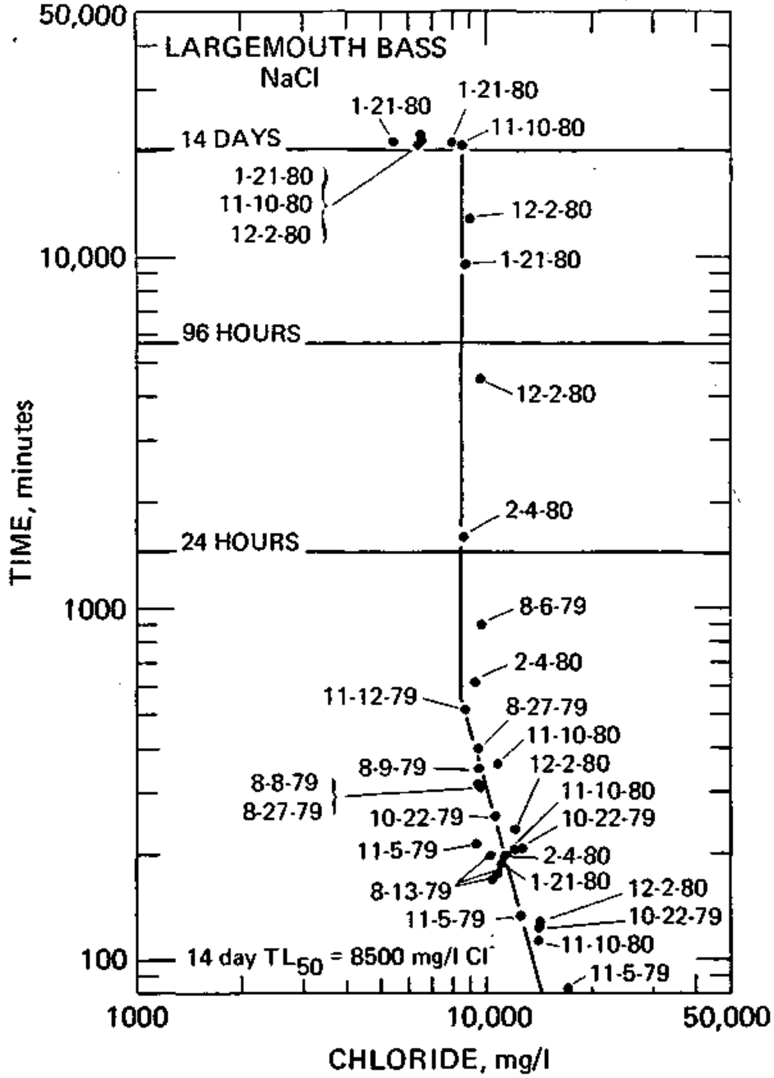

Figure 3. Acute toxicity curve for largemouth bass (Cl^-)

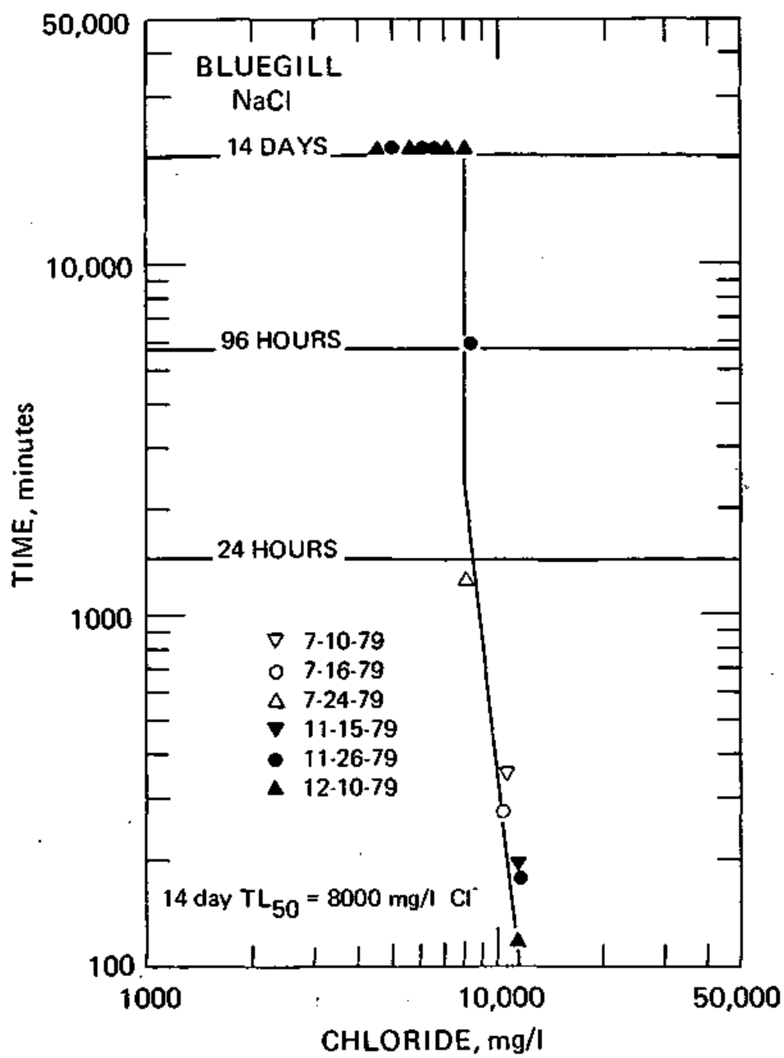

Figure 4. Acute toxicity curve for bluegill (Cl^-)

