Water Pollution Studies

Federal Aid Project F-243R-1

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Federal Aid in Fish and Wildlife Restoration

Job Progress Report

Colorado Division of Wildlife

Fish Research Section

Fort Collins, Colorado

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JOB PROGRESS REPORT

State: Colorado

Project No. F243R-1

Title: Water Pollution Studies

Period Covered: July 1, 1993 to June 30, 1994

Principal Investigator: Patrick H. Davies
Co-investigator: Stephen F. Brinkman

Objective: To develop quantitative chemical and toxicological data on the toxicity of pollutants to aquatic life, investigate water pollution problems in the field, and provide expertise in aquatic chemistry and aquatic toxicology.

STUDY PLAN 1: REGULATORY AND LEGAL ACTIVITIES

Objective: To provide technical assistance to regulatory and legal entities toward the development, implementation, and enforcement of water quality standards needed to protect or enhance the aquatic resources of Colorado.

Job 1. Water quality standards for the protection of aquatic life in Colorado

Job Objectives:

1. To apply research results and toxicological information from literature toward the development, enactment, and implementation of water quality standards and appropriate aquatic life use classifications.

2. To provide technical information and/or expert testimony in aquatic toxicology and aquatic chemistry in agency meetings, regulatory hearings, and/or court litigations as needed to protect aquatic resources of Colorado.

3. To develop or compile toxicological and chemical data on toxicants for which state or federal governments have not developed a standard.

ACCOMPLISHMENTS

CHRONIC SILVER STANDARD FOR AQUATIC LIFE

At the request of the Colorado Water Quality Control Division (WQCD), Davies prepared and submitted rebuttal testimony, in opposition to a petition by the national silver coalition to eliminate the chronic silver standard in Colorado. The Water Quality Control Commission has scheduled a hearing to consider this petition on July 11, 1994.

STUDY PLAN 2: LABORATORY STUDIES

Objective: To research and develop information on, or analytical tools in, aquatic chemistry and aquatic toxicology to better assess toxic responses of pollutants to aquatic life in laboratory and natural waters, such as the Arkansas River.

Job 1. Chemical Equilibria and Kinetic Effects on the Bioavailability and Toxicity of Metals to Aquatic Life

Job Objective: To develop analytical methods using Ion Chromatography, ion separation and/or ultrafiltration to measure toxic fractions and effects of chemical kinetics on toxicity of zinc, copper, lead, cadmium and/or silver to *Ceriodaphnia dubia*, rainbow trout, brown trout and/or fathead minnows in waters of different complexing capacity. Concurrently, investigate effects of chemical kinetics on results obtained from toxicity tests.

Job 2. Use of Biochemical Methods to Measure Disruption of Ion Regulation and Stress in Aquatic Organisms Exposed to Metals

Job Objective: To develop biochemical methods to measure effects on enzyme systems using electrophoresis or other methods to assess stress in rainbow and brown trout exposed to zinc, copper, lead and/or cadmium.

Job 3. Investigations on the Toxicity of Silver to Aquatic Organisms in Waters Different Complexing Capacity

Job Objective: To develop acceptable toxicant concentrations of silver for rainbow trout, brown trout, and/or fathead minnows in hard, high alkaline, and soft, low alkaline waters.

Job 4. Effects of Calcium Hardness, Inorganic and Organic Ligands and Sediments on Toxicity of Metals to Aquatic Organisms

Job Objective: To determine antagonistic effects of calcium hardness in low alkaline waters and the effects of specific inorganic and organic ligands and sediments on acute and long-term toxicity of zinc, copper, lead, cadmium, and/or silver to rainbow trout, brown trout and/or fathead minnows.

Job 5. Investigations on Enhanced Toxicity of Unionized Ammonia to Fish at Cold Water Temperatures

Job Objective: To determine effects of temperature on toxicity of unionized ammonia to rainbow trout and fathead minnows or other warmwater species at optimal and less than 5° C water temperatures.

Job 6. Effects of Episodic Exposure on Toxicity and Sensitivity of Aquatic Life to Intermittent Exposure to Metals

Job Objective: To determine toxic effects and organism sensitivity to intermittent exposure of zinc, copper, lead, and/or cadmium to rainbow trout, brown trout and/or fathead minnows, and their ability to acquire and/or lose tolerance.

Job 7. Investigations on Enhanced Toxicity of Water-Borne Metals to Aquatic Life Exposed to Dietary Sources of Metals

Job Objective: To determine effects of water-borne zinc, copper, cadmium, lead and/or manganese on their toxicity to rainbow and brown trout following and/or concurrent with exposure to dietary metals.

Job 8. Investigations on Effects and Interactions of Multiple Metal Exposure on Toxicity to Aquatic Life

Job Objective: To determine effects of exposure of rainbow trout and/or brown trout to zinc, copper, cadmium, lead, and manganese at different combinations found in Colorado's mining areas. Will require an ability to measure bioavailable forms on metals as outlined in Study Plan 2, Job1.ob B1.

Job 9. Investigations of Analytical Methods to Measure Rotenone Concentrations in the Field and Antimycin Concentrations in the Laboratory

Job Objective: To develop a field method to measure rotenone and a laboratory method for measuring antimycin at concentrations less than 5 ppb using cation exchange chromatography.

ACCOMPLISHMENTS

Acute and Chronic Toxicity of Lead to Brown Trout in Soft Water

METHODS

Thirty fingerling brown trout (*Salmo trutta*) were exposed to seven concentrations of lead (Pb) for a period of 7 months using modified, flow-through, proportional diluters (Mount and Brungs, 1967). Soft, low alkaline, dechlorinated city water with an alkalinity and hardness of 39.7 and 37.1 mg/liter, respectively, supplied the diluters. Lead, as $PbNO_3$, was delivered at a flow rate of two liters per 3-minute cycle to each of seven aquaria at nominal concentrations of 200, 150, 112, 64, 36, 20 and 0 μg Pb/liter. We also exposed ten rainbow trout for a period of 6 months to Pb concentrations of 112, 64 and 36 μg/liter in baskets suspended in the same aquaria used for brown trout to compare toxicity results with that found previously in a slightly softer, less alkaline water (Davies *et al.*, 1976).

We collected water samples from each aquarium daily for lead analysis. Seven-day aliquots of 5 mls were stored in 60 ml high density polyethylene (HDPE) bottles acidified with 10 drops of high purity (Ultrex) nitric acid. We analyzed Pb concentrations weekly using a Thermo Jarrell Ash, Series 4000 Atomic Absorption Spectrophotometer (AA) with Smith-Hieftje background correction and a Model 188 Controlled Temperature Furnace Atomizer (CTF). Alkalinity, hardness, pH, conductivity, temperature and dissolved oxygen were measured weekly in all aquaria (APHA, 1985). During experiments, we inspected aquaria daily for dead fish from which lengths and weights were collected. Feeding rates were adjusted monthly (Piper *et al.*, 1982). After 7 months of exposure, length and weight were recorded from survivors anesthetized with Metomidate.

We combined analytical and mortality data since Pb concentrations from replicate aquaria were not significantly different ($P > 0.05$). We also calculated grand means for water quality characteristics. Chronic values were calculated as the geometric mean of no-effect/effect concentrations.

Following termination of the chronic lead toxicity test, brown and rainbow trout were immediately frozen whole. These fish were later thawed and dissected using stainless steel instruments. The kidney, liver, gill arches, and opercular bones were weighed in polypropylene test tubes and dried to constant weight in a drying oven at 80° C. One half milliliter of trace metal grade nitric acid was added to each tube which was then predigested for 24 hours. Test tubes were heated at 80° C in a constant temperature water bath for four hours after which one half milliliter of trace metal grade hydrogen peroxide was added. Tubes were heated an additional four hours then diluted to a final volume of five milliliters with deionized water. System blanks were included in all steps. Lead concentrations were determined using an air-acetylene flame on an Instrumentation Laboratory Video 22 atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

The no-effect/effect concentrations based on mortality of brown and rainbow trout exposed to Pb in water with an alkalinity of 40 mg/liter was between 60 and 105 µg/liter giving a chronic value of 79 µg/liter (Table 1). However, rainbow trout were more sensitive to the toxic effects of Pb at a given concentration. For example, at 105 µg/liter 80% of rainbow trout died whereas only 42% of brown trout died (Table 1). Pb toxicity to fish is greatly influenced by alkalinity. Alkalinity is a measure of the bicarbonate and carbonate concentrations. Bicarbonates in natural waters quickly cause complexation and precipitation of Pb thus significantly reducing its toxicity to aquatic life. For comparison, the no-effect/effect concentration for rainbow trout exposed to Pb in water of 26 mg/liter alkalinity was between 7.2 and 14.6 µg/liter or a chronic value of 10.2 µg/liter (Davies *et al.*, 1976). Consequently, with only a 1.5 factor increase in alkalinity, 26 versus 40 mg/liter, we found an eight fold increase in the chronic value, 10 versus 79 µg Pb/liter, for rainbow trout.

Table 1. Analytical and mortality data from combined replicate, long-term exposure of brown and rainbow trout to lead.

Pb μg/L	198	140	105	60	34	19	0.5
Brown Trout							
No. Dead	59	54	25	2	2	0	1
N	59	59	60	57	56	60	59
% Mort.	100	91.5	41.7	3.5	3.6	0.0	1.7
				^			
Rainbow Trout							
No. Dead			16	1	0		
N			20	18	19		
% Mort.			80.0	5.5	0.0		
				^			

^ no-effect/effect concentrations based on mortality.

A significant number of rainbow trout exposed to Pb developed blacktails, spinal abnormalities (lordoscoliosis), eroded caudal fins and paralysis from neurological damage, as was reported in earlier studies (Davies *et al.*, 1976). For example in the current study, rainbow trout exhibited these abnormalities in 100% and 53% of the fish exposed to 60 and 34 μg/liter, respectively. Only 5% of the brown trout exposed to 60 μg/liter demonstrated these symptoms. No sublethal effects occurred at 34 μg/liter. Consequently, the no-effect/effect concentration based on neurological effects would lie between 34 and 60 μg/liter with a chronic value of 45 μg/liter as compared to 79 μg/liter based on mortality.

