WATER POLLUTION STUDIES

Federal Aid Project #F-243R-3

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Federal Aid in Fish and Wildlife Restoration

Job ~~Final~~ Progress Report

Colorado Division of Wildlife

Fish Research Section

Fort Collins, Colorado

August 1996

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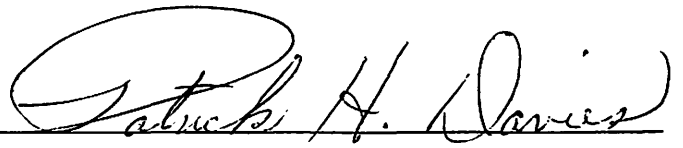
Prepared by: 

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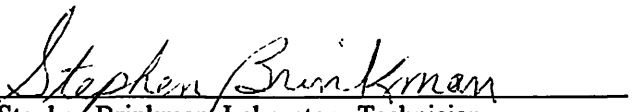

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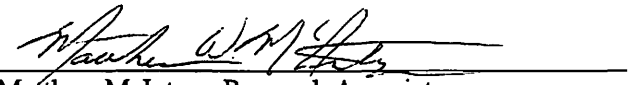

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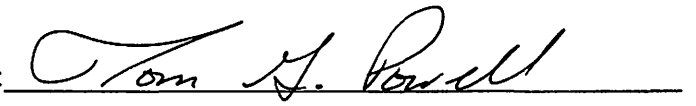
Approved by: 

Date: 8/19/96

The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Division of Wildlife policy by the Director or Wildlife Commission.

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JOB PROGRESS REPORT

State: Colorado

Project No. F243R-2

Title: Water Pollution Studies

Period Covered: July 1, 1995 to June 30, 1996

Principal Investigator: Patrick H. Davies
Co-investigators: Stephen F. Brinkman, Matthew McIntyre

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

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.

ACCOMPLISHMENT:

In cooperation with the Colorado Water Quality Control Commission, Colorado Attorney General's Office, Colorado Department of Health and Environment, and attorney for the modification of the manganese (Mn) water quality standard on the West Fork of Clear Creek, the Colorado Division of Wildlife Aquatic Toxicology Research Laboratory entered into a cooperative research study with ENSR corporation on the toxicity of Mn to brown trout in waters of different hardness. Little information is available on the chronic toxicity of Mn to brown trout in hard waters. State officials believed that brown trout was the species of concern in the West Fork of Clear Creek. ENSR conducted their tests in a water hardness of 30 mg/liter. We investigated the toxicity of Mn in water hardnesses of 150 and 450 mg/liter. Early-life-stage (ELS) tests

were conducted to determine the toxicity of Mn to brown trout and to evaluate the extent to which water hardness affects the chronic toxicity of Mn. Water hardness significantly affected Mn chronic toxicity, with toxicity decreasing with increased hardness. Decreased survival was the predominate effect noted in the 30 mg/liter hardness experiment. Significant growth and mortality effects were found in both the 150 and 450 mg/liter hardness experiments. IC_{25} values, representing a 25 percent inhibition concentrations, were 4.67, 5.59, and 8.68 mg Mn/liter at water hardness levels of 30, 150, and 450 mg/liter, respectively. The IC25 endpoints are based on both survival and growth effects. Study results suggest that site specific standard for Mn concentrations corresponding to $e^{0.2064(\ln \text{hardness})+7.7092}$ will not be toxic to brown trout in the West Fork of Clear Creek. This standard should not be applied in water containing more sensitive species of aquatic life. USEPA has raised concerns regarding this standard providing adequate protection of brook trout which are the predominate fish species in the West Fork of Clear Creek. We have agreed to repeat these studies on brook trout when they become available this fall.

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

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

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 of Different Complexing Capacity

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

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

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

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

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

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 Job 1.

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

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

Toxicity of Manganese to Early-Life Stage Brown Trout (Salmo trutta) in Water Hardnesses of 150 and 450 mg/liter

INTRODUCTION

Manganese (Mn) is distributed throughout soils, surface waters, and aquatic sediments. Concentrations of Mn in fresh waters range widely from below detection to very high levels, and is frequently elevated in surface waters in the vicinity of metal mining operations. Mn is generally present in Colorado's

mineral belt which extends from the mountains northwest of Denver to Durango in the southwest corner of the state. Consequently, it is a common constituent of mining and smelting effluents, tailings, and abandoned mine drainage.

The United State Environmental Protection Agency (EPA) has not promulgated national water quality criteria for Mn. In 1976, Colorado promulgated a water quality standard of 1000 mg/liter for Mn from limited, chronic, toxicity data on rainbow trout in soft water (Davies and Goettl 1976). Water alkalinity and hardness have long been recognized as major factors influencing the toxicity of metals to aquatic life (Sprague 1985 and Davies 1986) with the latter specifically considered by regulatory agencies in the development of national ambient criteria for a number of metals (USEPA 1986).

To specifically address the lack of chronic toxicity data on Mn in different water hardnesses to other fish species, Colorado Division of Wildlife (CDOW) undertook cooperative research studies with ENSR Corporation to determine the chronic toxicity of Mn to brown trout in waters of 30 (ENSR), 150 and 450 mg/liter (CDOW). Early-life-stage (ELS) toxicity tests were conducted using continuous-flow diluters and flow-though diluters at multiple concentrations of Mn.

MATERIALS AND METHODS

ELS Test Organisms

Brown trout were obtained as "eyed" eggs from CDOW's Bellvue Research Hatchery; these eggs were obtained from wild fish spawned at Delaney Butte Reservoir. Acclimation to temperature and test water quality conditions, feeding and maintenance of test organisms were performed according to ASTM guidance. Laboratory dilution/control water for each test was obtained from different sources as follows:

Medium hardness: (150 mg/liter, as $CaCO_3$) water was obtained by mixing dechlorinated municipal water (Fort Collins, CO originating from Horsetooth Reservoir) with water obtained from a well located at the CDOW Research Facility. Neither source received additional treatment prior to use.
High Hardness: (450 mg/liter, as $CaCO_3$) water was obtained from the same water described for the medium hardness test. The water received no filtration or adjustment of hardness or alkalinity prior to use.

Eggs were held on hatching trays submersed in continuous-flow dilution waters at 12°C for two days prior to initiating tests. Both tests were conducted concurrently with eggs from the same production lot. After hatching and yolk-sac absorption, hatched organisms were fed 0.5 ml per chamber of concentrated suspension of brine shrimp nauplii (Argent Chemical Laboratories, Redmond, WA or Aquatic Ecosystems, Inc., Apopka, FL) four times daily (twice a day on week-ends). As the tests progressed, the amount of food fed to all chambers increased dependent on the amount of uneaten food left in the chambers one hour after feeding. The amount of food added to the test chambers was documented and all chambers containing live fish, within each test, were fed an equal amount. Test aquaria were routinely cleaned using a sweep net to remove excess feed and fish feces.

Toxicant

Reagent grade manganese chloride ($MnCl_2 \cdot 4H_2O$) was used as the toxicant. Chemical stocks for toxicity test were prepared by dissolving a calculated amount of crystalline $MnCl_2$ in deionized water to equal the nominal stock solution concentration. The resulting stock solution was delivered to the test diluters as described below; a new stock solution was prepared as needed during the tests.

Chronic ELS Test Methods

ELS tests were conducted under guidance methodology provided in ASTM Method E1241-92, *Standard Guide for Conducting Early Life-Stage Test with Fishes* (ASTM 1993). Continuous-flow proportional diluters (after Benoit et al. 1982) with a dilution factor of 0.5 were used for each test. The diluters were constructed of glass joined with silicone adhesive (Silicone II®) and silicone stoppers. Test solutions were delivered to the test chambers through ⅜ in. Tygon® R-3603 tubing. The diluter systems were constructed to deliver seven test concentrations and a dilution water control to the test chambers. A flow-splitting chamber was used for each test concentration (treatment) to promote mixing of the test solution and to equally allocate the test concentration among four replicate test chambers. The flow rate to each of the replicate chambers was 22.5 ml per minute. Test solutions delivered to each chamber overflowed CPVC standpipes and discharged into the water bath containing the 32 test chambers. The flow rate through the individual chambers was sufficient to provide a minimum of 1 liter of test solution per 0.5 g of fish over a 24-hr period based on the average weight of fish in the control aquaria upon termination of the tests. The test chambers were glass aquaria (L 19.5 X W 10.0 X H 15.5 cm) containing approximately 2.2 liters of test solution. This fulfilled the loading protocol requiring ≤5 g of fish per liter.

A Marriotte bottle was used to deliver an appropriate flow of $MnCl_2$ stock solution to each of the diluters. The stock solution was metered into the diluters at a rate of 4.0 and 5.0 mls/minute respectively for the 150 and 450 hardness experiments. Nominal high concentrations of manganese (Mn) for 150 and 450 hardness experiments were 80 and 100 mg/liter, respectively. The diluter systems were calibrated prior to initiating the tests, and daily thereafter, with adjustments made as necessary.

Embryo cups were constructed of clear PVC pipe (6 cm O.D. by 7.5 cm long) with a Nitex® screen attached with silicone adhesive. Cups were suspended in the test solution of each of the randomly positioned test chambers with nylon line, and oscillated 2.5 to 4.0 cm vertically in the test water by means of a 2 rpm rocker-arm assembly. Fifteen embryos were randomly assigned to each of the embryo cups consisting of four replicates per treatment (i.e., 60 organisms per treatment). The test temperature of the source water was maintained at 12°C with the water bath temperature held at the same temperature ± 1°C using a Remcor recirculating chilling unit (Remcor Products Company, Glendale Heights, IL). The tests were conducted under dim lighting of 10 to 18 foot candles and a 16-hr light/ 8-hr dark photoperiod.

During the tests, dead organisms were removed and counted daily. Upon hatching, fish were released from the egg cups into the test chambers. The number of live eggs, hatches or partial hatches were counted in each test chamber and recorded on a daily basis. The test were continued for 30 days after the day on which the majority of the control organisms had reached the swim-up stage (test day 32). This gave a study duration of 62 days. Upon terminating the tests, fish in each of the test chambers were anesthetized with metomidate and counted. The fish were blotted dry and total lengths in mm and weights (grams) were obtained.