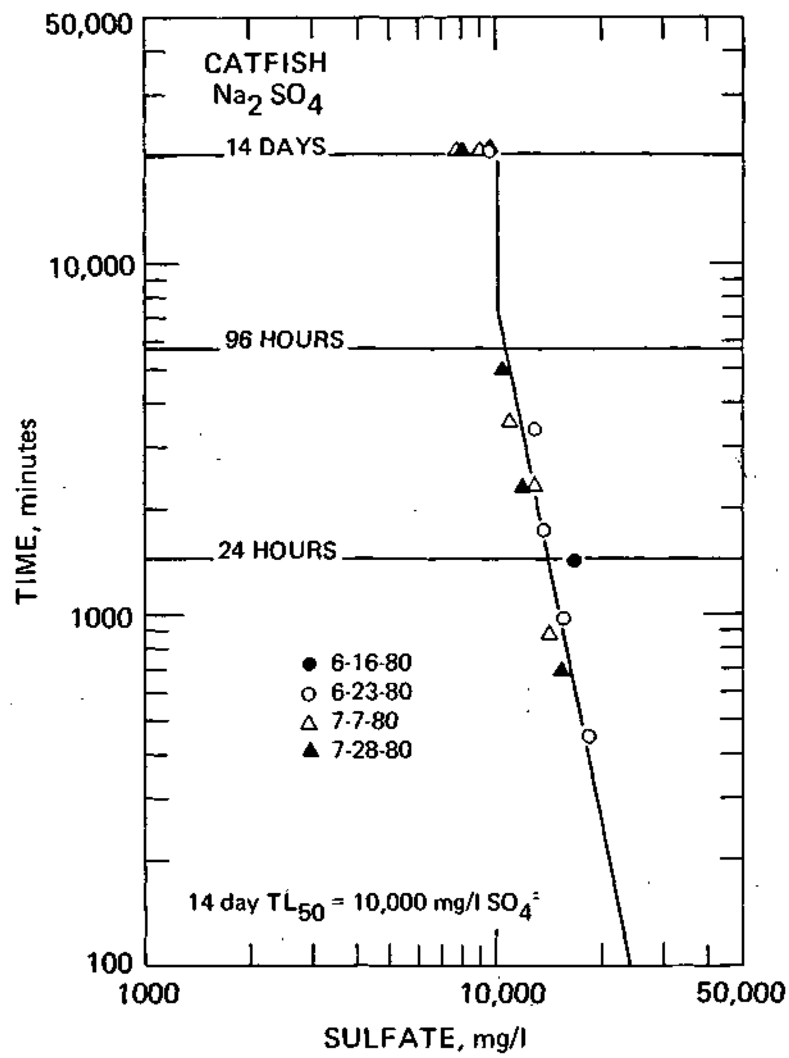

Figure 5. Acute toxicity curve for channel catfish ($SO_4^=$)

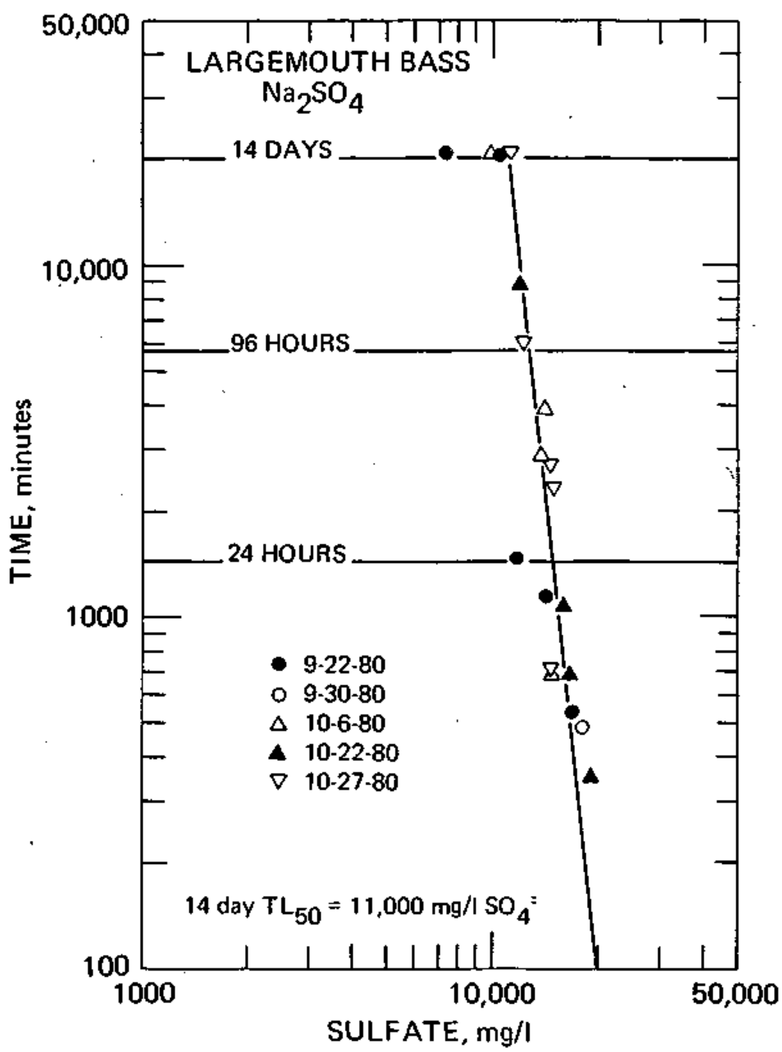

Figure 6. Acute toxicity curve for largemouth bass ($SO_4^{=}$)

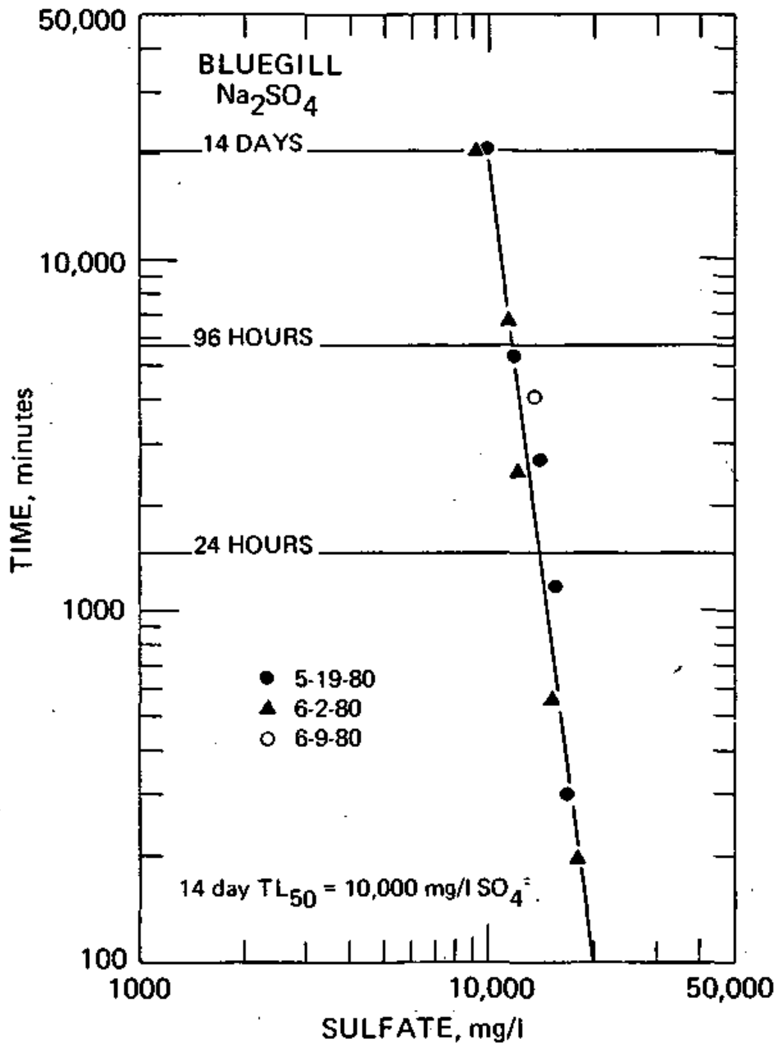

Figure 7. Acute toxicity curve for bluegill ($SO_4^{=}$)

Summary of TL50s for Figures 5, 6, and 7

(Milligrams per liter)

Time (hours)	*Catfish*	*Bass*	*Bluegill*
24	14,000	15,000	14,000
96	11,000	13,000	12,000
336 (14 days)	10,000	11,000	10,000

Total Dissolved Solids

The assessment of the effects of total dissolved solids on fishes consists basically of considering the chloride and sulfate concentrations in terms of total dissolved solids for the bioassays performed. Two conditions are considered. In one case the total dissolved solids are principally made up of sodium chloride; in the other case they principally consist of sodium sulfate.

