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Acute Toxicity of Copper to Some Fishes in High Alkalinity Water

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INTRODUCTION

There is a continuing need to develop information that will be useful to agencies and persons involved in the promulgation of regulations, the enforcement of rules, and the management of waste treatment facilities as such activities relate to the water quality of streams and lakes. The study reported herein deals with the acute toxicity of copper to certain fishes in waters of relatively high alkalinity. Rule 203(f) of the Water Pollution Regulations of Illinois limits *total* copper concentrations in the waters of streams and lakes to 0.02 mg/1. Rule 408(a) permits a maximum concentration of *total* copper in effluent discharges to 1.0 mg/1.

In addition to these rules establishing maximum permissible limits, Rule 203(h) states:

> 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.

Here the median tolerance limit (TLm) is the concentration at which 50 percent of the test specimens survive. It is also referred to as TL50 or LC50 (lethal concentration). Changes of the 48-hour TLm to 96-hour TLm have been considered and will most likely be made. For assessing the acute toxicity of heavy metals on fish a 96-hour time period of fish exposure to the selected toxicant is too short. During the course of this study an exposure time of 14 days (336 hours) was used.

The levels of copper and its distribution in natural water bodies in Illinois are not well known. Recent investigations suggest that background levels may be higher than expected. From 219 water samples collected from five tributary streams and outlets of Rend Lake,[1] analyses on particulate material showed copper concentrations exceeding the water quality standard (0.02 mg/1) from 17 to 26 percent of the time. During the problem assessment of nonpoint sources of pollution in eight urban areas, as part of the 208' water quality management program, the average concentration of copper in urban stormwater runoff generally exceeded the water quality standard. Adverse effects, if any, of these excursions in copper levels have not been noted. In light of these documented excesses, in terms of the current water quality standard, the observations developed during this study seem pertinent.

Scope of Study

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

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 contains basically a literature review, description of methodology, fish reactions, results, and summary. All data developed from the bioassays are included in the appendices. A description of the chemistry of copper 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 offered in a manner that may be useful to the practitioners in the water quality field.

Acknowledgments

This study was conducted under the general supervision of Ralph L. Evans, Head of the Water Quality Section, and Dr. William C. Ackermann, Chief, Illinois State Water Survey. Several persons in the Water Quality Section assisted in the study. Dave Hullinger provided guidance for the analysis of copper. Julie Anderson and Robert Duffner performed analyses, lent direction to the operation and maintenance of the dilution apparatus, and occasionally maintained continuous 24-hour observations of the aquaria. Mr. Maurice Whitacre of the Department of Conservation offered advice on the maintenance of test specimens and indeed supplied most of them.

LITERATURE REVIEW

Copper Chemistry

In an aqueous system, metal constituents may be classified as soluble (free ions and metal complexes with organic or inorganic ligands) and insoluble (colloidal particulates of metal complexes, aggregates or hydrous metal oxides, and the metal complexes that are absorbed on suspended particulates).[2] Organic and inorganic debris, alkalinity, hardness, and pH are a few of the water properties that determine the proportions of soluble and insoluble copper. For analytical purposes, soluble copper is that portion that will pass through a 0.45 μm pore size membrane filter, while the insoluble copper will not. Total copper is the combination of soluble and insoluble copper.

Stiff[3] filtered 18 different water sources and found that in polluted waters, amino acids and polypeptides play a significant role in the complexation of copper. Because of the amino-acid complexes, inert acid complexes, and hexanol extractable complexes found in these samples, only 0.2 to 1.4 percent of the soluble copper existed as cupric ions. In another nine samples, the soluble copper accounted for 12 to 57 percent of the total copper.

Bicarbonates, as well as organic substances, can lessen the solubility of copper. Andrew[4] added copper to Lake Superior water. Although all of the copper was soluble at low concentrations, only 77 percent of the copper was soluble at the higher concentrations. When a bicarbonate was added, a 2 to 6 percent further decrease of soluble copper occurred. Using copper concentrations greater than 0.5 mg/1 , Shaw[5] found that bicarbonates form insoluble complexes. The cupric carbonate reacted with hydroxides and bicarbonates to form malachite, which quickly precipitated. Mancy,[2] however, stated that the precipitate complex depends upon the carbonate concentration. At low carbonate concentrations, the cupric ion precipitates as $Cu(0H)_2$ and evolves to tenorite (CuO). At higher levels, the precipitate evolves to malachite ($Cu_2(0H_2)C0_3$) or azurite ($Cu_3(0H_2)C0_3)_2$.

Brungs[6] found that as the alkalinity of a surface water increased, more copper was required to maintain the soluble copper concentration. The total copper in the experimental water varied from 1.6 to 21 mg/1, and the soluble copper was more stable at 0.60 to 0.98 mg/1. Whenever the alkalinity increased, much of the total copper was complexed with carbonates. Likewise, Pagenkopf[7] believes that a sizable portion of the total copper is complexed by carbonates and hydroxides. Therefore, alkalinity, and not hardness, is responsible for determining copper solubility. He states that hardness has only a minor, indirect effect on solubility, which occurs when the calcium and magnesium ions bind with the bicarbonates. This binding eliminates a few of the carbonates that might complex with copper.

Although hardness does not greatly affect copper complexation, Lloyd[8] showed that it may affect copper toxicity. When rainbow trout were maintained in hard water, and then transferred to soft water containing heavy metals, they survived longer than trout reared and tested in soft water. However, trout that were acclimated to the soft water for 5 days prior to the introduction of heavy metals, succumbed to the toxicant as rapidly as trout reared in soft water. It seems that calcium provides a temporary, internal protection against heavy metals. Sauter,[9] however, stated that hardness cannot protect fish that are exposed to copper for long periods of time. Channel catfish, walleye, and brook trout tested for 30 days in hard water had comparable maximum acceptable toxicant concentrations (MATC) to fish tested in soft water.

Copper complexation and toxicity are also influenced by pH. Figure 1 shows that at a pH of 7, about 55 percent of the total copper will be cupric ions. However, at a pH of 8, the cupric ion portion drops to only 10 percent of the total copper. When Shaw[5] compared copper values at a pH of 6.5 and 7.5, he found an 8 to 29 percent decrease of cupric ions at the higher pH. Stiff,[11] using a 1.0 mg/1 total copper solution with a pH of 8.57 and an alkalinity of 250 mg/1, observed that some of the copper precipitated. Therefore, the soluble copper was only 0.81 mg/1 and the cupric ion concentration was 0.0004 mg/1. He repeated the experiment, using the same alkalinity, but lowering the pH to 6.69. No precipitation occurred. The cupric ion level increased to 0.12 mg/1 .

Organic debris, bicarbonates, and higher pH seem to ***decrease*** the amounts of soluble copper. Andrew,[4] however, found that some substances (pyrophosphate) form soluble complexes with copper, thereby ***increasing*** the percentage of soluble copper. When a nominal total copper concentration of 127.6 µg/1 was added to Lake Superior water, the measured soluble copper was 97.8 µg/1. When pyrophosphate was added, the soluble copper increased to 123.8 yg/1.

