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16 ABSTRACT

The chronic effects of hexavalent chromium on the fathead minnow (Pimephales promelas) were investigated. Survival was affected only at the high test concentration of 3.95 mg Cr/L. All chromium concentrations, including 0.018 mg/L, the lowest tested, retarded the early growth of first-generation fish, but this effect was only temporary. Growth of second-generation fish was not affected at concentrations of 1.0 mg/L or lower. Reproduction and hatchability of eggs were not affected at any chromium concentration tested.

The maximum acceptable toxicant concentration (MATC) for fathead minnows in hard water (209 mg/L as $CaCO_3$ at pH 7.7) was based on survival and lies between 1.0 and 3.95 mg Cr/L, respectively. The application factor (MATC/96-hr LC50) is between 0.03 and 0.11.

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Chronic Toxicity of Hexavalent Chromium to the Fathead Minnow *(Pimephales promelas)*

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Abstract. The chronic effects of hexavalent chromium on the fathead minnow *(Pimephales promelas)* were investigated. Survival was affected only at the high test concentration of 3.95 mg Cr/L. All chromium concentrations, including 0.018 mg/L, the lowest tested, retarded the early growth of first-generation fish, but this effect was only temporary. Growth of second-generation fish was not affected at concentrations of 1.0 mg/L or lower. Reproduction and hatchability of eggs were not affected at any chromium concentration tested.

The maximum acceptable toxicant concentration (MATC) for fathead minnows in hard water (209 mg/L as $CaCO_3$ at pH 7.7) was based on survival and lies between 1.0 and 3.95 mg Cr/L, respectively. The application factor (MATC/96-hr LC50) is between 0.03 and 0.11.

Hexavalent chromium is a trace metal in natural water, and its presence at high concentrations usually indicates discharge from industrial and municipal effluents. In a 5-year study of 1,577 samples from rivers and lakes of the United States, Kopp and Kroner (1969) found dissolved chromium in 24.5% of the samples; the mean concentration was 9.7 μg/L, and the range was 1 to 121 μg/L based on total chromium. Most measurements of environmental samples of chromium are on the basis of total chromium. Criteria developed for chromium (U.S. Environmental Protection Agency 1976) for both domestic water supply and for freshwater aquatic life were developed on the basis of total chromium.

Because of its widespread use and toxicity, chromium can be a hazard to aquatic life and is one of the 65 toxic pollutants listed in a consent decree court order (National Resources Defense Council *et al.* vs Train 1976). A review of the environmental effects of chromium (Towill *et al.* 1978) indicates that the environmentally important forms are trivalent and hexavalent chromium. Hexavalent chromium is the more toxic form to mammals and fish.

Most 96-hr LC50 values for hexavalent chromium for fish range from 10 to 130 mg/L. Adverse effects are produced, however, at much lower concentrations with long-term exposure. Olson and Foster (1956) studied long-term effects of hexavalent chromium on chinook salmon *(Oncorhynchus tshawytscha)*

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and rainbow trout (*Salmo gairdneri*). They reported that survival was affected at 77 μg/L, and growth was reduced at 13 μg/L. Benoit (1976) examined the chronic effects of hexavalent chromium on brook trout (*Salvelinus fontinalis*) and rainbow trout and found that brook trout alevin survival was decreased at 350 μg/L, and growth in weight was retarded at 10 μg/L during the 8-month exposure. Sauter *et al.* (1976) studied the effects of hexavalent chromium on the eggs and fry of seven fish species. These embryo-larval studies gave estimated maximum acceptable toxicant concentration (MATC) values that varied from 51 to 105 μg/L for rainbow trout to more than 2,167 μg/L for walleye (*Stizostedion vitreum vitreum*).

The present chronic toxicity study is one of a series of studies designed to evaluate the direct effects of metals on fish. The objective of this study was to determine the MATC (Mount and Stephan 1967) of hexavalent chromium in hard water for the fathead minnow (*Pimephales promelas*). The MATC is established on the basis of a chronic exposure; survival, growth, and reproduction are used as measures of effect. In addition, acute toxicity studies were made to estimate an application factor (Mount and Stephan 1967) for chromium toxicity.

