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Effect of Zinc Exposure on Subsequent Acute Tolerance to Heavy Metals in Rainbow Trout

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Fish usually show increased tolerance to metals in solution if previously given an opportunity to acclimate to near lethal concentrations of the metal (Dixon and Sprague 1981a; McCarter and Roch 1983; Bradley et al. 1985; Chapman 1985), and tolerance has been correlated with an increase in tissue metallothionein (MT) (Dixon and Sprague 1981b; McCarter et al. 1982). The purposes of the tests described in this paper were: (1) to investigate the potential for increased tolerance to zinc; (2) to evaluate correlations between tolerance and liver MT level; (3) to go beyond simple one-step acclimation by increasing the acclimation level as tolerance developed; (4) to measure loss of tolerance following transfer of acclimated fish to control water; and (5) to determine if zinc acclimation led to increased tolerance to copper and cadmium.

MATERIALS AND METHODS

Trout used in these tests were juvenile rainbow trout (Oncorhynchus mykiss--formerly Salmo gairdneri); 1981 tests used the marine anadromous steelhead form and 1982 tests used the freshwater resident form. Both groups were obtained as eggs (embryos) from hatcheries of the Oregon Department of Fish and Wildlife. Embryos were brought to the US Environmental Protection Agency's Western Fish Toxicology Station (WFTS) in Corvallis, Oregon, and reared in vertical flow incubators until hatch, then transferred to troughs and subsequently transferred to 1700-L fiberglass tanks. Fish were fed Oregon Moist Pellet (OMP) diet of appropriate size. At the start of the 1981 test series the 7-mo-old trout had a mean (SD) wet weight of 4.95 (1.3) grams and total length of 8.6 (1.2) cm. At the

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start of the 1982 test series, the 5-mo-old trout had a mean (SD) wet weight of 3.0 (0.9) grams and total length of 7.0 (0.6) cm. Throughout rearing and testing, maximum loading guidelines (USEPA 1975) were observed.

Rearing, acclimation, and tests were conducted in WFTS well water that was aerated and temperature controlled by chilling or heating. Temperature in rearing and acclimation tanks and in one aquarium during each test were continuously recorded using 7-d thermographs. During tests, dissolved oxygen, pH, total alkalinity, and total hardness were determined near the beginning and end of each test from one test aquarium receiving the control water and one receiving the highest toxicant treatment. No differences were observed between these two treatments in any of these parameters and mean values were: temperature 12.6 C; DO 10 mg/L; alkalinity 25 mg/L as $CaCO_3$; hardness 33 mg/L as $CaCO_3$; and pH 6.6. Background metal concentrations in WFTS well water are low (Zn 5 ug/L, Cu 3 ug/L, Cd <1 ug/L).

Tanks in which the fish were acclimated prior to testing were outdoors (under cover) and received natural lighting. Tests were conducted under a 12:12 light:dark photoperiod including ramped 30-min dawn and dusk periods. During acclimation, fish were fed daily with OMP at 1.8% body weight (wet/wet). Feeding was terminated 1-d before fish were transferred to test aquaria, and fish were not fed during the toxicity tests.

Metal stock solutions were prepared from reagent grade metal chlorides ($ZnCl_2$, $CdCl_2 \cdot 2.5H_2O$, or $CuCl_2 \cdot 2H_2O$) dissolved in WFTS well water and acidified with 0.05 mL concentrated nitric acid per L of stock solution. Water samples for metal anyalysis were taken from each treatment aquaria on the 2nd and 4th day of each test and from the acclimation tank twice weekly. Metal analyses were by flame (Zn) or graphite furnace (Cd and Cu) atomic absorption of samples acidified with one volume percent of concentrated nitric acid and with precisions of $\pm$3 ug/L (Zn), $\pm$ 0.5 ug/L (Cu), and $\pm$ 0.1 ug/L (Cd).

During zinc acclimation, trout were distributed between two fiberglass tanks (950 and 570 L) and two identical tanks were used to hold control fish. All four tanks were continuously supplied with aerated, temperature-adjusted well water. Zinc stock solution for the acclimation tank was pumped at 70 mL/min from a 450-L polyethylene tank into a 2-L constant head box, and from there 60 mL/min of stock solution was introduced into a metered flow of dilution water and mixed in a

75-L constant head chamber. The zinc-acclimation solution was then split between the two acclimation tanks.

Each zinc acclimation concentration for each subject population was: 1) near its lethal threshold (LC1); 2) based upon mortality data and calculated 96-h LC10 from a just-completed zinc toxicity test; and 3) was always below 0.5 of its 96-h LC50. Mortality during acclimation ranged from <1 to 3%. All toxicity tests were conducted using 3 L/conc/min versions of the continuous flow diluter described by Garton (1980). Three such units were used, each dosing six pairs of aquaria; two units were used when comparing zinc tolerance of unacclimated and acclimated fish; the third unit was used when copper, cadmium, and zinc tolerance tests were conducted simultaneously.

