Metal Toxicity to Embryos and Larvae of Seven Freshwater Fish Species—I. Cadmium

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ABSTRACT

The embryos and larvae of seven freshwater fish were exposed to low concentrations of cadmium in soft water. All species were killed or their growth retarded by concentrations ranging from about 4 to 12 µg Cd/liter. The larvae were consistently more sensitive than the embryos. The agreement between these results and those from life-cycle chronic toxicity studies indicates that embryo and larval exposures will give reliable estimates of the chronic toxicity of cadmium to additional fish species. A 60-day exposure period appears to be appropriate for determining larval sensitivity to cadmium.

INTRODUCTION

The results of several life-cycle tests have indicated that exposures to cadmium of early life stages of fish might provide good estimates of the chronic toxicity of this metal. Cadmium reduced hatchability and caused larval[1] deformities in fathead minnows at 57 µg/liter, the lowest test concentration determined to be unsafe in a chronic exposure in hard water (PICKERING and GAST 1972). This response was observed both for embryos spawned at 57 µg/liter by chronically exposed parents and for newly spawned embryos transferred to this concentration from the control tanks. Crippling, poor survival, and reduced growth occurred among larval bluegills exposed in hard water to 80 µg/liter of cadmium, which also killed adult fish after several months (EATON 1974). Cadmium significantly reduced the growth of juvenile brook trout exposed to 3.4 µg/liter in soft water, a concentration that also caused a high mortality among adult males at spawning time (BENOIT *et al.* 1976). In all three of these species the next lower exposure concentration of cadmium in the chronic test produced no adverse effect as compared to the controls.

In a chronic exposure of flagfish to cadmium in soft water, embryos and 30-day-old larvae were unaffected at 16 µg/liter, but juvenile survival and growth were inhibited between 30 and 60 days at this concentration (SPEHAR 1976). The number of spawned embryos was reduced at the next lower concentration (8.1 µg/liter).

[1]The terms larval (larva) or juvenile refer to the major portion of the life stage represented during corresponding exposure periods, but may include a segment of another stage.

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Therefore, based on the results of the four chronic tests, it seemed likely that embryo, larval, and juvenile stages of other fish might also be sensitive to cadmium and in many cases might indicate the same degree of sensitivity observed in life-cycle chronic exposures. Tests of early life stages also provide the opportunity to test species that are nearly or actually impossible to deal with in the laboratory as adults, but for which the embryos can be hatched and the newborn reared to juveniles under exposure conditions. In the present study the sensitivity to cadmium of seven fish species was examined in this manner.

MATERIALS AND METHODS

Physical Conditions

The water for all tests was pumped directly from Lake Superior and filtered through sand. A proportional diluter (MOUNT and BRUNGS 1967) was used to deliver toxicant-bearing and control water to test tanks. The diluter was modified to deliver six toxicant concentrations plus control water. The lowest concentration was 0.4 µg/liter, and each succeeding concentration was increased by a factor of three. The nominal and measured test concentrations are shown in Table 1.

Test containers for embryos were screen-bottomed glass jars 6 cm in diameter, which were suspended and oscillated in the larval test chambers (NATIONAL WATER QUALITY LABORATORY 1972). After hatching, the young fish were released into the glass larval test chambers (15 cm wide by 30 cm deep by 30 cm long), which contained approximately 10 liters of water. Each toxicant and control water flow was split and delivered to four larval chambers, two of which were used to expose replicate groups of one species and two to expose replicate groups of another species. The estimated replacement time for 90 percent of the water (SPRAGUE 1969) in each chamber ranged from 6 to 7 hours for each of the tests. Control of the test-water temperature was minimal, the intent being only to keep temperature fluctuations resulting from incoming water within a desirable range. The means and ranges of daily temperature measurements are given in Table 2.