The results for the chloride-oriented total dissolved solids (TDS-Cl^-) are included in figures 8, 9, and 10 for catfish, bass, and bluegill, respectively. The sulfate-based total dissolved solids (TDS-$SO_4^=$) results are similarly depicted in figures 11, 12, and 13.

From an examination of figures 8, 9, and 10 it is apparent that all three species of fish exhibit a similar sensitivity to TDS-Cl^- at water temperatures of about 20 C. The TL50 concentrations range from 13,000 to 15,000 mg/1 total dissolved solids. Catfish is the most sensitive; bass is the most tolerant.

An examination of figures 11, 12, and 13 shows that there is more variability in TL50s among the fishes when exposed to TDS-$SO_4^=$ at about 20°C. The TL50 concentrations range from 14,000 to 17,500 mg/1 total dissolved solids. Here again the catfish is more sensitive; the bass and bluegill are about equally tolerant.

From this assessment it appears that total dissolved solids concentrations are not a sensitive indicator of acute toxicity for fishes. The tolerance to total dissolved solids varies with the species of fish and depends upon the principal anion comprising the dissolved solids.

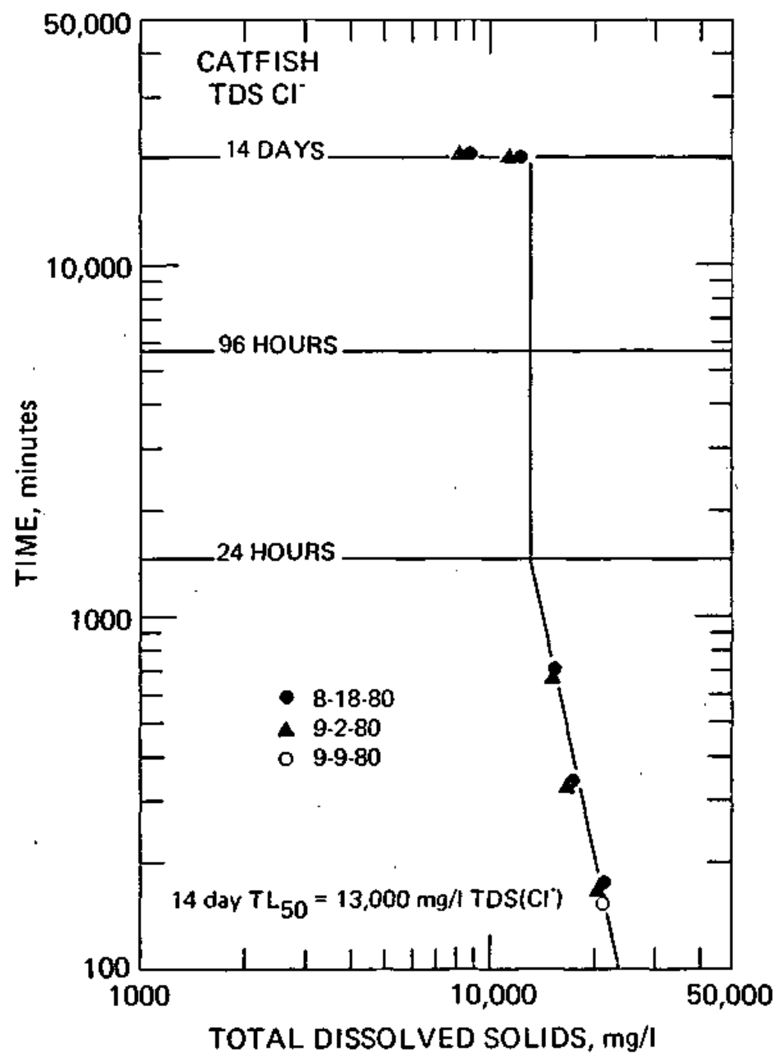

Figure 8. Acute toxicity curve for channel catfish (TDS-Cl⁻)

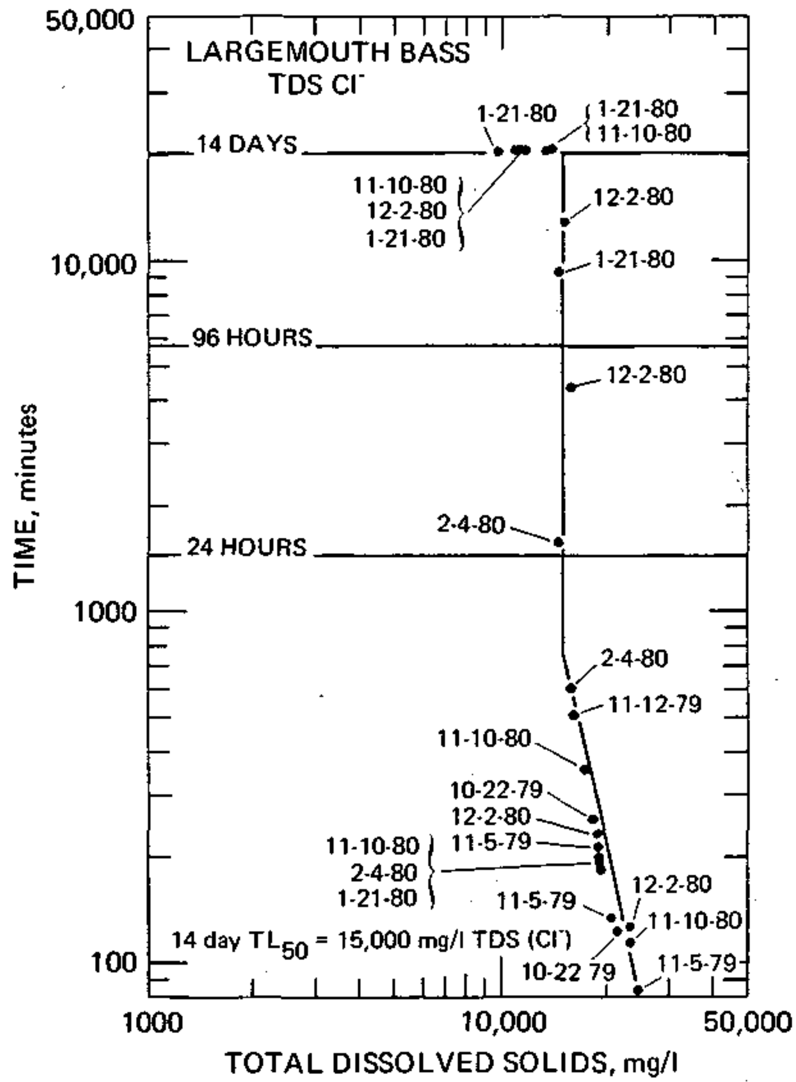

Figure 9. Acute toxicity curve for largemouth bass (TDS-Cl⁻)