Methods and Experimental Conditions

Exposure System

The design of this study was similar to that described by Mount and Stephan (1967). A serial diluter with a dilution factor of 0.25 was used to provide the control and five chromium concentrations. This dilution factor was used to obtain a wide range of concentrations, because long-term effects on survival were anticipated (Olson and Foster 1956). The randomly arranged all-glass exposure chambers (60 × 30 × 30 cm) contained a 15- × 15-cm compartment in the outlet corner for second-generation 30-day survival and growth studies. In addition, stainless steel chambers (60 × 15 × 20 cm) were used for the 30- to 60-day second-generation studies. The water volume of each adult spawning chamber was maintained at 30 L, and the flow rate averaged 200 L per day. The dilution water was a mixture of pond water originating from a spring and carbon-filtered, demineralized Cincinnati tap water. Six spawning substrates, consisting of half-tiles, were placed in each chamber, and eggs from these tiles were incubated in nylon-screen-bottom cups suspended from a rocker-arm that oscillated two times per minute. Light and photoperiod were provided by cool-white fluorescent ceiling lights and 60-W incandescent light bulbs suspended above each chamber.

Biological System

The chronic exposure was started in November when thirty-five 4-week-old juveniles reared from four groups of eggs spawned in the laboratory were randomly assigned to each exposure concentration. After nine weeks of exposure the number of fish was reduced to 20, except for the high concentration in which only 13 fish survived. Excess males were removed during the spawning season, which began in June, to reduce territorial conflict. The fish were fed a dry trout food daily and cladocerans weekly. Live organisms in the water supply supplemented this diet.

During the spawning season all spawning substrates were examined for eggs in the early afternoon. These eggs were removed and counted, and some were placed in egg cups for hatchability determinations. Usually, 100 eggs from each spawning, 25 eggs per cup, were exposed. Hatchability was calculated as the percentage of larvae hatched seven days after the spawning.

Fifty of these second-generation larvae from each concentration were placed in the larval growth chambers for 30 days from time of spawning. The fish were then measured and transferred

to the stainless steel chambers for an additional 30 days. All larval studies were started from eggs that were spawned within three days of each other

Physical and Chemical Conditions

Routine water analyses were made weekly with procedures described by the American Public Health Association *et al* (1965) Oxygen was measured in all chambers, and hardness, pH, alkalinity, and acidity in two chambers The mean and standard deviation for hardness, alkalinity, and acidity were 209 ± 5 mg/L, 159 ± 20 mg/L, and 9.5 ± 3.5 mg/L as $CaCO_3$, respectively. The pH varied from 7 5 to 8 2 The pH measurements were converted to ion concentration, and the mean ion concentration was calculated. Encoding this to pH, the mean pH was 7.73. The mean of the pH measurements, as such, was 7 75 Dissolved oxygen had a mean concentration and standard deviation of 7 5 ± 1 5 mg/L Dissolved oxygen concentrations in the two high chromium concentrations were corrected for the positive influence of dichromate in the Winkler titration

Reagent-grade potassium dichromate was introduced from a constant-level funnel maintained by a float valve via a toxicant-metering system as described by Mount and Warner (1965) Each weekday, water samples were measured for hexavalent chromium with the diphenylcarbazide method (American Public Health Association *et al.* 1965) A calibration curve was prepared at periodic intervals, and the reproducibility of the curve was excellent The curve was linear, complying with Beer's law, with the mean absorbance readings of 0 782, 0.548, 0.394, 0.156, and 0 078 for 200, 140, 100, 40, and 20 μg Cr/L, respectively. Higher test concentrations were quantitatively diluted to maintain a small range of concentrations Control blanks showed no positive interferences Mean chromium test concentrations ranged from 3.95 to 0.018 mg/L (Table 1)

No attempt was made to conduct the chronic study at a constant temperature. Mean weekly temperature was 16°C during the first fall and winter months and then slowly increased to 24°C in June, where it remained until the end of the test. The usual weekly fluctuation was about 3°C, and the minimum and maximum temperatures were 13°C and 27°C, respectively. Temperature was recorded continuously with a 7-day indicating and recording thermograph. A natural photoperiod (Evansville, Ind) was maintained, and adjustments were made bimonthly. For spawning purposes, a 16-hr light cycle was maintained from late June to the end of the test.