Chronic Test Methods for Juvenile Brown Trout

Initially two juvenile, brown trout chronic toxicity tests, of the same production lot used in the ELS studies, were conducted at the same hardnesses of 150 and 450 mg/liter. The fish were acclimated to water quality characteristics of the two waters for a minimum of two weeks. The purpose of these experiments on juvenile fish was to evaluate if non-embryonically exposed brown trout are more sensitive to the toxic effects of manganese than ELS exposed fish. We performed earlier studies on the toxicity of Mn to both rainbow and brown trout which suggested increased tolerance of ELS exposed fish compared to those not embryonically exposed (Davies and Brinkman 1994). The 150 and 450 hardness waters were as described for the ELS studies. Manganese chloride ($MnCl_2 \cdot 4H_2O$) stock solution was pumped to two modified (Mount and

Brungs 1967) diluters at a rate of 5.5 ml per 5 minute cycle. The nominal high concentration for both diluters was at 20 mg/liter with a dilution ratio of 100, 75, 56, 32, 18, 10, and 0 (control) percent. Toxicant solutions were delivered to each of the 92-liter, test aquaria at a rate of 2 liter per cycle. Fifteen brown trout, averaging 42 mm total length, were randomly placed into each of the test aquaria. The tests were terminated after 56 days.

A second set of juvenile, brown trout experiments were initiated following unexpectedly higher mortality and growth effects of Mn in the 450 hardness water than in the 150 hardness water. Toxicant exposure, fish, water hardness, and delivery systems were the same as those used in the original tests except for an additional test conducted in relatively soft water. The low hardness (50 mg/liter, as $CaCO_3$) water was obtained from dechlorinated municipal water (Fort Collins, CO originating from Horsetooth Reservoir). Manganese chloride stock solutions were pumped at a rate of 3.75, 5.0, and 5.0 ml/cycle to provide nominal high Mn concentrations of 15, 20, and 20 mg/liter, respectively for each of the 50, 150, and 450 hardness experiments.

Aquaria were checked for dead fish every 2 to 3 hours during the day and once on weekends; time of death, total length (mm), and weight (g) were recorded. Fish were not fed during the acute phase of the experiment; 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). Water samples for Mn analyses were collected daily during the initial 96-hr acute phase of the experiments. After the acute phase, 5 ml aliquot were taken daily and pooled in weekly samples stored in 60 ml HDPE bottles acidified with Ultrex nitric acid. Concentrations were determined using the same flame, atomic absorption spectrophotometer (AAS) system described below. Water quality characteristic were measured weekly as also described below.

Acute Test Methods for Brown and Rainbow Trout

Sufficient mortality occurred during the first 96 hrs of the 50 day tests conducted in 50 and 150 mg/liter water hardness to report acute toxicity data. Exposure systems and number of fish per treatment were reported in the chronic methods for juvenile fish above. Acute toxicity effects of Mn on brown trout in water hardnesses of 50 and 450 mg/liter were previously reported (Davies and Brinkman, 10/94 and 8/95). We lacked acute Mn toxicity data on brown and rainbow trout in 150 mg/liter water hardness. Acute toxicity tests were conducted on 130 mm brown trout collected as eggs from wild trout at Delaney Butte reservoir and 138 mm rainbow trout from the Crystal River Hatchery. Fish were acclimated to 150 hardness water for 12 days prior to initiating the experiments.. Pumps delivered manganese sulfate ($MnS0_4 \cdot H_20$) at 5 ml/2.5 minute cycle. Nominal concentrations of Mn delivered by modified Mount and Brungs proportional diluters were 50, 38, 25, 18, 9, 5, and 0 mg/liter to 92 liter aquaria containing 20 trout per treatment. Fish mortalities and associated length and weight data were collected at two hour intervals during the day. Ninety-six hour LC50s were determined by the probit method (Finney 1971) and/or the trimmed Spearman-Karber method (Hamilton et al. 1977 and 1978). Fish were not fed during acute experiments. Water samples for dissolved and total Mn were collected daily, acidified and analyzed; water quality characteristics were measured twice during the experiments, as described below.

Water Quality Analyses

Alkalinity, pH, temperature, dissolved oxygen, and specific conductance were measured and recorded in all test chambers at the beginning and end of the experiments. Similar measurements were taken weekly during the tests in alternating replicates. Hardness measurements were made only in control aquaria because of Mn interference with the EDTA hardness titration. Water quality parameters were analyzed using *Standard Methods* (APHA 1985). Anion concentrations were determined from control aquaria twice during

each study using a Dionex 4005i Ion Chromatograph (Dionex Instruments, Sunnyvale, CA) with an AS4A ion exchange column.

Manganese Analysis

Water samples from alternating replicate of each treatment in the ELS experiments were collected for total, acid-soluble and dissolved Mn on a weekly basis. Samples were preserved with Ultrex®, triple distilled, nitric acid to pH <2 at collection. Dissolved Mn samples were filtered through a 0.4 μm Nuclepore® filter using a positive pressure syringe prior to acidification. Water samples were analyzed for Mn using an Instrumentation Laboratory Video 22, flame, AAS (Allied Analytical Systems, Franklin, MA).

Statistical Analyses

Analysis of survival and growth data using hypothesis testing was performed using SAS® computer software (SAS 1989). Survival data were transformed by arcsine squareroot prior to analysis (Snedecor and Cochran 1980). Body weight in each replicate was determined as the mean wet (blotted dry) weight per surviving fish. Normality and homogeneity assumptions of survival and weight were evaluated by the Shapiro-Wilk's test and Bartlett's test, respectively ($\rho \leq 0.10$). If the data satisfied the assumptions of normal distribution and homogeneity of variance, analysis of variance (ANOVA), followed by Dunnett's multiple comparison test was used to compare ($\rho \leq 0.05$) organism performance in the experimental treatments with that observed in the control. If the data did not meet the normality and homogeneity assumptions, Steel's Many-One Rank Test was used to make the comparisons ($\rho \leq 0.05$). Response means for each test treatment were compared to that in the concurrent control groups for a given study. Groups in which a statistically significant decrease in survival occurred were first identified. The lowest Mn concentration associated with a statistically significant effect was designated the *lowest observed effect concentration* (LOEC). The highest Mn concentration associated with no statistically significant effect was designated the *no observed effect concentration* (NOEC).

ELS IC_{25} Concentration

The inhibition concentration (IC_{25} value), the concentration estimated to cause a 25% reduction in organism performance compared with the control, was also calculated (USEPA 1993) using terminal weight data. Mean treatment weights were determined as the combined weight of all surviving organisms divided by the total number of viable eggs at the start of the experiments. Thus, if an organism died, a weight value of zero was assigned. We also calculated chronic values (i.e., the geometric mean of the LOEC and NOEC concentrations).

RESULTS

ELS Chronic Toxicity Tests

Elevated concentrations of Mn caused eggs to hatch prematurely, reduced growth and survival of brown trout (Table 1.) The effect/no-effect concentrations (defined by the LOEC AND NOEC), chronic value, and IC_{25} concentrations for ELS brown trout exposed to manganese were 5.59 and 8.68 mg/liter, respectively, for the 150 and 450 hardness waters (Table 2). Based on mortality, chronic values were 6.23 and 11.9 mg Mn/liter at hardnesses of 150 and 450, respectively. Whereas, based on weight, corresponding chronic values of 3.50 and 6.28 mg/liter of Mn were calculated (Table 2). For both waters, the IC_{25} fell between the chronic values calculated for reduced growth and lethality.

Manganese toxicity

Hatching: Generally, hatching success was not affected by manganese exposure over the series of tested concentrations, ranging in all test groups from 86.6 to 98.2 percent (Table 1). No statistically significant difference in total hatch percentages were detected among exposure groups in the two studies. Control group total hatch percentages ranged from 89.6 to 93.3 percent. Higher Mn exposures accelerated hatching -- mean time to hatch was significantly reduced (by approximately 1.5 days), relative to control groups, for those eggs exposed to the highest Mn concentrations in both the 150 and 450 mg/l hardness tests (Table 1).

Survival: Larval survival decreased with increasing Mn concentrations in both experiments (Table 1). LOEC's, expressed in terms of trout mortality only (i.e., excluding growth differences) were 8.81 and 16.2 mg/liter, respectively in the 150 and 450 hardness tests. Mean control group mortality in the 150 hardness test was 13.3 percent and 56.2 percent in the 450 hardness test. During the final 10 days of the experiments, a fine filamentous algae developed in some replicates of the control and lower treatment levels. The algae did not form in aquaria containing higher concentrations of manganese or in all aquaria of the lower concentration exposures (Table 3 and Table 4). Where present, the filamentous growth apparently killed fish by clogging the gills with subsequent suffocation or was directly toxic. Control tanks in the 450 hardness test were the most seriously affected, causing loses of 56 percent of the fish (Figure 1). The low survival percentage was below acceptable criterion for the study (80 percent survival); however, it was not found to be indicative of generalized poor health of test population. Survival in the 2.54, 4.55, and 8.68 mg/liter treatments was 90.0, 90.8, and 83.3 percent, respectively, thus substantially exceeding that observed in the control group and that required by the study acceptance criterion. Consequently, test results were considered suitable for analysis. Statistical analyses to assess hatching, survival and growth did not include data from the control tanks in the 450 hardness experiment, or one replicate from the 4.55 mg Mn/L treatment (Table 4). In the 150 hardness test data, one replicate was rejected from the control, 2.78, and 4.41 mg/L treatments (Table 3) where presence of the algae was observed.

Mortality in the higher Mn concentrations was observed sooner after hatching than that occurring at lower levels of exposure in each of the hardness experiments (Figure 1). The number of days required to reach 30 percent mortality, for example decreased steadily among increasing concentration groups. This time interval was typically on the order of 15-20d post-hatch in the highest exposure levels, while time intervals among lower exposure groups were on the order of 34-40d. The slopes of the respective mortality response curves, however were generally consistent among all but the lowest concentration groups (Figure 1).

Growth: Brown trout growth, as measured by terminal body weight, was significantly affected at lower Mn concentrations than those affecting survival in both the 150 and 450 hardness tests. (Table 1). Effects on growth in this test was a more sensitive endpoint than survival for testing Mn toxicity to brown trout. LOEC's, expressed in terms of trout weights (excluding survival differences), in the 150 and 450 mg/liter hardness tests were 4.41 and 8.68 mg/liter, respectively.

Table 1. Exposure concentrations, mean fish weight, hatching data, and mortality data for early-life-stage tests with brown trout exposed to manganese for 62 days in 150 and 450 water hardness.