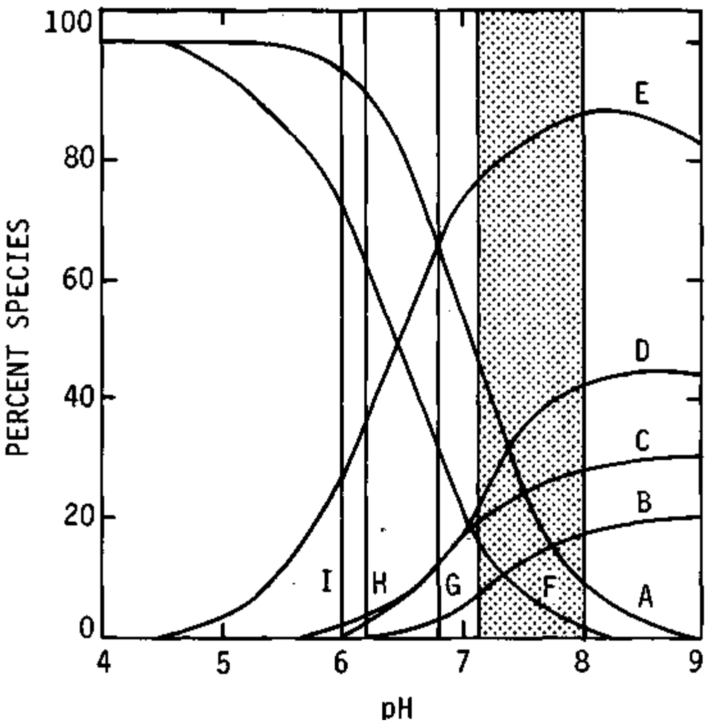

Figure 1. Speciation of copper (II) (total concentration 2 ppm) and carbonate as a function of pH

Note: A=Cu^{2+}; B=$Cu_2(OH)_2^{2+}$; C=$CuOH^+$; D=$CuCO_3$; E=HCO_3^-; F=H_2CO_3; G=pH at which $Cu(OH)_2$ will precipitate; H=pH at which $Cu_3(OH)_2(CO_3)_2$ will precipitate; and I=pH at which $Cu_2(OH)_2CO_3$ will precipitate (from reference 10)

Because copper may be complexed by organic and inorganic substances, carbonates, and other miscellaneous aqueous matter, it may exist in many different forms. Because of the diversity of copper complexes, the toxicity (if any) of each constituent should be determined.

Pagenkopf[7] stated that the cupric ion is primarily responsible for copper toxicity. Andrew's[4] work also points this out. As previously mentioned, the Lake Superior water with pyrophosphate contained higher levels of soluble copper than either Lake Superior water or the water with added bicarbonates. However, this pyrophosphate solution was about 10 times less toxic than the other solutions. This is puzzling until one realizes that the cupric ion concentration of the pyrophosphate solution was about 10 times less than the cupric ion concentration of the other solutions.

Copper Toxicity

Any water high in alkalinity, pH, and organic substances will have low cupric ion concentrations. Therefore, by knowing the characteristics of a certain body of water and deciding which complexes will form, one should be able to predict the toxicity of the copper. However, many of the reports about copper toxicity ignore water characteristics and their effects on cop-

Table 1. Summary of Toxicity Data from Other Sources
(Concentrations in mg/l)

Organism	*Size*	*Alka-linity*	*Copper*	*Reference*
Rainbow trout	yearling	250	48 hour TL50 0.75	32
Rainbow trout	12-16 centimeters	250	48 hour TL50 0.27	33
Rainbow trout	4.42 grams	low	24 hour TL50 0.43	36
Brook trout	0-60 days	178	MATC/60 days 0.006	9
Brook trout	0-60 days	28	MATC/60 days 0.004	9
Atlantic salmon	3 years old	4	96 hour LC50 0.125	37
Silver salmon	yearling	78	72 hour LC50 0.19	18
Silver salmon	yearling	36	72 hour LC50 0.48	18
Coho salmon	yearling	74	96 hour LC50 0.067	38
Walleye	0-60 days	187	MATC/60 days >0.071	9
Walleye	0-60 days	35	MATC/60 days 0.013-0.021	9
Channel catfish	0-60 days	173	MATC/60 days 0.015	9
Channel catfish	0-60 days	34	MATC/60 days 0.016	9
Channel catfish	8.8 grams	low	24 hour TL50 2.6	36
Fathead minnow	20-69 millimeters	90	96 hour TL50 0.69	6
Fathead minnow	20-69 millimeters	166(A)	96 hour TL50 0.77(A)	6
Fathead minnow	20-69 millimeters	205(A)	96 hour TL50 0.81(A)	6
Fathead minnow	adults	154	96 hour TL50 0.450	6
Fathead minnow	adults	30	96 hour TL50 0.080(A)	39
Bluegill	35 grams	43	96 hour TL50 1.10	20
Bluegill	?	low	96 hour TL50 1.25	40
Bluegill	1 inch or more	60-170	no mortality at 1.0	41
Bluegill	1-9 grams	4	96 hour TL50 0.74	42
Bluegill	0.64 grams	low	24 hour TL50 2.5	36
Pumpkinseed sunfish	?	low	96 hour TL50 2.4	43
Golden shiner	2.56 grams	low	24 hour TL50 0.27	36
Golden shiner	--	45	partial mortality at 1.0	41

Note: A = an average of results; MATC = maximum acceptable toxicant concentrations

per complexation. This is probably the reason for differing toxicity results. Table 1 lists the results of some toxicity studies employing copper as the toxicant.

Sauter et al.[9] found that the eggs of brown trout, channel catfish, and walleye were more resistant to copper than other life stages. King salmon eggs also hatched in copper concentrations where the fry did not survive.[12] Ninety-seven percent of the fertilized rainbow trout eggs that Shaw[13] tested at copper concentrations of 1 mg/l hatched. Beyerle and Williams, attempting to control bluegill populations in ponds, heavily dosed the spawning nests with copper sulfate. A population reduction did not occur.

Eggs are not as sensitive to copper as fish. Therefore, if fish would avoid copper, copper toxicity would not be such a major problem. Jones[15] found that the stickleback detected and avoided copper. However, the fish could not detect copper as readily as lead, mercury, and zinc, even though copper is more lethal than lead or zinc. Timms et al.[16] using a copper gradient found that 8 of 10 goldfish and 6 out of 7 channel catfish changed their orientation toward the ions. However, none of the 7 largemouth bass altered their orientation. Geckler et al.,[17] studying copper in a stream, observed that fish avoided the higher copper concentrations.

Fish either can not or will not avoid copper concentrations that might be detrimental. Holland et al.[18] reported some of the effects copper has on fish. Copper salts combine with proteins present in the mucus of the fish's mouth, gills, and skin, preventing aeration of the blood. Death sometimes results. Turnbull et al.[19] also noted a copper precipitate clinging to fish. Bluegill exhibited several weeks of periodic muscle spasms.[20] Baker,[21] working with flounders, observed neuromuscular disorders just prior to death. Brook trout that were exposed to copper had increased cough frequencies.[22] Grande[23] noticed that salmon fry darkened and refused to eat. Loss of appetite was also noticed in brook trout.[22] Perhaps the feeding inhibition prevented fathead minnow fry in copper solutions from growing as rapidly as the control fry.[24] Mummichogs developed lesions along the lateral line.[25] O'hara[26] stated that bluegill's oxygen consumption increased about 3 to 6 hours after copper was introduced.

