Bull. Environm. Contam. Toxicol. 19, 608-616 (1978)

Metal Toxicity to Embryos and Larvae of Eight Species of Freshwater Fish—II: Copper

J. M. McKim, J. G. Eaton, and G. W. Holcombe

U.S. Environmental Protection Agency
Environmental Research Laboratory—Duluth
6201 Congdon Boulevard, Duluth, Minn. 55804

ABSTRACT

Fish larvae and early juveniles of all species tested (brook trout, rainbow trout, brown trout, lake trout, northern pike, white sucker, herring, and smallmouth bass) were more sensitive to copper than the embryos. Embryo survival was affected only at the higher concentrations tested, for all species except the rainbow trout. The concentrations of copper that caused significant effects on the larval standing crop were similar for all species (31.7-43.5 µg Cu/l) except the northern pike, which seemed to be considerably more resistant (104.1 µg Cu/l). Copper concentrations shown to have no significant effects on the early developmental stages of these species are considered close estimates of the copper concentrations that would have no measurable adverse effects during a complete life cycle toxicity test under similar test conditions.

INTRODUCTION

The chronic toxicity of copper to fish has been extensively investigated with both partial and complete life-cycle tests on all developmental stages of three species of freshwater fish: fathead minnow (Pimephales promelas) (MOUNT, 1968; MOUNT and STEPHAN, 1969); brook trout (Salvelinus fontinalis) (McKIM and BENOIT, 1971, 1974); and bluegill (Lepomis macrochirus) (BENOIT, 1975). In all these long-term studies the most sensitive stage, or one of the most sensitive stages, in the life cycle was the embryo-larval or early juvenile period of development. In addition, several investigators (GRANDE, 1966; HUGHES, 1968; HAZEL and MEITH, 1970; O'REAR, 1972) have studied the sensitivity of embryos, larvae, and early juveniles of freshwater fish to copper in acute exposures and have found that newly hatched larvae are more sensitive to copper than embryos or early juveniles.

Since the embryo-larval and early juvenile life stages were the most sensitive, or among the most sensitive, to copper during life-cycle toxicity tests, the use of shorter exposures with only these sensitive life stages should allow the estimation of copper concentrations that would have no measurable adverse effects on the tested species. This procedure would allow a more rapid indication of environmentally acceptable copper concentrations for many different species of fish and also could be used with species that cannot be tested through an entire life cycle in the laboratory.

0007-4861/78/0019-0608 $1.80

The present study was designed to determine the copper concentrations that had no measurable adverse effects on embryos, larvae, and early juveniles of eight species of freshwater fish, and thus to provide an estimate of the copper concentrations that would not cause adverse effects in life-cycle tests with these species.

MATERIALS AND METHODS

Physical

The proportional diluter and exposure tank system used were identical to those described by EATON et al. (1977). The estimated replacement time for 90% of the water in each exposure chamber in each of the eight tests was approximately 5.3 hr (SPRAGUE, 1969). Water was obtained directly from Lake Superior and was used without prior treatment. Temperatures of the test water approximated those that might be expected for each species during the early stages of development. Temperature was measured once daily in each test, and a summary is presented in Table 1.

A copper sulfate ($CuSO_4$, anhydrous, reagent grade) stock solution (19 liters) acidified with 10 drops of 6 N HCl to prevent precipitation was prepared and introduced from a Mariotte bottle to the diluter. Daily water samples (100 ml) from each tank were composited over a 5-day period for the analysis of total copper. The cupferron-methyl isobutyl ketone extraction method with atomic absorption analysis was used for analysis of copper (ARTHUR and LEONARD, 1970). Two calibration curves were constructed by the addition of known amounts of copper to both glass-distilled water and Lake Superior waters; the curves compared well. The method with one extraction allowed a 98-100% recovery of copper. The nominal and measured copper concentrations from each exposure are shown in Table 2.