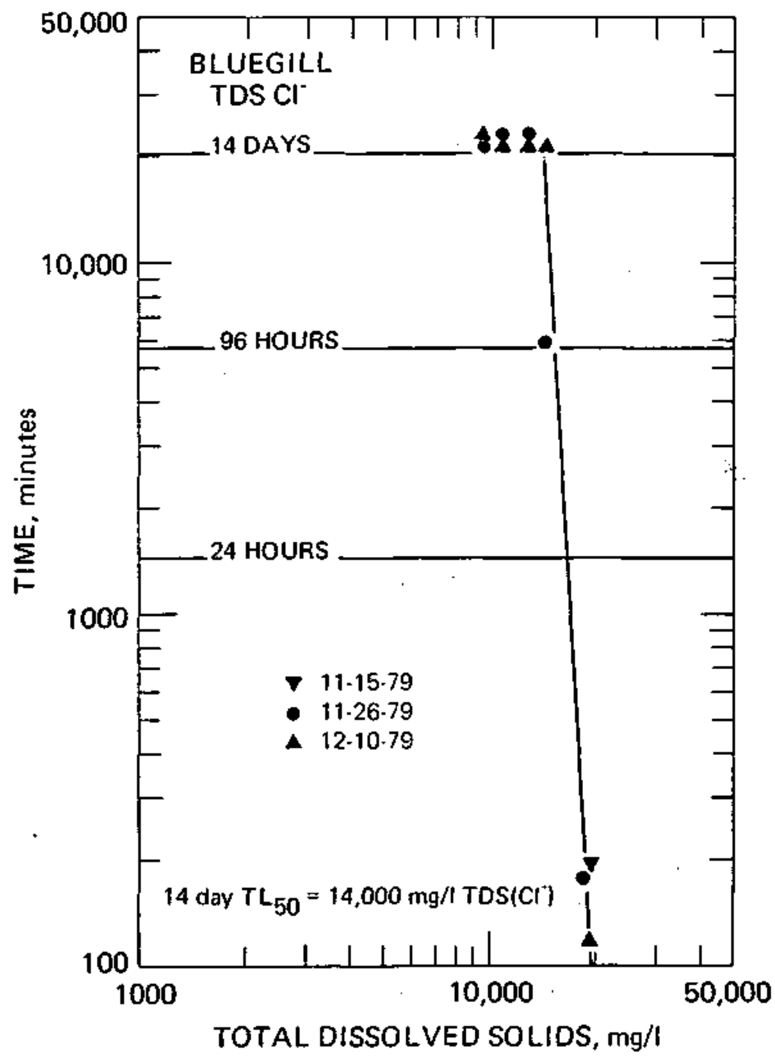

Figure 10. Acute toxicity curve for bluegill (TDS-Cl⁻)

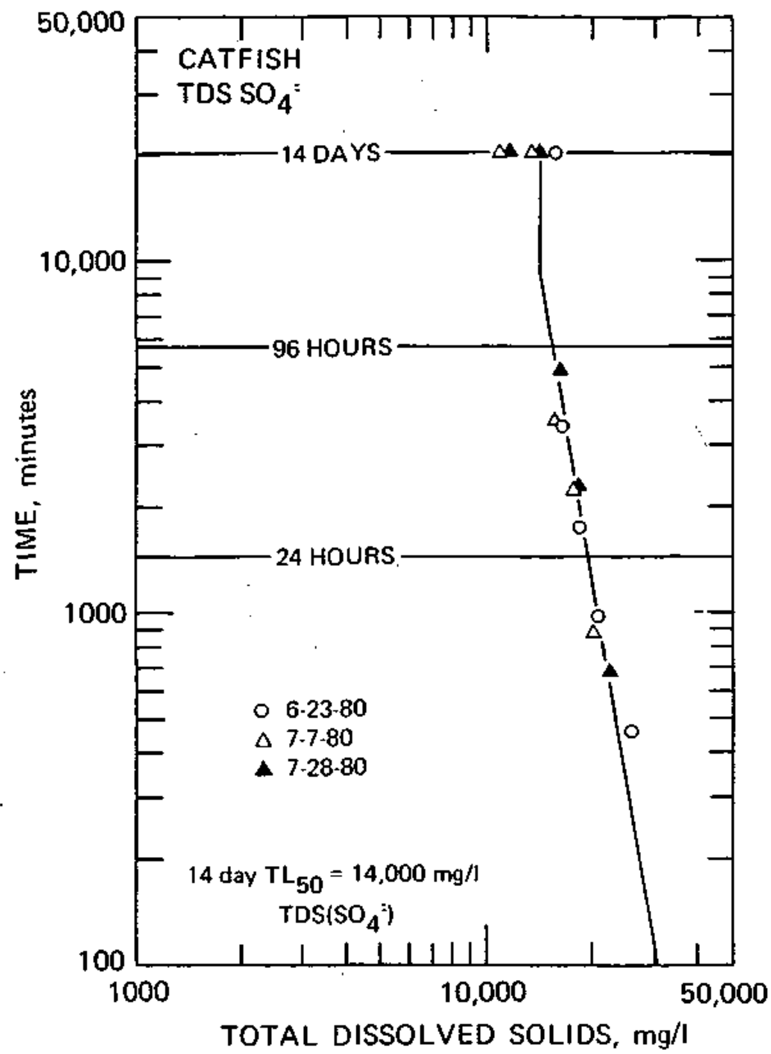

Figure 11. Acute toxicity curve for channel catfish (TDS-$SO_4^{=}$)

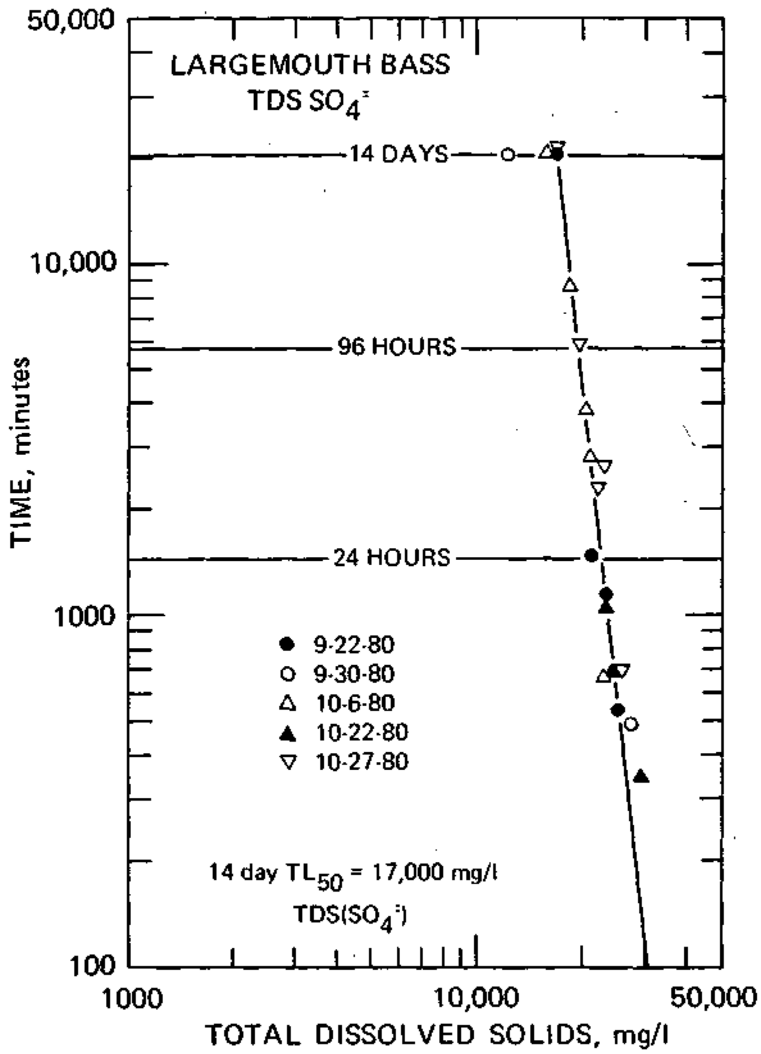

Figure 12. Acute toxicity curve for largemouth bass (TDS-$SO_4^{=}$)