Mean Mn mg/L	Mean Wt. ± SD g	Mean Time to Hatch (days)	Total Hatch %	Mortality %
HARDNESS 150 mg/L				
<0.02 (Control)	0.442 ± 0.074	8.95 ± 0.06	93.3	13.3
2.78 ± 0.58	0.405 ± 0.080	9.08 ± 0.25	95.6	11.8
4.41 ± 0.98	0.368 ± 0.066*	9.00 ± 0.14	88.8	15.9
8.81 ± 1.53	0.350 ± 0.062*	9.05 ± 0.34	89.8	36.1*
13.9 ± 2.69	0.325 ± 0.076*	9.12 ± 0.10	93.3	61.0*
28.3 ± 4.52	0.249*	8.92 ± 0.10	90.3	98.4*
54.6 ± 9.60	---	8.52 ± 0.36	96.5	100*
74.9 ±11.6	---	8.05 ± 0.44*	86.6	100*
HARDNESS 450 mg/L				
<0.02 (Control)	---[1]	9.18 ± 0.48	89.6	56.2[2]
2.54 ± 0.31	0.378 ± 0.061	9.30 ± 0.22	98.2	10.0
4.55 ± 0.70	0.383 ± 0.066	9.02 ± 0.17	93.0	9.2
8.68 ± 1.21	0.309 ± 0.064*	9.10 ± 0.16	91.7	16.7
16.2 ± 2.12	0.209 ± 0.058*	9.38 ± 0.15	95.0	66.6*
29.9 ± 3.32	---	9.18 ± 0.22	91.5	100*
55.7 ± 5.55	---	8.70 ± 0.29	91.7	100*
100.8 ± 9.72	---	7.60 ± 0.50*	91.3	100*

* Significantly different from controls ($\rho \leq 0.05$).

[1] The mean weight for this test group was 0.325 g. Because of survival patterns observed (see footnote 2) treatment group mean weights were compared with the mean weight measured in the 2.54 mg/L group, as opposed to the control group.

[2] Due to low control survival, statistical evaluations of data were performed against the 2.54 mg/L exposure group.

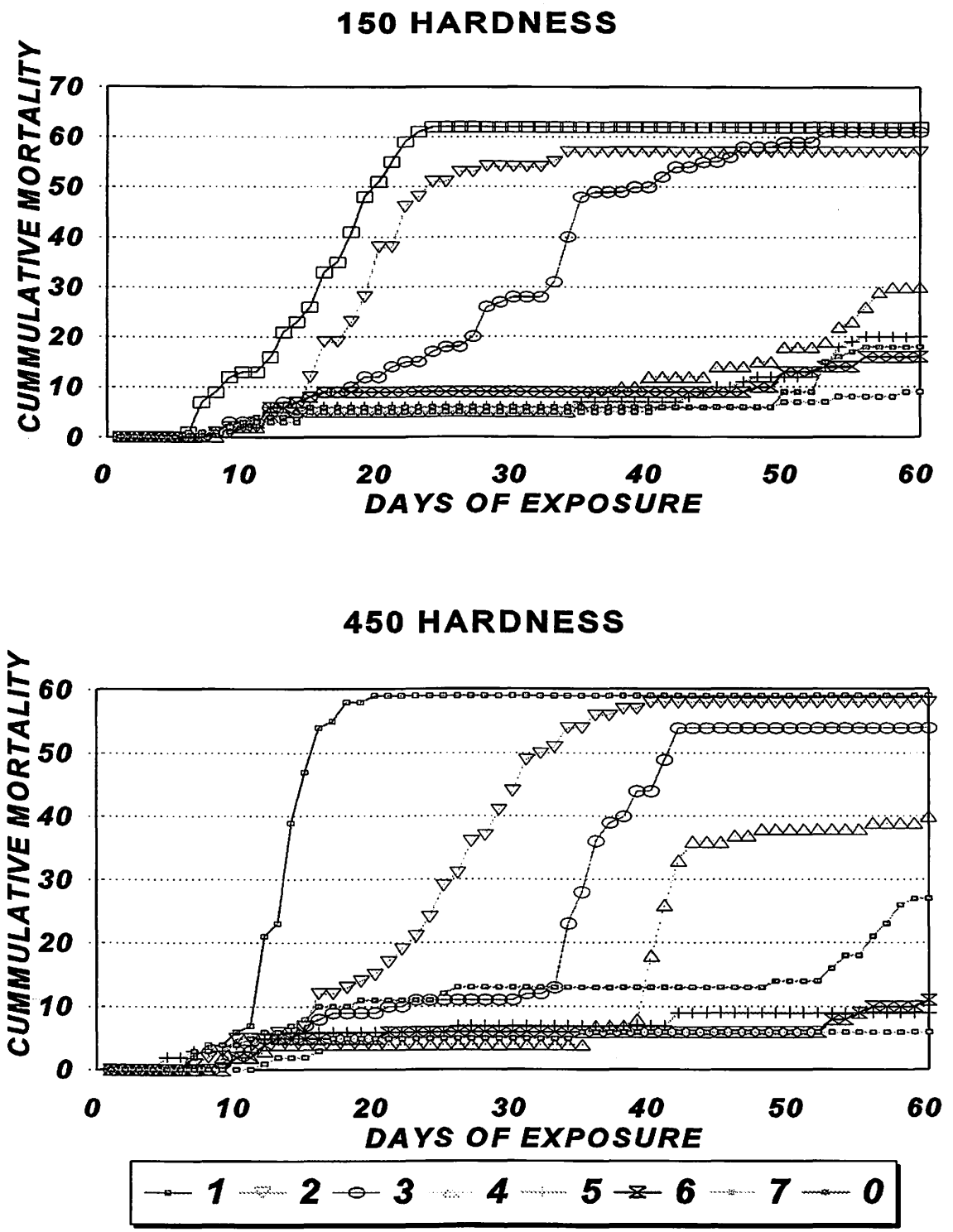

Figure 1. Cumulative mortality of brown trout over time for different Mn exposures in 150 and 450 hardness.

Table 2. NOEC and LOEC, chronic values (mg/L), and IC_{25} (25 percent inhibition concentrations) for early-life-stage test with brown trout exposed to manganese for 62 days in 150 and 450 hardness waters.

BASIS OF EFFECT	LOEC mg/L	NOEC mg/L	CHRONIC VALUE mg/L
HARDNESS 150 mg/L			
Growth	4.41	2.78	3.50
Mortality	8.81	4.41	6.23
IC_{25} = 5.59 mg/L (95% C.I. 4.2 to 6.8)			
HARDNESS 450 mg/L			
Growth	8.68	4.55	6.28
Mortality	16.2	8.68	11.9
IC_{25} = 8.68 mg/L (95% C.I. 7.1 to 10.2)			

Table 3. Average mortality (%) and weight of surviving brown trout (g) in replicate aquaria and treatment following 62 days exposure in 150 mg/L water hardness.

NOMINAL Mn (mg/L)	MEASURED Mn (mg/L)	REP-A	REP-B	REP-C	REP-D	TREATMENT MEAN (S.D.)
		% MORTALITY				
Control (0.0)	**<0.02**	26.7	13.3	0.0	80.0[a]	13.3 (13.4)
1.25	**2.78**	50.0[a]	13.3	15.4	6.7	11.8 (4.54)
2.50	**4.41**	7.7	20.2	20.2	50.0[a]	15.9 (7.10)
5.00	**8.81**	33.3	50.0	46.7	14.3	36.1 (16.2)*
10.0	**13.7**	46.7	86.7	53.3	57.1	61.0 (17.7)*
20.0	**28.3**	100	100	100	93.8	98.4 (3.13)*
40.0	**54.6**	100	100	100	100	100 (0.00)*
80.0	**74.9**	100	100	100	100	100 (0.00)*
		MEAN WEIGHT OF SURVIVING ORGANISMS (g)				
0.00	**<0.02**	0.4836	0.4258	0.4261	0.2903[a]	0.4422 (0.0735)
1.25	**2.78**	0.3749[a]	0.3860	0.4566	0.3811	0.4046 (0.0801)
2.50	**4.41**	0.3659	0.3806	0.3567	0.3429[a]	0.3677 (0.0658)*
5.00	**8.81**	0.3336	0.3837	0.3508	0.3448	0.3504 (0.0624)*
10.0	**13.7**	0.2952	0.3295	0.3911	0.2875	0.3253 (0.0762)*
20.0	**28.3**	---	---	---	0.2490	0.2490*
40.0	**54.6**	---	---	---	---	---
80.0	**74.9**	---	---	---	---	---

[a]Data were not used to calculate mean or perform ANOVA.
*Significantly lower than the control ($\rho <= 0.05$).

Table 4. Average mortality (%) and weight of surviving brown trout (g) in replicate aquaria and treatment following 62 days exposure in 450 mg/L water hardness.

NOMINAL Mn (mg/L)	MEASURED Mn (mg/L)	REP-A	REP-B	REP-C	REP-D	TREATMENT MEAN (S.D.)
		% MORTALITY				
Control (0.0)	**<0.02**	100[a]	21.4[a]	50.0[a]	53.3[a]	56.2[a]
1.56	**2.54**	0	7.1	18.8	14.3	10.0 (8.25)
3.12	**4.55**	13.3	0	14.3	60.0[a]	9.2 (7.98)
6.25	**8.68**	26.7	6.7	6.7	26.7	16.7 (11.6)
12.5	**16.2**	100	60	33.3	73.3	66.6 (27.8)*
25	**29.9**	100	100	100	100	100 (0.0)*
50	**55.7**	100	100	100	100	100 (0.0)*
100	**100.8**	100	100	100	100	100 (0.0)*
		MEAN WEIGHT OF SURVIVING ORGANISMS (g)				
Control (0.0)	**<0.02**	---	0.3848[a]	0.3155[a]	0.2747[a]	0.3250[a]
1.56	**2.54**	0.3493	0.3938	0.3738	0.4008	0.3779 (0.0613)
3.12	**4.55**	0.3753	0.3406	0.44	0.2625[a]	0.3827 (0.0659)
6.25	**8.68**	0.3265	0.3246	0.3246	0.2511	0.3089 (0.0636)*
12.5	**16.2**	---	0.1835	0.2157	0.2285	0.2086 (0.0584)*
25	**29.9**	---	---	---	---	---
50	**55.7**	---	---	---	---	---
100	**100.8**	---	---	---	---	---

[a] Data were not used to calculate mean or perform ANOVA.
* Significantly lower than the 2.54 mg/l (pseudo-control) exposure ($\rho <= 0.05$).