Copper also creates morphological changes in fish. Benoit[20] examined the gills, livers, and kidneys of bluegill that were exposed to copper. These organs contained higher levels of copper than organs of control fish. However, the brain, spleen, gonads, and muscle tissue had no more copper than those from the controls. This correlates with Baker's[21] study of the winter flounder. He found gross morphological changes in the gills, fatty metamorphosis of the lever, and necrosis of the kidney. There were no changes in the somatic muscles, heart, stomach, duodenum, intestine, spleen, or brain.

Changes in the blood also occur. After 6 days of exposure to copper, the blood of the brown bullhead showed glucose and hematocrit increases. After 30 days, the chloride and protein decreased.[27] In spite of all the external and internal changes induced by copper, most of the fathead minnows were still capable of caring for their young.[24]

Some work has also been done on the effects of mixing copper with other toxicants. Because many mining operations release discharges consisting of copper and zinc, an understanding of this mixture is desirable. Because salmon will sometimes avoid a copper-zinc mixture, spawning may be reduced. Saunders and Sprague[28] found that many salmon prematurely withdrew from the copper-zinc polluted spawning streams to seek cleaner water. Sprague et al.[29] showed that even young salmon will avoid copper-zinc mixtures in streams and in the laboratory. Sprague and Ramsay[30] and Lloyd[31] stated that the toxicity of a low concentration of a copper-zinc mixture can be calculated by adding the toxicities of the individual metals. However, at high levels, the toxicity is synergistic instead of additive.

In mixtures of copper-phenol, copper-zinc-phenol, and copper-zinc-nickel, Brown and Dalton[32] found that the toxicity equaled the summation of the fraction toxicity of each chemical. Herbert and Van Dyke[33] noted that the summation rule also applies to ammonia-copper mixtures.

EQUIPMENT AND METHODS

A modification of a proportional dilutor by Mount and Brungs[34] 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 of 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 two 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 copper study.

Two header boxes were used. The first one, consisting of a steel barrel lined with fiberglass, 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 copper bioassay:

	mg/l		mg/l*
Chemical oxygen demand	ND	Fluoride	0.79
Ammonia-N	0.07	Silica	9.84
Nitrate-N	4.6	Magnesium	32.7
Phosphate-P	0.24	Iron	0.12
Sulfate	120	Zinc	0.07
Chloride	117	pH	8.21
Copper	0.009	Hardness	260
		Alkalinity	255

ND = not detected

*Except pH

Stock Solutions and Chemical Analyses

The copper stock solutions were prepared by dissolving American Chemical Society analytical reagent grade hydrous cupric sulfate in deionized water. Because of the high alkalinity (250 mg/l) of the dilution water, precipitation occurred as the stock solution was added to the test chambers. As previously discussed, this insoluble copper is not as toxic to fish as soluble copper. To analyze for the soluble copper, the particulate had to be removed. At least four times during the first 2k 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 copper. The other 25 ml of the sample was analyzed for total copper. All copper analyses were determined by a Perkin-Elmer atomic absorption spectrophotometer, model 305A.

Because of the large range of values between the soluble and total copper (see table 2), the absorption curve was not always linear. Therefore, two curves were used, one for the soluble copper and one for the total copper. At least four duplicate standards were plotted for each daily curve. Correlation coefficients for the daily standards were never lower than 0.9992. The correlation coefficient for all the standards from June 15, 1977» to March 20, 1978, was 0.923 for soluble copper and 0.900 for total copper.

Table 2 shows that much of the copper in the test chambers formed insoluble compounds, especially at the higher concentrations. To further understand this relationship between soluble and insoluble copper, flasks of dilution water (having an alkalinity of 269 mg/l and a pH of 8.3) and deionized water were spiked with copper. Table 3 gives the results. Although the copper spikes with deionized water were comparable to the nominal levels, the spikes in dilution water were lower. This decrease might be due to the copper precipitate that lined the sides and bottom of the flasks. Only 45 to 71 percent of the total copper in the dilution water was soluble.

Hardness and alkalinity were determined in the control chambers and two other test chambers every day. Dissolved oxygen levels, measured by a Yellow Springs Instrument Model 54 oxygen meter, and pH were measured daily in all test chambers. The water temperature was monitored continuously by a Yellow Springs Instrument Model 46 Tele-Thermometer with output recorded on a Cole-Parmer Mark VII recorder. Hardness determinations were by EDTA titrametric method with Eriochrome Black T as an indicator. Alkalinity and pH were determined by a Leeds and Northrup meter, with 0.02N H_2SO_4 as a tritrant 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

The bluegill ***(Lepomis maorookirus)*** used in this investigation were obtained from the hatchery maintained by the Illinois Department of Conservation at Carbondale. In all, 340 bluegill with an average weight of 0.435 grams,

Table 2. Comparison of Total and Soluble Copper

Tanks	Total copper (mg/l)	SD	Soluble copper (mg/l)	SD	Total copper (mg/l)	SD	Soluble copper (mg/l)	SD
	June 15, 1977				*June 20, 1977*			
7-11	19.6	1.2	4.3	0.38	17.8	2.2	4.0	0.45
1-2	15.9	1.7	3.1	0.24				
4-6	11	0.65	2.0	0.15				
3-10	5.7	0.52	1.06	0.69				
5-8	2.9	0.51	0.73	0.12				
	June 28, 1977				*July 13, 1977*			
7-11	18.5	1.3	3.9	0.5	27.5	2.6	5.8	0.63
1-2	17.4	1.6	3.2	0.4	24.4	1.9	4.7	0.1
4-6	12.7	0.8	2.0	0.1	18.8	2.5	2.5	0.1
3-10	7.0	0.8	1.1	0.1	9.7	1.0	1.5	0.2
5-8	3.6	0.3	0.8	0.09	5.4	1.4	1.0	0.3
	August 17, 1977				*August 29, 1977*			
7-11	35.6	2.5	7.7	1.1	36.0	0.9	7.8	0.6
1-2	30.4	3.2	7.2	1.6	29.7	0.7	5.1	0.5
4-6	27.2	3.1	4.2	0.5	24.2	0.6	4.2	0.4
3-10	13.9	4.6	1.5	0.3				
5-8	6.6	0.8	0.9	0.1				
	October 5, 1977				*October 24, 1977*			
7-11	31.0	2.8	5.7	0.3	39.0		5.7	0.3
1-2	28.9	3.9	4.4	0.3	22.5		4.6	0.3
4-6	19.9	0.76	2.7	0.1	17.6		2.8	0.25
3-10	7.0	0.3	1.1	0.07	6.8		1.2	0.1
5-8	3.5	0.2	0.6	0.04	2.8		0.68	0.04

Table 3. Variations in Solubility of Copper

(Concentrations in mg/l)

Nominal copper	*Spikes/deionized water*	*Spikes/well water unfiltered*	*Percent recovery*	*Spikes/well water filtered*
0.5	0.501	0.420	85	0.290
0.5	0.485	0.428		0.315
1	1.01	0.956	94	0.526
1	0.97	0.916		0.542
2	2.02	1.83		1.06
2	2.02	1.84	93	1.01
2	2.03	1.89		
3	3.02	2.87		1.64
3	3.03	2.88	94	1.44
3	2.96	2.74		
4	4.01	3.82		
4	3.95	3.86	93	2.01
4	4.02	3.51		1.84
5	4.98	4.44	89	1.94
5	5.01			2.32
10	10.0	7.67	79	3.71
10		8.13		3.67
40	40.03	23.4	59	10.53