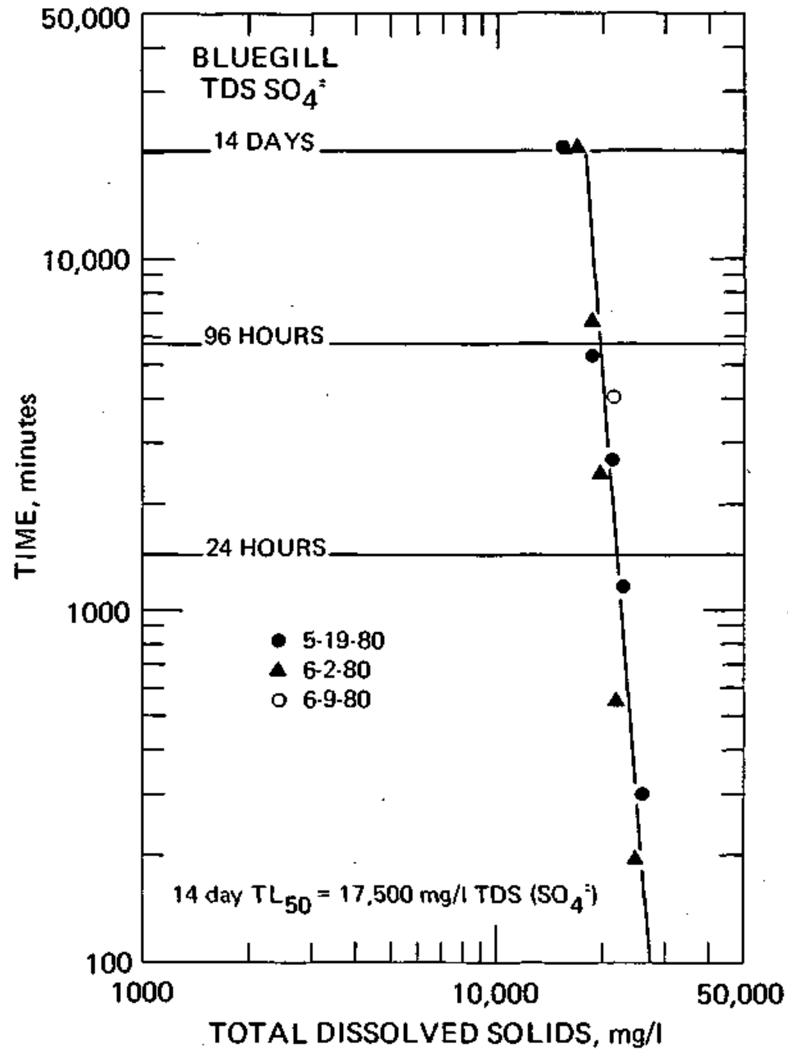

Figure 13. Acute toxicity curve for bluegill (TDS-$SO_4^=$)

SUMMARY AND CONCLUSIONS

In developing this summary the factor of 1/10 has been applied to the observed TL50s produced by this study.

- Channel catfish fingerlings, largemouth bass fingerlings, and bluegill fry were subjected to varying concentrations of chlorides and sulfates at water temperatures of about 20 C in waters relatively high in alkalinity and the salts of calcium and magnesium.

- Median tolerance limits (TL50) were developed from bioassays performed over a period of 14 days. Resultant toxicity curves permitted the comparison of 24-hr and 96-hr bioassays with the 14-day bioassays.

- The TL50 concentration for chloride ranged from 800 to 850 mg/1. Largemouth bass was the most tolerant of the three species.

- For chloride, there was not a perceptible difference in TL50 concentrations between bioassays with time lengths of 24 hrs, 96 hrs, and 14 days.

- The TL50 concentration for sulfate ranged from 1000 to 1100 mg/1. Largemouth bass was the most tolerant of the three species.

- For sulfate runs there was a difference in TL50 concentrations for time lengths of 24 hrs, 96 hrs, and 14 days. The shorter runs produced more liberal values. For example, the TL50 96-hr concentrations of sulfate ranged from 1100 to 1300 mg/1.

- The TL50 concentration for total dissolved solids where chloride was the principal constituent ranged from 1300 to 1500 mg/1. Channel catfish was the most sensitive of the three fish species.

- The TL50 concentration for total dissolved solids comprised mainly of sulfate ranged from 1400 to 1750 mg/1. Channel catfish was the most sensitive of the three fish species.

The current regulations governing the maximum permissible concentrations of chloride and sulfate in Illinois surface waters (500 mg/1) are more than adequate for the protection of aquatic life. In fact maximum permissible concentrations of 800 mg/1 chloride and 1000 mg/1 sulfate are more reasonable standards based on the results of this study.

The use of total dissolved solids as an indicator for the protection of aquatic life has little merit without considering the constituent concentrations of the dissolved solids.

In terms of relative acute toxicity, fishes are more tolerant to sulfates than chlorides; and generally the channel catfish is more sensitive than largemouth bass or bluegill to total dissolved solids.

The uniform application of the 1/10 factor to all toxic substances is a questionable practice. For some substances it may be too conservative, and for others too liberal. A thorough study of its utility would be worthwhile.

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. *Standard methods for the examination of water and wastewater.* American Public Health Association, New York, NY, 1193 p., 14th edition.

Butts, Thomas A., Jack W. Williams, and Ralph L. Evans. 1976. *Water quality evaluation of the Rector Creek — North Fork Saline River drainage.* Prepared for Soil Conservation Service, U.S. Department of Agriculture, Champaign, IL, by Illinois State Water Survey, 94 p.

Harmeson, Robert H., and T. E. Larson. 1969. *Quality of surface water in Illinois, 1956-1966.* Illinois State Water Survey Bulletin 54, 185 p.

Harmeson, Robert H., T. E. Larson, Laurel M. Henley, R. A. Sinclair, and J. C. Neill. 1973. *Quality of surface water in Illinois, 1966-1971.* Illinois State Water Survey Bulletin 56, 100 p.

Illinois Pollution Control Board rules and regulations. 1977. Chapter 3: Water Pollution (as amended through July 1, 1979). Springfield, IL, 63 p.

Larson, T. E., and B. O. Larson. 1957. *Quality of surface water in Illinois.* Illinois State Water Survey Bulletin 45, 135 p.

Mount, D. I., and W. A. Brungs. 1967. *A simplified dosing apparatus for fish toxicity studies.* Water Research v.1:21-29.

Nienkerk, Monto M., and Ronald C. Flemal. 1976. *Regional distribution of the major dissolved solids in the streams of Illinois.* Water Resources Center, University of Illinois at Urbana-Champaign, Research Report 109, 56 p.