Water Chemistry

Acid soluble and dissolved manganese concentrations in the two studies typically exceeded target values, especially at lower levels of exposure (Table 5). Concentrations in the control waters were below analytical detection (<0.02 mg/liter). Variability in measured concentrations with in treatment groups was generally low, and the data showed adequate separation of the eight exposure concentrations comprising each study. Dissolved Mn averaged 96.2% of total, acid soluble Mn, which did not vary substantially among treatment levels or exposure systems (Table 5). Water hardness and alkalinity did not noticeably affect the relative fractions of dissolved to acid soluble Mn. Water quality parameters (hardness, alkalinity, pH, conductivity, temperature and dissolved oxygen) varied little over the course of testing (Table 6). Mean hardness values were consistent with targets values, and dissolved oxygen concentrations were indicative of acceptable water flow and organism loading in the test chambers. Fluoride, chloride, nitrate, phosphate, and sulfate concentrations were consistent with expectations for the medium hard and hard waters used, except for fluoride in the 150 hardness water which reflects fluoridation of the Fort Collins municipal water that was mixed with the well water source to give the desired hardness.

Table 5. Nominal, total acid soluble, and dissolved Mn concentration data for early-life-stage tests with brown trout in 150 and 450 mg/L water hardness.

Nominal Mn mg/L	Mean ± SD Total Acid Soluble Mn (mg/L)	n	Mean ± SD Dissolved Mn (mg/L)	n	Dissolved to Total Mn Ratio (%)
150 HARDNESS					
Control	<0.02 ± 0.01	20	<0.02 ± 0.02	7	---
1.25	2.78 ± 0.58	16	2.84 ± 0.18	7	102.0
2.50	4.41 ± 0.98	16	4.31 ± 0.25	7	97.7
5.00	8.81 ± 1.53	16	8.69 ± 0.52	7	98.6
10.0	13.86 ± 2.69	16	15.09 ± 4.09	7	108.9
20.0	28.29 ± 4.52	13	27.50 ± 2.38	7	97.2
40.0	54.58 ± 9.60	8	52.10 ± 2.66	4	95.4
80.0	74.90 ± 11.63	12	71.95 ± 5.32	4	96.0
450 HARDNESS					
Control	<0.02 ± 0.03	17	<0.02 ± 0.01	7	---
1.56	2.54 ± 0.31	15	2.41 ± 0.46	6	94.9
3.12	4.55 ± 0.70	16	4.26 ± 0.76	7	93.6
6.25	8.68 ± 1.21	16	8.28 ± 0.91	7	95.4
12.5	16.21 ± 2.12	15	15.07 ± 1.09	7	93.0
25.0	29.88 ± 3.32	8	27.88 ± 1.67	4	93.3
50.0	55.74 ± 5.55	8	49.16 ± 2.01	4	88.2
100.0	100.82 ± 9.72	12	93.36 ± 5.89	4	92.6

Table 6. Summary of water quality characteristics for laboratory dilution waters used in manganese early-life-stage tests with brown trout.

Parameter	150 Hardness			450 Hardness		
	Mean	Range	n	Mean	Range	n
Hardness (mg/L as $CaC0_3$)[a]	151.8	146-172	23	449.6	441-456	18
Alkalinity (mg/L as $CaC0_3$)	113.8	103-156	60	315.5	307-324	57
pH	7.85	7.0-8.5	344	7.78	7.0-8.3	327
Conductivity (µS/cm)	259	249-300	35	708	660-839	35
Temperature (°C)	11.8	10.3-12.8	315	12.0	11.0-13.0	330
Dissolved oxygen (mg/L)	8.72	7.0-10.2	347	8.75	7.3-9.6	333
Fluoride (mg/L)	0.96			<0.5		
Chloride (mg/L)	8.98			21.34		
Nitrate (mg/L)	5.44			21.81		
Phosphate (mg/L)	<0.5			<0.5		
Sulfate (mg/L)	51.38			155.05		

[a] Data represents measurements in control tanks only.

Chronic Toxicity Tests with Juvenile Brown Trout

A summary of Mn toxicity data to juvenile brown trout is reported in Table 7. Mn significantly affected brown trout mortality and growth during the 56 day chronic tests in water hardnesses of 150 and 450 mg/liter (Tables 8 and 9). As was reported in the ELS studies, concentrations of total acid soluble Mn were virtually identical to dissolved Mn (Table 10); therefore, concentration effect data is reported as total (acid soluble) Mn. A chronic toxicity effect significantly different from control fish, occurred at the same level of Mn exposure for both mortality and growth in the 150 hardness test. This chronic value (CV), i.e., concentration assumed to be safe, was 8.79 mg/liter (Table 7), with lethal effects (Table 8), and effects on length and weight (Table 9) occurring at the same concentration. In both cases, the "lowest observed effect concentration" (LOEC) was 11.9 mg/liter.

Similar comparisons in the 450 hardness test, show effects of Mn on growth to be a more sensitive indicator, giving a chronic value of 2.70 mg/liter based on growth effects compared to a chronic value of 4.98 mg Mn/liter based on mortality (Tables 7 to 9). Contrary to normal expectations, toxic effects of Mn in the 450 hardness water, based on both growth (CV of 2.70 mg/liter) and mortality (CV of 4.98 mg/liter), were considerably lower than that occurring in the less complexing 150 hardness water with a CV of 8.79 mg/liter. Generally, harder more alkaline water is less toxic due to binding or complexation of toxic metals by negatively charged anions or ligands, such as bicarbonates, which reduce metal bioavailability. Information collected on water quality characteristics (Table 11) and anions measured in these same waters during the ELS experiments (Table 6) , show that the 450 hardness water is approximately three time higher in bicarbonates (reflected in the alkalinity concentrations) and considerably higher in anions than found in the 150 hardness water. We cannot explain or provide any reasons for the higher toxicity in the harder water.

We ran a second set of tests to see if we could replicate results obtained in the initial juvenile brown trout experiments, especially in the 450 hardness water. Effects of Mn on juvenile brown trout in water harnesses of 50, 150, and 450 mg/liter showed no difference in toxicity level between the 150 and 450 hardness waters (Tables 7 and 12). "Lowest observed effect concentrations" (LOECs), based on mortality, were 15.0 mg Mn/liter in both the 150 and 450 hardness waters during the 50 day exposure period. As expected, mortality (73.3 percent) was considerably higher in the 150 hardness test at 20 mg Mn/liter than the 6.7 percent mortality observed in the 450 hardness water. A chronic value of 3.37 mg/liter was calculated in the 50 mg/liter hardness water (Table 7), based on NOEC- LOEC of 2.48 and 4.58 mg/liter, respectively (Table 12). Unlike the earlier juvenile experiments, no significant effect on brown trout growth was found between treatment groups in any of the different hardness waters. The fish used in these studies were larger, and had greater variability. Average lengths and weights, and standard deviations within the test groups are presented in Table 13. Water quality characteristics and anion concentrations for the three waters are reported in Tables 14 and 15, respectively.

Table 7. Chronic toxicity of manganese (expressed as NOEC and LOEC, and chronic values) to juvenile brown trout initially exposed as 42 and 63 mm fish for 56 days in 150 and 450 mg/L hardness waters, and 50 days in water hardnesses of 50, 150, and 450 mg/L, respectively.

Basis of Effect	Brown Trout Size (mm)	Exposure (days)	NOEC (mg/L)	LOEC (mg/L)	Chronic Value (mg/L)
	50 HARDNESS				
Mortality	63	50	2.48	4.58	3.37
	150 HARDNESS				
Mortality	42	56	6.51	11.9	8.79
Growth	42	56	6.51	11.9	8.79
Mortality	63	50	11.2	15.0	13.0
	450 HARDNESS				
Mortality	42	56	3.69	6.72	4.98
Growth	42	56	1.97	3.69	2.70
Mortality	63	50	11.2	15.0	13.0

Table 8. Exposure concentrations and mortality from toxicity test on juvenile brown trout exposed to manganese in water hardnesses of 150 and 450 mg/L for 56 days.

	1	2	3	4	5	6	CONTROL
NOMINAL Mn (mg/l)	20.0	15.0	11.2	7.2	3.6	2.0	0
	HARDNESS 150						
TOTAL Mn (mg/l)	22.26	16.15	11.87	6.51	3.68	1.99	<0.02
DISSOLVED Mn (mg/l)	22.34	16.22	11.95	6.46	3.64	1.99	<0.02
MORTALITY (%)	71	75	30	0	5	0	0
	HARDNESS 450						
TOTAL Mn (mg/l)	21.54	13.87	11.89	6.72	3.69	1.97	<0.02
DISSOLVED Mn (mg/l)	21.39	13.87	11.94	6.72	3.70	1.96	<0.02
MORTALITY (%)	95	85	32	26	0	0	0

Table 9. Lengths (mm) and weights (g) of juvenile brown trout surviving exposure to manganese in water hardnesses of 150 and 450 mg/L for 56 days.

MEASURED TOTAL Mn (mg/l)	MEAN LENGTH (S.D.) (mm)	MEAN WEIGHT (S. D.) (g)
	HARDNESS 150	
<0.02	71.2 (5.7)	3.4206 (0.8640)
1.99	72.2 (5.6)	3.7306 (0.9247)
3.68	70.9 (4.9)	3.5814 (0.8177)
6.51	67.0 (9.5)	3.1652 (1.5140)
11.87	54.6 (8.4)*	1.5237 (0.8461)*
16.15	47.8 (7.3)*	1.0858 (0.3754)*
22.26	47.2 (4.9)*	0.9925 (0.3122)*
	HARDNESS 450	
<0.02	76.2 (3.9)	4.4082 (0.8076)
1.97	74.2 (5.0)	3.8620 (0.8690)
3.69	65.3 (6.9)*	2.7324 (0.8595)*
6.72	60.2 (6.7)*	2.1173 (0.7687)*
11.89	56.7 (6.5)*	1.6807 (0.6785)*
13.87	54.0 (7.1)*	1.554 (0.6074)*
21.54	50*	1.178*

*Significantly lower than control ($p<0.05$)

Table 10. Manganese exposure concentrations for toxicity test conducted on juvenile brown trout exposed in water hardnesses of 150 and 450 mg/liter for 56 days.

Nominal Concentration (mg Mn /L	Measured Total Acid Soluble Concentration (mg Mn /L)			Measured Dissolved Concentration (mg Mn /L)		
	Mean	Std. Dev.	n	Mean	Std. Dev.	n
	HARDNESS 150					
20.0	22.26	1.90	10	22.34	1.57	10
15.0	16.15	1.33	10	16.22	1.35	10
11.2	11.87	1.03	10	11.95	0.87	10
7.2	6.51	0.38	10	6.46	0.36	10
3.6	3.68	0.22	10	3.64	0.17	10
2.0	1.99	0.11	10	1.99	0.09	10
Control	<0.02	0	10	<0.02	0.007	10
	HARDNESS 450					
20.0	21.54	1.66	10	21.39	1.43	10
15.0	13.87	1.25	10	13.87	1.40	10
11.2	11.89	0.97	10	11.94	0.86	10
7.2	6.72	0.43	10	6.72	0.43	10
3.6	3.69	0.20	10	3.70	0.21	10
2.0	1.97	0.13	10	1.96	0.12	10
Control	<0.02	0	10	<0.02	0	10

Table 11. Water quality characteristics of toxicity test conducted on juvenile brown trout exposed to manganese in water hardnesses of 150 and 450 mg/L for 56 days.

