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The Acute Toxicity of Zinc to Rainbow and Brook Trout Comparisons in Hard and Soft Water

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THE ACUTE TOXICITY OF ZINC TO RAINBOW AND BROOK TROUT

Comparisons in Hard and Soft Water

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FOREWORD

Zinc is a ubiquitous pollutant and occurs in water from natural sources as well. Regulatory agencies face increasing difficulty trying to establish acceptable limits for such pollutants and at the same time not setting limits far below ambient concentrations which seemingly cause little harm to the aquatic environment.

Several important factors are known that make zinc biologically less active and therefore less toxic. Complexes of zinc in mineral form or with organic materials may reduce its availability. Inherent water quality characteristics such as pH, hardness, and alkalinity also change the biological activity of zinc.

The work described in this report further elucidates the subtleties of these latter effects. Such information should make the task of specifying acceptable limits easier for a variety of natural waters.

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ABSTRACT

The means and ranges of the 96-hour LC50's derived from three tests were 0.55 (0.37 to 0.76) and 2.5 (1.9 to 3.0) mg Zn/liter for rainbow and 2.0 (1.6 to 2.4) and 6.0 (5.0 to 7.0) mg Zn/liter for brook trout in soft and hard water, respectively. Based on overall means, brook trout were approximately 2.7 times more resistant than rainbow trout. Zinc toxicity to both species increased with increasing pH, and decreased with increasing hardness and alkalinity.

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ACKNOWLEDGMENTS

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SECTION 1

INTRODUCTION

Zinc is widely used in industry and is a common water pollutant; thus, knowledge of its toxicity to aquatic organisms is important. Numerous studies have documented the acute toxicity of zinc to various species of fish tested under conditions of stable water quality. Several of these studies include work on salmonid species (1-5). Another study by Nehring and Goettl (6) compared the acute toxicity of zinc to four different salmonid species. Acute tests comparing the toxicity of zinc to rainbow trout under conditions where water quality parameters were varied have also been conducted (7-9). A study has been conducted comparing the effects of varying pH and hardness on the toxicity of zinc to fathead minnows (*Pimephales promelas*) (10). Cairns et al. (11) studied the effects of variable pH, temperature and zinc solubility in a test on the bluegill sunfish (*Lepomis macrochirus*). Both Skidmore (12) and Pagenkopf (13) have reviewed the current state of knowledge of the toxicity of zinc to fish and the relationship to water quality.

This study included three separate exposures of rainbow trout (*Salmo gairdneri*) and brook trout (*Salvelinus fontinalis*) to zinc (as the sulfate) in hard and soft water to determine acute lethality, to compare toxicity differences between the two species, and to determine the effects of water quality changes on the toxicity of zinc to each species. The concentration of zinc which was lethal to 50 percent of the fish exposed at that concentration in 96 hours (96 hour LC50) was calculated for each species of fish in both hard and soft water for each of the three tests. Tests were conducted using the same nominal zinc levels and with the same stock of fish at different ages to yield information on the effects of size of the fish on susceptibility to zinc. In addition, two methods of artificially hardening water were used to compare resulting changes in zinc toxicity. Information gained from this study was used to compare and supplement data obtained by other investigators, and to estimate the effect of differing water quality conditions on the determination of an application factor (14) for zinc.

SECTION 2

CONCLUSIONS

Results of these acute tests indicated that rainbow trout were approximately 3.7 and 2.4 times more sensitive to zinc than were brook trout in soft and hard water, respectively. Based on overall means, rainbow trout were approximately 2.7 times more sensitive to zinc than brook trout under the same experimental conditions.

Increasing the mean water hardness from approximately 44 to 170 mg/l (as $CaCO_3$) using $Ca(NO_3)_2 \cdot 4H_2O$ did not change alkalinity, but did decrease the toxicity of zinc by a factor of 2.5 and 2.1 for rainbow and brook trout, respectively.

Increasing both hardness (from 47 to 179 mg/l as $CaCO_3$) and alkalinity (from 42 to 170 mg/l as $CaCO_3$) using a water hardener, decreased zinc toxicity by a factor of 5.7 to 6.8 and 3.3 to 3.9 for rainbow and brook trout, respectively.