Other chemical characteristics of the water were determined according to standard methods of the AMERICAN PUBLIC HEALTH ASSOCIATION et al. (1965). The results of these weekly analyses were: [mean, standard deviation, range, (N)] dissolved oxygen = 11.4 $\pm$ 1.2 mg/liter, 8.2-12.8, (86); hardness = 45.4 $\pm$ 0.8 mg/liter as $CaCO_3$, 44-50, (77); alkalinity = 42.4 $\pm$ 1.0 mg/liter as $CaCO_3$, 40-45, (88); and acidity = 1.9 $\pm$ 0.6 mg/liter as $CaCO_3$, 1.1-4.2, (88). The pH range of 88 analyses was 7.3-7.9.

Biological

Eight species of freshwater fish were exposed to various concentrations of copper in these studies utilizing methods described by EATON et al. (1977). Two species (northern pike and brown trout) were exposed during all of their embryo stage and for 30-60 days after hatching. The remainder of the species were exposed from early eyed and late eyed embryo stages to hatching and 30-60 days after hatching (Table 1). Experiments were also conducted with brown trout beginning with early eyed and late eyed

TABLE 1

Sources of embryos, dates of exposure, and test temperatures

Species	Source of embryos	Exposure date	Mean exposure temperature, ± SE and range (°C)
Rainbow trout (*Salmo gairdneri*)	Eyed embryos: Eyed embryos obtained from Cedar Island, Wisconsin, Hatchery on 4-25-69.	5-1-69 to 6-16-69	10.8 ± 0.12 (8.0-13.0)
White sucker (*Catostomus commersoni*)	Eyed embryos: Eggs stripped from fish from Greenwood Lake, Cook County, Minnesota, on 6-8-69.	6-13-69 to 7-22-69	14.9 ± 0.59 (9.0-21.0)
Lake herring (*Coregonus artedi*)	Eyed embryos: Eggs stripped from fish netted from Lake Superior on November 1969.	1-12-69 to 3-5-70	5.9 ± 0.09 (4.0-7.0)
Brook trout (*Salvelinus fontinalis*)	Eyed embryos: Eggs from laboratory fish, stripped and fertilized on 10-30-69.	12-23-69 to 3-10-70	5.6 ± 0.09 (4.0-7.0)
Lake trout (*Salvelinus namaycush*)	Eyed embryos: Embryos from Jordan River Hatchery, Michigan, obtained from French River, Minnesota, Hatchery on 1-5-70.	1-7-70 to 4-7-70	5.5 ± 0.10 (4.0-11.0)
Brown trout (*Salmo trutta*)	Green eggs: Eggs stripped from fish from Cedar Island Trout Farm, Brule, Wisconsin, on 12-3-69.	12-3-69 to 4-13-70	5.7 ± 0.09 (4.0-9.5)
Brown trout	Early eyed embryos: Embryos from same fish as above.	1-13-70 to 4-21-70	5.5 ± 0.0 (4.0-9.5)
Brown trout	Late eyed embryos: Embryos from same fish as above.	2-19-70 to 4-28-70	5.6 ± 0.14 (4.0-9.5)
Northern pike (*Esox lucius*)	Green eggs: Eggs obtained from fish taken from Cutfoot Sioux, Itasca County, Minnesota, on 4-26-71. Fish stripped and fertilized on 4-30-71.	4-30-71 to 6-4-71	15.6 ± 0.08 (14.0-16.4)
Smallmouth bass (*Micropterus dolomieui*)	Eyed embryos: Embryos taken from the nests of wild smallmouth bass in Two Island Lake near Grand Marais, Minnesota. Collected on 6-15-71.	6-15-71 to 7-20-71	20.0 ± 0.14 (16.0-21.0)

TABLE 2.

