The Effects of Zinc on Rainbow Trout (*Salmo gairdneri*) in Hard and Soft Water

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This research was performed to determine the effects of zinc sulfate on rainbow trout (Salmo gairdneri Richardson) in hard and soft water. Acute bioassays were used to determine TL_{50} values for zinc, i.e., that concentration of zinc lethal to 50% of the exposed fish. Chronic flow-through bioassays, in which exposure data collected over at least one generation of the test organism reflects the effects of a toxicant on growth, reproductive capacity, spawning behavior, viability of eggs, and the growth of fry, were used to evaluate the effects of zinc on rainbow trout throughout its life cycle. The results of these bioassays are expressed as "maximum acceptable toxicant concentrations" (MATC's). Well water (hardness = 330 mg/liter as $CaCO_3$) was used for the hard water experiments and dechlorinated tap water (hardness = 25 mg/liter as $CaCO_3$) was used for the soft water experiments.

The hard water chronic bioassay began with two-gram fingerlings and continued through sexual maturity as two-year-old fish. The soft water chronic bioassay began with eyed eggs and continued until, but not through, sexual maturity of the fish. Survival of fish from eggs and fry acclimated to sub-lethal concentrations was compared with survival of non-acclimated fish.

Because of the importance of zinc in the environment, toxicological information on zinc is available in the literature. BRUNGS (1969) developed an MATC for zinc using fathead minnows (Pimephales promelas, Rafinesque). However, most other work has dealt with the short-term, acute effects of zinc (LLOYD, 1960; GOODMAN, 1951; and MOUNT, 1966). There is a lack of information in the literature concerning chronic exposure of rainbow trout to zinc.

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METHODS

Acute bioassays--96-hour acute bioassays using juvenile rainbow trout were conducted in both hard and soft waters. Replicated, continuous-flow tests with 10 fish per replicate were conducted at 15 C according to the methods of the AMERICAN PUBLIC HEALTH ASSOCIATION *et al*. (1971). An acute bioassay was also conducted in soft water at 11 C using eyed eggs.

Chronic bioassays--The exposure system was similar to those used by BRUNGS (1969) and McKIM and BENOIT (1971). A modified proportional diluter (MOUNT and BRUNGS, 1967) delivered two liters of test solution to each aquarium every three minutes, resulting in a 95% replacement time of 20 hours (SPRAGUE, 1969). The test aquaria were 265-liter fiberglass tanks with acrylic plastic covers. All tanks were aerated throughout the experiments to maintain adequate dissolved oxygen levels. Fish were sacrificed periodically to adjust population density as crowding occurred in the tanks.

Reagent-grade zinc sulfate was used as the toxicant source. Daily, acidified water samples were composited and analyzed weekly by atomic absorption flame spectrophotometry. All zinc concentrations were significantly different ($P = 0.05$) for both experiments. Zinc concentration data for the bioassays are given in Tables 1 and 2.

Weekly water-quality determinations were performed according to the methods of the AMERICAN PUBLIC HEALTH ASSOCIATION *et al*. (1971) and analysis of variance tests ($P = 0.05$) revealed no difference in water quality between test aquaria. The average temperature for the hard water experiment was 16.2 C with a range of 14.3 to 18.0 C. The average temperature for the soft water experiment was 12.7 C with a range of 4.0 C to 18.0 C. Water quality data for both experiments are presented in Table 3.

TABLE 1

Zinc concentrations and mortality data--
hard water chronic bioassay.

Analyzed zinc concentration (µg/liter) Mean	N	Mortality
2200 ± 530[a]	75	23.0%
1055 ± 260	76	10.0%
640 ± 170	76	6.4%
320 ± 90	76	0.0%
170 ± 70	75	0.0%
30 ± 26	76	0.0%

[a]Standard deviation

TABLE 2

Zinc concentrations and mortality data--
soft water chronic bioassay.