Adult brook trout (*Salvelinus fontinalis*) exposed to 475 μg Pb/liter for 38 weeks in a water with an alkalinity and hardness of 43 and 44 mg/liter, respectively, showed no adverse effects (Holcombe et al., 1976). All second generation brook trout exposed to this concentration of Pb died before reaching sexual maturity. Second generation fish exposed to 235 μg Pb/liter were severely deformed (scoliosis). At 119 μg Pb/liter no effects on growth or survival were observed, but 34% of the fish had scoliosis and blacktails. Scoliosis and blacktails were not manifested in fish exposed to 58 μg Pb/liter or less (Holcombe et al. 1976). Consequently, corresponding chronic values for brook trout based on mortality and spinal abnormalities would be 162 and 83 μg Pb/liter, respectively, compared to 79 and 45 μg/liter for brown trout at a similar alkalinity and hardness.

Statistical data for Pb exposure concentrations and corresponding mortality of brown trout for the replicate experiments are reported in Tables 2 and 3. Water quality characteristics are given for each replicate (Tables 4 and 5).

Lead tissue content of the kidney, liver, gill arches and opercular bone are plotted against lead exposure concentration for brown and rainbow trout in Figures 1 and 2 respectively. As expected, increasing exposure concentrations of lead resulted in increasing tissue levels. Regression analyses were performed to determine the relationships of exposure concentration to lead concentrations in various tissues. These results are presented in Table 6. Regressions were performed on untransformed data with the exception of log transformation of lead content in bone of rainbow trout.

Rainbow trout accumulated lead to the greatest extent in the kidney followed by bone, gill then liver tissue. These results agree very well with previous studies of lead on rainbow trout (Goettl and Davies 1979) and brook trout (Holcombe et al. 1976). Brown trout exhibited similar accumulations of lead in bone, gill and liver tissue as rainbow trout. However, accumulation of lead in kidney tissue was significantly lower in brown trout than rainbow trout ($p < 0.01$).

Table 2. Pb concentrations, statistics and brown trout mortality for replicate A.

	TANK 1	TANK 2	TANK 3	TANK 4	TANK 5	TANK 6	CONTROL
MEAN	201.4	144.7	105.5	60.5	33.9	19.0	0.7
STD DEV	18.3	25.3	11.4	8.5	6.3	3.7	1.2
N	17	31	31	31	31	30	31
% MORT	100	93.3	30.0	3.7	3.6	0.0	3.3

Table 3. Pb concentrations, statistics and brown trout mortality for replicate B.

	TANK 1	TANK 2	TANK 3	TANK 4	TANK 5	TANK 6	CONTROL
MEAN	194.6	135.5	104.0	58.9	34.5	18.8	0.4
STD DEV	24.9	18.0	15.1	9.5	7.0	4.7	0.8
N	21	31	31	31	31	31	31
% MORT	100	90.0	53.3	3.3	3.6	0.0	0.0

Table 4. Water quality characteristics for replicate A.

	Alkalinity mg/L	Hardness mg/L	pH	D.O. mg/L	TEMP. °C	COND. µS/cm
MEAN	39.5	37.1	7.39	7.89	14.1	84.7
STD DEV	3.0	4.3	0.20	0.93	2.3	3.2
N	160	160	160	160	160	160

Table 5. Water quality characteristics for replicate B.

	Alkalinity mg/L	Hardness mg/L	pH	D.O. mg/L	TEMP. °C	COND. µS/cm
MEAN	40.0	37.1	7.41	7.95	13.9	86.6
STD DEV	3.0	4.8	0.18	0.93	2.2	3.3
N	152	152	152	152	152	152

Table 6. Linear regression parameters of Pb content of various tissues versus exposure concentration.

SPECIES	TISSUE	p	R^2
Brown Trout	Bone	<0.0001	0.851
Brown Trout	Gill	<0.0001	0.741
Brown Trout	Kidney	<0.0001	0.723
Brown Trout	Liver	<0.0001	0.691
Rainbow Trout	Bone	<0.0113	0.269
Rainbow Trout	Gill	<0.0003	0.469
Rainbow Trout	Kidney	<0.0007	0.426
Rainbow Trout	Liver	<0.0001	0.521

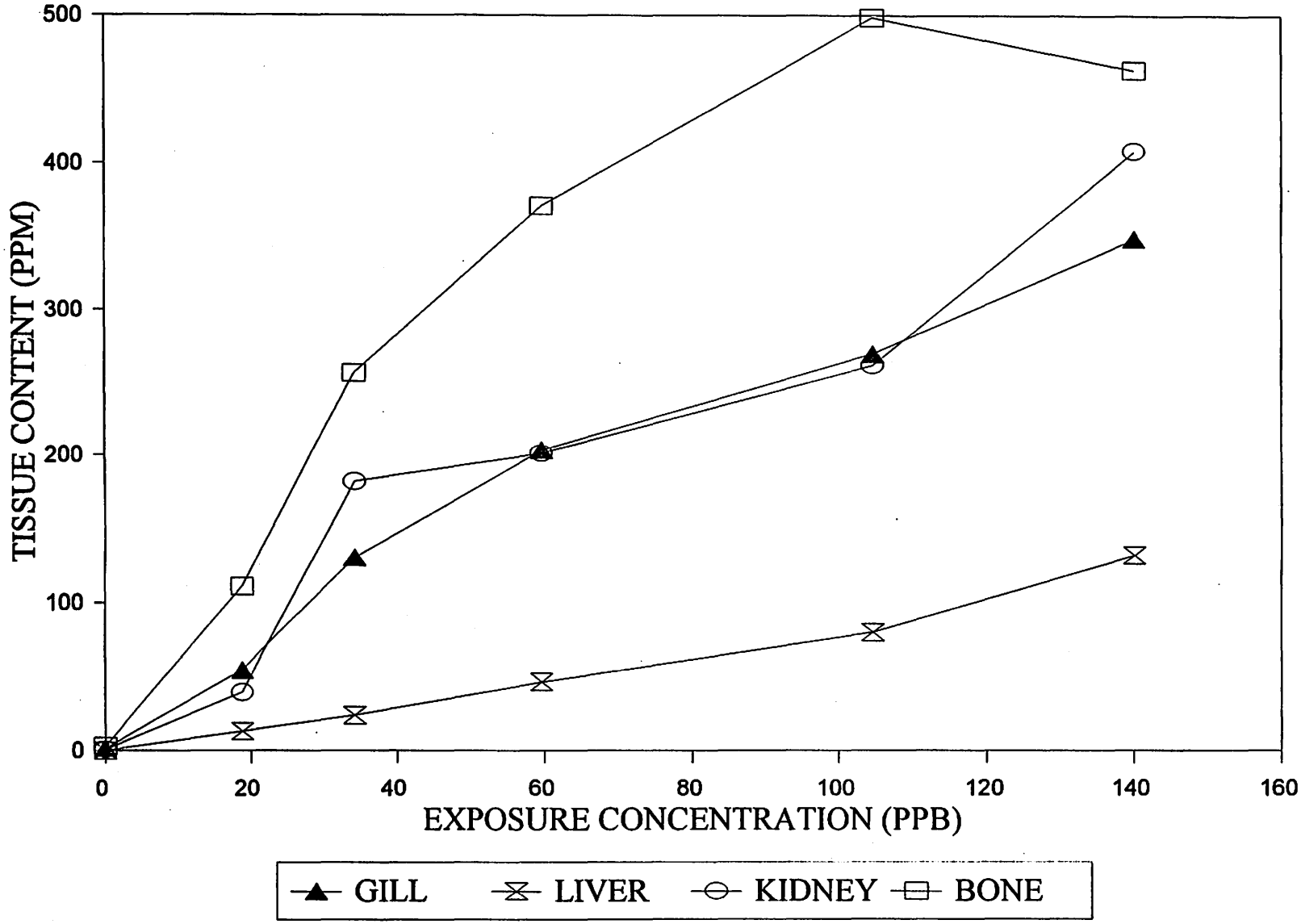

Figure 1. Lead content in tissues of brown trout exposed to different concentrations of lead.