Mean copper concentrations measured in embryo and larval-juvenile exposure chambers (µg/liter)

Species	Nominal concentration						
	Control	4	12	39	111	333	1,000
Rainbow trout	1.7 ± 0.58[a] (5)[b]	5.3 ± 0.80 (5)	11.4 ± 1.68 (5)	31.7 ± 3.48 (5)	121.6 ± 24.04 (8)	351.1 ± 67.76 (8)	1,076.5 ± 47.13 (8)
White sucker	3.0 ± 1.11 (5)	6.0 ± 1.01 (5)	12.9 ± 2.29 (4)	33.8 ± 9.41 (5)	118.0 ± 12.11 (4)	317.6 ± 24.64 (5)	934.0 ± 119.23 (5)

Species	Nominal concentration						
	Control	4	8	20	39	111	500
Herring	3.3 ± 0.81 (10)	4.1 ± 0.79 (11)	7.7 ± 1.20 (11)	22.6 ± 4.88 (11)	43.0 ± 8.67 (11)	102.8 ± 23.68 (11)	456.5 ± 80.59 (11)
Brook trout	3.2 ± 0.82 (10)	4.0 ± 0.86 (11)	7.6 ± 1.29 (11)	22.3 ± 4.94 (11)	43.5 ± 8.13 (11)	101.5 ± 23.34 (11)	456.1 ± 80.68 (11)
Lake trout	3.2 ± 1.01 (10)	4.5 ± 0.67 (12)	9.2 ± 3.69 (13)	22.0 ± 6.18 (12)	42.3 ± 8.32 (13)	120.1 ± 21.53 (13)	454.4 ± 72.27 (13)
Brown trout	3.2 ± 0.71 (15)	4.0 ± 0.74 (16)	7.6 ± 1.16 (16)	22.0 ± 4.31 (16)	43.2 ± 7.63 (16)	102.3 ± 21.22 (15)	469.4 ± 77.69 (16)
Brown trout	3.1 ± 0.71 (10)	3.9 ± 0.87 (11)	7.9 ± 1.22 (11)	23.0 ± 4.63 (11)	46.5 ± 6.34 (11)	104.6 ± 18.95 (10)	498.4 ± 67.52 (11)
Brown trout	2.9 ± 0.20 (6)	3.5 ± 0.31 (6)	7.4 ± 1.08 (6)	20.8 ± 2.57 (6)	43.8 ± 4.81 (6)	100.6 ± 11.10 (5)	511.5 ± 63.11 (6)
Northern pike	1.4 ± 0.68 (5)	3.2 ± 0.76 (5)	6.5 ± 1.04 (5)	19.3 ± 1.24 (5)	34.9 ± 1.87 (5)	104.4 ± 6.88 (3)	485.3 ± 21.97 (3)
Smallmouth bass	2.1 ± 0.64 (6)	3.9 ± 0.75 (6)	7.3 ± 0.32 (6)	19.4 ± 1.22 (6)	36.9 ± 5.42 (5)	103.8 ± 5.04 (5)	517.4 ± 19.39 (4)

[a] Standard deviation.

[b] Number of weekly composites analyzed.

embryos to determine the effects of exposure duration and stage of embryo development on toxicity. All tests began with 50 embryos per duplicate test chamber. Fall spawning salmonids were exposed longer in most cases because of their slower development rate at lower test temperatures.

Juvenile fish were fed either live brine shrimp nauplii or commercial trout food, according to their preference, three times each day.

Survival and growth of embryo-larval and early juvenile fish were measured. Size at test termination was determined by wet-weight measurements on surviving fish in each duplicate chamber. Standing crop (biomass at a single point in time) was determined for each exposure concentration and compared to control standing crop with procedures described by McKIM et al. (1975). Standing crop data were plotted as the ratio of the average experimental standing crop to the average control standing crop by species (expressed as a percentage change) and copper concentration.

RESULTS AND DISCUSSION

Larvae and early juvenile stages of all species tested were more sensitive to copper than the embryos. Embryo survival was affected only at the higher concentrations of copper tested in all species except rainbow trout. Embryo mortality was almost complete at the following concentrations: northern pike, 500 µg/liter; rainbow trout, 37 µg/liter; white sucker, 333 µg/liter; brook trout, 555 µg/liter; lake trout, 555 µg/liter; brown trout (green eggs and early eyed), 111 µg/liter; brown trout (late eyed), 555 µg/liter; and herring, 555 µg/liter. Copper had no effect on smallmouth bass embryos at any concentration tested. Although the effect of copper on embryos did not directly influence standing crop, it probably produced subtle changes during the embryo stage that indirectly influenced survival and growth of larvae and early juveniles.