Both the LC50's and the slopes of the probit mortality curves were significantly correlated with hardness, alkalinity and pH.

Susceptibility to zinc appeared to decrease slightly with an increase in the size of exposed fish but there was no significant correlation of LC50's or slopes of the mortality curves with weight of the fish.

Zinc was slightly more toxic to rainbow and brook trout in water hardened with $Ca(NO_3)_2 \cdot 4H_2O$ than in water hardened to the same degree with a water hardener (18). It appeared that this difference was correlated with changes in alkalinity and possibly with zinc complexation or precipitation by carbonate.

SECTION 3

RECOMMENDATIONS

It appears that if a source of hard water is unavailable, a water hardener (18) should be used to artificially harden water for use in toxicity tests rather than $Ca(NO_3)_2 \cdot 4H_2O$. The latter method does not correspondingly increase the alkalinity with increasing hardness as usually occurs in natural waters.

When fish that are being held in soft water are used in a hard water toxicity test, the fish should be acclimated to the hard water conditions for at least one week or until they are actively feeding to prevent placing a double stress on the fish.

Better control and more accurate measurement of variables such as pH, hardness, and alkalinity, coupled with measurements of complexation or precipitation, are needed to adequately interpret such metal toxicity tests in the future.

SECTION 4

MATERIALS AND METHODS

EXPOSURE SYSTEM

The exposure systems for these tests consisted of two, two liter/cycle, proportional diluters (15) with dilution factors of 0.65 and adjusted to cycle almost simultaneously. Each diluter had a series of flow splitting chambers (16) which divided each of the six concentrations equally into two duplicate stainless steel tanks. One diluter operated with soft water and one with artificially hardened water. Both rainbow and brook trout were exposed during each test, each species in one set of duplicate tanks in each system so that exposure conditions would be identical between species for each type of water. The tanks measured 92 by 36 by 41 cm deep with a 31 cm standpipe which yielded a tank volume of 100 liters. Flow rates for both diluters averaged 650 ml/minute with a 90 percent replacement time of six hours (17). Tanks were covered with fiberglass mesh screens and were surrounded by curtains to minimize disturbance of the fish. A constant 12 hour photoperiod was used during all tests and a combination of Sylvania Gro-Lux and Duro-Test Vita Lite fluorescent lamps was the source of illumination.

WATER CHARACTERISTICS

Water for all tests was obtained directly from Lake Superior, passed through an ultraviolet sterilizer to kill pathogens, and heated to 15 ± 1°C (Table 1) by a hot water heat exchange system. Water used in the three soft water exposures was unaltered Lake Superior water. The hard water used in tests #1 and #2 (Table 1) was produced by passing Lake Superior water through a water hardener (18). For test #3, Lake Superior water was hardened by dripping calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$) dissolved in distilled water into the diluter headbox from a Mariotte bottle. In this way, calcium hardness was increased without increasing the alkalinity. Hardness, alkalinity, specific conductance, and pH measurements were made daily on the test water in one or more tanks of each diluter system and in most cases measurements were made on samples from all tanks on one day during each test. Water chemistry information from all tests is contained in Table 1. Dissolved oxygen measurements were made twice during each test in each diluter and ranged from 9.4 to 10.2 mg/liter. All water characteristics were measured using methods described by the American Public Health Association (19).

TOXICANT SOLUTIONS

Zinc sulfate (granular, hydrated $ZnSO_4 \cdot 7H_2O$, reagent grade), manufactured by Mallinckrodt Chemical Company, was dissolved in distilled water in a