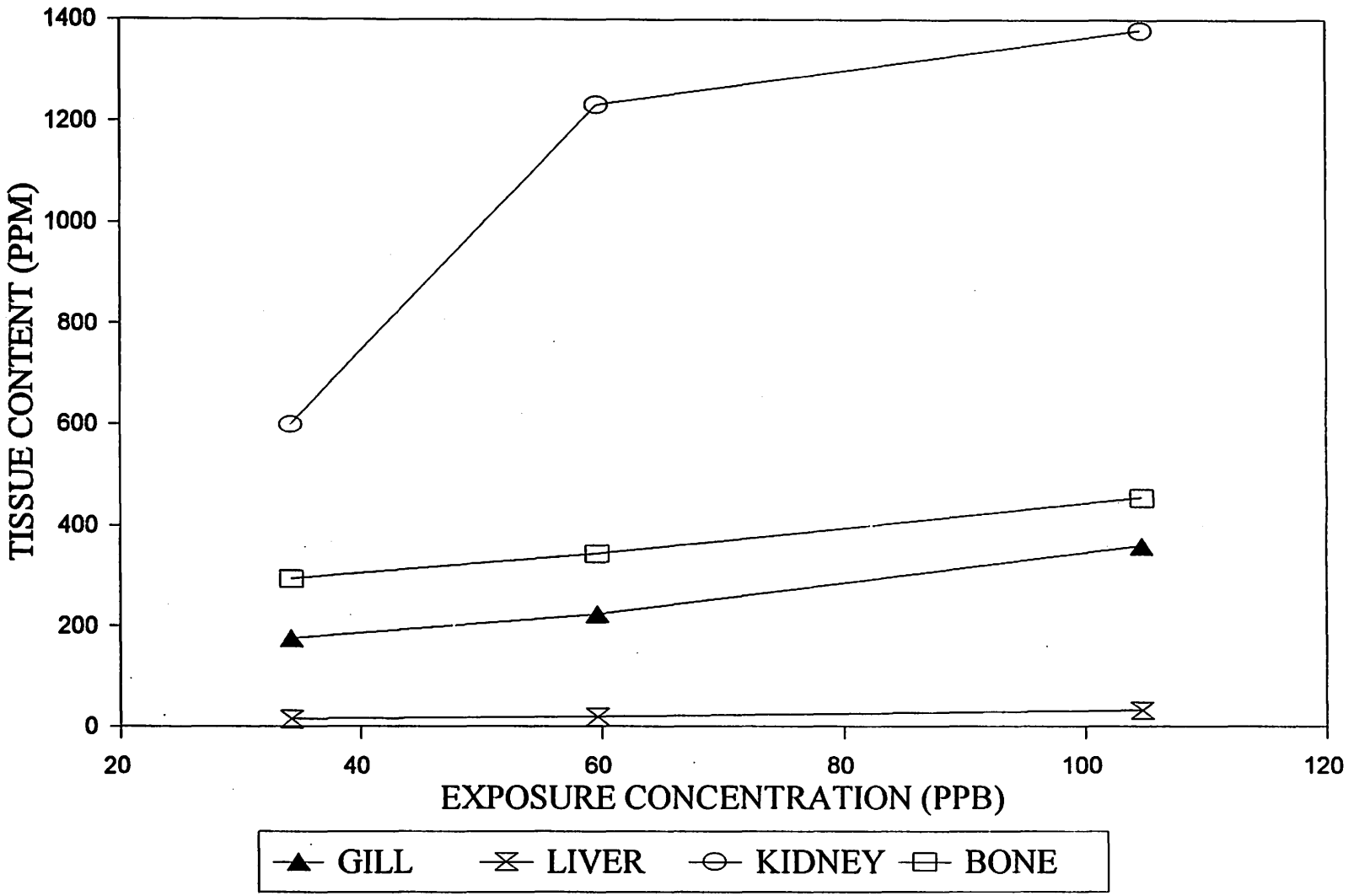

Figure 2. Lead content in tissues of rainbow trout exposed to different concentrations of lead.

Toxicity of Copper Sulfate and Cutrine to Rainbow Trout under Acute, Chronic, and Episodic Exposures

ABSTRACT

Following a fish kill on the Big Thompson River above Loveland, Colorado in 1992, concerns were raised regarding Northern Colorado Water Conservancy District's use of copper sulfate to control algae in irrigation canals. A series of experiments were performed to investigate the toxicity of copper (Cu) to rainbow trout in very soft, low alkaline water similar to the Big Thompson River under different conditions of exposure. Information was lacking on: 1) Toxicity of copper to rainbow trout exposed for only a brief period of time, e.g. 30 minutes; 2) Effect of increasing exposure time from 30 to 60 minutes on toxicity; 3) Toxicity of Cu in very soft, low alkaline water of 10 mg/liter alkalinity and hardness; 4) Differences in toxicity of Cu from continuous versus weekly, episodic exposures; and 5) Possible use of Cutrine as a less toxic alternative to copper sulfate.

The most important factor controlling the short-term toxicity of Cu is length of exposure. Following exposure of rainbow trout to 40 mg Cu/liter for 30, 45, and 60 minutes, 110, 5.6 and 1.3 hours were required to achieve 100 percent mortality. The 96-hr LC50, following exposure of rainbow trout to copper sulfate for 30 minutes was 25.8 mg Cu/liter compared to 1.93 mg/liter for Cutrine following the recommended 3 hour exposure period. Ninety-six hour LC50s from continuous exposure of rainbow trout to copper sulfate and Cutrine were 4.92 and 7.20 μg Cu/liter.

Repeated episodic exposure of rainbow trout to copper sulfate and Cutrine resulted in increased mortality through six, weekly episodic events. Mortality essentially ceased after six episodes. Effect/no effect concentrations for rainbow trout exposed to copper sulfate for 30 minutes for eighteen weeks were 2.14 and 1.03 mg Cu/liter, giving a chronic value of 1.48 mg/liter. The effect/no effect concentration following weekly exposure episodes of 3 hours to Cutrine were 0.26 and (an estimated) 0.1 mg/liter for a chronic value of 0.16 mg Cu/liter. This demonstrates that copper sulfate is a much safer alternative than Cutrine for controlling algae in soft, low alkaline waters. Chronic values for rainbow trout continuously exposed to copper sulfate and Cutrine were 1.77 and 2.47 μg Cu/liter. This also shows how extremely toxic Cu is in low alkaline waters.

INTRODUCTION

Following a fish kill in the Big Thompson River above Loveland, Colorado on August 27, 1992, concerns were raised about the use of copper sulfate by the Northern Colorado Water Conservancy District to control algae in the Charles Hanson Feeder Canal. Investigations at the time of the fish kill failed to establish the cause and suggested that copper (Cu) was not responsible. However, information was lacking on: (1) Concentrations of Cu and its duration in waters of the canal and Big Thompson River resulting from the District's treatment with copper sulfate, (2) Toxicity of copper to fish exposed for only a short period of time, (3) Toxicity of Cu in waters of very low alkalinity and hardness, i.e. about 10 mg/liter, which is characteristic of the canal and Big Thompson during spring runoff and summer conditions, (4) Toxicity of Cu from episodic exposures characterized by the District's weekly application of copper sulfate, and (5) Information on the use of "Cutrine" as a possible, less toxic, alternative to copper sulfate for controlling algae.

Much to the credit of employees and management of the Northern Colorado Conservancy District, they commissioned research by Colorado Division of Wildlife (DOW) and Colorado State

University (CSU). This research was to evaluate their use of copper sulfate to control algae in irrigation canals and its possible impacts on fish of the Big Thompson River. Information was to be developed on the toxicity of Cu to fish following repeated, short periods of exposure at water quality conditions characteristic of the Hanson Canal.

This is a report on findings from research conducted at the DOW Aquatic Toxicology Laboratory in Fort Collins, Colorado on acute, episodic, and long-term toxicity of Cu, as copper sulfate and Cutrine, to rainbow trout (*Oncorhynchus mykiss*) in soft, low alkaline waters.

MATERIALS AND METHODS

Copper Exposure and Water Quality Characteristics

Data collected from the Charles Hanson Canal and Big Thompson River established that following the addition of copper sulfate to the canal below Flatiron Reservoir Cu concentrations followed a typical bell-shaped curve (Figure 3). Elevated concentrations of Cu at any particular point along the canal or Big Thompson River did not to exceed 30 minutes. Measurements of important water quality characteristics in the Charles Hanson Canal and Big Thompson River revealed very soft, low alkaline, and low ionic strength waters during spring runoff and summer flow conditions. Alkalinity and hardness were typically about 10 mg/liter, as calcium carbonate, with pH and conductivity between 6.5 to 7.0, and 2.0 to 2.5 mS/m, respectively. Similar water quality characteristics were created for laboratory experiments by mixing Fort Collins city water obtained from the Cache La Poudre River with reverse osmosis (RO) water under constant flowing conditions. RO water, Continental Water Systems, Model ROSLV1060, was provided on demand at a flow rate of up to 10 gpm. The two waters were mixed to desired water quality characteristics at valves supplying water to proportional diluters (Mount and Brungs 1967) which delivered six different Cu concentrations and control water containing no Cu. Colorado River strain, rainbow trout were acclimated to this low alkaline water for about 3 weeks prior to initiating experiments.

Information on Copper Sulfate and Cutrine Used in Experiments

Copper sulfate used in experiments was 99.0 % copper sulfate pentahydrate with 1.0 % inert ingredients. This was the same product used by the District in its algae control operations.

"Cutrine-Plus" (Copper Ethanolamine complexes) is a chelated form of copper that is frequently used as an alternative to copper sulfate to control algae because it is thought to be less toxic. In theory Cu is chemically bound to ethanolamine and is not free or biologically available to fish. However, the manufacturer warns that the product should not be used in water containing fish if the hardness does not exceed 50 ppm. This suggests that in low alkaline waters Cu in Cutrine will become free or bioavailable, i.e. toxic to fish. We conducted experiments on the toxicity of Cutrine to rainbow trout to compare its toxicity to that of copper sulfate in a water of 10 ppm alkalinity and hardness. One of the principal differences in the use of Cutrine is that a contact time of three hours is recommended to effectively control algae compared to about 30 minutes for copper sulfate. The liquid formulation of Cutrine-Plus used in our experiments contained 0.909 lbs of elemental Cu per gallon.