The effect of copper on the standing crop of six species of fish exposed during the embryo, larval, and early juvenile stages of development is shown in Figures 1, 2, and 3.

Northern pike, rainbow trout, and white suckers were exposed for approximately 30 days after hatching (Fig. 1). The northern pike standing crop was significantly reduced at copper concentrations of 104.4 µg/liter and above. Rainbow trout and suckers were considerably less tolerant of copper, however, and their standing crops were significantly affected at copper concentrations of 31.7 and 33.8 µg/liter and above, respectively.

Brook trout and lake trout, exposed for approximately 60 days after hatching, were similar in their sensitivity to copper (Fig. 2). The standing crop of both species was reduced significantly at water copper concentrations of 43.5 µg/liter and above. Three groups of brown trout embryos, each group exposed to copper for a different length of time, were also exposed to copper for approximately 60 days

after hatching (Fig. 3). Copper concentrations causing significant reductions in standing crop were dependent on the duration of embryo exposure; those exposed longest were the most sensitive. This response was opposite that seen in brown trout exposed to cadmium, where the longest embryo exposure produced the least effect on standing crop (EATON et al., 1977).

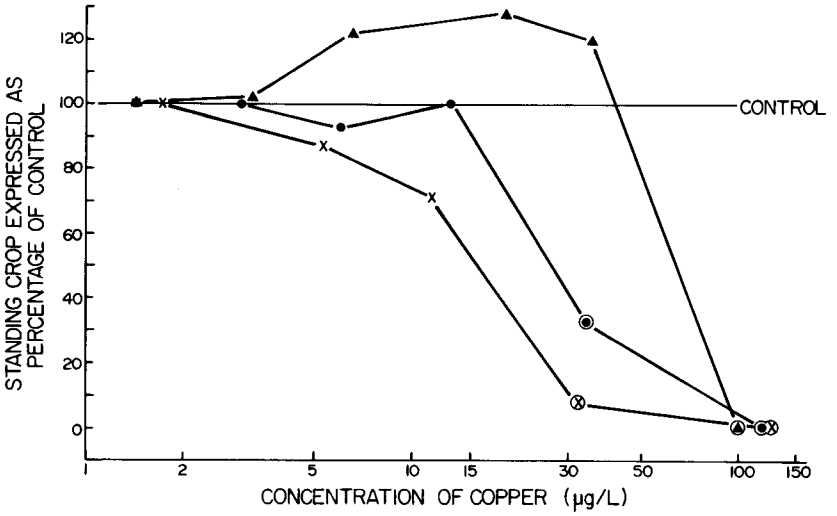

Figure 1. Ratio of experimental to control standing crop of embryo-larval-early-juvenile rainbow trout (X) (embryo exposure 11 days; larval-early-juvenile exposure 35 days), white sucker (●) (embryo exposure 13 days; larval-early-juvenile exposure 27 days), and northern pike (▲) (embryo exposure 6 days; larval-early-juvenile exposure 34 days) exposed to various copper concentrations. (Circled points are signficantly lower than controls, P = 0.05.)