Analyzed zinc concentration (µg/liter) Mean	N	Egg mortality	Mortality before feeding	Mortality after feeding
547 ± 134[a]	48	3.3%	16.9%	45.1%
260 ± 96	48	4.3%	13.1%	6.9%
140 ± 51	48	3.0%	9.8%	1.7%
71 ± 31	48	3.0%	10.1%	1.4%
36 ± 17	48	3.6%	12.0%	1.5%
11 ± 9 (Experimental control)	48	2.0%	5.8%	2.6%
2 ± 0.1 (Hatchery control)	5	3.8%	9.5%	2.2%

[a]Standard deviation

TABLE 3

Average water quality for hard and soft water chronic bioassays

Characteristic	Hard water chronic Mean	N	Soft water chronic Mean	N
Alkalinity	238 ± 13.85[a]	76	25 ± 4.65[a]	60
Conductivity	1383 ± 117.56	360	133 ± 13.98	60
Dissolved oxygen	6.8 ± 1.07	456	6.8 ± 1.38	60
Hardness	333 ± 27.25	73	26 ± 3.70	60
pH	7.81 ± 0.39	456	6.80 ± 0.17	60
Temperature	16.2 ± 0.79	456	12.7 ± 3.40	60

[a]Standard deviation

Hard water chronic bioassay--Chronic exposure of rainbow trout to zinc in hard water began 1 July 1969 by placing 30 2-g fingerlings into the exposure tanks. All aquaria were checked daily for dead fish. Total weight of the fish in each aquarium was obtained quarterly during the first year, and monthly thereafter, to evaluate the effects of zinc on growth. From these data, feeding rates were calculated for each test aquarium.

After 21 months of exposure to zinc, 6 fish remained in each aquarium; aggressive behavior was observed in all tanks and eggs were found in several aquaria. Pans of gravel were placed in each aquarium in an effort to induce spawning. The substrates were removed daily and checked for eggs, however none were found. The experiment was terminated a month later when no natural spawning had occurred.

Soft water chronic bioassay--Chronic exposure of rainbow trout to zinc in soft water began 27 April 1972 with eyed eggs. The eggs, in plastic hatching trays, were incubated in the 265-liter tanks. Water temperatures averaged 12.5 C until hatching was complete six weeks later. To assess any growth effects caused by zinc, total weight of the fish was obtained monthly;

for fish under 60 mm, a modification of the photographic technique of MARTIN (1967) was used with weight data from sacrificed fish. These data were also used to project daily feeding rates (HORAK, 1969).

Prior experiences at this laboratory indicated that it might be possible to acclimate rainbow trout to zinc in order to create some type of resistant mechanism within the fish. To explore this idea further, several hundred fish from the same batch of eggs used in the soft water chronic bioassay were marked with fluorescent pigment (PHINNEY et al., 1967). Two weeks after marking, the fish were added to the zinc exposure tanks. Dead fish were collected daily and examined under a black light for identification of marked fish.

RESULTS AND DISCUSSION

Acute bioassays--The 96-hour TL_{50} values for juvenile rainbow trout in hard and soft water at 15 C were 7210 and 430 µg/liter, respectively. Obviously, the acute toxicity of zinc to rainbow trout decreases as water hardness increases. LLOYD (1965) and MOUNT (1966) report similar findings with rainbow trout and fathead minnows. MOUNT (1968) and MOUNT and STEPHAN (1969) obtained similar results with fathead minnows exposed to copper. The acute bioassay with eyed eggs resulted in a TL_{50} value of 2720 µg/liter zinc, which indicates that the eyed egg is one of the more resistant stages in the rainbow trout's life cycle. SKIDMORE (1965, 1966) found that the eggs of zebrafish (*Brachydanio rerio* Hamilton-Bachman) were less sensitive to zinc than were the juvenile fish.

Chronic bioassays--MATC's of zinc in hard and soft water are established on the basis of death during the juvenile portion of the rainbow trout's life cycle. Death of juvenile fish appears to be the most sensitive criterion for establishing MATC values.

Hard water chronic bioassay--The MATC for rainbow trout exposed to zinc in a water hardness of 330 mg/liter was between 640 µg/liter zinc where there was 6.4% zinc-caused mortality, and 320 µg/liter where there were no zinc-caused mortalities. Many deaths occurred during the first two weeks, and again after four months of exposure in aquaria having zinc concentrations of 2200, 1050, and 640 µg/liter zinc. Mortality data are presented in Table 1.

There was no difference in growth rates (P = 0.05) between the zinc concentrations. Within sample variation of fish sizes was conspicuous throughout the experiment.