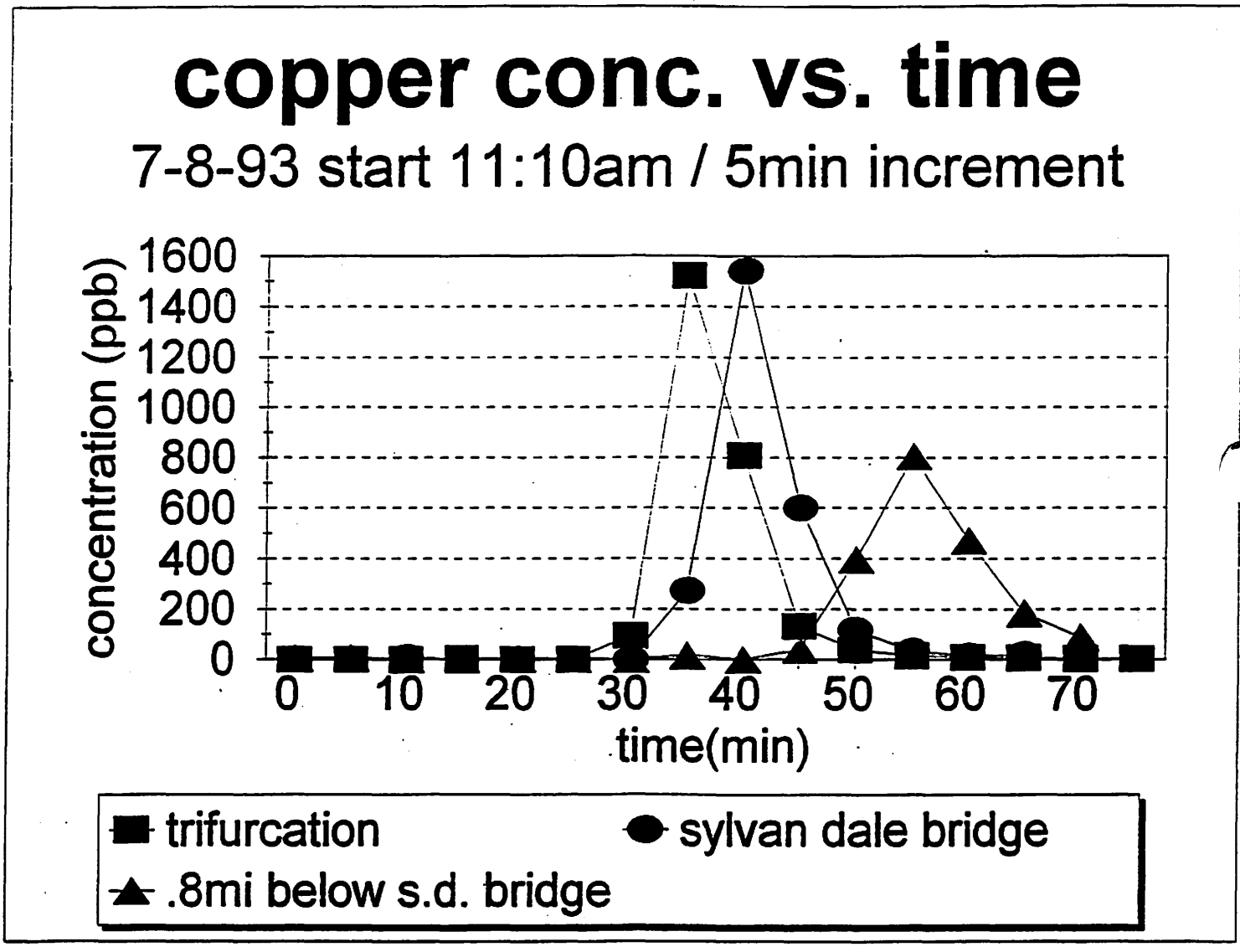

Figure 3. Duration and concentration of copper in the Charles Hanson Feeder Canal and Big Thompson River following the application of copper sulfate for algae control.

Range Finding and Acute Toxicity with Copper Sulfate

A series of experiments were conducted to determine toxicity of Cu to kill rainbow trout over exposure periods of 30 minutes in water of 9.6 mg/liter - alkalinity, 9.0 mg/liter - hardness, pH - 6.9, conductivity of 2.0 mS/m, temperature - 13.3° C, and dissolved oxygen - 7.4 mg/liter. Ten rainbow trout, averaging 60 mm total length and weighing 2.2 g, were placed in 2 liter beakers containing Cu concentrations of 573, 295, 147, 77, and 34 mg/liter. The beakers were aerated during the 30 minute exposure period after which fish were placed into 60 liter aquaria containing intermittent flowing water of the same water quality characteristics with no added copper. Fish deaths over the next 5 days were variable, but all fish exposed to 34 mg/liter died within 126-hr. This defined the upper concentration of for determining a 96-hr LC50 and testing the effect of weekly 30 minute episodic exposures on rainbow trout survival.

Under the same water quality conditions and following the same procedures, we evaluated effects of increased exposure time on Cu toxicity. Ten rainbow trout were exposed to copper sulfate at a nominal concentration of 40 mg Cu/liter for 45 and 60 minutes. Observations were made hourly to evaluate effects of increased exposure time on the death rate of rainbow trout.

Acute and Chronic Toxicity of Copper Sulfate and Cutrine During Episodic Exposures

Twenty rainbow trout acclimated to low alkaline water were placed into 92-liter aquaria. Low alkaline water with no Cu added flowed intermittently from a proportional diluter. Fish were netted weekly and placed into 9.5-liter buckets containing 5 liters of aerated Cu solutions. Exposure solutions were of similar water quality characteristics as maintenance aquaria. The buckets were placed into aquaria with flowing water to maintain temperature. Fish were exposed to copper sulfate at nominal concentrations of 20, 10, 5, 2, 1, and 0.5 mg Cu/liter for 30 minutes. Similar procedures were followed with Cutrine except exposure was for three hours at nominal concentrations of 10, 5, 2, 1, 0.4, 0.2 and 0.0 mg Cu/liter. Following exposure rainbow trout were immediately placed back into appropriate aquaria containing no Cu. Observations were made daily for dead fish. Mortality data collected during 96-hr following the first episodic exposure were used to calculate a LC50. The LC50 refers to the lethal concentration of Cu that kills 50 percent of the fish within 96-hr following episodic exposure. Water samples were collected for measuring Cu concentrations and water quality characteristics during each episodic event. The experiments were terminated after 18 weeks of episodic exposure. Chronic values i.e., those concentrations presumed to have no adverse effect, were calculated as the geometric mean of effect/no effect concentrations. The probit method was used to calculate 96-hr LC50 concentrations (Finney 1971).

Acute and Chronic Toxicity of Copper Sulfate and Cutrine During Continuous Exposure

A modified Mount and Brungs (1967) proportional diluter intermittently delivered Cu concentrations to 92-liter aquaria containing 20 rainbow trout approximately 60 mm in length. During the first set of acute tests, Cu (as copper sulfate) was added at nominal concentrations of 50, 37.5, 28, 16, 9, 5, and 0 μg Cu/liter. Nominal concentration of Cutrine was twice this level of Cu. Two liters of Cu solutions were delivered to each aquarium every 15 minutes. Acute data were also collected during the first 96-hr of chronic tests on copper sulfate and Cutrine. In both experiments, nominal concentrations of exposure were 20, 15, 11.2, 6.4, 3.6, 2.0 and 0.0 μg Cu/liter. After two weeks a seventh level of exposure at a nominal concentration of 1.0 μg/liter was added to both experiments following death of all fish at 20 and 15 μg/liter. Mortality data collected during the first

96-hr of the experiment were used to calculate LC50 concentrations using the probit method (Finney 1971). Water samples consisting of 5 ml aliquots were collected daily and pooled for seven days for Cu analysis. Experiments were terminated after 18 weeks of continuous exposure. We used the geometric mean of the effect/no effect concentrations to calculate chronic values.

Copper and Water Quality Analyses

Cu concentrations were determined using a Thermo Jarrell Ash, Model SH 4000 Atomic Absorption Spectrophotometer (AA) with Smith-Hieftje background correction using Model 188 Furnace Atomizer with autosampler. Samples were acidified with high purity, Ultrex, nitric acid. Alkalinity, hardness, pH, dissolved oxygen, temperature and conductivity were determined weekly in exposure and maintenance aquaria in accordance with *Standard Methods* (APHA 1985).

RESULTS

Acute Toxicity of Copper Sulfate with Increased Exposure Time

Most important to the short-term toxicity of copper sulfate is the length of exposure. Mortality for a given concentration of copper sulfate varied greatly over a 30 minute difference in exposure time. The times required to attain 100 percent death of fish exposed to 34 mg Cu/liter for 30, 45, and 60 minutes were 110, 5.6, and 1.3 hours, respectively (Figure 4). Water quality characteristic were: alkalinity - 9.6 mg/liter, hardness - 9.0 mg/liter, pH 6.4, dissolved oxygen - 7.4 mg/liter, temperature - 13.3° C, and conductivity - 2.0 mS/m. Ten rainbow trout for each exposure period averaged 60.5 mm total length and weighed 2.20 g.

Acute Toxicity of Copper Sulfate and Cutrine During Single Episodic Exposure

Copper concentrations and associated mortality of the first episodic exposure is shown in Table 7. The 96-hr LC50 of rainbow trout following exposure to copper sulfate for 30 minutes was 25.8 mg Cu/liter compared to 1.93 mg Cu/liter following 3-hr exposure to Cutrine (Table 8). Increased exposure time significantly increased the toxicity of Cutrine compared to copper sulfate. Water quality characteristics during both experiments were: alkalinity - 10.1 mg/liter, conductivity - 2.0 mS/m, with dissolved oxygen and temperature at about 8.0 mg/liter and 13° C, respectively. Fish averaged about 58 mm total length and weighed 2.1 g.

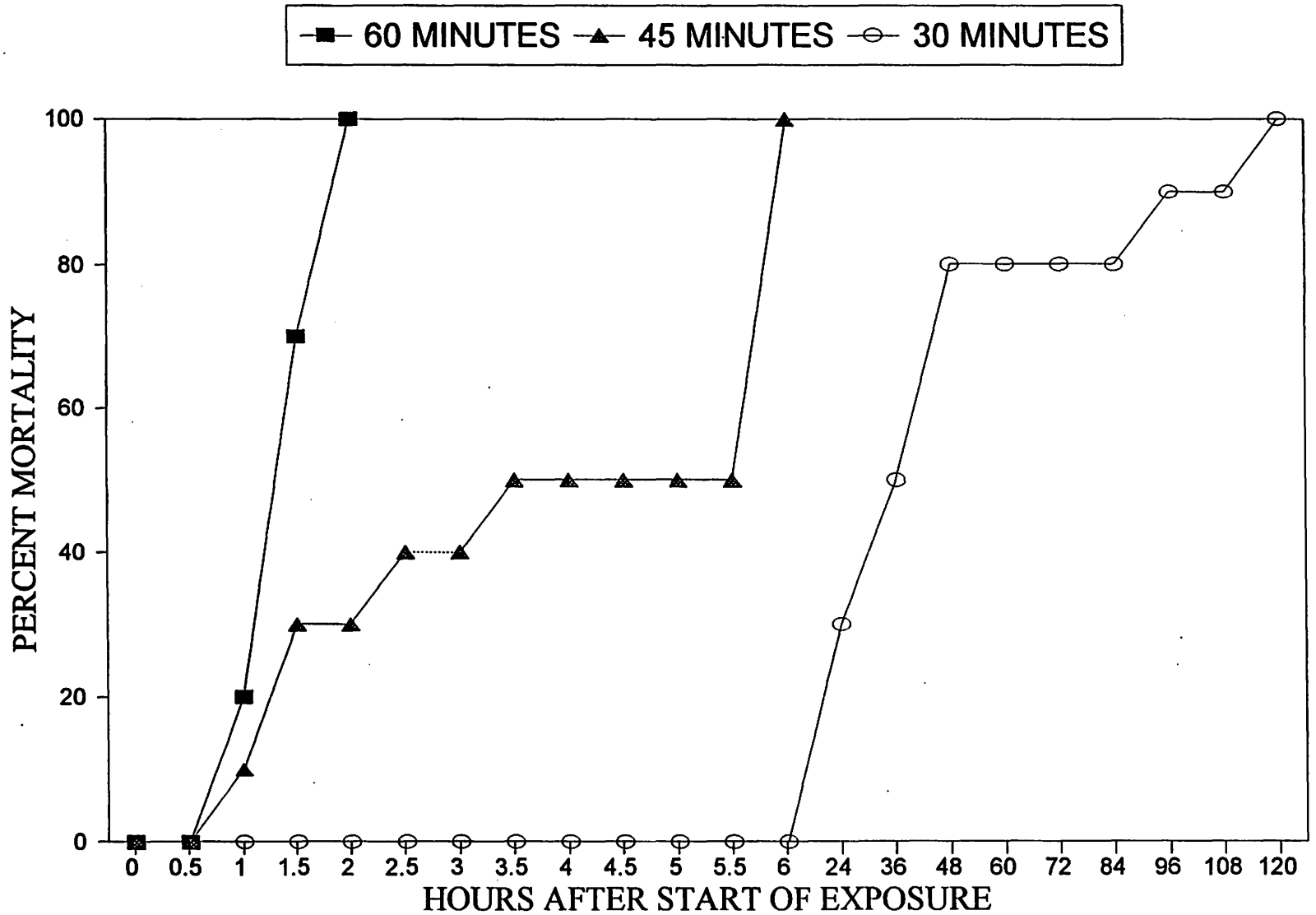

Figure 4. Mortality of rainbow trout exposed to 34 mg Cu/l for 30, 45, and 60 minutes.