TABLE 1. EXPOSURE DATES, WATER QUALITY CHARACTERISTICS, AND TEST CONDITIONS OF THE THREE ACUTE ZINC EXPOSURES

Test number and exposure dates	Water type	pH	Hardness (mg/liter as $CaCO_3$)	Alkalinity (mg/liter as $CaCO_3$)	Specific conductance (micromhos)	Temperature (°C)
#1						
6-28-73 through 7-2-73	Soft	7.63 (7.53-7.69)* (14)‡	46.8±1.4† (12)‡	41.8±0.6† (12)‡	72.4 (64.9-80.5)* (14)‡	14.9 (14.7-15.0)* (5)‡
	Hard§	7.41 (7.32-7.46) (14)	177.6±4.6 (24)	170.2±0.7 (24)	261.8 (248.0-269.0) (14)	14.9 (14.4-15.4) (5)
#2						
8-1-73 through 8-5-73	Soft	7.58 (7.39-7.70) (14)	47.0±1.5 (12)	42.8±0.2 (12)	74.3 (65.4-93.0) (14)	15.2 (14.8-15.5) (5)
	Hard§	7.17 (6.94-7.45) (14)	179.0±5.0) (12)	170.1±1.3 (14)	256.4 (227.0-280.0) (14)	15.7 (15.3-16.5) (5)
#3						
12-17-73 through 12-21-73	Soft	7.38 (7.19-7.50) (15)	44.4±0.5 (4)	42.5±0.7 (4)	68.8 (65.5-73.1) (15)	15.0 (14.9-15.2) (5)
	Hard#	7.31 (7.14-7.43) (15)	169.7±4.0 (9)	43.0±0.3 (9)	274.5 (256.0-312.0) (15)	14.8 (14.6-14.9) (5)

* - Mean and range.

† - Mean and standard deviation.

‡ - Number of measurements.

§ - Water hardened with a water hardener (Lemke 18).

\# - Water hardened using $Ca(NO_3)_2 \cdot 4H_2O$.

Mariotte bottle and introduced to the diluters by a toxicant metering device (20). Nominal zinc concentrations were different for the soft and hard water diluters, but were kept the same throughout the three tests for each water type. Measured zinc concentrations (Table 2) varied somewhat due to slight changes in the toxicant metering device. At the start of each test, after fish had been placed in them, the tanks were spiked with a calculated volume of solution from the Mariotte bottle to bring the zinc concentrations up to approximate nominal levels. Daily water samples were taken from one set of duplicate tanks in both diluters and analyzed for zinc daily during each test. Zinc concentrations in the test solutions were measured by direct aspiration of acidified samples (10^{-2}M $HClO_4$) into a Perkin-Elmer 403 Atomic Absorption Spectrophotometer with an air-acetylene flame (21). On several occasions zinc concentrations were also measured by differential pulse polarography using a PAR Model 174 Polarographic analyzer with a dropping mercury electrode to determine an approximation of "dissolved" zinc. Calibration curves constructed by the method of known additions of zinc to control water were used in both methods of zinc analysis. Comparisons of results from the two methods of analysis showed that between 90 to 100 percent of the total zinc present in the test water was dissolved.

BIOLOGICAL METHODS

The juvenile rainbow and brook trout used in the three tests were obtained from Cedar Bend Trout Farm in Scandia, Minnesota. The species were kept in separate fiberglass holding tanks in unaltered Lake Superior water maintained at 15°C prior to being transferred to the test tanks. Fifty trout of each species were placed by random assignment in the respective duplicate tanks of both diluter systems for tests #1 and #2. In test #3, only 30 brook trout were used per tank in one set of duplicates in both diluters to avoid overcrowding; only 11 rainbows were used per tank in the other sets of duplicates because of an inadequate supply of fish. Exposure dates and the approximate size of each species of trout at the time of exposure are given in Tables 1 and 3, respectively. For all tests, the fish were allowed to acclimate to test water conditions before zinc exposure was started. Acclimation was considered adequate when all fish were actively feeding in the test tanks, which required one week in test #1 and three weeks in tests #2 and #3. Feeding was discontinued 48 hours prior to zinc exposure and fish were not fed during the tests. Mortality was the effect used throughout this series of exposures. Procedures used regarding aeration, tank cleaning, and checking for and removing dead fish followed the basic flow-through acute toxicity test method described by the Committee on Methods for Toxicity Tests with Aquatic Organisms (22).