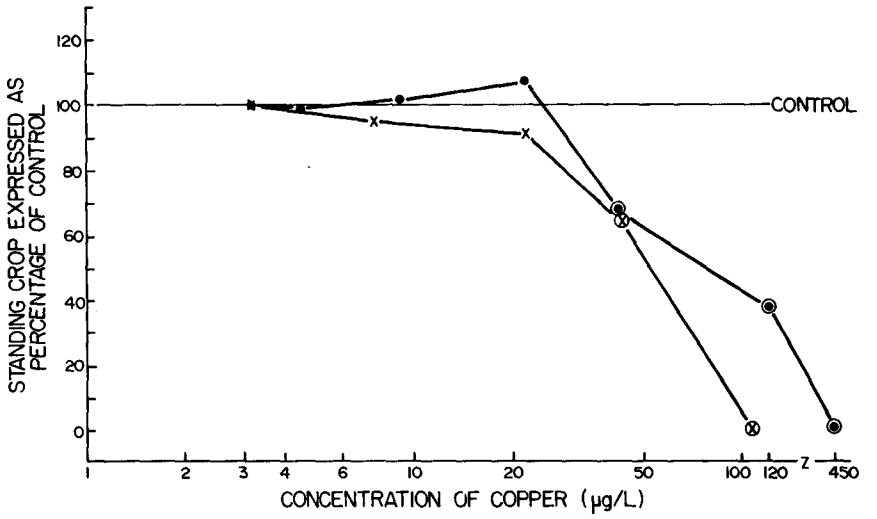

Figure 2. Ratio of experimental to control standing crop of embryo-larval-early-juvenile brook trout (X) (embryo exposure 16 days; larval-early-juvenile exposure 60 days) and lake trout (●) (embryo exposure 27 days; larval-early-juvenile exposure 66 days) exposed to various copper concentrations. (Circled points are significantly lower than controls, P = 0.05.)

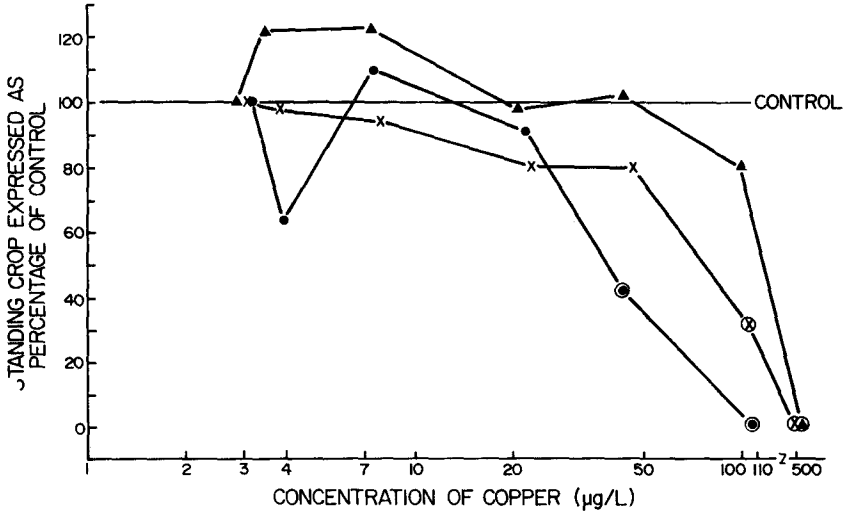

Figure 3. Ratio of experimental to control standing crop of embryo-larval-early juvenile brown trout exposed to various copper concentrations (●) (embryo exposure 72 days; larval-early-juvenile exposure 55 days), (X) (embryo exposure 36 days; larval-early-juvenile exposure 61 days), and (▲) (embryo exposure 8 days; larval-early-juvenile exposure 63 days). (Circled points are significantly lower than controls, P = 0.05.)

High mortality among the controls of the other two species tested, lake herring and smallmouth bass, did not allow statistical treatment of the data. However, mortality data for these species excluding the controls showed that copper concentrations of 102.8-103.8 µg/liter and above would have reduced standing crop 30 days after hatching.