Table 7. Acute toxicity data for rainbow trout exposed to a single episodic event of copper sulfate and Cutrine in water with an alkalinity of 10 mg/liter.

EXPOSURE	1	2	3	4	5	6	C
Copper Sulfate							
Cu (mg/L)	34.0	21.0	10.8	5.1	1.9	1.0	0.0
Exposure Time (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
% Dead	90	25	5	5	0	0	0
Cutrine							
Cu (mg/L)	10.3	5.0	1.9	1.0	0.4	0.2	0.0
Exposure Time (hr)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
% Dead	85	75	70	30	0	5	0

Table 8. 96-hr LC50s and 95% confidence intervals for rainbow trout exposed to a single episodic event of copper sulfate and Cutrine for 30 minutes and 3 hours, respectively, at an alkalinity of 10 mg/liter.

	COPPER SULFATE	CUTRINE
96-hr LC50	**25.8 mg/L**	**1.93 mg/L**
95% C.I.	19.8 - 42.8	1.36 - 2.81

Acute Toxicity of Copper Sulfate and Cutrine During Continuous Exposure

Acute toxicity data collected during the first 96-hr of the copper sulfate and Cutrine chronic experiments (Table 9) resulted in 96-hr LC50s of 4.92 and 7.20 μg Cu/liter, respectively (Table 10). Water quality characteristics for both experiments were very similar (Table 11). Rainbow trout averaged 58 mm total length and weighed 1.95 g. Data from the first set of acute tests for both copper sulfate and Cutrine were not used since fish deaths of 55 and 70 percent occurred in the lowest concentrations tested, 5.8 and 11.8 μg/liter, respectively.

Table 9. Acute toxicity data for rainbow trout continually exposed to copper sulfate and Cutrine at an alkalinity of 10 mg/liter.

Exposure	1	2	3	4	5	6	C
Copper Sulfate							
Cu (μg/L)	21.0	16.4	11.8	6.8	4.2	2.2	0.7
STD DEV	1.3	1.6	0.7	0.4	0.8	0.2	0.7
N	4	4	4	4	4	4	4
% DEAD	95	85	95	65	60	5	0
Cutrine							
Cu (μg/L)	17.8	14.4	11.8	7.0	5.9	3.4	0.5
STD DEV	2.3	3.4	3.8	2.2	4.5	2.0	0.3
N	4	4	4	4	4	4	4
% Dead	90	95	80	70	30	0	0

Table 10. 96-hr LC50s and 95% confidence interval for rainbow trout continuously exposed to copper sulfate and Cutrine at an alkalinity of 10 mg/liter.

	COPPER SULFATE	**CUTRINE**
96-hr LC50	**4.92 μg/L**	**7.20 μg/L**
95% C.I.	(3.81 - 6.11)	(6.12 - 8.30)

Table 11. Water quality characteristics during acute toxicity experiments for rainbow trout exposed to copper sulfate and Cutrine.

	Alkalinity mg/L	Hardness mg/L	pH	D.O. mg/l	Temp. °C	Conductivity mS/m
Copper Sulfate						
Mean	10.8	11.8	6.59	8.64	14.49	2.55
S.D.	0.3	0.4	0.05	0.10	0.17	0.08
N	11	11	11	11	11	11
Cutrine						
Mean	11.0	10.9	6.51	8.49	14.94	2.60
S.D.	0.3	00.4	0.04	0.16	0.41	0.10
N	11	11	11	11	11	11

Chronic Toxicity of Copper Sulfate and Cutrine During Weekly Episodic Exposures

Repeated weekly episodic exposure of rainbow trout to copper sulfate and Cutrine caused increased mortality over time following the initial exposure of 0.5 and 3.0 hrs, respectively (Figures 5 and 6). Mortality generally increased for a period of six weeks following exposure to weekly episodic events. One additional mortality occurred in two different concentrations following the 8th and 17th exposure event.

Toxic effects from weekly episodic events were seen at Cu concentrations as low as 2.14 mg/liter for copper sulfate and 0.26 mg/liter for Cutrine in a low alkaline water of 10 mg/liter (Table 12). Again we the see the result of increased exposure time on the toxicity of Cutrine. A chronic value of 1.48 mg Cu/liter was calculated for copper sulfate (Table 13). A chronic value for Cutrine could not be precisely calculated since significant mortality occurred at the lowest concentration tested. If, however, we assume no effect at 0.10 mg Cu/liter (a realistic assumption), we could estimate a chronic value of 0.16 mg Cu/liter for Cutrine in water with an alkalinity of 10 mg/liter (Table 13). One rainbow trout death occurred at a Cu concentration of 0.48 mg/liter following the 6th episodic event in the copper sulfate test. This, however, was not considered a metal-related mortality since no mortalities occurred in aquarium 5 which had double the concentration of Cu. Water quality characteristics were determined weekly during episodic exposure and in maintenance aquaria (Table 14 and 15). Mean total length and weight of rainbow trout after 18 weeks of episodic exposure to copper sulfate and Cutrine were 86 and 89 mm, and 6.43 and 7.13 g, respectively.

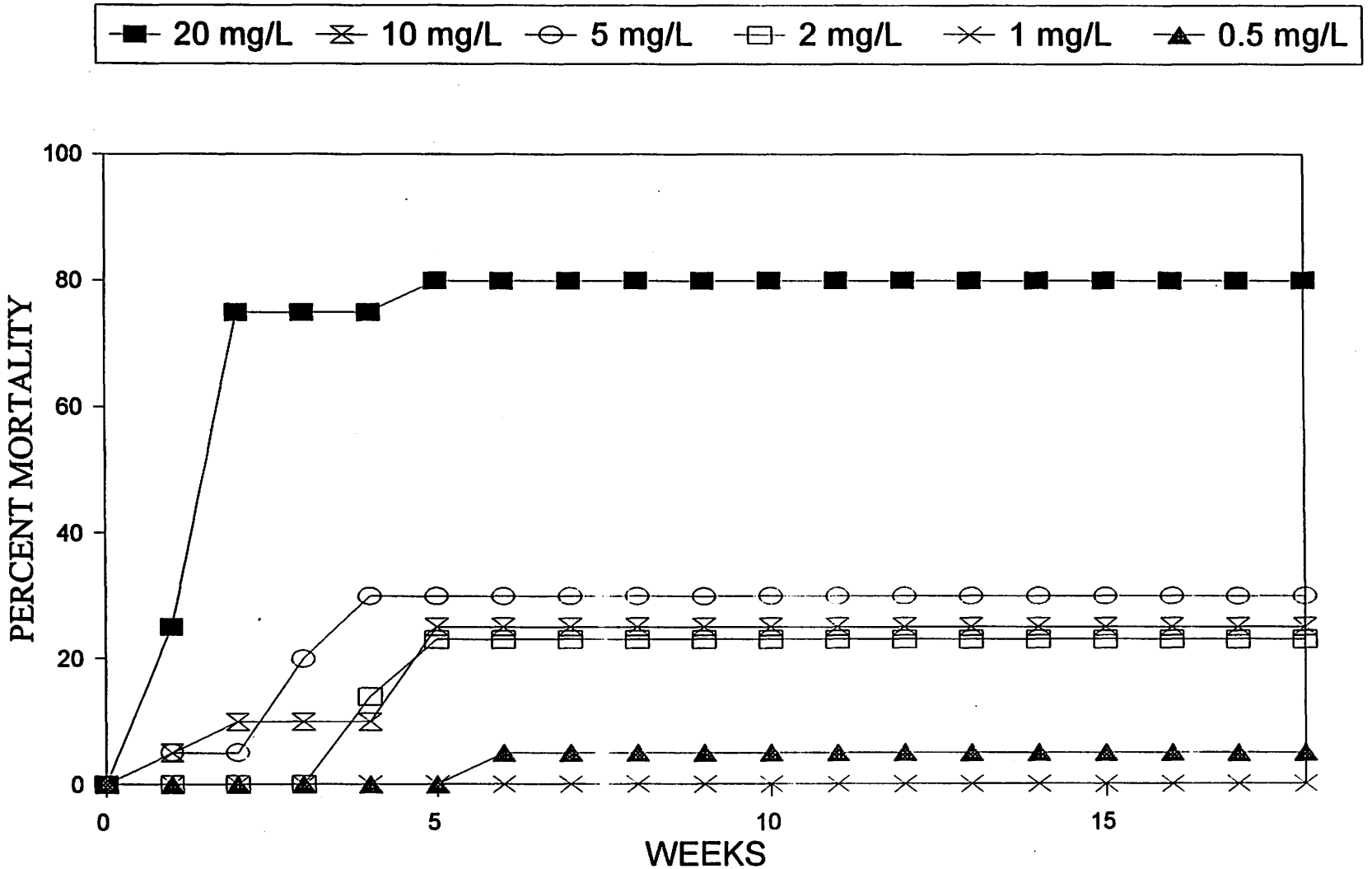

Figure 5. Percent mortality of rainbow trout exposed to copper sulfate following repeated weekly exposure of 30 minutes each.