STATISTICS

The 96 hour LC50's and the slope of the percent mortality vs. concentration curves for each species in each test were determined using the probit method (23). The computer program used (24) fits a linear curve to the probit of the percent mortality vs. log zinc concentration by iteration of maximum likelihood. Stepwise multiple regression (25) of LC50 and the slope of the mortality curves vs. the experimental variables: species, weight of fish, pH, hardness and alkalinity were also performed. For comparative purposes similar calculations were performed using data from Mount (10).

TABLE 2. MEASURED ZINC CONCENTRATIONS IN THE SOFT AND HARD WATER EXPOSURES DURING EACH OF THE THREE TESTS, AND PERCENT MORTALITY OF RAINBOW AND BROOK TROUT OCCURRING AT EACH CONCENTRATION IN 96-HOURS

Test #1					
Soft			Hard		
Zinc conc. in mg/liter	96-hr rainbow mortality (%)	96-hr brook mortality (%)	Zinc conc. in mg/liter	96-hr rainbow mortality (%)	96-hr brook mortality (%)
2.84$\pm$0.17*	100†	78†	7.39$\pm$0.42*	100†	78†
1.90$\pm$0.14	98	66	4.80$\pm$0.24	100	12
1.25$\pm$0.10	96	44	3.21$\pm$0.30	90	10
0.84$\pm$0.10	80	16	2.21$\pm$0.17	16	0
0.56$\pm$0.12	74	2	1.47$\pm$0.15	6	2
≤0.01 (control)	2	0	≤0.01 (control)	0	2

Test #2					
Soft			Hard		
Zinc conc. in mg/liter	96-hr rainbow mortality (%)	96-hr brook mortality (%)	Zinc conc. in mg/liter	96-hr rainbow mortality (%)	96-hr brook mortality (%)
2.45$\pm$0.14*	96†	52†	9.64$\pm$0.07*	100†	96†
1.55$\pm$0.11	90	46	6.58$\pm$0.18	100	28
1.00$\pm$0.05	82	6	3.83$\pm$0.08	94	4
0.64$\pm$0.03	68	8	2.34$\pm$0.15	8	0
0.41$\pm$0.02	32	0	1.51$\pm$0.03	0	0
≤0.01 (control)	0	0	≤0.01 (control)	0	0

Test #3					
Soft			Hard		
Zinc conc. in mg/liter	96-hr rainbow mortality (%)	96-hr brook mortality (%)	Zinc conc. in mg/liter	96-hr rainbow mortality (%)	96-hr brook mortality (%)
2.93$\pm$0.10*	100‡	73.3§	7.86$\pm$0.28*	100‡	96.7§
1.83$\pm$0.10	100	20	4.84$\pm$0.44	100	36.6
1.32$\pm$0.09	90.9	0	3.31$\pm$0.26	100	10
0.86$\pm$0.16	36.4	0	2.19$\pm$0.14	54.5	3.3
0.53$\pm$0.04	36.4	0	1.49$\pm$0.15	27.3	0
≤0.01 (control)	0	0	≤0.01 (control)	0	0

* Mean and standard deviation of five daily water samples.

† Percent mortality of 50 fish exposed.

‡ Percent mortality of 11 fish exposed.

§ Percent mortality of 30 fish exposed.

TABLE 3. ZINC TOXICITY (LC50) AND SLOPE OF THE PROBIT MORTALITY CURVE IN SOFT AND HARD WATER IN THE THREE ACUTE TESTS

Species tested	Test no.	Mean Fish wt.* (g.)	LC50 (mg/1)	Slope (probit/log conc.)
			Soft water	
Rainbow trout	1	3.9	0.370 (0.195–0.494)†	2.98
Rainbow trout	2	4.9	0.517 (0.410–0.610)	2.87
Rainbow trout	3	28.4	0.756 (0.538–0.944)	4.66
Brook trout	1	3.0	1.55 (1.38–1.75)	3.68
Brook trout	2	3.9	2.12 (1.41–14.4)	3.22
Brook trout	3	19.0	2.42 (2.20–2.72)	8.15
			Hard water	
Rainbow trout	1	3.9	2.51 (1.65–3.71)	9.42
Rainbow trout	2	4.9	2.96 (2.77–3.15)	13.84
Rainbow trout	3‡	28.4	1.91 (1.53–2.28)	7.37
Brook trout	1	3.0	6.14 (0–∞)	5.21
Brook trout	2	3.9	6.98 (3.87–9.97)	9.08
Brook trout	3‡	19.0	4.98 (4.49–5.58)	6.90

* Mean live weight of 50 fish weighed in aggregate.