The concentrations of copper that caused significant reductions in standing crop were similar for the brook trout, rainbrow trout, lake trout, brown trout, and white sucker. The concentration causing such an effect in northern pike, however, differed by a factor of three from the others. These concentrations were also similar to copper concentrations that caused measurable effects on embryo-larval and early juvenile stages in previous life-cycle toxicity tests. A life-cycle toxicity test with fathead minnows exposed to copper in soft water similar to that used in the present study showed effects on embryo-larval and early juvenile stages at 18.4, but not at 10.0 µg Cu/liter (MOUNT and STEPHAN, 1969). Life-cycle toxicity tests with brook trout (McKIM and BENOIT, 1971, 1974) and bluegills (BENOIT, 1975) in the same dilution water as the present study showed effects on the early developmental stages at 17.4 and 40.0, but not at 9.5 and 20 µg Cu/liter, respectively. The bluegill did not differ in copper sensitivity from the species reported in the present study, but the fathead minnow and the brook trout in the earlier studies were somewhat more sensitive than those in the present tests. Embryo-larval fish from previously exposed and unexposed parents differ little in copper sensitivity (MOUNT and STEPHAN, 1969; McKIM and BENOIT, 1971, 1974), so this factor was not considered in explaining differences in copper toxicity. The embryo-larval and early juvenile brook trout exposure appeared to underestimate the toxicity of copper, in comparison with life-cycle exposure, by a factor of about two. However, when dealing with chronic effects of a toxicant on several groups of fish under different experimental conditions, this variation may not be unreasonable.

The brook trout, lake trout, and brown trout were exposed for both 30 and 60 days. In all cases, the effect concentrations were lower in the 60-day copper exposures than in the 30-day exposures. The northern pike, rainbow trout, and white suckers were exposed for only 30 days and at much higher temperatures. Effect concentrations were established for these species on the basis of growth, not survival. Previous life-cycle toxicity studies with fathead minnows, brook trout, and bluegills exposed to copper indicated that at temperatures of 9-24° C exposure of embryo-larval and early juvenile stages for more than 30 days was not necessary to establish the lowest effect concentration (MOUNT and STEPHAN, 1969; McKIM and BENOIT, 1971, 1974: BENOIT, 1975; BENOIT, personal communication). Until further data are available, we suggest a minimum duration for embryo-larval or early juvenile exposures of 60 days for estimating chronic copper toxicity with all species.

The copper concentrations reported here to have no statistically significant effect on early developmental stages of brook trout, lake trout, brown trout, rainbow trout, white sucker, and northern pike are considered to be close estimates of the copper concentrations that would have no measurable effects during a partial or complete life-cycle toxicity test with these species under similar test conditions.

ACKNOWLEDGMENTS

We thank E. Leonard for analysis of copper concentrations in test chambers, D. Olson for routine analysis of other water characteristics, T. Thorslund for aid in statistics and automation of data analysis, E. Hunt and N. Joyce for special assistance when needed, and other members of the Environmental Research Laboratory-Duluth for review of the manuscript.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, and WATER POLLUTION CONTROL FEDERATION: Standard methods for the examination of water and wastewater. 11th ed. New York, N.Y. 1965. 626 p.

ARTHUR, J. W., and E. N. LEONARD: J. Fish. Res. Board Can. 27, 1277 (1970).

BENOIT, D. A.: Trans. Am. Fish Soc. 104, 353 (1975).

EATON, J. G., J. M. McKIM, and G. W. HOLCOMBE: Bull. Environ. Contam. Toxicol. (In press) (1977).

GRANDE, M.: Third Int. Conf. Water Poll. Res. 1:97-111 (1966).

HAZEL, G. R., and S. J. MEITH: Calif. Fish Game 56, 121 (1970).

HUGHES, J. S.: Proc. S.E. Assn. Game & Fish Comm. 21:230-234 (1968).

McKIM, J. M., J. W. ARTHUR, and T. W. THORSLUND: Bull. Environ. Contam. Toxicol. 14, 1 (1975).

McKIM, J. M., and D. A. BENOIT: J. Fish. Res. Board Can. 28, 655 (1971).

McKIM, J. M., and D. A. BENOIT: J. Fish. Res. Board Can. 31, 449 (1974).

MOUNT, D. I.: Water Res. 2, 215 (1968).

MOUNT, D. I., and C. E. STEPHAN: J. Fish. Res. Board Can. 26, 2449 (1969).

O'REAR, C. W., Jr.: Proc. 26th Ann. Conf. S.E. Assn. Game & Fish Comm. Oct. 22-25, 484 (1972).

SPRAGUE, J. B.: Water Res. 3, 793 (1969).