Acute Toxicity

Side-by-side static and flow-through bioassays were conducted twice in which immature (1 g) fathead minnows and dilution water from the same source were used. The static bioassays were conducted according to the methods recommended by the American Public Health Association *et al* (1965) Duplicate series of five fish per 10/L were used at concentrations with a dilution factor of 0 56 The flow-through bioassays were conducted with a dilution factor of 0.5; 10 fish were used in each exposure chamber for the two consecutive tests. Later, a third flow-through test was conducted with 10 fish per duplicate exposure chamber. All tests were conducted at 25°C The LC50 values were calculated on the basis of nominal concentrations in the static tests and measured concentrations in the flow-through tests

Statistics

Growth data were analyzed by one-way analysis of variance and Duncan's multiple-range test (Dixon 1974). The LC50 values and 95% confidence limits were determined with a computer program based on a moving average angle method (Harris 1959).

Results

Survival

Mortality occurred in the high test concentration of 3.95 mg Cr/L. The first fish died after three weeks of exposure. After nine weeks, 37% had survived (Table

Table 1. Survival and growth of fathead minnows exposed to hexavalent chromium

Measured concentration mg/L[a]	First generation						Second generation			
			Final (412 days)							
	9 weeks		Males		Females		30 days		60 days	
	Survival %	Weight g[a]	Length mm[c]	Weight g[c]	Length mm[c]	Weight g[c]	Survival %	Length mm[a]	Survival %	Length mm[a]
3.95[b] ± 0.20	37	—	64.7	3.7	54.5	2.0	38	9.7 ± 0.8	12	11.5 ± 1.6
1.00 ± 0.07	94	0.11 ± 04[d]	69.9	4.4	55.4	1.9	98	15.4 ± 1.8	98	24.7 ± 3.5
0.26 ± 0.016	86	0.15 ± .07[d]	71.8	4.9	58.5	2.1	94	15.1 ± 1.3	94	26.5 ± 3.2
0.066 ± 0.008	97	0.14 ± .04[d]	71.0	5.4	55.2	2.0	84	13.7 ± 1.2	82	23.9 ± 3.6
0.018 ± 0.002	97	0.15 ± .04[d]	67.3	4.4	54.4	1.8	98	11.9 ± 1.2	80	24.6 ± 3.7
Control	100	0.19 ± .08	68.0	4.6	55.7	1.9	74	15.1 ± 2.4	72	24.7 ± 3.6

[a] Mean and standard deviation
[b] Each concentration value is the mean of 217 measurements
[c] Mean
[d] Significantly different (P = 0.05) from control

1), and only 13% survived to the termination of the exposure. Survival of second-generation fish also was greatly reduced at 3.95 mg Cr/L (Table 1). After 30 days, survival was 38%, and after 60 days only 12% survived. Survival of both first- and second-generation fish at concentrations of 1.0 mg/L and lower was similar to that of the control fish.

Survival of the eggs that were spawned and incubated at all concentrations was not adversely affected by chromium (Table 2). Hatchability varied from 86 to 96%.

The 96-hr LC50 values of hexavalent chromium for the two static bioassays were 39.7 and 32.7 mg/L. These two values were not significantly different ($P = 0.05$), so the data were combined giving a 96-hr LC50 value of 36.2 mg/L (27.2 – 45.4 mg/L). The 96-hr LC50 values of the two consecutive flow-through bioassays were 37.7 mg/L (29.5 – 57.5 mg/L) and 37.0 mg/L (27.4 – 52.6 mg/L). The 96-hr LC50 value of the third flow-through bioassay was 35.9 mg/L (29.1 – 45.9 mg/L). The mean of the three flow-through tests was 36.9 mg/L.