The cadmium stock solution was prepared by dissolving an appropriate amount of $CdCl_2$ in 10 liters of distilled water. Approximately 2 ml of this stock was metered into the diluter each time it cycled to create the desired test-chamber concentrations. Composites of test water were collected by taking a 50-ml sample from one of the two replicates for each concentration every weekday. Cadmium analysis of the composite was performed by APDC-MIBK extraction (MANSELL 1965) followed by atomic absorption spectrophotometry that provided a detection sensitivity of about 0.1 µg Cd/liter. The method of known additions of cadmium to control water was used to construct calibration curves. Analysis of 12 spiked samples at the 2-µg/liter level of cadmium demonstrated a 99 percent mean recovery with a standard deviation of ±6%. All analyses were for total cadmium.

TABLE 1

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

Species	Nominal concentration						
	Control	0.4	1.2	3.7	11.1	33.3	100
Brook trout (*Salvelinus fontinalis*)	0.16 ± 0.13[a] (23)[b]	0.48 ± 0.20 (23)	1.1 ± 0.2 (22)	3.8 ± 0.9 (22)	11.7 ± 1.1 (15)	32.0 ± 6.9 (15)	103 ± 10 (15)
Brown trout (*Salmo trutta*)	0.17 ± 0.14 (19)	0.42 ± 0.25 (20)	1.1 ± 0.4 (20)	3.8 ± 0.8 (18)	11.7 ± 1.1 (18)	34.2 ± 8.3 (19)	107 ± 10 (18)
Brown trout late eyed eggs (*Salmo trutta*)	0.19 ± 0.13 (10)	0.58 ± 0.19 (10)	1.1 ± 0.3 (10)	3.7 ± 0.9 (10)	11.1 ± 0.8 (9)	32.0 ± 5.6 (9)	96 ± 3 (9)
Lake trout (*Salvelinus namaycush*)	0.26 ± 0.13 (10)	0.67 ± 0.18 (10)	1.4 ± 0.4 (10)	4.4 ± 0.7 (9)	12.3 ± 0.9 (9)	33.4 ± 3.2 (9)	106 ± 10 (9)
Northern pike (*Esox lucius*)	0.06 ± 0.02 (4)	0.30 ± 0.09 (4)	1.1 ± 0.2 (4)	4.0 ± 0.6 (4)	12.9 ± 2.2 (4)	34.7 ± 5.7 (5)	104 ± 10 (5)
White sucker (*Catostomus commersoni*)	0.09 ± 0.09 (8)	0.40 ± 0.10 (8)	1.1 ± 0.2 (8)	4.2 ± 0.7 (8)	12.0 ± 0.4 (8)	28.9 ± 5.0 (8)	107 ± 12 (5)
Smallmouth bass (*Micropterus dolomieui*)	0.13 ± 0.10 (5)	0.48 ± 0.11 (5)	1.0 ± 0.2 (5)	4.3 ± 0.7 (5)	12.7 ± 1.8 (5)	25.7 ± 2.2 (5)	107 ± 8 (2)
Lake Superior coho salmon sac fry (*Oncorhynchus kisutch*)	0.06 ± 0.01 (4)	0.37 ± 0.06 (4)	1.3 ± 0.5 (4)	3.4 ± 0.5 (4)	12.1 ± 1.3 (4)	37.3 ± 2.3 (3)	104 ± 5 (3)
West Coast coho salmon (*Oncorhynchus kisutch*)	0.21 ± 0.14 (13)	0.60 ± 0.20 (13)	1.4 ± 0.3 (13)	4.1 ± 0.8 (12)	12.5 ± 1.2 (12)	34.4 ± 3.4 (12)	106 ± 8 (12)

[a] Standard deviation; the apparent variability and high standard deviations of control values are due to their being near the detectability limit for cadmium.

[b] Number of weekly composites analyzed.