† 95 percent confidence (fiducial) limits.

‡ Water hardened using $Ca(NO_3)_2 \cdot 4H_2O$.

SECTION 5

RESULTS

The percent mortality resulting at all zinc concentrations in 96 hours for both species of trout in the three tests is shown in Table 2. A summary of the 96-hr LC50's calculated from the mortality data, the slope of the mortality curves (probit vs. log conc.), and size of test fish is shown in Table 3. The means and ranges of the 96-hr LC50's derived from the three tests were 0.55 (0.37 to 0.76) and 2.5 (1.9 to 3.0) mg Zn/liter for rainbow and 2.0 (1.6 to 2.4) and 6.0 (5.0 to 7.0) mg Zn/liter for brook trout in soft and hard water, respectively.

Based on overall means, brook trout were approximately 2.7 times more resistant to zinc toxicity (higher LC50) than rainbow trout, under the same experimental conditions. Linear correlations of LC50 and the slope of mortality curves with the experimental variables (Table 4), showed significant correlations with the covariants; pH, hardness, and alkalinity. No correlation was found with fish weight. Zinc toxicity in both species increased (decreasing LC50) with increasing pH, and decreased with increasing hardness and alkalinity. Slope of the mortality curve for both species decreased with increasing pH, and for rainbow trout increased with increasing hardness and alkalinity. With brook trout the relationship of slope to hardness and alkalinity was non-significant. On the basis of these correlations, multiple regression techniques (least squares) were used to fit equations for each species and the two species combined, relating LC50 to the significant variables pH, hardness, and alkalinity:

$$\text{Rainbow Trout LC50 (mg Zn/l)} = 12.39 - 1.65\,(\text{pH}) + 0.0081\,(\text{hardness}) + 0.0056\,(\text{alkalinity})$$

$$\text{Brook Trout LC50 (mg Zn/l)} = 26.50 - 3.42\,(\text{pH}) + 0.018\,(\text{hardness}) + 0.010\,(\text{alkalinity})$$

$$\text{Combined LC50* (mg Zn/l)} = 11.16 - 1.47\,(\text{pH}) + 0.0074\,(\text{hardness}) + 0.0047\,(\text{alkalinity}).$$

* In order to combine LC50's from both species into a single regression equation, brook trout LC50's were divided by 2.7 which is the ratio of the mean LC50's for the two species.

TABLE 4. CORRELATION COEFFICIENTS OF EXPERIMENTAL VARIABLES, LC50, AND SLOPE OF MORTALITY CURVES FOR RAINBOW (UPPER RIGHT) AND BROOK TROUT (LOWER LEFT), RESPECTIVELY (N=6)

	LC50	Slope	pH	Hardness	Alkalinity	Fish wt.	
LC50	-	0.97	-0.83	0.96	0.86	N.S.*	Rainbow trout
Slope	N.S.	-	-0.87	0.87	0.87	N.S.	
pH	-0.83	-0.93	-	N.S.	N.S.	N.S.	
Total hardness	0.96	N.S.	N.S.	-	N.S.	N.S.	
Total alkalinity	0.85	N.S.	N.S.	N.S.	-	N.S.	
Fish wt.	N.S.	N.S.	N.S.	N.S.	N.S.	-	
Brook trout							

* Not significant, P=0.05, to be significant at P=0.05 with N=6, r must be greater than 0.73.