Statistic	Hardness[a] (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	pH (S.U.)	Temperature (°C)	Dissolved Oxygen (mg O_2/l)	Conductivity[a] (μS)
	HARDNESS 150					
Mean	145.4	104.0	7.73	12.4	9.31	266.4
Std. dev.	8.5	5.3	0.17	0.5	0.24	25.2
n	12	14	14	14	14	14
	HARDNESS 450					
Mean	438.6	307.3	7.84	12.8	8.98	705.7
Std. dev.	10.3	6.3	0.18	1.2	0.64	37.5
n	9	16	16	16	16	16

[a] Data represents measurements in control tanks only.

Table 12. Exposure concentrations and mortality from toxicity test on juvenile brown trout exposed to manganese in water hardnesses of 50, 150 and 450 mg/L for 50 days.

	1	2	3	4	5	6	CONTROL
	HARDNESS 50						
NOMINAL Mn (mg/l)	15.0	11.2	8.4	5.4	2.7	1.5	0.0
TOTAL Mn (mg/l)	14.7	10.9	7.37	4.58	2.48	1.35	<0.02
Std. dev.	0.85	0.43	0.58	0.15	0.12	0.05	0.0
MORTALITY (%)	86.7	73.3	46.7	28.6	6.7	6.7	6.7
	HARDNESS 150						
NOMINAL Mn (mg/l)	20.0	15.0	11.2	7.2	3.6	2.0	0
TOTAL Mn (mg/l)	21.2	15.4	11..2	6.20	3.45	1.86	<0.02
Std. dev.	1.07	0.50	0.47	0.23	0.11	0.08	0.0
MORTALITY (%)	73.3	20.0	7.1	0.0	0.0	0.0	6.7
	HARDNESS 450						
NOMINAL mg/L	20.0	15.0	11.2	7.2	3.6	2.0	0
TOTAL Mn (mg/l)	20.2	13.8	11.6	6.18	3.42	1.84	<0.02
Std. dev.	1.50	1.03	0.97	0.48	0.18	0.12	0.0
MORTALITY (%)	6.7	13.3	0.0	0.0	0.0	0.0	0.0

Table 13. Lengths (mm) and weights (g) for juvenile brown trout surviving exposure to manganese in water hardnesses of 50, 150 and 450 mg/L for 50 days.

MEAN LENGTH (S.D.) (mm)	MEAN WEIGHT (S. D.) (g)
HARDNESS 50	
87.0 (10.0)	7.428 (2.669)
HARDNESS 150	
79.4 (9.8)	5.396 (1.940)
HARDNESS 450	
81.8 (9.0)	5.803 (2.000)

Table 14. Water quality characteristics for chronic toxicity tests conducted on juvenile brown trout exposed to manganese in water hardnesses of 50, 150 and 450 mg/L for 50 days.

Statistic	Hardness[a] (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	pH (S.U.)	Temperature (°C)	Dissolved Oxygen (mg O_2/l)	Conductivity[a] (μS/cm)
	HARDNESS 50					
Mean	52.7	36.1	7.54	13.4	8.80	97.9
S. D.	2.6	1.3	0.12	0.6	0.31	9.1
	HARDNESS 150					
Mean	148.7	103.6	7.65	13.3	8.83	250.6
S. D.	5.1	1.5	0.14	0.4	0.28	12.1
	HARDNESS 450					
Mean	434.6	311.3	7.72	12.9	8.93	679.8
S. D.	8.2	3.8	0.15	0.2	0.26	7.3

[a] Data represents measurements in control tanks only.

Table 15. Anion concentrations for chronic toxicity tests conducted on juvenile brown trout exposed to manganese in water hardnesses of 50, 150 and 450 mg/L for 50 days.

Statistic	Fluoride (mg /L)	Chloride[a] (mg /L)	Nitrate (mg/L)	Phosphate (mg/L)	Sulfate (mg /L)
	HARDNESS 50				
Mean	0.7	1.9	<0.5	<0.5	13.9
Std. dev.	0.4	0.1	0.1	0.0	0.7
	HARDNESS 150				
Mean	<0.5	6.2	5.0	<0.5	50.8
Std. dev.	0.0	6.3	0.18	1.2	0.64
	HARDNESS 450				
Mean	<0.5	18.2	22.7	<0.5	146.0
Std. dev.	0.0	1.2	5.0	0.0	3.0

[a] Data represents measurements in control tanks only.

Acute Toxicity Tests with Brown and Rainbow Trout

The acute toxicity of Mn to juvenile brown trout was approximate three times greater at a water hardness of 50 mg/liter than that occurring at 150 mg/liter. The 96-hr LC50s, respectively, were 8.48 and 24.3 mg Mn/liter (Table 16 and 17). Acute toxicity effects were not significantly different for 63 versus 130 mm brown trout where 96-hr LC50s were 24.3 and 29.3 mg Mn/liter, respectively. LC50s for similar size brown and rainbow trout were not significantly different in 150 mg/liter water hardness (Table 16). Length, weight, and water quality information are reported in Table 18 through 20.

DISCUSSION

ELS Exposed Brown Trout

The early life stage (ELS) test exposes test organisms over three life stages, these being embryo (egg), larva (sac-fry), and alevin (swim-up fry). The latter two stages are recognized as being especially sensitive to metal exposures, and the results of the test are intended to provide a suitable estimate of chronic (multi-life-stage) toxicity to the test species. In general, ELS test results have been shown to be comparable to those of whole life-cycle tests (McKim 1977). For some metals, such as zinc and cadmium, we have determined that ELS exposure, especially during the egg and larval stage, actually conditions fish to tolerate (i.e., acclimate) to metals, such that greater toxicity is found with fish that have not been exposed during their early stages of development. (Sinley el al. 1974 and Davies 1986). For zinc and cadmium, non-ELS exposed fish were four times more sensitive than ELS exposed fish. This relationship to stage of exposure may have important significance to stocked fish and fish that could potentially migrate through a metal impacted area, such as occurs with alevins and juveniles migrating downstream from nursery areas or fish migrating upstream to spawn.

This study, along with ENSR's findings on the toxicity of Mn to brown trout in soft water (hardness of 30 mg/liter), demonstrates a significant relationship between water hardness and alkalinity and Mn toxicity. Brown trout embryos were shown to be tolerant of Mn exposure. Nearly all mortality was observed after

hatching. Exposure had some limited effect on hatch rate, with mean time to hatch showing a significant reduction, relative to controls, among the highest Mn exposure groups in the 150 and 450 mg/liter hardness tests. Hatch times differed by no more than 1.6 days; consequently, it is unclear what ecological significance may be associated with the observed effect. Water hardness had no effect on hatching rates or mean time to hatch. Control fish in the 30, 150 and 450 mg/liter hardness studies had mean hatch times of 9.15, 8.95, and 9.18 days, respectively.

IC_{25} values were calculated as the Mn concentration associated with a 25 percent reduction in both survival and growth of exposed fish relative to controls. By calculating final treatment mean weights, (dividing the total mass of surviving fish measured at the end of the test by the number of viable eggs at test initiation) any mortalities occurring during testing were accounted for in the final mean weight estimate. Weight values of 0 g were assigned to individuals that died during testing , and these individuals would thus have been included in the mean treatment group weight determination at the end of the test..

In addition to the chronic effect of Mn on growth and mortality of brown trout in 150 and 450 harness waters (Table 2), the experiment by ENSR in 30 mg/liter water hardness gave LOEC and NOEC of 7.38 and 3.94 mg Mn/liter, respectively. For each of the three hardness experiments, chronic toxicity endpoints were calculated as a chronic value (an assumed safe concentration calculated as the geometric mean of the NOEC/LOEC concentrations), and as an interpolated point estimates called the IC_{25} value. IC_{25} values increased with water hardness (4.67, 5.59, and 8.68 mg/liter Mn. NOEC/LOEC values for the 150 mg/liter hardness test (2.78/4.41 mg/liter, based on growth) were lower than those of the 30 mg/liter hardness test (3.94/7.38 mg/liter); but values in the 150 hardness test, based on mortality, (4.41/8.81 mg/liter) were higher. In the 30 mg/liter hardness test, NOEC/LOEC values were based on mortality, since no growth effect was observed among treatment groups. The discrepancy between trends in the two endpoint types is also a function of two primary differences between the respective calculation methods. NOEC/LOEC estimates are constrained by the value of the exposure concentrations used. The chronic value, as a geometric mean of the NOEC and LOEC values, is dependent upon the selection of toxicant exposure concentrations and may not accurately reflect the true effect level, especially if the concentrations are set widely apart.

In order to overcome the difficulties in statistically deriving the NOEC using hypothesis testing, the USEPA (1991) developed an alternative statistical procedure for estimating effect concentrations referred to as the inhibition concentration (IC). The IC is interpolated from a dose-response data set and provides an estimate of the toxicant concentration that would cause a given percent reduction (e.g., 25 percent or IC_{25}) in a biological response measurement among test organisms. Because the IC_{25} value need not equal pre-established exposure concentrations and can be defined to correspond to a specified level of effect, EPA recommended it as a preferred statistical method for determining meaningful estimates of the true no-effect concentration in toxicity tests.

Given these considerations, IC_{25} estimates, as opposed to chronic values, were considered appropriate for analyzing and interpreting the Mn exposure/response data and for setting Mn water quality standards for the West Fork of Clear Creek. IC_{25} values calculated from the three hardness toxicity tests were regressed against the mean test water hardness values providing a regression equation for calculating safe Mn concentrations for brown trout in water of different hardness.

$$IC25_{\textit{(at specific hardness)}} = e^{(0.2064[\ln \text{hardness}]+7.7092)}$$

The site specific, hardness equation developed from these studies is protective of brown trout in the West Fork of Clear Creek. However, it should not be applied to streams containing species of fish that may be more sensitive than brown trout.