TABLE 2

Sources of embryos, dates of exposure, and test temperatures

Species	Source of embryos	Exposure date	Mean exposure temperature and range (°C)
Brook trout	Embryos from fish from Cedar Island, Wisconsin, hatchery, stripped and fertilized on 10/24, and held in incubator until 12/4/70.	12-4-70 to 5-3-71	9.7(8.0-11.1)
Brown trout	Embryos from fish obtained by electroshocking in French River, Minnesota; stripped and fertilized on 10/27/71.	10-29-70 to 2-16-71	9.7(8.0-10.8)
Brown trout late eyed eggs	Embryos from same fish as above, held in incubator until 2/1/71.	2-1-70 to 4-5-71	10.0(9.0-11.1)
Lake trout	Embryos obtained from Green Lake, Michigan, via personnel at Jordan River, Michigan, hatchery.	12-18-70 to 3-2-71	9.6(8.0-11.2)
Northern pike	Embryos from fish trapped by Minnesota Department of Natural Resources personnel at Cut Foot Sioux Lake, Minnesota; stripped and fertilized on 4/30/71.	4-30-71 to 6-4-71	15.9(15.0-16.7)
White sucker	Embryos stripped from fish trapped in Cook County, Minnesota, and fertilized on 5/19/71.	5-19-71 to 7-10-71	18.1(13.1-21.0)
Smallmouth bass	Embryos removed from nests of wild fish in Two Island Lake, Cook County, Minnesota, on 6/15/71.	6-15-71 to 7-20-71	20.2(16.3-21.0)
Lake Superior coho salmon sac fry	Embryos from salmon netted by Minnesota Department of Natural Resources personnel at French River, Minnesota; stripped and fertilized on 12/9/70 and held in incubator until 3/4/71.	3-4-71 to 3-31-71	10.1(8.5-10.7)
West Coast coho salmon	Eyed embryos obtained from Environmental Protection Agency Western Fish Toxicology Station, Corvallis, Oregon.	12-24-70 to 3-16-71	9.7(8.0-11.2)

Weekly analyses of other water characteristics in various exposure chambers were conducted on a rotational basis. The means and ranges (in parentheses) of these measurements were: dissolved oxygen, 10.3 mg/liter (8.0-12.2); hardness, 45 mg/liter as $CaCO_3$ (44-46); alkalinity, 41 mg/liter as $CaCO_3$ (39-43); acidity, 3.0 mg/liter as $CaCO_3$ (1.7-4.7); and pH, 7.6 (7.2-7.8). Thirty measurements in all were made for each water characteristic.

Biological Conditions

The sources of the embryos and dates of the exposures are shown in Table 2. Most tests were started by placing 50 embryos in one or more incubation jars (depending on the size of the embryos) for each replicate of each exposure chamber. The embryos of the suckers, pike, and bass were placed in test chambers within a few minutes to 2 days after fertilization. Brook trout, lake trout, and west coast coho salmon were introduced during the eyed-embryo stage of development. Brown trout exposures were started with newly fertilized embryos in one test and late eyed embyros from the same source in another test to determine the influence of duration of embryo exposure on organism sensitivity. Lake Superior coho salmon were introduced while in the sac-fry stage approximately 1 week after hatching.

The larvae or juveniles were released into rectangular chambers after complete hatch in each incubation jar and remained there until termination of the test. The larval-juvenile exposures lasted approximately 30, 60, or 120 days from the time of complete hatch. Subsamples of 10 fish from each chamber were sacrificed for determinations of wet weight (growth) at intervals of approximately 30 and 60 days during 60- and 120-day exposures. Salmonids were exposed longer because of their slower development at the colder test temperatures. The exact duration of embryonic and post-hatch exposure is given in Table 3. In addition to growth, observations were recorded daily on embryo and larval-juvenile mortality. Fish were fed either live brine shrimp nauplii or commercial trout food, according to their preference, three times each day.