Reed, Paula, Dorothy Richey, and Donald Roseboom. 1980. *Acute toxicity of zinc to some fishes in high alkalinity water.* Illinois State Water Survey Circular 142, 21 p.

Richey, Dorothy, and Donald Roseboom. 1978. *Acute toxicity of copper to some fishes in high alkalinity water.* Illinois State Water Survey Circular 131, 24 p.

Roseboom, Donald P., and Dorothy L. Richey. 1977. *Acute toxicity of residual chlorine and ammonia to some native Illinois fishes.* Illinois State Water Survey Report of Investigation 35, 42 p.

Toler, L. G. 1980. *Some chemical characteristics of mine drainage in Illinois.* U.S. Geological Survey, Open-File Report 80-416, Champaign, IL, 47 p.

Appendix A. Observations of Percent Bass Mortality, Chloride Bioassays*

Date: 8/6/79
Average Weight: 0.72 grams
Water Temperature: 20.8°C

Chloride (mg/1)	9718	9584
T.D.S. (mg/1)	DNA	DNA
% Mortality		
10	434	938
20	521	1010
30	576	1067
40	630	1110
50	632	1134
60	675	1164
70	788	1177
80	864	1178
90	866	1570
100	2131	2131

Date: 8/8/79
Average Weight: 0.26 grams
Water Temperature: 21°C

Chloride (mg/1)	9665	9713
T.D.S. (mg/1)	DNA	DNA
% Mortality		
10	208	295
20	261	302
30	294	217
40	295	218
50	297	326
60	302	331
70	334	361
80	371	364
90	382	368
100	394	382

* Time of mortality is in minutes
DNA = data not available

Date: 8/9/79
Average Weight: 0.35 grams
Water Temperature: 21.8°C

Chloride (mg/1)	9587	9587
T.D.S (mg/1)	DNA	DNA
% Mortality		
10	214	274
20	271	318
30	312	319
40	336	331
50	364	342
60	365	371
70	373	385
80	431	423
90	467	433
100	502	573

Date: 8/13/79
Average Weight: 0.38 grams
Water Temperature: 21.1°C

Chloride (mg/1)	10199	10442	10413	10510	10753	10947	7593	7638
T.D.S. (mg/1)	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
% Mortality								
10	153	130	125	133	113	115		
20	154	144	133	136	115	118		
30	160	178	138	162	173	170		
40	177	215	142	171	174	178		
50	184	224	161	172	201	181		
60	205	225	173	177	216	185		
70	226	235	174	187	219	193		
80	227	246	228	243	239	210		
90	280	270	229	254	241	217		
100	312	312	230	260	280	237		

Date: 8/27/79
Average Weight: 0.72 grams
Water Temperature: 21.6°C

Chloride (mg/1)	9262	9493	9480	9393	6119	6127
T.D.S (mg/1)	DNA	DNA	DNA	DNA	DNA	DNA
% Mortality						
10	201	255	240	243		
20	. 310	310	241	244		
30	313	343	248	274		
40	324	355	260	299		
50	362	386	279	311		
60	378	413	315	312		
70	488	454	353	359		
80	494	559	521	370		
90	744	731	529	401		
100	818	1005	530	461		

Date: 10/22/79
Average Weight: DNA
Water Temperature: 20.6°C

Chloride (mg/1)	14075	14075	12647	12375	10549	10490
T.D.S (mg/1)	21313	21481	DNA	DNA	17881	18192
% Mortality						
10	95	95	144	185	182	161
20	119	111	161	199	204	162
30	120	125	173	213	237	218
40	125	126	177	219	242	222
50	127	130	197	252	253	267
60	128	131	198	257	267	321
70	129	132	202	261	274	322
80	139	135	204	295	284	337
90	148	136	219	315	385	406
100	149	137	220	331	406	528

-Appendix A. Continued

Date: 11/5/79
Average Weight: 2.11 grams
Water Temperature 20.5°C

Chloride (mg/1)	15308	14745	12346	12934	11621	11809
T.D.S (mg/1)	24393	24529	20492	20488	18975	18780
% Mortality						
10	69	73	103	88	163	184
20	71	74	107	125	207	186
30	72	82	128	126	209	187
40	73	83	130	138	216	199
50	77	84	131	145	222	204
60	78	85	139	153	226	208
70	80	86	164	154	253	217
80	90	87	165	160	261	223
90	91	88	170	173	271	225
100	101	89	182	182	273	241

Date: 11/12/79
Average Weight: 2.02 grams
Water Temperature: 20.5°C

Chloride (mg/1)	9847	9647
T.D.S (mg/1)	16111	16178
% Mortality		
10	317	345
20	331	358
30	337	405
40	357	425
50	427	564
60	531	571
70	715	674
80	806	701
90	843	739
100	1475	949

. Appendix A. Continued

Date: 1/21/80
Average Weight: 3.59 grams
Water Temperature: 20.4°C

Chloride (mg/1)	10973	11067	8582	8938	8077	7955	6439	6534	5400	5358
T.D.S (mg/1)	19158	19136	14663	14677	13679	13639	11632	11729	9811	9741
% Mortality										
10	169	152	7230	9028	8305	9030				
20	171	165	7310	9028						
30	174	175	8304	9028						
40	177	178	8305	9028						
50	181	189	9028	9038						
60	194	191	9788	10016						
70	199	192		10608						
80	206	195		10608						
90	236	196								
100	240	242								

Date: 2/4/80
Average Weight: 4.39 grams
Water Temperature: 20.1°C

Chloride (mg/1)	11371	11184	9078	9499	8709	8725
T.D.S (mg/1)	18869	18814	15858	15858	14662	14624
% Mortality						
10	112	172	300	269	712	466
20	138	183	466	369	976	805
30	170	186	467	466	1148	849
40	186	191	575	488	1379	1014
50	204	203	611	527	1911	1030
60	222	205	651	620	1911	1370
70	223	236	757	635	2890	2517
80	234	237	903	1212	3440	
90	239	251	933	1427	5690	
100	279	252	1200	2755		

. Appendix A. Concluded

Date: 11/10/80
Average Weight: 1.92 grams
Water Temperature: 19.5°C

Chloride (mg/1)	14126	14065	11924	11924	10665	10763	8452	8462	6170	6140
T.D.S (mg/1)	23240	23289	18942	1826	16880	17344	13960	13865	10932	10889
% Mortality										
10	94	97	161	175	366	256				
20	110	101	172	176	367	256				
30	113	106	177	194	368	266				
40	114	108	180	194	372	283				
50	120	110	193	204	394	289				
60	121	116	198	207	400	289				
70	124	117	199	210	415	320				
80	126	123	221	245	415	468				
90	126	128	225	253	421	468				
100	133	133	320	253	531	646				

Date: 12/2/80
Average Weight: 2.26 grams
Water Temperature: 18.5°C

Chloride (mg/1)	14432	14371	12597	11558	9726	9626	9131	9038	6418	6363
T.D.S. (mg/1)	23409	23437	18984	18976	15866	15805	15093	14965	11342	11278
% Mortality										
10	89	127	185	228	761	1016	6888	6888		
20	95	133	200	239	784	4702	8938	8084		
30	100	137	205	240	2846	5410		11031		
40	109	139	205	245	4606	5410		11031		
50	109	144	212	246	5410	5410		11031		
60	110	157	215	257	5410	6888		16028		
70	110	159	220	264	5410	6888				
80	133	159	241	267	8084	6888				
90	134	165	248	281						
100	145	187	253	313						