Table 4. Test Conditions for Bluegill and Catfish Bioassays

	Soluble copper (mg/l)	*Average temperature (°C)*	*Average fish weight (grams)*	*pH range*	*Average alkalinity (mg/l)*	*Average hardness (mg/l)*	*Percent dissolved oxygen saturation*
Bluegill							
6/15/77	3.1-0.7	23.7	0.142	7.44-8.18	253	230	89.4
6/20/77	4.1	23.8	2.423	7.45	228	218	89.0
6/28/77	3.9-0.8	24.2	1.769	7.36-8.10	250	257	88.3
7/13/77	4.7-1.0	24.3	1.153	7.29-7.98	252	247	90.3
8/17/77	4.2-0.9	24.0	0.545	7.36-8.14	237	280	97.2
8/29/77	5.1-4.3	24.0	0.934	7.22-7.40			
1/18/78	3.1-2.6	24.4	0.380	7.58-7.69	240	362	90.0
3/7/78	5.7-3.1	24.0	1.250	7.28-7.62	250	221	86.0
Catfish							
10/5/77	5.4-0.6	22.3	4.422	7.28-8.20		263	80.0
10/24/77	5.7-0.6	22.6	4.076	7.30-8.12	252	319	84.9
11/9/77	3.0-0.5	22.4	5.150	7.72-8.25	270	284	84.7
11/30/77	1.4-0.2	22.2	5.705	8.04-8.10	273	315	83.7

and 370 blueglll with an average weight of 1.454 grams were tested at about 24°C. A total of 480 channel catfish *(letalurus punctatus)* , obtained from Fender's Fish Hatchery in Baltic, Ohio, were tested at about 22.5°C. All test specimens were conditioned to the 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 and B provide approximate mortality times for 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

The bluegill in soluble copper concentrations of 3.6 mg/1 or greater became very aggressive, as dominant individuals chased and nipped their tankmates. Such aggression occurred about every 8 minutes and lasted from 5 to 45 seconds. Afterwards, both the submissive and the aggressive fish, having flared operculums, floated at the water surface. The aggressors did not survive in the copper any longer than the submissive fish.

Except for the moments of aggression, the bluegill were very sluggish, usually hovering at the water surface. Copper precipitate coated the gills, making respiration difficult. A few fish blew bubbles.

At soluble copper concentrations below 3 mg/1, only about 15 percent of the fish hovered at the surface. Usually by the end of the bioassay, these fish did not appear stressed.

In soluble copper concentrations greater than 4.0 mg/1, bluegill ignored food. Table 5 shows the eating patterns of bluegill exposed to concentrations of soluble copper less than 4.0 mg/1.

The channel catfish were also very sluggish and exhibited respiratory problems. Many of the catfish hovered near the surface, gulping air and water. A few caudal fins hemorrhaged, and some spines became sigmoid shaped. Muscle twitches often occurred. Table 5 gives the eating patterns of the catfish exposed to soluble copper concentrations of about 1 mg/1 and less.

Most of the bluegill and catfish mortality occurred in the first 7000 minutes of each bioassay. Toward the end of each bioassay, the fish were eating better and showing fewer signs of stress. This could indicate that some of the fish were acclimating to copper. Most of the fish were observed for 3 weeks following each bioassay. Only about 3 percent mortality occurred during this time and those that died were usually the fish that still refused to eat.

Table 5. Eating Patterns of Fish Exposed to Soluble Copper of Given Concentrations (mg/1)

Time (hours)	*3.8*	*3.2*	*2.8*	*2.0*	*1.5*
Bluegill (1.45 grams)					
0-71	ignored food				
0-72		ignored food	ignored food	ignored food	ignored food
73-140		ignored food	ignored food	eating fair	eating good
141-183		nuzzled food	nuzzled food	eating good	eating as well as controls
184-336		eating poor	eating fair	eating good	
336-500		eating fair	eating fair	eating good	
	1.05	*0.70*	*0.27*	*0.22*	
Channel catfish (4.5 grams)					
0-72	ignored food	ignored food	ignored food	ignored food	
73-140	ignored food	ignored food	ignored food	nuzzled food	
141-183	ignored food	nuzzled food	ignored food	eating poor	
184-336	ignored food	eating poor	eating fair	eating good	
336-500	ignored food	eating poor	eating good	eating good	
501-560	eating poor	eating fair	eating good	eating as well as controls	

No mortalities occurred in the control tanks. The fish behaved normally, showing none of the symptoms observed for the fishes exposed to copper. 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 2 demonstrates the procedure, showing that 50 percent mortality occurred in 345 minutes at the soluble copper concentration of 4.96 mg/1. The acute toxicity curve was developed by plotting the median lethal times against the corresponding copper concentrations on log-log paper. The arrow in figure 3 indicates the point that represents the condensation or the median lethal

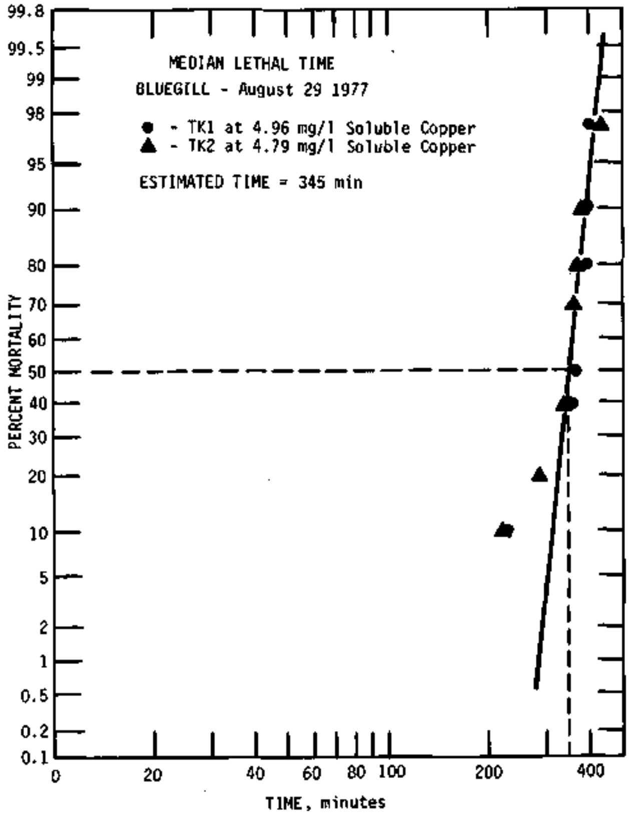

Figure 2. Percent mortality for bluegill

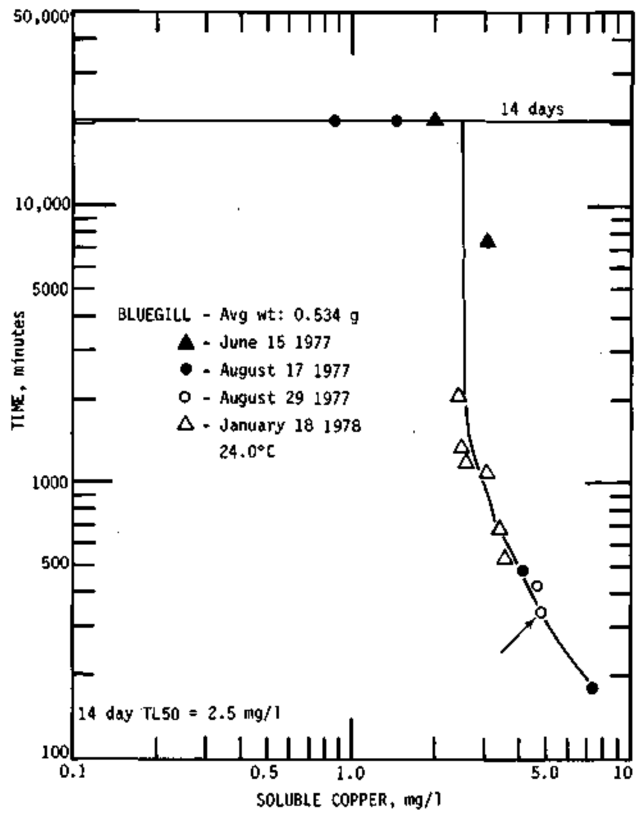

Figure 3. Acute toxicity curve for bluegill

time of figure 2. 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. Figure 3, 4, and 5 give the TL50s for this study.