Growth

All concentrations of chromium reduced growth of first-generation fish after nine weeks of exposure (Table 1). Analysis of variance indicated that chromium had a significant effect ($P = 0.01$) on the weight of fish. Duncan's multiple range test indicated that the weight of fish exposed to 1.0 mg/L was significantly different ($P = 0.05$) from the three low concentrations and the control. The three low-test concentrations were homogeneous and significantly different from the control. At the end of the chronic exposure, 412 days, the length and weight of the females exposed to chromium were similar to control fish (Table 1). At the sublethal concentrations of chromium, the length and weight of the males were similar to that in the control (Table 1). The length and weight of the males in the lethal concentration of 3.95 mg/L was less than that in the control. However, small excess males were removed from the sublethal concentrations and control to reduce territorial conflict. So it was not possible to statistically analyze these growth data. The mean length of the second-generation fish exposed to 3.95 mg/L was reduced more than 50% below the control after 60 days' exposure. However, the final lengths of the fish at 1.0 mg/L and lower were similar to that of the control fish.

Reproduction

Sublethal concentrations of chromium did not adversely affect egg production, and even at the highest concentration, which caused some deaths, the surviving fish spawned (Table 2).

Discussion

The results of this study clearly show that the high concentration of 3.95 mg Cr/L was lethal to the fathead minnow. This concentration had an adverse effect on survival of both first- and second-generation fish. The first-generation fish started dying after three weeks of exposure, and 63% were killed after nine

Table 2. Spawning results and hatchability of fathead minnow eggs in the chronic exposure to hexavalent chromium

Mean measured concentration mg/L	Number of males	Number of females	Total number of eggs	Total number of spawnings	Mean eggs per female	Percentage hatch	Number of eggs incubated
3.95	6	2	711	3	356	86	135
1.00	8	9	10,189	43	1,132	95	600
0.26	7	8	7,285	36	910	93	625
0.066	7	8	4,889	21	611	93	364
0.018	9	11	2,559	15	269	96	547
Control	4	7	2,632	17	376	95	525

weeks. The fish in this concentration were visually smaller than fish in other concentrations, and the smaller ones were the first to die. Fish continued to die for about two more months, but no adult fish died during the spawning season. Second-generation and first-generation fish were very similar in their sensitivity to the lethal effects of chromium.

Fathead minnows tested in hard water were more resistant to long-term lethal effects of chromium than salmonoids exposed in softer water. Olson and Foster (1956) studied the effects of long-term exposure to chromium on the various developmental stages of rainbow trout and chinook salmon. They found that exposure to 0.17 mg/L caused the death of rainbow trout fry within a few days, and fish continued to die over a 2-month period to a maximum mortality of 94%. For chinook salmon, they found that after the end of the fry stage significantly fewer fish survived at 0.18 mg/L, and mortality increased during the fingerling stage. Benoit (1976) reported that mortality of alevin rainbow trout exposed to 0.34 mg/L for 12 weeks was 100%, and mortality of young brook trout was 72% under similar conditions. Sauter *et al.* (1976) reported that exposure to 0.82 mg/L for 60 days significantly reduced survival of rainbow trout fry when compared to controls. They also found that concentrations between 1.4 and 11.6 mg/L appeared to reduce survival of lake trout fry, but because of variability among replicates only concentrations of 6.0 and 11.6 mg/L significantly reduced survival ($P = 0.05$).

Fathead minnow embryos were not sensitive to chromium exposure. Survival of eggs that were spawned and incubated in all concentrations of chromium was similar to survival of control eggs. Hatchability varied from 86 to 96%, all within the normal variability found at our laboratory. Benoit (1976) found that hatching success of brook trout eggs spawned and incubated in 0.35 mg Cr/L and control embryos transferred to 0.76 and 1.56 mg Cr/L was as good as control eggs. Sauter *et al.* (1976) reported on the toxicity of chromium to the eggs and fry of seven fish species. In all tests, survival and/or growth of fry were affected at concentrations lower than those that affected hatchability.

The 96-hr LC50 values calculated during the present study were similar to 96-hr LC50 values reported by Adelman and Smith (1976). They found a mean 96-hr LC50 value of chromium for the fathead minnow of 26 mg/L when the desired concentrations were immediately obtained, and a mean 96-hr LC50 value of 48 mg/L when the desired concentrations were not obtained for 3 to 4 hr. Ruesink and Smith (1975) reported a 96-hr LC50 value of 37 mg/L for the fathead minnow tested at 25°C. However, the fathead minnow tested in hard

water had a lower 96-hr LC50 value than that reported by Benoit (1976) for the brook trout exposed in soft water. Pickering and Henderson (1966) found that hexavalent chromium was more toxic to the fathead minnow and bluegill (*Lepomis macrochirus*) in soft water than in hard water of high alkalinity and pH. Trama and Benoit (1960) reported that hexavalent chromium was less toxic when potassium chromate was used than when potassium dichromate was used. They attributed this difference in toxicity to the greater concentration of hydrochromate ions in the more acidic dichromate salt solution.