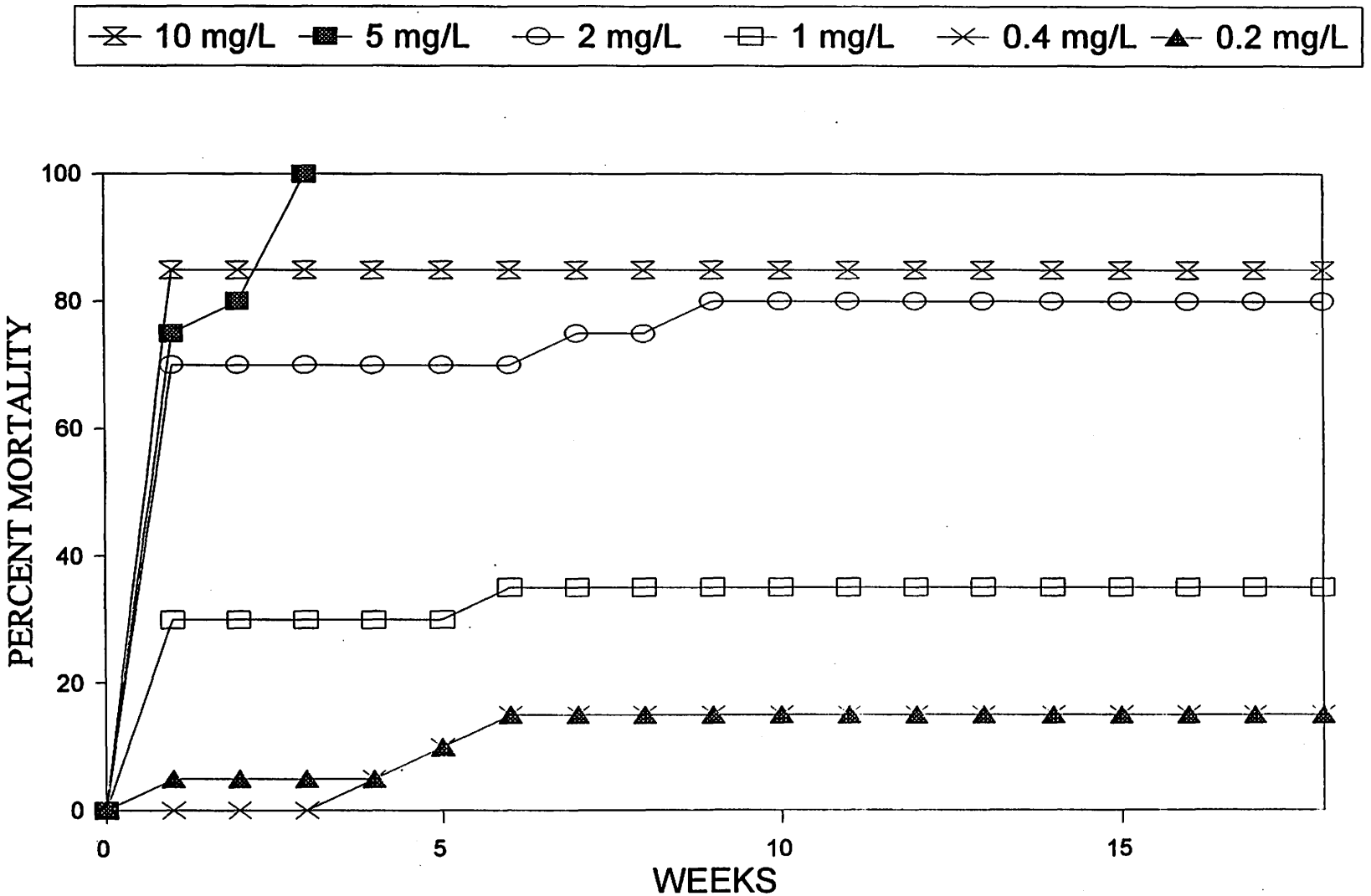

Figure 6. Percent mortality of rainbow trout exposed to Cutrine following repeated weekly exposures of 3 hours each.

Table 12. Chronic toxicity of Cu to rainbow trout exposed to 18 weekly episodic events of 30 minute and 3 hour, respectively, for copper sulfate and Cutrine at an alkalinity of 10 mg/liter.

Exposure	1	2	3	4	5	6	C
Copper Sulfate							
Cu (mg/L)	21.1	10.5	5.46	2.14	1.03	0.48	0.00
STD DEV	2.5	1.8	0.90	0.41	0.23	0.22	0.00
N	17	17	18	18	18	18	18
% DEAD	85	25	30	23	0	5	0
Cutrine							
Cu (mg/L)	10.5	5.97	2.03	1.02	0.51	0.26	0.00
STD DEV	0.7	1.12	0.28	0.18	0.21	0.09	0.00
N	18	3	18	18	18	16	17
% Dead	85	100	80	65	15	15	0

Table 13. Effect/no effect concentrations, and chronic value determined or estimated for copper sulfate and Cutrine following weekly episodic exposure of rainbow trout in water of an alkalinity of 10 mg/liter.

	Effect/ No effect	**Chronic Value**
COPPER SULFATE	2.14 - 1.03 mg/L	1.48 mg/L
CUTRINE	0.26 - (est) 0.1 mg/L	(est) 0.16 mg/L

Table 14. Water quality characteristics in maintenance aquaria during weekly episodic exposure of rainbow trout to copper sulfate.

MAINTENANCE AQUARIA						
	Alkalinity mg/L	**Hardness mg/L**	**pH**	**D.O. mg/L**	**Temp. °C**	**Conductivity mS/m**
Mean	**10.1**	**11.4**	**6.81**	**8.05**	**13.5**	**2.50**
S.D.	0.5	0.6	0.26	0.53	1.1	0.15
N	61	61	61	61	61	61

WEEKLY EPISODIC EXPOSURE						
	Alkalinity mg/L	**Conductivity mS/m**	**D.O. @ start mg/L**	**D.O. @ end mg/L**	**Temp. @ start °C**	**Temp. @ end °C**
Mean	**10.1**	**2.09**	**8.66**	**6.69**	**11.5**	**12.5**
S.D.	0.1	0.06	0.48	0.59	1.0	1.0
N	18	16	17	16	17	16

Table 15. Water quality characteristics in maintenance aquaria and during weekly episodic exposure of rainbow trout to Cutrine.

MAINTENANCE AQUARIA						
	Alkalinity mg/L	**Hardness mg/L**	**pH**	**D.O. mg/L**	**Temp. °C**	**Conductivity mS/m**
Mean	**10.1**	**11.3**	**6.77**	**8.15**	**13.8**	**2.47**
S.D.	0.7	1.2	0.22	0.54	1.0	0.20
N	67	67	67	67	67	67

WEEKLY EPISODIC EXPOSURE						
	Alkalinity mg/L	**Conductivity mS/m**	**D.O.@ start mg/L**	**D.O. @ end mg/L**	**Temp.@ start °C**	**Temp. @ end °C**
Mean	**10.2**	**2.05**	**8.59**	**6.69**	**11.1**	**14.0**
S.D.	0.4	0.07	0.67	1.00	1.4	1.1
N	16	12	17	16	17	16

Chronic Toxicity of Copper Sulfate and Cutrine From Continuous Exposure to Cu

Long-term toxic effects of continuous exposure occurred at Cu concentrations of 2.1 and 3.2 μg/liter for copper sulfate and Cutrine, respectively, (Table 16). Chronic values for copper sulfate and Cutrine were 1.77 and 2.47 μg Cu/liter, respectively (Table 17). Again we see reduced toxicity from some complexation of Cu ion by ethanolamine in Cutrine. Water quality characteristics were determined weekly during eighteen weeks of continuous exposure to copper sulfate and Cutrine (Table 18). Mean total length and weight of rainbow trout after 18 weeks of continuous exposure to copper sulfate and Cutrine were 92 and 94 mm, and 7.81 and 8.27 g, respectively.

Table 16. Chronic toxicity of Cu to rainbow trout continuously exposed to copper sulfate and Cutrine at an alkalinity of 10 mg/liter for 18 weeks.

EXPOSURE	1	2	3	4	5	6	7	C
COPPER SULFATE								
Cu (μg/L)	21.0	16.4	11.8	6.3	3.7	2.1	1.5	0.6
STD DEV	1.3	1.6	0.8	1.3	1.1	0.7	0.8	0.7
N	4	4	9	24	25	21	21	23
% DEAD	100	100	100	74	65	5	0	0
CUTRINE								
Cu (μg/L)	17.8	14.4	8.5	5.1	3.2	1.9	1.3	0.5
STD DEV	2.3	3.4	2.5	1.6	2.1	1.1	0.7	0.5
N	4	4	24	24	24	24	20	23
% DEAD	100	100	95	75	30	0	0	0

Table 17. Effect/no effect concentrations, and chronic values for copper sulfate and Cutrine following continuous exposure of rainbow trout with an alkalinity of 10 mg/liter for 18 weeks.

	Effect/ No effect	**Chronic Value**
COPPER SULFATE	2.1 - 1.5 μg/L	1.77 μg/L
CUTRINE	3.2 - 1.9 μg/L	2.47 μg/L

Table 18. Water quality characteristics during continuous exposure of rainbow trout to copper sulfate and Cutrine in chronic experiments.

	Alkalinity mg/L	Hardness mg/L	pH	D.O. mg/l	Temp °C	Conductivity mS/m
COPPER SULFATE						
Mean	10.2	11.4	6.92	8.30	13.5	2.47
S.D.	0.5	0.8	0.26	0.44	1.1	0.15
N	54	54	54	54	54	54
CUTRINE						
Mean	10.2	11.0	6.81	8.21	13.7	2.51
S.D.	0.7	0.7	0.30	0.38	1.7	0.17
N	57	57	57	57	57	57

DISCUSSION

The most important factor influencing the short-term acute toxicity of Cu is length of exposure (Figure 4). Above an initial exposure of 30 minutes, the toxicity rate increases dramatically with an additional 15 to 30 minutes of exposure. This was also very evident in comparing 96-hr LC50 concentrations following the first episodic exposure of 30 minutes and 3 hours, for copper sulfate (LC50 - 25.8 mg/liter) and Cutrine (LC50 - 1.93 mg/liter), respectively (Table 2). Clearly, in soft waters, use of copper sulfate is a better alternative to Cutrine for minimizing impacts on aquatic life.