When applicable, the U.S. graphical method was also used to determine the 14 day TL50.[35] 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 copper concentration. The line was drawn, and the concentration that would be lethal to 50 percent of the fish was determined. Figure 6 demonstrates the procedure. The TL50 shown is very close to the 1.2 mg/1 determined by the acute toxicity curve for channel catfish shown in figure 5; however, for a different bioassay on channel catfish, as plotted in figure 7, the results are slightly lower than depicted in figure 5.

By comparing figures 3 and 4, one can see that at about.24°C the 0.5 gram fish having TL50 of 2.5 mg/1 soluble copper are more sensitive than the larger bluegill with a TL50 of 3.7 mg/1. However, the channel catfish tested at about 22.0°C are more sensitive than either size of the bluegill. This sensitivity may be temperature dependent. Cairns et al.[36] found that at 15°C, bluegill

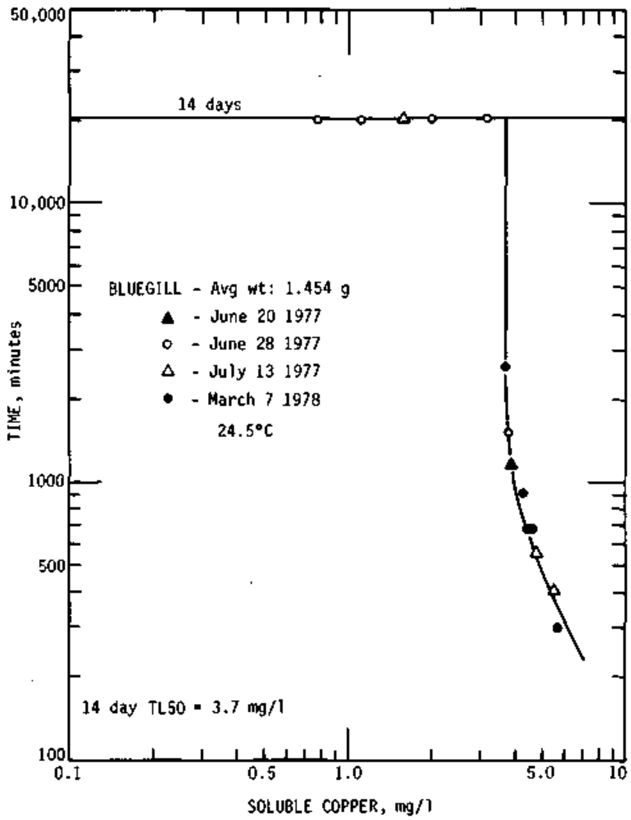

Figure 4. Acute toxicity curve for bluegill

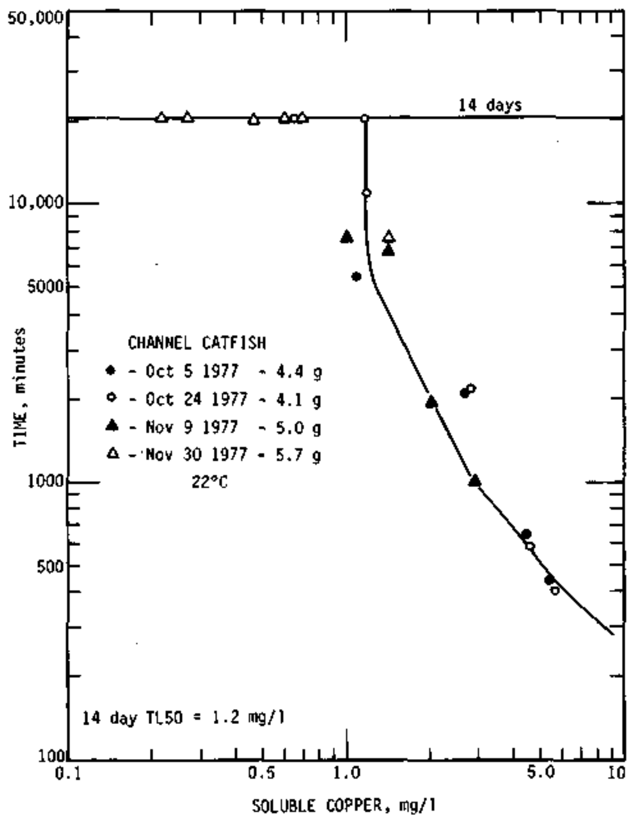

Figure 5. Acute toxicity curve for channel catfish

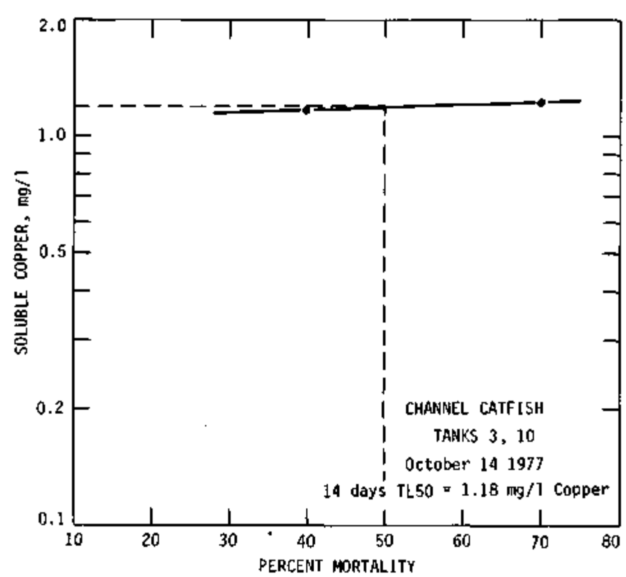

Figure 6. The 96-hour TL50 for channel catfish by U. S. Graphical method, October 14, 1977

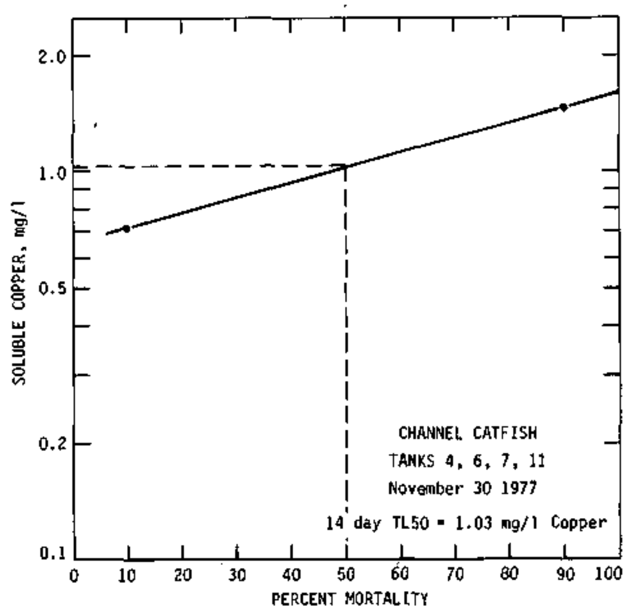

Figure 7. The 96-hour TLSO for channel catfish by U. S. Graphical method, November 30, 1977

having a 2k hour TL50 of 2.5 mg/1 were more sensitive than catfish having a TL50 of 2.6 mg/1. At 30°C the channel catfish were more sensitive than the bluegi11.