SECTION 6

DISCUSSION

Previously published zinc LC50's for rainbow trout exposed in soft water agree closely, even in static exposures, with the range of LC50's observed for rainbow trout (0.37 to 0.76 mg Zn/liter) in the present study. In a series of soft water (hardness = 25 mg/liter as $CaCO_3$) exposures, Goettl et al. (9) observed 96-hr LC50's for rainbow trout ranging from 0.24 to 0.83 mg Zn/liter depending on size of fish and test conditions. Chapman (5) reported 96-hr LC50's for steelhead trout in soft water (hardness = 25 mg/liter as $CaCO_3$) ranging from 0.093 to 0.82 mg Zn/liter depending on which life stage was exposed. Nehring and Goettl (6) reported a 14-day LC50 of 0.41 mg Zn/liter for rainbow trout in soft water (hardness = 40 mg/liter as $CaCO_3$). Herbert and Shurben (8) found the 48-hr LC50 for rainbow trout in soft water (hardness = 44 mg/liter as $CaCO_3$) to be 0.91 mg Zn/liter in a static bioassay. Soft water LC50 values obtained from zinc exposures of salmonid species other than rainbow trout were also within the range of those obtained for rainbows in the present study. Sprague (4) obtained a 24-hr LC50 of 0.65 mg Zn/liter exposing Atlantic salmon (*Salmo salar*), Rabe and Sappington (3) derived a 24-hr LC50 of 0.42 mg Zn/liter using cutthroat trout (*Salmo clarki*), Chapman (26) found a 96-hr LC50 of 0.7 mg Zn/liter with sockeye salmon (*Oncorhynchus nerka*), and Nehring and Goettl (6) calculated 14-day LC50s of 0.67 and 0.64 mg Zn/liter for cutthroat and brown trout (*Salmo trutta*), respectively.

The hard water LC50's for rainbow trout (1.9 to 3.0 mg Zn/liter) in the present study were generally lower than those derived by other investigators. Herbert and Shurben (8) and Brown (2) reported 48-hr LC50's of 3.86 and 3.5 mg Zn/liter, respectively in water with a total hardness of 320 mg/liter as $CaCO_3$. Solbe (1) found a 48-hr LC50 of 4.76 mg Zn/liter in very hard water (hardness = 500 mg/liter as $CaCO_3$), and Ball (27) obtained a 120-hr LC50 of 4.6 mg Zn/liter in hard water. Goettl et al. (9) derived 96-hr LC50's between 1.19 and 7.21 mg Zn/liter in water with a total hardness of 350 mg/liter as $CaCO_3$ depending on size of fish and test conditions. Differences in toxicity of zinc to rainbow trout between the present study and those found by other investigators are believed due mainly to differences in total hardness and slight variations in pH between exposures.

The range of 96-hr LC50's for brook trout from the present study was 1.6 to 2.4 mg Zn/liter in soft water, while Nehring and Goettl (6) observed a 14-day LC50 of 0.96 mg Zn/liter for brook trout in soft water (hardness = 40 mg/liter as $CaCO_3$). Considering the longer exposure in the earlier work, these results are in reasonable agreement.

The relationship of zinc toxicity to carbonate alkalinity has not been reported previously, probably because this parameter has not been varied independently of calcium hardness. Use of the water hardener (18) and additions of calcium (as the nitrate) indicate that calcium hardness and carbonate alkalinity are equally important factors governing the toxicity of zinc to fish. Except for the work of Mount (10) and Cairns et al. (11), pH likewise has not been widely recognized as having such a large effect on zinc toxicity.

These tests present an ideal situation for comparison of toxicity differences between species since both species were exposed in the same diluter system under identical experimental conditions for both hard and soft water. Rainbow were found to be more sensitive to zinc than brook trout in both hard and soft water. The mean ratio of LC50's for the two species was 2.7. A series of acute tests comparing zinc toxicity differences between species was also conducted by Nehring and Goettl (6). They found rainbows to be 2.5 times more sensitive to zinc in soft water than were brook trout. The inverse relationship of decreased zinc toxicity with increased water hardness found for both species of fish in the present study agreed with previous reports of Cairns and Scheier (28) and Pickering and Henderson (29) on bluegills (Lepomis macrochirus), Sinley et al. (30) on rainbow trout, and Mount (10) using fathead minnows. Zitko and Carson (31) also observed decreased zinc toxicity with increased water hardness on Atlantic salmon and concluded this was caused by a competition for active sites between magnesium and zinc.