Non-ELS Exposed Brown Trout

For juvenile non-ELS exposed brown trout, we calculated a chronic value (CV) of 3.37 mg/liter, based on morality defined by NOEC/LOEC of 2.48 and 4.58 mg/liter in a water hardness of 50 mg/liter. Compared to the NOEC/LOEC range of 3.94 to 7.38 mg/liter, giving a CV of 5.39 mg/liter for the ELS data found by ENSR in a water hardness of 30 mg/liter, data suggests that non-ELS exposed brown trout are more sensitive than ELS exposed fish. But as mentioned in the previous section, this method of estimating chronic effects precludes determination of a specific percent estimate of inhibition. Earlier studies (Davies and Brinkman 1994) in 40 mg/liter water hardness, with fingerling brown trout exposed to increasing concentrations of Mn from 0 to 0.80 mg/liter for four months, gave a CV of 4.19 mg/liter. A CV of 2.70 mg/liter was obtained from toxicity tests with Mn on four month old, non-ELS exposed brown trout (Table 21), which again suggest a greater sensitivity of Mn to brown trout not previous exposed. Similar results were found for ELS rainbow trout similarly exposed to increasing concentrations of Mn over a four month period (Davies and Brinkman 1994). A CV of 1.57 mg/liter was determined for the ELS exposed group compared to a CV of 0.79 for the non-ELS exposed group (Table 21). Goettl and Davies (1978) reported effect/no-effect concentrations for juvenile rainbow trout exposed to Mn giving a CV of 1.08 mg/liter in a water hardness of 34 mg/liter (Table 21).

Ninety-six hour LC50s for previously exposed and unexposed brown trout in a water hardness of 40 mg/liter were significantly different - 9.06 mg/liter compared to 3.77 mg/liter, respectively (Davies and Brinkman 1994). This reflects significant acclimation or increased tolerance of the pre-exposed group (Table 22). In contrast, we found a 96-hr LC50 of 8.48 mg Mn/liter for unexposed, juvenile brown trout in 50 mg/liter hardness water (Table 16). Davies and Brinkman (1994) reported only a slight (non significant) difference in 96-hr LC50s in the acute toxicity of Mn to ELS exposed and non-exposed juvenile rainbow trout - 3.32 and 4.83 mg/liter, respectively (Table 22). Rainbow trout appear to be more sensitive than brown trout to the Mn toxicity in soft waters. In harder more alkaline water acute toxicity differences are only slight, if at all (Table 16). A 96-hr LC50 of 49.9 mg/liter (Table 22) was determined for brown trout in 450 mg/liter hardness water (Davies and Brinkman 1995).

Table 16. 96-hr LC50s and 95% confidence intervals for brown and rainbow trout exposed to Mn (as total) in waters of 50 and 150 mg/liter hardness.

Species	Method	96-hr LC50 mg/L	95% C.L. mg/L
50 Hardness			
Juvenile Brown Trout (63 mm length)	Probit	**8.48**	6.87 - 10.7
150 Hardness			
Juvenile Brown Trout (63 mm length)	Probit	**24.3**	19.3 - 106.9
Brown Trout (130 mm length)	Probit Spearman-Karber	**29.3** **26.9**	24.4 - 35.4 21.9 - 33.1
Rainbow Trout (138 mm length)	Probit Spearman-Karber	**20.7** **20.2**	18.1 - 23.3 18.6 - 22.1

Table 17. Exposure concentrations (mg/liter) and % mortality from acute toxicity tests on brown and rainbow trout exposed to total and dissolved manganese in water hardnesses of 50, and 150 mg/L.

	1	2	3	4	5	6	Control
	63 mm, Juvenile Brown Trout - 50 HARDNESS						
Nominal Mn mg/L	15.0	11.2	8.4	5.4	2.7	1.5	0
Total Mn (mg/L) (Std. dev.)	14.2 (0.39)	10.7 (0.24)	7.14 (0.26)	4.50 (0.08)	2.48 (0.05)	1.32 (0.05)	<0.02 (0.00)
% Mortality	80.0	66.7	33.3	21.4	0.0	0.0	0.0
	63 mm, Juvenile Brown Trout - 150 HARDNESS						
Nominal Mn mg/L	20.0	15.0	11.2	7.2	3.6	2.0	0
Total Mn (mg/L) (Std. dev.)	21.2 (1.19)	15.6 (0.56)	11.5 (0.54)	6.35 (0.13)	3.52 (0.05)	1.92 (0.05)	<0.02 (0.00)
% Mortality	40.0	13.3	7.1	0.0	0.0	0.0	0.0
	130 mm, Brown Trout - HARDNESS 150						
Nominal Mn mg/L	50.0	37.5	28.0	18.0	9.0	5.0	0.0
Diss. Mn (mg/L) (Std. dev.)	53.3 (1.44)	37.4 (2.00)	27.4 (0.60)	14.6 (1.76)	8.10 (0.53)	4.58 (0.20)	<0.02 (0.00)
Total Mn (mg/L) (Std. dev.)	52.0 (0.46)	38.0 (1.72)	28.3 (1.08)	15.0 (0.91)	8.45 (0.27)	4.76 (0.39)	<0.02 (0.00)
% Mortality	80.0	55.0	70.0	10.0	0.0	0.0	0.0
	138 mm, Rainbow Trout - 150 HARDNESS						
Nominal Mn mg/L	50.0	37.5	28.0	18.0	9.0	5.0	0.0
Diss. Mn (mg/L) (Std. dev.)	52.0 (1.40)	37.6 (0.42)	28.5 (1.31)	16.1 (1.30)	8.34 (0.73)	4.37 (1.23)	<0.02 (0.00)
Total Mn (mg/L) (Std. dev.)	51.4 (0.60)	37.8 (0.88)	27.8 (0.52)	16.0 (0.91)	8.61 (0.16)	4.34 (0.69)	<0.02 (0.01)
% Mortality	100	95.0	100	10.0	0.0	0.0	0.0

Table 18. Lengths (mm) and weights (g) for brown and rainbow trout surviving acute exposure to manganese in water hardnesses of 50 and 150 mg/liter.

Fish Species	MEAN LENGTH (S.D.) (mm)	MEAN WEIGHT (S.D.) (g)
HARDNESS 50		
Juvenile Brown Trout (63 mm)	66.1 (7.4)	2.892 (0.916)
HARDNESS 150		
Juvenile Brown Trout (63 mm)	60.3 (8.5)	2.153 (0.872)
Brown Trout (130 mm)	129.9 (18.1)	24.56 (11.03)
Rainbow Trout (138 mm)	138.4 (16.5)	29.86 (12.25)

Table 19. Water quality characteristics of acute toxicity tests conducted on brown and rainbow trout exposed to manganese in water hardnesses of 50 and 150 mg/liter.

Statistic	Hardness[a] (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	pH (S.U.)	Temperature (°C)	Dissolved Oxygen (mg O_2/l)	Conductivity[a] (μS/cm)
	63 mm, Juvenile Brown Trout - HARDNESS 50					
Mean	50.6	34.0	7.59	12.6	9.04	90.0
Std. dev.	0.3	0.8	0.05	0.1	0.06	4.21
	63 mm, Juvenile Brown Trout - HARDNESS 150					
Mean	144.1	101.0	7.75	12.8	9.05	250.5
Std. dev.	1.6	1.4	0.03	0.2	0.06	0.7
	130 mm, Brown Trout - HARDNESS 150					
Mean	150.5	106.5	7.65	15.0	8.24	275.8
Std. dev.	1.8	2.5	0.15	0.3	0.24	3.9
	138 mm, Rainbow Trout - HARDNESS 150					
Mean	148.7	103.7	7.45	15.1	8.18	278.8
Std.dev.	2.7	1.1	0.16	0.2	0.46	11.5

[a] Data represents measurements in control tanks only.

Table 20. Anion concentrations for acute toxicity tests conducted on brown and rainbow trout exposed to manganese in water hardnesses of 50 and 150 mg/liter.

Statistic	Fluoride (mg /L)	Chloride (mg /L)	Nitrate (mg/L)	Phosphate (mg /L)	Sulfate (mg/L)
	63 mm, Juvenile Brown Trout - HARDNESS 50				
Mean **Std. dev.**	0.2 0.3	1.8[a] ---	0.2 0.3	<0.5 0.0	12.8 0.1
	63 mm, Juvenile Brown Trout - HARDNESS 150				
Mean **Std. dev.**	<0.5 0.0	5.7[a] ---	5.4 0.1	<0.5 0.0	48.7 0.1
	130 mm, Brown Trout - HARDNESS 150				
Mean **Std. dev.**	0.6 0.0	7.4 0.1	6.2 0.1	<0.5 0.0	54.2[a] 0.2
	138 mm, Rainbow Trout - HARDNESS 150				
Mean **Std.dev.**	0.6 0.0	7.3 0.0	5.9 0.0	<0.5 0.0	52.6[a] 0.0

[a] Data represents measurements in control tanks only.

Table 21. Chronic toxicity of manganese (expressed as NOEC and LOEC, and chronic values) to ELS, pre-exposed and non-exposed brown and rainbow trout in waters of different hardness.

Source	Trout	Hardness (mg/L)	NOEC (mg/L)	LOEC (mg/L)	Chronic Value (mg/L)
Goettl and Davies 1978	98 mm, Rainbow non-exposed	34	0.77	1.53	1.08
Davies and Brinkman 1994	138 mm, Brown non-exposed	40	2.03	3.59	2.70
Davies and Brinkman 1994	138 mm, Brown pre-exposed	40	3.59	4.88	4.19
Davies and Brinkman 1994	42 mm, Rainbow non-exposed	37	0.60	1.04	0.79
Davies and Brinkman 1994	52 mm, Rainbow ELS exposed	37	1.15	2.13	1.57

Table 22. 96-hr LC50s and confidence intervals for brown and rainbow trout exposed to Mn in waters of different hardnesses.

Source	Trout Species	Hardness mg/L	96-hr LC50 mg/L	95% C.I. mg/L
Davies and Brinkman 1994	138 mm, Brown pre-exposed	40	9.06	7.43 - 10.83
Davies and Brinkman 1994	138 mm, Brown non-exposed	40	3.77	3.17 - 4.41
Davies and Brinkman 1994	52 mm, Rainbow ELS exposed	37	3.32	2.97 - 3.72
Davies and Brinkman 1994	42 mm, Rainbow non-exposed	37	4.83	4.18 - 5.58
Davies and Brinkman 1995	116 mm, Rainbow non-exposed	454	49.9	43.6 - 57.4

Acute toxicity of cadmium to boreal toads subjected to a 24 hour static renewal toxicity test for ten days.