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 bluegi11 fry and channel catfish fingerlings in water with high alkalinity, the soluble copper should be no higher than 0.27 and 0.12 mg/1, respectively.

SUMMARY

- Bluegi11 and channel catfish were subjected to varying concentrations of copper 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 copper precipitated. Copper complexes also occurred in the soluble copper, reducing the cupric ion concentration.

- Acute toxicity curves were developed for each species permitting assessment for 14-day TL50.

- The 14-day TL50 at 24°C was 2.5 mg/1 soluble copper for 0.5 gram bluegill and 3.7 mg/1 soluble copper for 1.5 gram bluegill.

- In the case of the channel catfish, apparently the more sensitive species to copper, the 14-day TL50 at 22.0°C was 1.2 mg/1 soluble copper.

- For the protection of the fishes investigated and in conjugation with the water pollution regulation of Illinois, the soluble copper levels in Illinois streams having water characteristics similar to that of the dilution water used should not exceed 0.12 mg/1.

REFERENCES

1 Illinois State Water Survey. 1978. *Water quality assessment of Rend Lake and its tributaries.* Prepared for the Greater Egypt Regional Planning and Development Commission, 83 p.

2 Mancy, K. H., and H. E. Allen. 1977. *A controlled bioassay system for measuring toxicity of heavy metals.* Office of Research and Development, Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota.

3 Stiff, M. J. 1971. *The chemical states of copper in polluted fresh water and a scheme of analysis to differentiate them.* Water Research v.5(8): 585-599.

4 Andrew, R. W. , K. E. Biesinger, and G. E. Glass. 1977. *Effects of inorganic complexing on the toxicity of copper to,* Daphnia Magna. Water Research v. II(3):309-315.

5 Shaw, T. L., and V. M. Brown. 1974. *The toxicity of some forms of copper to rainbow trout.* Water Research v. 8(6):377-382.

6 Brungs, William A. 1976. *Acute and chronic toxicity of copper to the fathead minnow in a surface water of variable quality.* Water Research v. 10:37-43.

7 Pagenkopf, Gordon. 1974. *Effect of complexation on toxicity of copper to fishes.* Journal Fisheries Research Board of Canada v. 31(4):462-465-

8 Lloyd, Richard. 1975. *Factors that affect the tolerance of fish to heavy metal poisoning.* U. S. Public Health Service Publication No. 999-WP-25.

9 Sauter, S., K. S. Buxton, K. J. Macek, and S. R. Petrocelli. 1976. *Effects of exposure to heavy metals on selected fresh water fish: Toxicity of copper, cadmium, chromium and lead to eggs and fry of seven fish species.* Environmental Research Laboratory, U. S. Environmental Protection Agency, Duluth, Minnesota.

10 Sylva, R. N. 1976. *The environmental chemistry of copper (II) in aquatic systems.* Water Research v. 10:789-792.

11 Stiff, M. J. 1971. *Copper bicarbonate equilibria in solution of bicarbonate ion at concentrations similar to those found in natural water.* Water Research v. 5(5):171-176.

12 Hazel, Charles, and Stephen Meith. 1970. *Bioassay of king salmon eggs and sac fry in copper solutions.* California Fish and Game v. 56(2): 121-124.

13 Shaw, T. and V. M. Brown. 1971. *Heavy metals and the fertilization of rainbow trout eggs.* Nature v. 230, March 26.

14 Beyerle, G. B., and J. E. Williams. 1967. *Attempted control of bluegill reproduction in lakes by the application of copper sulfate crystals to spawning nests.* Progressive Fish Culturist, July, pp. 150-155.

15 Jones, J. R. E. 1947. *A further study of the reactions of fish to toxic solutions.* Journal of Experimental Biology, v. 25.

16 Timms, A. M., H. Kleerekoper, and J. Matis. 1972. *Locomotor response of goldfish, channel catfish, and largemouth bass to a 'copper polluted' mass of water in an open field.* Water Resources Research v. 8(6): 1574-1580.

17 Geckler, J. R., W. B. Horning, T. M. Neiheisel, Q. H. Pichering, and E. L. Robinson. 1976. *Validity of laboratory tests for predicting copper*

toxicity in streams. Environmental Research Laboratory, U. S. Environmental Protection Agency, Duluth, Minnesota.

18 Holland, G. A., J.E. Lasater, E. D. Neumann, and W. E. Eldridge. 1960. *Toxic effects of organic and inorganic pollutants on young salmon and trout.* Washington State Department of Fisheries, Research Bulletin No. 5.

19 Turnbull, H., J. G. DeMann, and R. M. Weston. 1954. *Toxicity of various refinery materials to fresh water fish.* Industrial and Engineering Chemistry v. 46(2):324-333.

20 Benoit, D. A. 1975. *Chronic effects of copper on survival growth and reproduction of the bluegill* (Lepomis macrochirus). Transactions American Fisheries Society v. 104(2):353-358.

21 Baker, Jeremy Y. 1969. *Histological and electron microscopical observations on copper poisoning in the winter flounder* (Pseudopleuronectes americanus). Journal Fisheries Research Board of Canada v. 26:2785-2793.

22 Drummond, R. A. 1973. *Some short-term indicators of sublethal effects of copper on brook trout*, Salvelinus fontinalis. Journal Fisheries Research Board of Canada v. 30(5):698-701.

23 Grande, M. 1967. *Effect of copper and zinc on salmonid fishes.* Proceedings International Conference on Advances in Water Pollution Research v. 1:97-111.

24 Mount, D. I. 1968. *Chronic toxicity of copper to fathead minnows* (Pimephales Promelas, Ratinesque). Water Research v. 2:215-223.

25 Gardner, G. R., and Gilles La Roche. 1973. *Copper induced lesions in estuarine telosts.* Journal Fisheries Research Board of Canada v. 30(3): 363-368.

26 O'hara, James. 1971. *Alterations in oxygen consumption by bluegills exposed to sublethal treatment with copper.* Water Research v.5(6):321-327.

27 Christensen, G. M., J. M. McKim, W. A. Brungs, and E. P. Hunt. 1972. *Changes in the blood of brown bullhead* flctalurus nebulosus (Lesuear)) *following short and long term exposure to copper (II).* Toxicology and Applied Pharmacology v. 23:417-427.

28 Saunders, R. L., and J. B. Sprague. 1967. *Effects of copper-zinc mining pollution on a spawning migration of Atlantic salmon.* Water Research v. 1:419-432.

29 Sprague, J. B., P. F. Elson, and R. L. Saunders. 1965. *Sublethal copper-zinc pollution in a salmon river - a field and laboratory study.* International Journal of Air and Water Pollution v. 9:531-543.