Data Handling

The results in each chamber are expressed as an estimate of the standing crop remaining at the end of each 30-, 60-, or 120-day exposure period. Standing crop was estimated as described by BEVERTON and HOLT (1957) by multiplying the proportion of fish surviving by their wet weight. Standing crop (biomass) is thought to provide a response measure with greater ecological relevance because it is less influenced by relatively small impacts on individual life stages where they are compensated for by responses at other times during the test. A one-way analysis of variance was used following log transformation to determine the exposure concentration at which standing crop was different from that of the controls at the 0.05 and 0.01 levels of significance.

TABLE 3

Duration of embryonic and larval-juvenile exposure and cadmium concentrations at which estimates of standing crop were determined to be significantly different from those of the respective controls

Species	Days of exposure		Lowest cadmium concentration[a] (µg/liter) at which standing crop was significantly different from controls		Highest cadmium concentration (µg/liter) at which standing crop was not significantly different from controls
	Embryos	Larvae-juveniles	(P=0.05)	(P=0.01)	(P=0.05)
White sucker	10	30	12.0	same	4.2
Northern pike	7	28	12.9	same	4.2
Smallmouth bass	3	30	12.7	-	4.3
Lake Superior coho salmon	0	27	3.4	same	1.3
West Coast coho salmon	20	27	12.5	same	4.1
		62	12.5	-	4.1
Lake trout	10	31	12.3	same	4.4
		64	12.3	same	4.4
Brook trout	24	31	0.48 and 11.7	11.7	3.8
		65	0.48 and 3.8	3.8	1.1
		126	3.8	same	1.1
Brown trout	50	33	11.7	same	3.8
		60	11.7	same	3.8
Brown trout late eyed embryos	2	29	11.2	same	3.7
		61	3.7	11.2	1.1

[a]Concentrations are means of all cadmium measurements made during tests of each species.

RESULTS

Larvae or juveniles were in all cases more sensitive than embryos. Only white sucker embryos were not able to tolerate the highest exposure concentrations (~100 µg/liter). All sucker embryos died at 107.2 µg/liter, but none died in the control tanks or in any concentration between 0.40 and 28.9 µg/liter. The three highest concentrations actually reduced mortality of smallmouth bass embryos by inhibiting a fungus that afflicted this species. Mortality ranged from 30 to 50% in the control and three lowest concentrations, but was only 15-18% in the three highest concentrations. Mortality of newly spawned brown trout embryos ranged randomly from 11 to 20% in all concentrations and the control. Embryo mortality in the other tests was 4% or less in any control or treatment tank and was randomly distributed. The greatest impact of the toxicant was on larval or juvenile survival. Only among brook trout juveniles was growth reduced at a concentration (3.8 µg/liter) lower than that causing death (11.7 µg/liter).

The concentrations at which estimates of standing crop differed from the controls at two levels of statistical significance are shown in Table 3. The high mortality of smallmouth bass embryos and larvae in lower concentrations and control chambers due to disease did not permit demonstration of a highly significant effect (P=0.01) at 12.7 µg/liter or above. West coast coho salmon juveniles in the two lowest concentrations grew substantially faster than controls between 30 and 60 days, and the resultant variability again permitted demonstration of an effect only at the 0.05 significance level.

Estimates of brook trout standing crop were different from the control at the 0.05 level of significance at 11.7 µg/liter after 30 days, at 3.8 µg/liter after 60 days, and at 0.48 µg/liter after both 30 and 60 days (Table 3). Standing crop was not significantly different from that of the control at the intermediate concentrations. We believe that only the reductions observed at 3.8 µg/liter or higher represent the response of the test fish to cadmium and that other undetermined factors are responsible for the result at 0.48 µg/liter. A reduction in brook trout standing crop resulting from juvenile growth inhibition became more pronounced with exposure time, and therefore the level at which a highly significant difference (P=0.01) occurred was reduced to 3.8 µg/liter after 60 and 120 days.