INTRODUCTION

A 24-hour static renewal cadmium toxicity test was conducted on Boreal toad (*Bufo boreas*) tadpoles as part of investigations into the decline of this species in Colorado. Cadmium was selected as a toxicant based on its high toxicity to aquatic organisms (Eisler 1985). The primary purpose of this experiment was to provide a range of cadmium exposure concentrations to use in a follow up chronic flow-through toxicity test.

METHODS

Exposure

Egg masses containing between 70 and 120 Boreal toad eggs were placed in 300 ml polyethylene beakers containing 200 milliliters of 1000, 500, 100, 50, or 0 μg Cd/l added as cadmium sulfate to dechlorinated Fort Collins city tap water. The beakers were placed in an incubator, covered with a watch glass and gently aerated with compressed air. Temperature of the incubator was maintained at 25°C during a 12 hour day and 15°C during a 12 hour night. Eggs/larvae/tadpoles were transferred to 300 ml polyethylene beakers containing 200 mls of freshly prepared solutions daily using a brine shrimp net. Mortalities were monitored daily and removed using a pipet. Tadpoles were fed daily with Mazuri amphibian feed beginning eight days after start of cadmium exposure. Duration of exposure was ten days.

Water Quality

Water quality parameters were measured weekly. Hardness and alkalinity were determined according to Standard Methods (APHA). pH was determined using an Orion Research pH meter 811 calibrated daily using pH 7.00 and 4.00 pH buffers. Conductivity was determined using a YSI Model 35 conductance meter. Anions were determined using a Dionex 4005i ion chromatograph.

Cadmium Analyses

Water samples for cadmium analysis were collected from freshly prepared solutions as well as 24 hour-old solutions to measure loss of cadmium from the solution as a result of sorption, precipitation or uptake by organisms. Water samples for cadmium analysis were preserved by acidification to pH<2 using Ultrex nitric acid.

Surviving tadpoles within an exposure concentration were pooled, dried with a paper towel and weighed in preweighed polypropylene centrifuge tubes. The tubes containing the tadpoles were placed in a drying oven at 80°C and dried to constant weight. The tadpoles were then digested with trace metal grade nitric acid and heated for eight hours in a water bath at 60°C. Trace metal grade hydrogen peroxide was added and the tubes heated an additional eight hours. The digests were then diluted to volume with deionized water.

Cadmium concentrations in water samples and tadpole digests were measured using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction.

Statistical Analyses

Median lethal concentrations were calculated using the trimmed Spearman-Karber method (Hamilton et al. 1977) based on initial exposure concentrations. All other statistical analyses were performed using Statistical Analysis System (SAS), PC version. Initial and 24 hour cadmium concentrations were compared using one-tailed t-test.

RESULTS

Water quality characteristics are presented in Table 23 . Mean cadmium concentrations of initial and 24 hour old exposure solutions are presented in Table 24. With the exception of the control, cadmium concentrations of 24 hour old solutions are significantly lower than the initial concentrations. Figure 2. shows mortality over time for the different cadmium exposure concentrations. Median lethal concentration (LC50) for 72 hours exposure was 815 μg Cd/L with a 95% confidence interval of 741-895 μg Cd/L. The 96 h LC50 was 582 μg Cd/L with a 95% confidence interval of 539-628 μg Cd/L. Mean wet weights, dry weights and cadmium content of tadpoles are reported in Table 25. The experiment was not replicated therefore statistical inferences are limited. However clearly the lowest observed effect concentration (LOEC) based on mortality and growth is 500 μg Cd/L with a no observed effect concentration (NOEC) of 100 μg Cd/L (Figure 2 and Table 25). The maximum allowable toxicant concentration (MATC) calculated as the geometric mean of LOEC and NOEC is 223 μg Cd/L.

DISCUSSION

The cadmium exposure concentrations declined substantially over the course of 24 hours. This result underscores the importance of collecting a water sample from solutions at the beginning and end of renewals. Boreal toad tadpoles appear to be very tolerant of aqueous cadmium exposure compared to salmonids. The 96 hour LC50 for boreal toads was 582 μg/L compared to 2.7 and 3.0 μg/L for rainbow trout, *Oncorhynchus mykiss*, in water with the same characteristics (Davies et al. 1993). Exposure to cadmium concentrations as high as 100 μg/L for 10 days did not result in significant mortality or reductions in growth. Exposure to aqueous cadmium resulted in substantial bioaccumulation of cadmium after ten days.

Table 23. Water quality parameters of exposure water.

Hardness (mg $CaCO_3$/l)	49.9
Alkalinity (mg $CaCO_3$/l)	36.6
pH (S.U.)	7.16
Conductivity (μS)	111.6
Fluoride (mg/l)	1.3
Chloride (mg/l)	3.0
Nitrate (mg/l)	<0.5
Phosphate (mg/l)	<0.5
Sulfate (mg/l)	30.6

Table 24. Mean, standard deviation, and ranges of cadmium concentrations (μg/l) in freshly prepared and 24 hour-old exposure solutions.

Nominal (μg/l)	0		50		100		500		1000	
	Initial	24 hours	Initial	24 hours	Initial	24 hours	Initial	24 hours	Initial	24 hours
Mean (μg/l)	<5	<5	45.9	31.9*	91.6	51*	457	295*	908	655*
Std Dev	0	0	1.7	4.2	4.8	14.3	12.1	61.3	10.5	145
Range	<5	<5	44-48	25-38	83-100	28-67	433-477	161-350	895-920	407-785

*Significantly less than initial ($p<0.01$)

Table 25. Mortality (%), mean wet and dry weight (mg) and cadmium content (μg/g dry weight) of tadpoles following ten day exposure to waterborne cadmium.

Nominal cadmium concentration (μg/l)	0	50	100	500	1000
Mortality (%)	1.1	0.0	6.2	27.1	100
Mean wet weight (mg)	10.9	10.0	10.9	5.8	--
Mean dry weight (mg)	0.651	0.579	0.592	0.397	--
Cadmium content (μg/g dry weight)	0.12	6.34	13.83	34.89	--

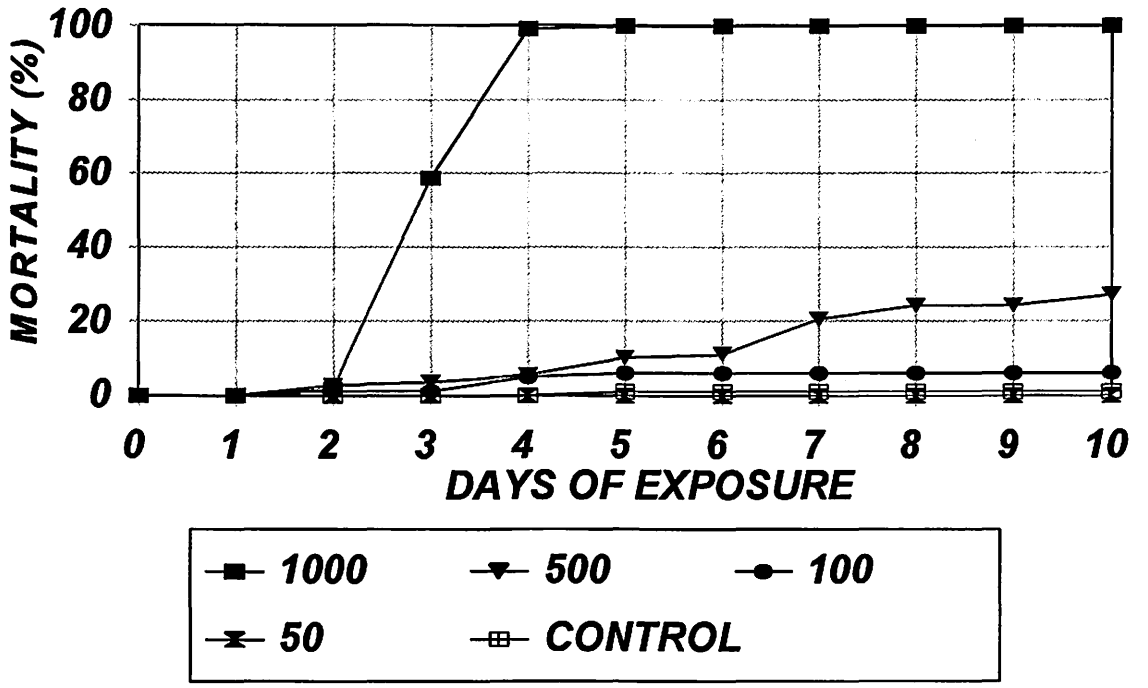

Figure 2. Mortality (%) of boreal toads exposed to waterborne cadmium over time.

Acute toxicity of copper to boreal toads subjected to a 24 hour static renewal toxicity test for ten days.

INTRODUCTION

A 24-hour static renewal copper toxicity test was conducted on Boreal toad (*Bufo boreas*) tadpoles as part of investigations into the decline of this species in Colorado. Copper can be quite toxic to toad tadpoles (John Goettl, Colorado Division of Wildlife, personal communication). The primary purpose of this experiment is to provide a range of copper exposure concentrations to use in a follow up chronic flow-through toxicity test.

MATERIALS AND METHODS

Exposure

Boreal toad (*Bufo boreas*) tadpoles approximately 10 d old were placed in 300 ml polyethylene beakers containing 200 milliliters of 100, 50, 20, 10, or 0 μg Cu/l added as copper sulfate pentahydrate to dechlorinated Fort Collins city tap water. The beakers were placed in an incubator, covered with a watch glass and gently aerated with compressed air. Temperature of the incubater was maintained at 25°C during a

12 hour day and 15°C during a 12 hour night. Tadpoles were transferred to 300 ml polyethylene beakers containing 200 mls of freshly prepared solutions daily using a brine shrimp net. Mortalities were monitored daily and removed using a pipet. Tadpoles were fed daily with Mazuri amphibian feed and frozen lettuce. Duration of exposure was ten days.

Water Quality

Water quality parameters were measured weekly. Hardness and alkalinity were determined according to Standard Methods (APHA). pH was determined using an Orion Research pH meter 811 calibrated daily using pH 7.00 and 4.00 pH buffers. Conductivity was determined using a YSI Model 35 conductance meter. Anions were determined using a Dionex 4000i ion chromatograph.

Copper Analyses

Water samples for copper analysis were collected from freshly prepared solutions. Water samples for copper analysis were preserved by acidification to pH < 2 using Ultrex nitric acid.

Surviving tadpoles within an exposure concentration were pooled, dried with a paper towel and weighed in preweighed polypropylene centrifuge tubes. The tubes containing the tadpoles were placed in a drying oven at 80°C and dried to constant weight. The tadpoles were then digested with trace metal grade nitric acid and heated for eight hours in a water bath at 60°C. Trace metal grade hydrogen peroxide was added and the tubes heated an additional eight hours. The digests were then diluted to volume with deionized water.