30 Sprague, J. B., and B. Ramsay. 1965. *Lethal levels of mixed copper-zinc solutions for juvenile salmon.* Journal Fisheries Research Board of Canada v. 22(2):425-431.

31 Lloyd, R. 1961. *The toxicity of mixtures of zinc and copper sulphates to rainbow trout (*Salmo gairdnerii Richardson). Annals of Applied Biology v. 49:535-538.

32 Brown, V. M., and R. A. Dalton. 1970. *The acute lethal toxicity to rainbow trout of mixtures of copper, phenol, zinc, and nickel.* Journal of Fish Biology v. 2.

33 Herbert, D. W. M., and J. M. Van Dyke. 1964. *The toxicity to fish to mixtures of poisons. Part II. Copper-ammonia and zinc-phenol mixtures.* Annals of Applied Biology v. 53:415-421.

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

35 Sprague, J. B. 1969- *Measurement of pollutant toxicity to fish. Part I. Bioassay methods for acute toxicity.* Water Research v. 3:793-821.

36 Cairns, J., Arthur Buikema, A. Heath, and Bruce Parker. 1978. *Effects of temperature on aquatic organism sensitivity to selected chemicals.* Center for Environmental Studies, Virginia Polytechnic Institute and State University, Bulletin 106.

37 Wilson, R. C. H. 1972. *Prediction of copper toxicity in receiving waters.* Journal Fisheries Research Board of Canada v. 29(10):1500-1502.

38 Lorz, H. W., and B. P. McPherson. 1977. *Effects of copper and zinc on smoltification of coho salmon.* Corvallis Environmental Research Laboratory, U. S. Environmental Protection Agency, Corvallis, Oregon.

39 Mount, D. I., and C. E. Stephan. 1969. *Chronic toxicity of copper to the fathead minnow (*Pinephales promelas) *in soft water.* Journal Fisheries Research Board of Canada v. 26(9).

40 Patrick, Ruth. 1968. *The relative sensitivity of diatoms, snails, and fish to 20 common constituents of industrial wastes.* Progressive Fish Culturist v. 30:137-140.

41 Eipper, Alfred W. 1959. *Effects of 5 herbicides on farm pond plants and fish.* New York Fish and Game Journal v. 6(1):46-56.

42 Trama, Francesco. 1954. *The acute toxicity of copper to the common bluegill.* Notulae Naturae No. 257:1-13.

43 Rehwoldt, R., G. Bida, and B. Nerrie. 1971. *Acute toxicity of copper, nickel and zinc ions to some Hudson River fish species.* Bulletin Environmental Contamination and Toxicology v. 6(5).

Appendix A. Observations of Percent Bluegill Mortality

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
						Tank number						
Soluble copper (mg/l)			3.1	3.1	2.0	1.9	1.05	1.06	0.70	0.76	Controls	
1300			10	10	0	10						
2830			20	20	0	10						
2950			20	30	0	10						
4250			20	40	0	20						
7150			30	40	0	20						
7500			30	50	0	20						
11,000			40	50	0	20	0	0	0	0	0	0
11,300			40	50	10	20	0	0	0	0	0	0
11,500			40	50	10	20	0	0	0	0	0	0

Date: 6/15/77
Average weight: 0.142 gram
Water temperature: 23.7°C
pH: 7.44-8.18
Alkalinity: 253 mg/l
Hardness: 230 mg/l

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
Soluble copper (mg/l)	3.9										Controls	
1060	10											
1065	40											
1160	50											
1300	60											
1360	70											
1506	80										0	0
2460	90										0	0
2465	100										0	0

Date: 6/20/77
Average weight: 2.423 grams
Water temperature: 23.8°
pH: 7.45
Alkalinity: 228 mg/l
Hardness: 218 mg/l

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
Soluble copper (mg/l)	3.8	4.0	3.2	3.2	2.0	1.9	1.1	1.1	0.76	0.78	Controls	
450	0	10										
1300	10	60	10	0								
1310	20	60	10	0								
1471	40	60	10	0								
1630	50	60	10	0								
2880	90	100	20	10								
2990	100		20	10	0	0	0	0	0	0	0	0
4330			20	30	0	0	0	0	0	0	0	0
20,160			20	30	0	0	0	0	0	0	0	0

Date: 6/28/77
Average weight: 1.769 grams
Water temperature: 24.2°C
pH range: 7.37-8.10
Alkalinity: 250 mg/l
Hardness: 257 mg/l

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
Soluble copper (mg/l)	5.4	5.5	4.7	4.8	2.7	2.8	1.5	1.6	1.03	0.93	Controls	
345	0	10										
367	0	30										
381	10	40										
409	20	50										
414	30	50										
440	60	70										
459	70	80										
475	80	100	20	10								
513	90		30	10								
575	100		40	50								
590			50	60								

Date: 7/13/77
Average weight: 1.153 grams
Water temperature: 24.3°C
pH range: 7.29-7.98
Alkalinity: 252 mg/l
Hardness: 247 mg/l

(Continued on next page)

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
						Tank number						
696			60	70						7/13/77 Continued		
700			70	100								
710			70									
1400			100		0	10	0	0	0	0	0	0
1532					0	20	0	0	0	0	0	0
1980					10	20	0	0	0	0	0	0
2880					20	20	0	0	0	0	0	0
2888					90	20	0	0	0	0	0	0
3275					100	20	0	0	0	0	0	0
15,840						20	0	0	0	0	0	0
Soluble copper (mg/l)												
	8.0	6.8			4.1	4.3	1.4	1.5	0.87	0.87	Controls	
150	10	10										
158	30	30										
165	50	30										
184	60	30										
190	60	40										
206	60	50										
209	80	60										
225	90	60										
230	90	70										
250	90	90										
295	100	90										
336		100										
388					0	10	0	0	0	0	0	0
440					10	10	0	0	0	0	0	0
490					20	20	0	0	0	0	0	0
541					40	30	0	0	0	0	0	0
563					40	50	0	0	0	0	0	0
570					60	60	0	0	0	0	0	0
600					60	80	0	0	0	0	0	0
625					70	80	0	0	0	0	0	0
675					80	80	0	0	0	0	0	0
710					90	90	0	0	0	0	0	0
1440					100	100	0	0	0	0	0	0
Soluble copper (mg/l)												
			5.0	4.8	4.6	4.1					Controls	
222			0	10								
228			10	10								
289			10	20								
345			10	40	0	10						
360			40	50	0	10						
370			50	70	0	10						
385			60	90	10	10						
390			80	90	10	10						

Date: 8/17/77
Average weight: 0.545 gram
Water temperature: 24°C
pH range: 7.36-8.14
Alkalinity: 237 mg/l
Hardness: 280 mg/l

Date: 8/29/77
Average weight: 0.934 gram
Water temperature: 24°C
pH range: 7.22-7.40

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	Tank number											
Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
Soluble copper (mg/l)			5.0	4.8	4.6	4.1					Controls 8/29/77 Continued	
405			100	90	10	10						
445				100	20	10						
491					40	10					0	0
495					70	40					0	0
505					80	40					0	0
510					80	60					0	0
530					90	60					0	0
550					100	60					0	0
560						80					0	0
600						80					0	0
1400						100					0	0

Date: 1/18/78
Average weight: 0.380 gram
Water temperature: 24.4°C
pH range: 7.58-7.69
Alkalinity: 240 mg/l
Hardness: 362 mg/l