Appendix B. Observations of Percent Bluegill Mortality, Chloride Bioassays*

Date: 7/10/79
Average Weight: 2.79 grams
Water Temperature: 22.5°C

Chloride (mg/1)	10690	10597
T.D.S (mg/1)	DNA	DNA
% Mortality		
10	281	200
20	307	313
30	314	337
40	315	338
50	340	342
60	354	343
70	410	389 \
80	474	465
90	482	471
100	545	529

Date: 7/16/79
Average Weight: 4.51 grams
Water Temperature: 23.5°C

Chloride (mg/1)	10704	9878
T.D.S. (mg/1)	DNA	DNA
% Mortality		
10	167	252
20	179	262
30	185	284
40	194	330
50	199	343
60	240	364
70	248	366
80	254	392
90	266	475
100	276	864

* Time of mortality is in minutes
DNA = data not available

Date: 7/24/79
Average Weight: 7.24 grams
Water Temperature: 21.7°C

Chloride (mg/1)	9103	9161
T.D.S (mg/1)	DNA	DNA
% Mortality		
10	680	1017
20	811	1042
30	858	1128
40	894	1187
50	902	1344
60	1133	1516
70	1187	1610
80	1798	1999
90	2536	3100
100	2536	

Date: 11/15/79
Average Weight: 2.13 grams
Water Temperature: 20.0°C

Chloride (mg/1)	11646	11446
T.D.S (mg/1)	19161	19036
% Mortality		
10	153	173
20	157	181
30	165	201
40	174	204
50	185	208
60	187	212
70	189	221
80	204	225
90	209	249
100	218	290

- Appendix B. Concluded

Date: 11/26/79
Average Weight: 2.31 grams
Water Temperature: 20.6°C

Chloride (mg/1)	11546	11546	8287	8244	6648	7376	6606	6622	5303	5277
T.D.S (mg/1)	18547	18549	14596	14609	13333	13283	11219	11251	9555	9434
% Mortality										
10	157	152	694	2339						
20	159	153	2596	2340						
30	160	514	2855	4053						
40	177	156	4279	4219						
50	186	167	6657	6657						
60	189	174	6658	9520						
70	197	187	11950	9521						
80	206	196								
90	214	201								
100	232	231								

Date: 12/10/79
Average Weight: 0.34 grams
Water Temperature: 20.4°C

Chloride (mg/1)	11184	11231	8212	8166	7425	7518	6150	6173	5151	5105
T.D.S. (mg/1)	19143	19119	14208	14207	13043	12956	11220	11285	9541	9378
% Mortality										
10	94	80	5470	429	17095	4274	12080			
20	96	91	7000	3442	19850	8606				
30	99	109	18430	3512		19850				
40	111	110								
50	128	114								
60	132	126								
70	136	145								
80	139	146								
90	142	149								
100	181	155								

Appendix C. Observations of Percent Catfish Mortality, Chloride Bioassays*

Date: 8/18/80
Average Weight: 1.54 grams
Water Temperature: 17.8°C

Chloride (mg/1)	13272	13783	11167	11103	9878	9954	7626	7795	5289	5175
T.D.S. (mg/1)	21144	21265	17231	17264	15428	15306	12396	12441	9206	8899
% Mortality										
10	150	150	241	303	500	568	7372	15735		
20	163	169	276	303	502	672	10153			
30	165	174	303	316	587	676				
40	172	179	346	323	709	724				
50	172	180	347	323	743	744				
60	177	180	356	356	743	749				
70	182	187	358	363	746	770				
80	184	191	369	425	747	770				
90	198	191	425	438	769	1065				
100	210	194	488	460	975	1180				

Date: 9/2/80
Average Weight: 2.37 grams
. Water Temperature: 19.9°C

Chloride (mg/1)	13151	13088	11389	10508	9439	9489	7704	7626	5185	5077
T.D.S. (mg/1)	20566	20618	16671	16718	15124	15086	12253	12108	8951	8721
% Mortality										
10	148	153	264	301	467	600				
20	152	153	287	303	490	604				
30	160	153	320	313	508	749				
40	167	163	325	320	549	778				
50	175	165	341	325	600	779				
60	180	167	343	325	631	782				
70	187	174	360	325	705	836				
80	187	174	365	339	793	852				
90	203	174	374	400	820	860				
100	210	190	385	414	890	927				

* Time of mortality is in minutes
DNA = data not available

Appendix C. Concluded

Date: 9/9/80
Average Weight: 3.51 grams
Water Temperature: 20.1°C

Chloride (mg/1)	13340	13592
T.D.S. (mg/1)	21287	21303
% Mortality		
10	128	117
20	149	147
30	152	153
40	160	155
50	162	156
60	163	157
70	164	159
80	166	164
90	166	164
100	174	164

Appendix D. Observations of Percent Bass Mortality, Sulfate Bioassays*

Date: 9/22/80
Average Weight: 1.24 grams
Average Temperature: 20.2°C

Sulfate (mg/1)	17484	16468	14031	14132	10984	12406	10210	9907	7870	7556
T.D.S (mg/1)	25351	25469	20668	20635	21820	21837	17073	16992	13499	13321
% Mortality										
10	275	402	957	866	1197	1171		2137		
20	416	411	1065	889	1220	1340		3819		
30	466	579	1129	956	1340	1377				
40	467	642	1130	1001	1386	1511				
50	526	661	1197	1171	1400	1607				
60	528	698	1220	1197	1438	1623				
70	588	730	1351	1220	1473	1666				
80	621	743	1367	1243	1717	1723				
90	622	771	1438	1438	1753	1753				
100	640	870	1511	1608	2292	1834				

Date: 9/30/80
Average Weight: 1.30 grams
Average Temperature: 21.6°C

Sulfate (mg/1)	18868	16547
T.D.S (mg/1)	27275	27277
% Mortality		
10	171	258
20	303	303
30'	343	357
40	431	420
50	621	431
60	701	572
70	832	638
80	876	700
90	941	741
100	1521	950

* Time of mortality
DNA = data not available

Date: 10/6/80
Average Weight: 1.33 grams
Average Temperature: 20.0°C

Sulfate (mg/1)	14567	14567	13964	13246	13712	14105	11969	11640	9953	10068
T.D.S. (mg/1)	23666	23639	21049	21516	20990	20944	18686	18471	16215	16104
% Mortality										
10	456	224	1620	1272	2162	1430	6027	6855	16044	15113
20	505	473	2228	1484	2162	2162	6855	6855		19905
30	537	515	2228	1639	3258	3070	6855	6855		
40	677	644	3352	2237	3321	3711	6855	6855		
50	703	843	3813	2237	4607	4689	6855	8292		
60	759	903	3813	2237	5190	5205	6855	10049		
70	822	950	4657	3018	5190	6027	8292	12998		
80	844	975	5190	4619	5190	6855	10059	13681		
90	948	1117	5190	5274	5741	6855	13681	16044		
100	976	1588	5190	6855	6024	6855	18155	16105		