Copper concentrations in water samples and tadpole digests were measured using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction.

Statistical Analyses

Median lethal concentrations were calculated using the trimmed Spearman-Karber method (Hamilton et al. 1977).

RESULTS

Water quality characteristics of exposure water are shown in Table 26. Measured copper concentrations and associated mortality are shown in Table 27. All mortalities occurred within 24 hours of exposure. The 24, 48, 72 and 96 hour median lethal concentration (LC50) is 57.11 μg Cu/L with a 95% confidence interval of 47-69 μg Cu/L. This experiment like the cadmium toxicity test was not replicated therefore statistical inferences are again limited. However clearly the lowest observed effect concentration (LOEC) based on mortality (Table 27) is 57.7 μg Cu/L with a no effect concentration (NOEC) of 24.2 μg Cd/L (Table 27). The maximum allowable toxicant concentration (MATC) calculated as the geometric mean of LOEC and NOEC is 37.4 μg Cu/L.

DISCUSSION

Copper appears to be much more toxic to boreal toad tadpoles than cadmium. Aqueous copper caused mortality at one tenth the concentrations of cadmium and in much shorter times (see "Acute toxicity of cadmium to boreal toads subjected to a 24 hour static renewal toxicity test for ten days.", this report). Copper exposure does not seem to reduce growth of boreal toad tadpoles to the same extent as cadmium exposure.

That all mortality occurred during the first 24 hours of exposure can be explained several ways. All sensitive individuals may have died during the first 24 hours while the more tolerant individuals remained. Alternatively, the surviving tadpoles may have acclimated to the copper exposure concentrations. Finally, the first 24 hours of exposure may have occurred during a very sensitive lifestage which the tadpoles quickly out grew.

Table 26. Water quality parameters of exposure water.

Hardness (mg $CaCO_3$/l)	49.9
Alkalinity (mg $CaCO_3$/l)	36.6
pH (S.U.)	7.16
Conductivity (μS)	111.6
Fluoride (mg/l)	1.3
Chloride (mg/l)	3.0
Nitrate (mg/l)	<0.5
Phosphate (mg/l)	<0.5
Sulfate (mg/l)	30.6

Table 27. Mean and standard deviation of copper exposure concentrations, associated mortality, mean wet and dry weights and copper content of boreal toads subjected to a ten day 24 h static renewal toxicity test.

Nominal (μg/l)	0	10	20	50	100
Mean Cu Concentration (μg/l)	<5.0	13.6	24.2	57.7	132
Std. Dev.	0	3.0	3.6	9.6	0
% Mortality	0	0	0	50	100
Mean Wet Weight (mg)	136.7	132.3	136.4	101.0	--
Mean Dry Weight (mg)	8.3	8.0	7.9	7.2	--
Cu Content (μg/g dry wt)	9.8	19.5	19.4	46.9	---

Acute toxicity of hydrogen peroxide to rainbow trout (*Oncorhynchus mykiss*)

INTRODUCTION

Because hydrogen peroxide (H_2O_2) is a strong oxidizer, it was hoped that it could be used as a replacement for potassium permanganate ($KMNO_4$) to detoxify rotenone. Use of hydrogen peroxide to detoxify rotenone would have several advantages over potassium permanganate. Hydrogen peroxide is colorless and the breakdown products are water and oxygen. The toxicity of potassium permanganate is very close to treatment levels (Davies 1983). This increases risks involved with using rotenone since too little $KMNO_4$ may not detoxify rotenone and too much is toxic. To determine the toxicity of H_2O_2, an intermittent flow-through test was conducted on rainbow trout (*Oncorhynchus mykiss*).

MATERIALS and METHODS

A modified Mount and Brungs (1967) diluter delivered six concentrations of hydrogen peroxide and control to 90 L aquaria using dechlorinated Fort Collins tap water as a source. Each aquaria received two liters of solution every 2.3 minutes. Ten adult rainbow trout (about 135 g each) supplied by Pitkin Hatchery in Pitkin, Co. were placed in each exposure aquaria. Aquaria were checked every six hours for mortalities during the first 24 hours of exposure. Mortalities were checked every 12 hours thereafter. Lengths (mm) and weights (g) were determined on all mortalities. Fish were not fed during the first 96 hours. After one week of exposure, surviving trout were sacrificed via terminal anesthesia with metomidate and lengths and weights determined. The 96 hour LC50 was calculated using the trimmed Spearman-Karber technique (Hamilton et al. 1977). Water hardness and alkalinity were measured according to Standard Methods (APHA 1985). Dissolved oxygen was measured using a YSI Model 58 dissolved oxygen meter. An Orion Research model 811 pH meter calibrated daily with two standardized buffers was used to measure pH. Conductivity was determined using a YSI Model 35 conductance meter. Hydrogen peroxide concentrations were measured daily in all aquaria during the first 96 hours. Hydrogen peroxide was measured using iodometry (Vogel 1985). Two toxicity experiments were performed. The first used hydrogen peroxide exposure concentrations as high as 8.5 mg/L but did not result in any mortality. A second toxicity experiment used nominal exposure concentrations of 30 and 15 mg/L. Approximately 24 hours after the start of the experiment, a new toxicant stock solution was made using a different lot of hydrogen peroxide. The new lot of hydrogen peroxide was considerably stronger than the previous lot causing the exposure concentration to increase to about 2.5 times nominal. This situation was quickly corrected but probably contributed to greater mortality than expected. After the pulse of high H_2O_2 concentrations a fish in the 16.8 mg/L exposure concentration was found on its side though it continued to gill. The fish remained on its side for the rest of the experiment but never fully died. At the end of the experiment, the fish was sacrificed and sent on ice to the Colorado State Fish Health Laboratory for examination to determine gill damage.

RESULTS

Concentrations of hydrogen peroxide and associated mortality of the two toxicity tests are shown in Table 28. Concentrations and associated mortality over time are shown in Table 29. This table shows the large increase of hydrogen peroxide concentrations that occurred at 46 hours and the associated mortality. Much of the mortality observed during the test occurred during or shortly after 46 hours of exposure when the concentrations of hydrogen peroxide were elevated. The 96 hour LC50 is 26.6 mg/L with a 95% confidence interval of 18.8-32.3 mg/L. The LC50 was calculated using the mean concentrations of hydrogen peroxide including the elevated concentrations. Water quality characteristics are shown in Table 30. The lengths and weights of trout used in the experiment are shown in Table 31. Following 96 hours of exposure, rainbow trout ate freely when fed trout pellets. Examination of the gills of the sacrificed fish sent to the Fish Health lab showed no abnormalities or damage.

DISCUSSION

According to the results of this toxicity test, rainbow trout can tolerate hydrogen peroxide concentrations as high as 8 mg/L for one week with no mortality. Exposure to 20 mg/L for one week resulted in only 30 percent mortality. Much of the observed toxicity occurred during or shortly after the sharp rise of hydrogen peroxide concentrations and mortalities would probably have been lower if concentrations remained constant. Therefore the LC50 from this experiment can be regarded as a fairly conservative estimate of toxicity. The low toxicity of hydrogen peroxide is expected since it is often used to treat ectoparasites in fishes. However, the toxicity of potassium permanganate is much greater. In soft water similar to that used in this experiment, Davies (1983) found that exposure to 6.8 mg/L $KMNO_4$ for 4 hours resulted in 100 % mortality. Toxicity in hard water is even greater since exposure to $KMNO_4$ as low as 2.0 mg/L resulted in 100% mortality in only 6 hours. These results suggest that H_2O_2 would be a safer alternate than $KMNO_4$ for detoxification of rotenone. However later experiments using small rainbow trout placed in beakers containing rotenone and different concentrations failed to demonstrate that hydrogen peroxide was capable of detoxifying rotenone.

Table 28. Measured hydrogen peroxide concentrations (mg/L) and associated mortality (%) during first 96 hours of toxicity test.

Nominal Concentration (mg/L)	Measured Concentration (mg/L)	Standard Deviation	Mortality (%)
30	37.43	17.65	90
16.8	19.74	9.22	20
8.0	8.30	0.29	0
6.0	6.33	0.32	0
4.5	4.52	0.08	0
2.9	2.81	0.13	0
1.4	1.88	0.25	0
0.8	1.04	0.10	0
0	<0.50	0.00	0

Table 29. Concentrations of hydrogen peroxide (mg/L) and associated mortality (%) over time for the second acute toxicity test using higher exposure concentrations. Note the elevated exposure concentration and resultant mortality at 46 hours.

Hours of Exposure	Nominal 30 mg/L		Nominal 16.8 mg/L		Control	
	Measured H_2O_2 (mg/L)	% Mortality	Measured H_2O_2 (mg/L)	% Mortality	Measured H_2O_2 (mg/L)	% Mortality
1	30.9	0	18.0	0	<0.5	0
3	29.4	0	17.2	0	<0.5	0
22	31.1	30	15.3	0	<0.5	0
46	77.3	80	40.5	10	<0.5	0
53	30.7	80	14.7	20	<0.5	0
72	28.8	80	15.7	20	<0.5	0
96	33.8	90	16.8	20	<0.5	0
120	26.2	90	16.0	20	<0.5	0
144	27.4	90	15.8	30	<0.5	0

Table 30. Water quality characteristics of exposure water during hydrogen peroxide toxicity test.

	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	pH (S.U.)	Dissolved Oxygen (mg/L)	Temperature (°C)	Conductivity (μS/cm)
Mean	47.1	33.4	7.99	9.33	12.1	86.7
Std Dev	1.0	17.0	0.21	0.24	1.2	6.6

Table 31. Mean lengths (mm) and weights (g) of rainbow trout subjected to acute hydrogen peroxide toxicity test.

	Length (mm)	Weight (g)
Mean	233.7	135.0
Standard Deviation	19.5	31.1

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 1. Water Quality Assistance to Other Personnel

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

Water quality characteristics and or metal analyses were performed for the following persons and agencies:

Will Clements, Colorado State University (CSU)
John Stednick, CSU
Barb Horn, Colorado Division of Wildlife (CDOW)
John Woodling, CDOW
John Goettl Jr., CDOW
Greg Policky, CDOW
Ken Kehmeier, CDOW
Del Nimmo, National Biological Survey (NBS)
Steve Corn, NBS
Therese Johnson, National Park Service
Chuck Peterson, Yellowstone National Park
Eldon Guymon, United States Forest Service
Robert Pistisano, Wyoming Game and Fish

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