The responses of the two groups of brown trout (embryos and late eyed embryos) were different from one another after 60 days of post-hatch exposure at the 0.05 significance level but not at the 0.01 level (Table 3). The brown trout first exposed as late eyed embryos, like the brook trout, exhibited greater sensitivity after 60 days of larval exposure than they did at 30 days.

DISCUSSION

Species differences in sensitivity in the present study were not great, being separated by only one concentration increment, or a factor of about four times. However, the greater sensitivity of two salmonid species (brook trout and Lake Superior coho salmon) might indicate that this group is somewhat more sensitive generally. The resistance of the embryos as compared to the larvae-juveniles in the present study contrasts sharply with the results of the chronic tests of PICKERING and GAST (1972), which demonstrated that fathead minnow embryos were the most sensitive life stage.

The results of the brook trout embryo and post-hatch exposures agree closely with those obtained by BENOIT *et al.* (1976) in a life-cycle chronic test on three consecutive generations of brook trout. Juvenile growth inhibition was the most sensitive response in both tests, and the lowest cadmium concentrations causing harmful effects were similar in the two experiments (3.4 and 3.8 µg/liter).

Few additional references to the toxicity of cadmium to salmonids can be found in the literature. BALL (1967) found the 7-day LC50 for rainbow trout to be 8-10 µg/liter in hard water (290 mg/liter as $CaCO_3$). CHAPMAN (1972) obtained a 96-hr LC50 of

0.95 µg/liter for juveniles of a migratory strain of rainbow trout (steelhead) in water of 20-25 mg/liter hardness. Slightly higher concentrations of 1.5-2 µg/liter caused deaths among swim-up-stage chinook salmon exposed as embryos and through 12 weeks post-hatch (CHAPMAN, personal communication).

The "safe" concentrations (4.1µg/liter and lower) obtained by SPEHAR (1976) in chronic exposures of flagfish in soft water agree closely with the no effect concentrations for non-salmonids in this study. However, reproduction rather than survival or growth of embryos, larvae, or juveniles was the most sensitive response in flagfish. Significantly high mortality and growth inhibition were recorded at 60 days post-hatch at 16 µg/liter but not at 8.1 µg/liter, and neither effect was evident at 30 days post-hatch (SPEHAR 1976). Thus, in terms of embryo, larval, or juvenile sensitivity, the fish in the present study were more sensitive than flagfish. The flagfish results serve as a warning that some species might be more sensitive in life-cycle chronic tests than embryo, larval, or juvenile exposures to cadmium would indicate.

The safe and lowest-effect cadmium concentrations were 37 and 57 µg/liter for fathead minnows (PICKERING and GAST 1972) and 31 and 80 µg/liter for bluegills (EATON 1974) exposed in life-cycle tests in hard water. The high hardness (~220 mg/liter) or related water quality characteristics such as alkalinity probably account for the lower toxicity as compared to the species tested in the present study. However, as mentioned previously, the embryos, larvae, or juveniles were as sensitive as any other life stage in both of the life-cycle tests.

The slight reduction in sensitivity (at $P=0.05$, but not at $P=0.01$) of brown trout resulting from longer exposure of embryos is in agreement with observations on flagfish. SPEHAR (1976) reported no significant mortality among flagfish larvae exposed to 31 µg/liter initially as embryos (time from fertilization to hatch is approximately 5 days), whereas those not exposed as embryos suffered 80-100% mortality within 30 days. Thus the extent of embryonic exposure apparently can influence the sensitivity of the organism to the toxicant.

The effect of cadmium on juveniles of brook trout and eyed embryos of brown trout was greater after exposure for 60 days than after exposure for 30 days. In coho salmon, lake trout, and the younger embryos of brown trout the longer exposure did not evoke greater sensitivity, nor did a 120-day exposure to cadmium increase the sensitivity of brook trout compared to that at 60 days. Therefore, based on the limited amount of information provided by this study and a few life-cycle chronic exposures, 60 days seems to be an appropriate duration of larval or juvenile exposure to estimate cadmium chronic toxicity.

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