Date: 10/22/80
Average Weight: 1.45 grams
Average Temperature: 20.2°C

Sulfate (mg/1)	18679	18989	16197	15886	15576	19609	13269	12959	11201	11459
T.D.S. (mg/1)	29573	29557	24343	24385	23930	23895	18937	18836	16369	16306
% Mortality										
10	241	265	365	375	540	852		652		
20	246	278	419	408	642	892		1503		
30	259	352	514	493	842	1057				
40	311	375	619	432	849	1057				
50	329	389	689	892	879	1179				
60	350	424	760	928	1179	1238				
70	354	430	812	959	1339	1284				
80	387	436	842	1130	1506	1311				
90	405	439	1033	1263	1560	1503				
100	537	535	1130	1351	1560	1558				

Date: 10/27/80
Average Weight: 1.77 grams
Average Temperature: 19.9°C

Sulfate (mg/1)	14839	14208	14907	13916	14804	14647	12166	11950	10479	10323
T.D.S. (mg/1)	25941	25986	23424	23421	22237	23043	19934	19752	17186	17183
% Mortality										
10	352	325	1541	2297	1571	911	3554	3554	8150	5236
20	528	618	1702	2297	1896	1267	4284	4572		5909
30	541	688	2253	2297	2253	2253	4572	5236		
40	631	722	2253	2297	2253	2253	5236	5874		
50	688	753	2253	2954	2253	2253	6773	6217		
60	893	755	2920	3146	2253	3003	6773	6217		
70	965	817	3141	3165	2857	3285	6773	6773		
80	1019	846	3213	3706	2900	3353		8150		
90	1063	933	3730	3706	2973	3800				
100	1235	1235	4329	4383	3610	4549				

Appendix E. Observations of Percent Bluegill Mortality, Sulfate Bioassays*

Date: 5/19/80
Average Weight: 0.65 grams
Average Temperature: 21.0°C

Sulfate (mg/1)	15806	17483	14789	16059	13533	14430	11861	11741	9801	9850
T.D„S. (mg/1)	26024	25963	22224	23589	21102	20973	18608	18594	15527	15400
% Mortality										
10	106	208	713	138	772	204	1060	715	920	
20	127	225	1060	355	1210	1302	4227	934	9314	
30	158	242	1077	557	1230	1652	4361	5934		
40	216	253	2709	576	1565	2235	5561	6985		
50	228	266	2829	655	1769	4130	6098	7253		
60	333	386	2836	810	5707	6985	8004	9237		
70	652	476	2985	825	6985	8500	8473	9237		
80	675	485	4211	908	7253	9237	9237	10628		
90	854	487	6810	1302	7253	9237	10628	17726		
100	880	743	6810	4130	9237	10628				

Date: 6/2/80
Average Weight: 0.59 grams
Average Temperature: 21.7°C

Sulfate (mg/1)	17296	18009	15565	14546	11956	12058	11131	11477	9730	9418
T.D.S. (mg/1)	23143	26611	21901	22057	19816	19409	18399	18437	15651	15460
% Mortality										
10	122	84	424	235	1422	768	712	1945		
20	156	123	500	345	1945	769	1270	1945		
30	201	145	501	346	1945	801	6700	2496		
40	214	167	598	348	2610	1031	8179	9121		
50	218	187	705	495	3387	1409	12483	10060		
60	231	188	712	503	4824	1945	12922	14701		
70	266	238	712	523	8179	1945	15800	15800		
80	268	239	713	801	8180	3387	15800	15800		
90	370	313	767	890	8891	9121	15918	15800		
100	480	399	1407	2402	9800	11213	16618			

* Time of mortality
DNA = data not available

Appendix E. Concluded

Date: 6/9/80
Average Weight: 1.09 grams
Average Temperature: 20.8°C

Sulfate (mg/1)	13483	13844
T.D.S. (mg/1)	21467	21456
% Mortality		
10	2359	2770
20	3350	3350
30	3350	3350
40	3925	3350
50	4346	3350
60	5227	4577
70	5227	4577
80	5227	5676
90	5227	5676
100	5804	6500

Appendix F. Observations of Percent Catfish Mortality, Sulfate Bioassays*

Date: 6/16/80
Average Weight: 1.01 grams
Average Temperature: 21.4ºC

Sulfate (mg/1)	15426	18205
T.D.S. (mg/1)	DNA	DNA
% Mortality		
10	250	310
20	280	378
30	290	434
40	333	464
50	407	497
60	407	517
70	424	517
80	464	549
90	464	549
100	655	826

Date: 6/23/80
Average Weight: 1.28 grams
Average Temperature: 20.8°C

Sulfate (mg/1)	16840	19245	15587	15377	13705	13496	13287	12555	9032	9959
T.D.S. (mg/1)	25968	25947	21105	20585	18625	17656	16329	16406	13877	13966
% Mortality										
10	322	328	513	740	619	1102	1333	914	9190	6309
20	339	386	619	889	889	1228	2071	2071	9959	9190
30	386	440	831	889	914	2071	2899	2071		10508
40	409	440	1000	934	1333	2071	3540	2629		14074
50	409	509	1039	946	1333	2071	3540	4970		
60	440	548	1041	1039	2071	2071	4003	4970		
70	440	551	1168	1043	2071	2071	4970	5618		
80	447	621	1401	1212	2071	3540	5822	5724		
90	457	646	1441	1212	2071	3540	5896	6291		
100	718	889	2071	1333	4970	5500	6377	9190		

* Time of mortality
DNA = data not available

Date: 7/7/80
Average Weight: 1.55 grams
Average Temperature: 21.0°C

Sulfate (mg/1)	13540	14564	12293	13165	10273	11628	8506	8442	6769	6860
T.D.S. (mg/1)	20436	20440	17807	18039	15621	16265	13131	13108	10911	10722
% Mortality										
10	708	584	2028	1541	2869	2028				
20	754	584	2028	2028	2990	2028				
30	793	584	2028	2028	3503	2028				
40	898	607	2028	2028	3503	2527				
50	961	708	2028	2028	3503	2782				
60	1130	816	2534	2028	4435	2869				
70	1130	926	2583	2028	4910	3503				
80	1146	932	2756	2819	4910	3503				
90	1311	1312	2869	2916	19801	4280				
100	1811	1454	3503	3503		4281				

Date: 7/28/80
Average Weight: 1.85 grams
Average Temperature: 19.4°C

Sulfate (mg/1)	15084	15584	11606	12021	10573	10156	8945	8803	7198	7019
T.D.S. (mg/1)	22954	22731	18292	18050	16209	16087	13625	13585	11180	11052
% Mortality										
10	460	649	738	2034	2744	2034				
20	461	689	977	2035	2815	3015				
30	493	776	1406	2036	3470	4005				
40	520	777	2034	2037	3471	5356				
50	521	826	2035	2641	3472	5690				
60	522	877	2036	3470	4257	6411				
70	528	878	2037	4200	4906	8964				
80	689	879	2038	5356	5690	12620				
90	826	1033	2039	5799	7880	13039				
100	960	1251	3470	9142	11131	13999				