Cu is extremely toxic to fish continuously exposed in low alkaline waters, below 20 mg/liter alkalinity and hardness. Rainbow trout exposed continuously to copper sulfate had a 96-hr LC50 of 4.92 μg Cu/liter. Similarly, a 96-hr LC50 of 7.20 μg Cu/liter was obtained for exposure to Cutrine. The 96-hr LC50 for Cutrine is significantly higher than copper sulfate. It appears that even in very low alkaline water, Cu in Cutrine is still partially bound to the ethanolamine complex, thus reducing its toxicity. The LC50 from continuous exposure to copper sulfate is over five thousand time greater than the LC50 from an exposure of 30 minutes (i.e., 4.92 μg Cu/liter versus 25.8 mg Cu/liter).

We did not achieve near the level of toxicity from repeated episodic events as from continuous chronic exposure. The chronic value for episodic exposure to copper sulfate was 1480 μg Cu/liter compared to 1.77 μg Cu/liter for continuous exposure. Similarly, for Cutrine, chronic values of 160 and 2.47 μg Cu/liter were obtained for episodic versus continuous exposure, respectively. It must be stressed that the estimated chronic value of 1.48 mg/liter for repeated, episodic exposures to copper sulfate assumes that the repeated exposures to Cu would not exceed a 30 minute period. Increases in the length of exposure would cause much greater mortality. As seen in Figure 4, it requires 110 versus 1.3 hours to achieve 100 percent mortality in rainbow trout exposed to 34 mg Cu/liter of copper sulfate for 30 and 60 minutes, respectively. Also notice that the chronic value of 0.16 mg/liter for repeated, episodic exposure to Cutrine is much lower than the 1.45 mg/liter obtained from copper sulfate. Again this occurs from the much greater exposure period of 3 hours

for Cutrine versus 30 minutes for copper sulfate. This again establishes use of copper sulfate compared to Cutrine as the preferred alternative for controlling algae. The episodic exposures of rainbow trout to copper sulfate and Cutrine continued for 18 weeks. However, for the most part, mortality ceased after 6 weekly, episodic events.

The results of these experiments suggest the 1992 fish kill in the Big Thompson River was not the result of the District's use of copper sulfate to control algae in the Charles Hanson Canal. However, the effect concentration for rainbow trout exposed weekly for 30 minutes to copper sulfate, at conditions similar to those encountered by the District, was 2.14 mg/liter (Table 13). Cu concentrations in the Charles Hanson Canal typically exceeded 2 mg Cu/liter and approached this concentration in the Big Thompson River below the canal discharge point. This is more than twice the District's target concentration of 1 mg/liter needed to effectively control algae.

RECOMMENDATIONS

1. We recommend that the District reduce its current application rate of copper sulfate by half. We also suggest that, at least initially, the District measure Cu concentrations in the canal below the point of addition to confirm that they do not significantly exceed their target of 1 mg/liter. This would require that samples for Cu analyses be collected at five minute intervals for about 45 minutes.

2. When applying copper sulfate it is very important to dump the entire amount at one instant to minimize the exposure window or duration of exposure and potential lethal impacts to fish.

3. Do not consider using Cutrine as an alternative to copper sulfate. Cutrine is much more toxic than copper sulfate in soft low alkaline waters, because of the recommended 3 hour exposure for Cutrine.

4. The District may also want to reconsider whether weekly applications of copper sulfate are necessary to control algae in the Hanson Canal. Results from CSU algae studies indicate that weekly applications may not be required.

Acute and Chronic Toxicity of Manganese to Exposed and Unexposed Rainbow and Brown Trout

INTRODUCTION

A series of toxicity test were conducted to determine if fish embryonically exposed to manganese (Mn) could acclimate and tolerate higher concentrations of Mn than unexposed fish. Acclimation occurred when rainbow trout were embryonically exposed to zinc and cadmium (Sinley et al. 1974 and Davies 1986, respectively). Colorado River strain, rainbow trout (*Oncorhynchus mykiss*) eggs were obtained from Bellvue Research Hatchery five days after eye-up. Forty five millimeter, Delaney Butte, brown trout (*Salmo trutta*) fingerlings were also obtained from the hatchery where they were hatched and reared in high alkaline, hard water at a temperature of 13° C. Since brown trout eggs were not available, we decided to evaluate if brown trout could acquire increased tolerance if exposed to slowly increasing concentrations of Mn.

METHODS AND MATERIALS

Acclimation Conditions for Exposed Groups

Eyed rainbow eggs and fingerling brown trout were acclimated to relatively soft water at a temperature of 6° C for a period of four days and two weeks, respectively prior to initiating exposure to Mn. The stock solution, composed of manganous sulfate dissolved in demineralized water, supplied a modified (Mount and Brungs 1967) diluter. Mn concentrations in 60 liter aquaria were increased from 0 to 0.14 mg/liter over a period of two days, after which Mn concentrations were increased to about 0.36 mg/liter. After five days, Mn was increased again to a final acclimation concentration of 0.80 mg/liter for four months. Under similar conditions, the unexposed group of rainbow trout eggs and fingerling brown trout were exposed to the same water quality conditions except no Mn was added. Dead eggs and rainbow sac fry were picked daily from both exposed and unexposed groups. We found no difference in egg and sac fry mortality between the two groups. Brown trout mortality was negligible in both groups.

Acute Toxicity Tests

We conducted two set of acute toxicity tests for both exposed and unexposed rainbow and brown trout in relatively soft water. Dechlorinated Fort Collins city water obtained from the Cache la Poudre River provided water with an alkalinity and hardness of about 40 mg/liter, as calcium carbonate. Acute toxicity data were collected during the acute phase of chronic experiments for both exposed and unexposed rainbow trout. An acute test was conducted on brown trout at nominal Mn concentrations of 30, 22.5, 16.8, 9.6, 5.4, 3.0 and 0.0 mg/liter. The proportional diluter delivered two liters of test solution per cycle to each aquarium. The adipose fin was removed from brown trout in the exposed group and 20 fish placed randomly into each of the experimental, 92 liter aquaria. Twenty unexposed fish without fin clip were similarly placed into the same aquaria.

Fish mortalities and associated length and weight information were recorded every two to four hours during acute experiments. Ninety-six hour, LC50s were determined by the probit method where less than 100 percent mortality occurred (Finney 1971), and otherwise, by the modified Spearman-Karber method (Hamilton et al. 1977 and Finney 1971). Fish were not fed during acute experiments. Water samples for Mn analyses were collected daily from all aquaria in 60-ml, high

density polyethylene (HDPE) bottles. Samples were acidified with 10 drops Ultrex triple-distilled nitric acid. We measured Mn concentrations using an Instrumentation Laboratory Video 22 atomic absorption spectrophotometer (flame AA) using an air-acetylene flame with Smith-Hieftje background correction. Water quality characteristics for alkalinity, pH, dissolved oxygen, temperature and conductivity were measured in all aquaria during acute tests consistent with Standard Methods (APHA 1985). Hardness was determined in control aquaria only due to manganese interference.

Chronic Toxicity Tests

We conducted four, four month experiments on the chronic toxicity of Mn to exposed and unexposed rainbow and brown trout. Twenty rainbow trout from each group were randomly placed into each aquarium of two diluter and toxicity testing systems with exposed and unexposed groups remaining distinct. Twenty exposed and unexposed brown trout were randomly placed into each aquarium of a single toxicity testing system. The adipose and right pelvic fin of exposed fish were removed to distinguish them from unexposed brown trout with no fins removed. Nominal concentrations of Mn were 6.00, 4.50, 3.36, 1.92, 1.08, 0.60 and 0 mg/liter in all three systems. Mn concentrations were delivered to aquaria using modified flow-through diluters (Mount and Brungs 1967). Water samples for determining Mn concentrations were collected daily during the initial 96-hr acute phase of the experiments. After the acute phase, 7-day aliquot of 5 mls each were collected and stored in 60 ml HDPE bottles acidified with Ultrex nitric acid. Mn concentrations were determined using the same flame AA system described above.

Alkalinity, pH, dissolved oxygen, temperature and conductivity were measured in all aquaria weekly according to Standard Methods (APHA 1985). Hardness was determined in control tanks only due to manganese interference. Feeding rates during the chronic phase of the experiments were adjusted as necessary based on weight of control fish and number of survivors in each aquarium (Piper et al. 1982). Experiments were terminated after 4-months and lengths and weights of survivors recorded. Fish were frozen for later analyses of Mn accumulation in tissues. We calculated chronic values from the geometric mean of effect and no effect concentrations.

RESULTS AND DISCUSSION

Acute Toxicity Tests

Ninety-six hour LC50s were only slightly different in the exposed and unexposed groups of rainbow trout. However, LC50s of previously exposed and unexposed brown trout were significantly different (Table 19). Mortality data for exposed and unexposed rainbow trout show similar toxicity patterns with unexposed brown trout showing significantly greater sensitivity than the exposed group (Table 20). Exposed brown trout juveniles appear to be significantly more resistant to acute Mn toxicity than embryonically exposed rainbow trout. Whereas, unexposed rainbow and brown trout show similar sensitivity to acute Mn exposure (Table 19). Differences in size of the rainbow and brown trout (Table 21) used in the acute experiments appear to have little influence on acute mortality as indicated by similarities in LC50s between the unexposed groups (Table 19). Water quality characteristics during the acute exposure of exposed and unexposed group of rainbow and brown trout were very similar (Table 22).

Table 19. 96-hr LC50s and 95 % confidence intervals for exposed and unexposed rainbow and brown for Mn in water with an alkalinity and hardness of 40 mg/liter.

ACUTE	EXPOSED	UNEXPOSED
Rainbow Trout		
96-hr LC50	3.32 mg/L	4.83 mg/L
95% C.I.	(2.97 - 3.72)	(4.18 - 5.58)
Brown Trout		
96-hr LC50	9.06 mg/L	3.77 mg/L
95% C.I.	(7.43 - 10.83)	(3.17 - 4.41)

Table 20. Acute toxicity data for exposed and unexposed rainbow and brown trout exposed to Mn in three separate acute experiments in soft water.