When comparing the 96-hr LC50 values with the size of fish exposed (Table 3) for both species in both soft and hard water (disregarding test #3 in hard water), it appears that zinc toxicity decreased as size increased. Although not statistically significant ($p \geq 0.05$), there appeared to be a slight trend toward decreased zinc toxicity with increased fish size. This trend, however, more closely correlated with a decrease in pH, probably as a result of increased CO_2 excretion from the larger fish. Goodman (32), Goettl et al. (9), and Chapman (5) have also found that, in general, smaller rainbow trout are more susceptible to zinc than larger ones.

Mount (10) also found that zinc was more toxic to fathead minnows with increasing pH and decreasing hardness. Using Mount's data, a significant correlation of LC50's with hardness and pH were found. The relationship with alkalinity was non-significant, since alkalinity was not varied independently in his tests. The multiple regression equation calculated from Mount's data was: Fathead minnow LC50 (mg Zn/l) = 47.87 - 6.04 (pH) + 0.072 (hardness). This indicated that the toxicity of zinc to fathead minnows (LC50) fluctuated more widely with pH or hardness changes than it did with either of the salmonid species tested in the present study. The LC50's for fathead minnows however, were much higher because a higher proportion of the zinc was in a precipitated or other non-available form (13).

Because of the effects of pH, hardness, and alkalinity on LC50 values for zinc, considerable caution should be exercised when comparing the sensitivities of species tested in different waters. Slight differences in experimental conditions, especially pH, result in changes in LC50 values that make direct comparisons of relative acute sensitivities impractical, unless the effect of water quality on the LC50 has been previously determined. For example, at a constant hardness and alkalinity the LC50 for trout varies by a factor of approximately two for each unit change in pH. This also indicates that when an application factor is determined for zinc, the water quality must be the same in the acute and chronic tests.

REFERENCES

1. Solbe, J. F., D. E. L. G. The Toxicity of Zinc Sulphate to Rainbow Trout in Very Hard Water. Water Res., 8:389-391, 1974.

2. Brown, V. M. The Calculation of the Acute Toxicity of Mixtures of Poisons to Rainbow Trout. Water Res., 2:723-733, 1968.

3. Rabe, F. W., and C. W. Sappington. The Acute Toxicity of Zinc to Cutthroat Trout (Salmo clarki). In: Biological Productivity of the Coeur d' Alene River as Related to Water Quality. 1970. Completion Rept. Water Resources Res. Inst. Univ. Idaho, Moscow, Idaho. pp. 1-16.

4. Sprague, J. B. Lethal Concentrations of Copper and Zinc for Young Atlantic Salmon. J. Fish. Res. Board Can., 21(1):17-26, 1964.

5. Chapman, G. A. Toxicities of Cadmium, Copper, and Zinc to Four Juvenile Stages of Chinook Salmon and Steelhead. Trans. Am. Fish. Soc. 107. In press.

6. Nehring, R. B., and J. P. Goettl, Jr. Acute Toxicity of a Zinc Polluted Stream to Species of Salmonids. Bull. Environ. Contam. Toxicol., 12(4): 464-469, 1974.

7. Lloyd, R. The Toxicity of Zinc Sulphate to Rainbow Trout. Ann. Appl. Biol., 48(1):84-94, 1960.

8. Herbert, D. W. M., and D. S. Shurben. The Toxicity to Fish of Mixtures of Poisons I. Salts of ammonia and zinc. Ann. Appl. Biol., 53:33-41, 1963.

9. Goettl, J. P., Jr., J. R. Sinley, and P. H. Davies. Water Pollution Studies. Colorado Div. Wild. Dept. Nat. Resource Proj. F-33-R, 1972. pp. 36-38.

10. Mount, D. I. The Effect of Total Hardness and pH on Acute Toxicity of Zinc to Fish. Air Water Pollut. Int. J., 10:49-56, 1966.