All aquaria contained male fish with viable sperm (CRUEA et al., 1969). Approximately half the female fish contained eggs which had begun to be reabsorbed and the other half contained undeveloped eggs. It is felt that ovarian development was not affected by the various zinc concentrations. BRUNGS (1969) reported the MATC range for fathead minnows as 30 µg/liter to 180 µg/liter zinc in water with a hardness of 200 mg/liter. This MATC was established on the basis of impaired egg production in the 180 µg/liter zinc concentration. It was evident that the zinc concentrations of this experiment did not impair egg production. The lack of reproductive information for this experiment is a result of inconsistent ovarian development among two-year-old rainbow trout and their failure to spawn in artificial substrates. Similar difficulties have been experienced during other chronic experiments using rainbow trout (DAVIES and EVERHART, 1973). Another problem closely related to inconsistent egg production is that of too few experimental animals at the time of sexual maturity, which, in this case, is a function of aquaria size. As a result of several bioassays with rainbow trout, we feel that a minimum of 10, and preferably 20, females are required for statistical validity of reproductive information.

Soft water chronic bioassay--The MATC for rainbow trout exposed to zinc in a water hardness of 25 mg/liter occurred between 260 µg/liter zinc, which caused appreciable mortality after the young fry were feeding normally, and 140 µg/liter zinc, where there were no zinc-caused deaths. The zinc concentrations of this experiment caused no appreciable eyed-egg mortality. Although the mortality in the control is lower than in any of the other exposure aquaria, eggs of the same lot from which the experimental eggs were taken exhibited a 3.8% mortality in the Bellvue Research Hatchery of the Colorado Division of Wildlife (Table 2). The slightly higher mortality in all zinc exposure tanks therefore is not considered to be caused by zinc.

Deaths continued to occur after hatching was complete. Death of sac fry occurred in all zinc concentrations and the control, the heaviest mortality occurring in the 547 and 260 µg/liter zinc concentrations. Virtually all death in the lower zinc concentrations and control (140 to 11 µg/liter zinc) ceased after swim-up fry were feeding normally, indicating a normal die-off until the fish accepted food. Fish continued to die in the 547 and 260 µg/liter zinc concentrations (45.1% and 6.9%, respectively, Table 2) after the fry were feeding normally.

Comparison of total length data indicated that, initially, fish in the 547 and 260 µg/liter zinc concentrations were smaller than fish in the lower zinc concentrations and the control. However, after three month's exposure this difference was no longer evident. Again, within sample variation was very pronounced.

The reproductive aspects of the MATC were not examined during this experiment because of the small numbers of fish remaining at sexual maturity and inconsistent egg production by two-year-old trout.

The chronic toxicity of zinc to rainbow trout also decreases as water hardness increases. The MATC obtained in hard water is approximately two-and-one-half times the MATC obtained in soft water. MOUNT (1968) and MOUNT and STEPHAN (1969) report similar findings from chronic exposure of fathead minnows to copper.

An MATC can also be established from the mortality data of Table 4. Such an MATC, for fish not exposed to zinc during the egg stage, would lie between 71 and 36 µg/liter zinc. Since all deaths occurred in the first five days of the 25-day subchronic exposure period for these fish, an acute mode of toxic action is also suggested. A log-probit plot (SPRAGUE, 1969) of the data yields a 120-hour TL_{50} value of 135 µg/liter zinc, which is the lower end of the MATC range for fish exposed to zinc from the eyed-egg stage. Obviously, fish which have been exposed to zinc as eggs are more resistant than fish which have not been exposed to zinc as eggs. BOYD and FERGUSON (1964) and FERGUSON and BINGHAM (1966) have demonstrated that the mosquitofish (Gambusia affinis) and the yellow bullhead (Ictalurus matalis) can become resistant to endrin.

The chronic toxicity of zinc to rainbow trout is also dependent upon the stage of the fish's life cycle during which exposure begins. The data of Table 4 indicates that fish not exposed to zinc as eggs may be as much as four times more susceptible to zinc than fish exposed to zinc as eggs. It is possible therefore that an MATC for zinc in hard water may be higher than the one reported here if exposure had begun with eggs rather than fry.

TABLE 4

Mortalities of unacclimated rainbow trout

Zinc concentration (μg/liter)	547	260	140	71	36	11
% Mortality	100	83	59	8	1	0

The MATC values for zinc in hard and soft water were obtained from chronic bioassays which did not yield valid reproductive data. Therefore, the results of these bioassays may not be directly comparable to the MATC's derived from other chronic exposures which include valid reproductive information. However, these MATC's may be quite useful where hatchery stocking programs exist in areas with zinc polluted streams. The information concerning differences in MATC values between exposed and not exposed as egg fish populations also begins to shed a bit of light on the presence of small fish populations in streams known to be polluted with zinc (GOETTL and SINLEY, 1970).

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