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
Soluble copper (mg/l)	3.1	3.1	3.6	3.7	3.4	3.1	2.5	2.4	2.7	2.6	Controls	
310			10	10								
355	10	0	10	20								
380	20	0	10	30								
400	20	0	10	40								
500	20	0	30	50	10	0	0	10				
530	30	10	60	60	10	0	0	10				
585	30	20	70	60	30	0	0	10				
660	30	20	70	60	40	0	0	10				
680	30	20	80	60	50	0	10	10				
715	30	30	80	60	60	0	10	20				
765	30	40	90	80	60	10	10	20				
875	40	60	90	80	60	10	10	20	10	20		
910	50	70	100	90	70	20	10	20	10	20		
925	50	70		90	80	20	10	20	10	20		
1038	50	70		100	80	30	20	20	10	30		
1059	50	70			90	30	30	20	10	30		
1170	80	70			90	30	30	20	30	30	0	0
1191	90	80			90	30	30	20	30	30	0	0
1210	90	80			90	50	40	20	40	30	0	0
1269	90	100			90	90	50	20	50	40	0	0
1294	90				100	90	60	20	50	40	0	0
1307	100					90	60	20	60	40	0	0
1450						100	70	30	60	40	0	0
1615							70	40	60	40	0	0
2750							90	60	60	60	0	0
3100							90	70	60	60	0	0
17,650							90	70	70	60	0	0
20,160							90	70	70	60	0	0

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Appendix A. Concluded

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
						Tank number						
Soluble copper (mg/l)	5.8	6.7	4.3	4.5	4.7	4.7	3.4	3.4	3.8	3.6	Controls	
177	10	0										
268	10	10										
280	20	10										
285	40	10										
310	50	20										
315	60	40										
329	60	50										
338	70	60										
340	90	70										
390	90	80										
450	100	100										
550					0	20						
560					10	30						
600			0	10	20	40						
605			0	40	20	40						
650			0	40	20	60						
660			0	50	30	70						
690			10	60	40	70						
720			20	60	50	80			10	0		
760			30	60	60	90			10	0		
780			30	60	70	90			10	0		
840			40	60	70	90			10	0		
900			40	60	70	100			10	0	0	0
960			50	70	70				10	10	0	0
990			60	80	70				10	10	0	0
1050			70	80	70				10	10	0	0
1140			90	80	70				10	10	0	0
1230			90	90	70				10	10	0	0
1480			90	100	80		0	10	20	20	0	0
2880			100		100		10	30	70	60	0	0
4400							10	40	80	70	0	0
10,450							70	60	100	100	0	0
11,450							90	90			0	0

Date: 3/7/78
Average weight: 1.25 grams
Water temperature: 24.0°C
pH range: 7.28-7.62
Alkalinity: 250 mg/l
Hardness: 221 mg/l

Appendix B. Observations of Percent Channel Catfish Mortality

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
Soluble copper (mg/l)	5.5	5.4	4.5	4.4	2.7	2.7	1.1	1.1	0.64	0.63	Controls	
351	10	0										
375	30	0										
428	50	30										
495	70	80										
540	90	100	10	20								
600	90	100	30	30								
700	100	100	70	70								
750			80	70								
1400			100	100	0	0						
1760					30	10						
1870					30	20						
2880					80	50	10	0				
3260					90	90	20	30				
4260					100	100	30	40				
6000							50	60	10	20	0	0
7100							50	70	10	30	0	0
8600							60	70	20	30	0	0
9100							60	80	20	30	0	0
10,400							70	80	20	30	0	0
11,400							70	80	20	40	0	0
14,400							80	90	20	40	0	0
16,000							80	90	30	40	0	0
17,300							80	90	40	40	0	0
20,160							80	90	40	40	0	0

Date: 10/5/77
Average weight: 4.422 grams
Water temperature: 22.3°C
pH range: 7.28-8.20
Hardness: 263 mg/l

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
Soluble copper (mg/l)	5.7	5.7	4.3	4.7	2.9	2.7	1.23	1.17	0.67	0.63	Controls	
308	10	0										
341	20	0										
365	40	10										
400	40	30										
425	60	40										
435	80	50										
490	90	70	20	20								
540	100	90	40	30								
655		100	60	50								
788			90	80								
1500			100	100	10	30						
1690					10	40						
1850					30	50						
2300					50	60						
2900					80	80						
3200					90	80						

Date: 10/24/77
Average weight: 4.076 grams
Water temperature: 22.6°C
pH range: 7.30-8.12
Alkalinity: 252 mg/l
Hardness: 319 mg/l

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Appendix B. Concluded

Time (minutes)	7	11	1	2	4	6	3	10	5	8	9	12
						Tank number						
Soluble copper (mg/l)	5.7	5.7	4.3	4.7	2.9	2.7	1.23	1.17	0.67	0.63	Controls	
4300					90	90	10	0				
5800					90	90	10	10	20	0	0	0
9000					100	90	20	10	30	0	0	0
10,800						90	30	20	30	0	0	0
11,700						90	60	20	40	0	0	0
13,000						100	70	30	50	0	0	0
14,960							70	30	60	10	0	0
15,100							70	40	60	10	0	0
20,160							70	40	60	10	0	0
Soluble copper (mg/l)		2.9	2.0	2.1	1.4	1.5	0.69	0.67	0.46	0.47	Controls	
1300		10	10	10								
1310		70	40	20								
1500		80	50	20								
1590		80	60	30								
1860		90	60	30								
2700		100	60	50								
4290			80	60								
5990			80	70	30	40						
7180			80	70	50	60				10	0	0
8600			80	70	50	70	10	20		10	0	0
8700			80	70	50	70	30	20		10	0	0
10,000			80	70	60	70	40	20	10	10	0	0
10,600			80	70	70	70	40	20	10	10	0	0
11,800			80	70	80	80	50	20	10	10	0	0
12,100			80	70	90	80	60	20	10	10	0	0
13,000			90	70	90	80	60	20	10	20	0	0
16,100			100	70	90	80	60	20	10	20	0	0
17,300				70	100	80	60	20	10	20	0	0
20,160				70		80	60	20	10	20	0	0
Soluble copper (mg/l)	1.5	1.4	1.05	1.03	0.70	0.73	0.27	0.27	0.22	0.22	Controls	
4220	10	0	10	10								
5900	70	20	40	30	10	10						
7100	80	40	50	50	10	10						
7400	90	40	60	60	10	10						
8700	90	50	60	60	10	10						
10,000	90	50	70	60	10	10	0	0	0	0	0	0
13,200	90	60	70	60	10	10	0	0	0	0	0	0
16,000	90	70	70	60	10	10	0	0	0	0	0	0
17,200	90	80	70	70	10	10	0	0	0	0	0	0
18,900	90	90	70	70	10	10	0	0	0	0	0	0
19,100	90	90	80	70	10	10	0	0	0	0	0	0
20,160	90	90	80	70	10	10	0	0	0	0	0	0

10/24/77 Continued

Date: 11/9/77
Average weight: 5.150 grams
Water temperature: 22.4°C
pH range: 7.72-8.25
Alkalinity: 270 mg/l
Hardness: 284 mg/l

Date: 11/30/77
Average weight: 5.705 grams
Water temperature: 22.2°C
pH range: 8.04-8.10
Alkalinity: 273 mg/l
Hardness: 315 mg/l