Decreased growth is considered an indication of sublethal toxicity. Growth of first-generation fish was significantly ($P = 0.05$) affected at all chromium concentrations after nine weeks of exposure. The mean weight of fish exposed to 1.0 mg/L was about 60% that of control fish, and the mean weight of fish in the three low concentrations was about 80% of the weight of controls. This adverse effect on growth was only temporary, however, as the final weight of these fish was similar to the weight of the controls, and the return to normal weight suggests recovery from the toxic effects of chromium. This effect of chromium on growth parallels that found by Benoit (1976). He found an adverse effect on growth rate of brook trout during eight months' exposure to concentrations as low as 0.01 mg/L. He suggested that these early effects on growth rate were only temporary, as trout exposed to 0.35 mg/L for 6 months weighed 25% less than controls, but after 12 to 24 months weight varied only 10 to 12% from controls.

Growth of second-generation fish at the sublethal concentrations of 1.0 mg/L and lower was similar to growth of the control fish. This lack of effect on growth of the second-generation fish suggests that they were more resistant than the first-generation fish to the toxic action of chromium. The nature of the acclimation is not known, however. Hermanutz (1978) found that second-generation flagfish (*Jordanella floridae*) were more tolerant to the effects of endrin and malathion on growth than were first-generation fish. Spehar (1976) found that flagfish previously exposed to zinc as embryos were more tolerant to the effects on growth than those not previously exposed; effect on second-generation fish was not studied. In the present study, first-generation fish were not exposed as embryos. Thus, it is not possible to determine if the increased resistance of the second-generation fish is due to the nature of the initiation of chromium exposure.

Reproduction was not adversely affected at any chromium concentration. Egg production at 1.0 mg Cr/L was the best of any exposure, although fecundity of the females was poor when compared to most tests conducted at this laboratory. Even at the high lethal concentration of 3.95 mg/L, the surviving fish spawned. Benoit (1976) also reported that brook trout spawned at a lethal concentration of 0.35 mg Cr/L.

Hexavalent chromium at a concentration of 1.0 mg/L is considered an acceptable concentration for fathead minnows in hard water. This concentration caused no effects in first- or second-generation fish. The mean weight of the first-generation fish after nine weeks of exposure was reduced at this concentration, but the effect was only temporary. Growth of second-generation fish was similar to growth of control fish. Chromium at a concentration of 3.95 mg/L was lethal to both first- and second-generation fish.

The application factor developed in this study is between 0.03 and 0.11,

based on a MATC of 1.0 – 3.95 mg Cr/L and a 96-hr LC50 value of 36.9 mg Cr/L. Benoit (1976) reported an application factor of 0.003 – 0.006 derived from a MATC of 0.20 – 0.35 mg Cr/L. He used survival as the endpoint and a 96-hr LC50 value of 59 mg Cr/L. Temporary adverse effects of chromium on growth were not used to derive the MATC in either of these studies. If these temporary effects on growth were used to derive the MATC, the application factor for chromium would be more similar, but much smaller. Macek and Sleight (1977) reported an application factor for hexavalent chromium of 0.02 – 0.05 based on an embryo-larval study. This application factor is similar to that found in the present study. The MATC for their study was based on reduced weight of rainbow trout after 60 days' exposure of the larval stage.

At present, it does not seem possible to recommend an application factor for chromium based on chronic studies reported here and by Benoit. The MATC for chromium, when the fathead minnow and brook trout are used as test animals, is based on lethal concentrations. In the acute tests, chromium in hard water killed fathead minnows at lower concentrations than chromium in soft water killed brook trout. In the chronic tests, chromium killed brook trout at lower concentrations. Death of the test animal is the most significant endpoint of toxicity, and as a criterion of toxicity is unambiguous and final. Reduced growth is also an endpoint. However, the ecological significance of a temporary effect on growth of first-generation fish is uncertain.

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