EXPOSURE	1	2	3	4	5	6	c
Rainbow Trout - Exposed Group							
Mn (mg/L)	6.61	4.77	3.45	1.93	1.06	0.56	<0.02
STD DEV	0.51	0.40	0.34	0.22	0.10	0.06	0.03
N	4	4	4	4	4	4	4
% DEAD	100	85	55	0	0	0	0
Rainbow Trout - Unexposed Group							
Mn (mg/L)	6.46	4.50	3.32	1.85	0.88	0.69	<0.02
STD DEV	0.40	0.36	0.31	0.20	0.17	0.32	0.02
N	4	4	4	4	4	4	4
% DEAD	85	15	40	0	0	0	0
Brown Trout - Both Groups							
Mn (mg/L)	32.0	23.5	17.6	9.73	5.31	2.86	<0.02
STD DEV	1.88	1.24	1.00	0.76	0.33	0.38	0.02
N	4	4	4	4	4	4	4
% DEAD EXPOSED	95	95	95	60	15	0	0
% DEAD UNEXPOSED	85	100	100	100	85	20	0

Table 21. Mean length and weight of rainbow and brown trout acutely exposed to Mn in soft water.

GROUP	LENGTH (mm)	WEIGHT (g)
EXPOSED RAINBOW TROUT	52.4	1'.41
UNEXPOSED RAINBOW TROUT	42.0	0.65
EXPOSED BROWN TROUT	138.7	28.87
UNEXPOSED BROWN TROUT	138.1	28.54

Table 22. Mean water quality characteristics during acute toxicity experiments on rainbow and brown trout exposed to Mn in soft water.

	Alkalinity mg/L	Hardness mg/L	pH	D.O. mg/l	Temp °C	Conductivity mS/m
RAINBOW TROUT - EXPOSED GROUP						
Mean	10.8	11.8	6.59	8.64	14.49	2.55
RAINBOW TROUT - UNEXPOSED GROUP						
Mean	10.8	11.8	6.59	8.64	14.49	2.55
BROWN TROUT - BOTH GROUPS						
Mean	11.0	10.9	6.51	8.49	14.94	2.60

Chronic Toxicity Tests

Effect/no effect concentration and chronic value data show about a 2X increase in tolerance of exposed rainbow trout eggs and 1.6 times for exposed brown trout fingerlings over unexposed fish chronically subjected to Mn (Tables 23 and 24). A 4X increase in tolerance occurred with rainbow trout embryonically exposed to zinc (Zn) and cadmium (Cd) (Sinley, et al. 1974 and Davies 1986, respectively). A 2X increased tolerance was observed with juvenile rainbow trout exposed to slowly increasing concentrations of Zn (Davies 1987). Chronic toxicity data on unexposed rainbow trout suggests that Colorado's current, chronic, water quality standard for Mn of 1.0 mg/liter may be too high. The standard appears to be adequate for acclimated rainbows and brown trout. Earlier work by (Goettl and Davies 1978) on the longterm toxicity of Mn to juvenile rainbow trout found an effect/no effect concentration and chronic value of 1.53 - 0.77 and 1.09 mg/liter, respectively.

Table 23. Effect/no effect concentrations and chronic values, in mg/liter, for Mn with exposed and unexposed rainbow and brown in soft water of 40 mg/liter alkalinity and hardness.

CHRONIC	EFFECT/NO EFFECT CONC.	CHRONIC VALUE
Rainbow Trout		
EXPOSED	2.13 - 1.15	1.57 mg/L
UNEXPOSED	1.04-0.60	0.79 mg/L
Brown Trout		
EXPOSED	4.88 - 3.59	4.19 mg/L
UNEXPOSED	3.59 - 2.03	2.70 mg/L

Table 24. Chronic toxicity data for exposed and unexposed rainbow and brown trout exposed to Mn in three separate acute experiments in soft water.

EXPOSURE	1	2	3	4	5	6	7
Rainbow Trout - Exposed Group							
Mn (mg/L)	6.61	5.08	3.74	2.13	1.15	0.61	<0.02
STD DEV	0.51	0.33	0.25	0.17	0.09	0.04	0.03
N	4	18	18	18	18	18	18
% DEAD	100	95	90	35	0	0	0
Rainbow Trout - Unexposed Group							
Mn (mg/L)	6.50	4.58	3.49	1.96	1.04	0.60	<0.02
STD DEV	0.35	0.36	0.18	0.12	0.12	0.14	0.03
N	5	5	18	18	18	18	18
% DEAD	100	100	95	60	10.5	0	0
Brown Trout - Both Groups							
Mn (mg/L)	6.68	4.88	3.59	2.03	1.11	0.59	<0.02
STD DEV	0.29	0.30	0.22	0.15	0.08	0.05	0.02
N	20	20	20	20	20	20	20
% DEAD EXPOSED	35	20	0	0	0	0	0
% DEAD UNEXPOSED	70	21	10	0	0	0	0

Water quality characteristics were not found to be significantly different among test aquaria. Consequently we reported grand means for these characteristics (Table 25). Length and weight data were determined for exposed and unexposed groups of rainbow and brown trout upon terminating the experiments (Table 26).

Table 25. Water quality characteristics during chronic toxicity experiments on rainbow and brown trout exposed to Mn in soft water.

	Alkalinity mg/L	Hardness mg/L	pH	D.O. mg/l	Temp °C	Conductivity mS/m
RAINBOW TROUT - EXPOSED GROUP						
Mean	41.0	36.8	7.56	8.08	15.2	9.03
S.D.	2.8	6.6	0.12	0.83	1.08	0.61
N	97	15	97	97	97	97
RAINBOW TROUT - UNEXPOSED GROUP						
Mean	41.0	36.8	7.56	8.17	15.0	8.83
S.D.	3.0	6.6	0.10	0.34	1.62	0.47
N	82	15	82	82	82	82
BROWN TROUT - BOTH GROUPS						
Mean	41.8	37.5	7.19	7.07	15.2	9.48
S.D.	3.3	6.6	0.16	0.69	1.0	0.76
N	119	15	119	119	119	119

Table 26. Mean length and weight of rainbow and brown trout surviving chronic exposure to Mn.

GROUP	LENGTH (mm)	WEIGHT (g)
EXPOSED RAINBOW TROUT	89.5	7.44
UNEXPOSED RAINBOW TROUT	87.1	7.02
EXPOSED BROWN TROUT	154.3	39.74
UNEXPOSED BROWN TROUT	151.4	39.22

STUDY PLAN 3: TECHNICAL ASSISTANCE

Objective:

To provide expertise, consultation, evaluation and training in aquatic toxicology and aquatic chemistry to Division of Wildlife personnel, and other state and federal agencies.

Job I. Water Quality Assistance to Other Personnel

Job Objectives:

1. To oversee the training and evaluation of metal analysis by laboratory technicians.

2. To assist Division and other state and federal personnel in the analysis and toxicological assessment of water quality data.

3. To develop and maintain a quality assurance program to evaluate the quality of analytical results for metals.

4. To collect and analyze metals concentrations in samples from the Arkansas River.

ACCOMPLISHMENTS

Stephen Brinkman trained and supervised laboratory assistants who continued analyses of water samples collected as part of the Rivers of Colorado Water Watch Network. Colorado State University graduate students conducting work as part of joint research projects with the Division of Wildlife were also trained. Samples collected from wells from potential sites for a new fish hatchery where also analyzed for metals and anions. Water samples associated with a fish kill in La Jara Creek were analyzed for rotenone. Rotenone analyses were also performed on a number of water samples from a reclamation project in Lake John. Qualitative analyses were performed on shotgun pellets and Canadian geese tissues at the request of Bill Adrian as part of an investigation regarding the use of toxic shot. Metal analyses were performed on water samples from the Colorado River as part of investigations of year class losses of Colorado River strain of rainbow trout. Metal analyses were also performed on water samples from the South Platte river collected by Rod Van Velson.

LITERATURE CITED

APHA. 1985. *Standard Methods for the Examination of Water and Wastewater*, 16th edn. American Public Health Association and American Water Works Association and Water Pollution Control Federation, Washington, D.C.

Davies, P. H. 1986. Toxicity and chemistry of metals in urban runoff. *In*: Urbonas, B. and Roesner, L.A. (eds), *Urban Runoff Quality-Impact and Quality Enhancement Technology*, pp. 60-78. Proceedings of Engineering Foundation Conference, 23-27 June 1986, Henniker, N.H. American Society of Civil Engineers, New York.

Davies, P. H. 1987. Zinc acclimation: exposure in feed versus water. Pages 10-12. *In*: Water Pollution Studies Job Progress Report. Federal Aid Project F-33-R. Colorado Division of Wildlife, Fort Collins, Colorado.

Davies, P. H., J. P. Goettl, Jr., J. R. Sinley, and N. F. Smith. 1976. Acute and chronic toxicity of lead to rainbow trout, *Salmo gairdneri*, in hard and soft water. Water Research 10:199-206.

Finney, D. J. 1971. *Probit Analysis*, 3rd edn. Cambridge Press, New York.

Goettl, J. P., Jr., and P. H. Davies. 1978. Manganese. Pages 34-36. *In*: Water Pollution Studies Job Progress Report. Federal Aid Project F-33-R-13 Colorado Division of Wildlife, Fort Collins, Colorado.

Goettl, J. P., Jr., and P. H. Davies. 1979. Lead Bioaccumulation. Pages 8-22. *In*: Water Pollution Studies Job Progress Report. Federal Aid Project F-33-R-14. Colorado Division of Wildlife, Fort Collins, Colorado.

Hamilton, M. A., R. C. Russo, and R. V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science and Technology 11(7):714-719.

Hamilton, M. A., R. C. Russo, and R. V. Thurston. 1978. Correction. Environmental Science and Technology 12:417.

Holcombe, G. W., D. A. Benoit, E. N. Leonard, and J. M. McKim. 1976. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). Journal of the Fisheries Research Board of Canada 33:1731-1741.

Mount, D. I. and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. Water Res., 1:21-29.

Piper, R. J., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. *Fish Hatchery Management*. US Fish and Wildlife Service, Dept. of Interior, Washington, DC.

Sinley, J. R., J. P. Goettl, Jr., P. H. Davies. 1974. The effects of zinc on rainbow trout (*Salmo gairdneri*) in hard and soft water. Bull. Env. Contam. Tox. 12(2):193-201.