11. Cairns, J., Jr., T. K. Balms, D. T. Burton, K. L. Dickson, R. E. Sparks, and W. T. Waller. The Effects of pH, Solubility, and Temperature Upon the Acute Toxicity of Zinc to the Bluegill Sunfish (Lepomis macrochirus Raf.). Trans. Kansas Acad. Sci., 47(1):81-92, 1971.

12. Skidmore, J. F. Toxicity of Zinc Compounds to Aquatic Animals with Special Reference to Fish. Q. Rev. Biol., 39:227-248, 1964.

13. Pagenkopf, G. K. Zinc Speciation and Toxicity to Fishes. In: Toxicity to Biota of Metal Forms in Natural Water. Proc. of workshop held at Duluth, MN, Oct. 7-8, 1975. Publ. International Joint Commission, Windsor, Ontario, 1976. pp. 77-91.

14. Mount, D. I., and C. E. Stephan. A Method for Establishing Acceptable Toxicant Limits for Fish--Malathion and the Butoxyethanol Ester of 2,4-D. Trans. Am. Fish. Soc., 96:185-193, 1967.

15. Mount, D. I., and W. A. Brungs. A Simplified Dosing Apparatus for Fish Toxicology Studies. Water Res., 1:21-29, 1967.

16. Benoit, D. A., and F. A. Puglisi. A Simplified Flow-Splitting Chamber and Siphon for Proportional Diluters. Water Res., 7:1915-1916, 1973.

17. Sprague, J. G. Measurement of Pollutant Toxicity to Fish. I. Bioassay methods for acute toxicity. Water Res., 3:793-821, 1969.

18. Lemke, A. E. A Water Hardener for Experimental Use. J. Am. Water Works Assoc., 61(8):415-416, 1969.

19. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. Standard Methods for the Examination of Water and Waste Water. 13th Ed. New York, N.Y., 1971. 874 pp.

20. McAllister, W. A., W. L. Mauck, and F. L. Mayer. A Simplified Device for Metering Chemicals in Intermittent-Flow Bioassays. Trans. Am. Fish. Soc., 101:555, 1972.

21. Perkin-Elmer. Analytical Methods for Atomic Absorption Spectrophotometry. September, 1976. 2 pp.

22. The Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. EPA-660/3-75-009, U.S. Environmental Protection Agency, Duluth, MN, 1975. 67 pp.

23. Finney, D. J. Probit Analysis. 3rd Ed., Cambridge Univ. Press. 1971. 333 pp.

24. Stephan, C. E., K. A. Busch, R. Smith, I. Burke, and R. W. Andrew. Computer Program for Calculating an LC50. In press.

25. Efroynisen, M. A. Edited by A. Ralston and H. S. Wilf. Multiple Regression Analysis, Mathematical Methods for Digital Computers, Part V, (77), 1960. 464 pp.

26. Chapman, G. A. Effects of Continuous Zinc Exposure on Sockeye Salmon During Adult-to-Smolt Freshwater Residency. Trans. Am. Fish. Soc., 107, In press.

27. Ball, I. R. The Relative Susceptibilities of Some Species of Freshwater Fish to Poisons - II. Zinc. Water Res., 1:777-783, 1967.

28. Cairns, J., Jr., and A. Scheier. The Effects of Temperature and Hardness of Water Upon the Toxicity of Zinc to the Common Bluegill (Lepomis macrochirus Raf.). Notul. Nat., 299, 1957. 12 pp.

29. Pickering, Q. H., and C. Henderson. The Acute Toxicity of Some Heavy Metals to Different Species of Warm-Water Fishes. Air Water Pollut. Int. J., 10:453-463, 1966.

30. Sinley, J. R., J. P. Goettl, Jr., and P. H. Davies. The Effects of Zinc on Rainbow Trout (Salmo gairdneri) in Hard and Soft Water. Bull. Environ. Contam. Toxicol., 12(2):193-201, 1974.

31. Zitko, V., and W. G. Carson. A Mechanism of the Effects of Water Hardness on the Lethality of Heavy Metals to Fish. Chemosphere, 5:299-303, 1976.

32. Goodman, J. R. Toxicity of Zinc for Rainbow Trout (Salmo gairdnerii). Calif. Fish and Game, 37(2):191-194, 1951.