All tests used 10 fish per aquarium and, except for the unreplicated Cd-Cu-Zn cross-tolerance tests and the 3- and 7-d deacclimation tests, used two replicate aquaria per treatment. Mortality was observed and recorded several times a day and dead fish were removed every 24 h. Estimates of LC10 concentrations from baseline tests prior to acclimation were obtained using logit analysis; except as noted, LC50 calculations were by the trimmed Spearman-Karber procedure (Hamilton et al. 1977). Significant differences between various acclimation treatments and controls were calculated based upon the standard error of the difference procedure described by Sprague and Fogels (1977). Throughout the data presentation in this paper, LC50 values that differed significantly from controls ($p=0.05$) are marked with an asterisk.

Rainbow trout from the 1982 series were taken from the acclimation or control tanks, anaesthetized with 2-phenoxyethanol, and frozen in polyethylene bags. Within 3 mo, the livers were dissected out, rinsed in 0.7% NaCl, and MT-like proteins determined by the method of Piotrowski et al (1973) as modified by Kotsonis and Klaasen (1977). In this method, ^{203}Hg in the fraction of liver homogenate below 10 kilo-Daltons is assumed bound to MT-like proteins. Changes in ^{203}Hg activity in this fraction are reported as changes in MT.

RESULTS AND DISCUSSION

In the 1981 test series, acclimation to a zinc concentration of 50 ug/L increased the tolerance of juvenile steelhead to zinc by a factor of 3 to 5 fold (Table 1). Maximum tolerance was achieved within 7 d with no further change noted after 2 or 3 wks acclimation. This general pattern of a rapidly attained plateau of tolerance is consistent with several other studies of acclimation to copper or zinc (Dixon and Sprague 1981a; McCarter and Roch 1983; Bradley et al. 1985), but the magnitude of increase was about twice that reported by these other investigators.

Table 1. Comparisons of tolerance to zinc in steelhead acclimated to 50 ug Zn/L for indicated periods.

Acclimation Period	96-h LC50(95%C.L.) [ug/L] Controls	Acclimated	Increased Tolerance
0	132(106-163)		
7 d	95(57-159)	450(384-527)*	4.7
14 d	141(101-197)	485(425-550)*	3.4
21 d	107(83-139)	421(374-474)*	3.9

* Significantly different from controls (p=0.05)

In order to further evaluate the relationship between acclimation time and tolerance,the first tests in 1982 series included acclimation periods of 3 to 28 d and included MT measurements. Results from these tests again indicated a very rapid development of zinc tolerance that remained reasonably stable throughout the 28-d acclimation period (Table 2). Near-maximal tolerance was seen after a 3-d acclimation, and the magnitude of increase in tolerance was only 2 to 3 fold. Increase in MT levels in livers of acclimated fish was consistent, but the magnitude of increase was less than the increase in tolerance.

Table 2. Comparisons of tolerance to zinc and liver MT levels in rainbow trout acclimated to 80 ug zinc/L for indicated periods.

Acclim. Period	96-h LC50(95%C.L.) [ug/L] Controls	Acclimated	Increased Tolerance	MT
0	212(179-252)			
3 d	344(273-436)	>798	>2.3	1.21
7 d	297(238-371)	924(772-1107)*	2.9	1.34
28 d	469(369-595)	1030(937-1132)*	2.2	--

* Significantly different from controls (p=0.05)

As a result of the significant increase in tolerance observed after only a 3-d acclimation, we later conducted a test to determine the influence of only 1 or 2-d acclimation. These results indicated no increase in tolerance and suggested that such short-term acclimation might result in decreased tolerance to zinc (Table 3; based upon probit analysis because all noncontrol treatments had >50% mortality). Because the effects of 1 and 2-d acclimation were measured some 5 mo after the effects of 3-d acclimation, the profound difference between 2 and 3-d acclimation may be somewhat sharper than would occur if they had been investigated in the same time frame.

Table 3. Comparisons of zinc tolerance in rainbow trout acclimated to 100 ug Zn/L for 1 or 2 days.

Acclimation	96-h LC50(95%C.L.) [ug/L]		Increased
Period	Controls	Acclimated	Tolerance
0	181(82-245)		
1 d	-	149(60-208)	0.84
2 d	-	113(31-190)	0.62

Tests of the effect of gradually increasing acclimation concentrations and loss of tolerance following sudden cessation of Zn exposure are summarized in Table 4. Acclimation at 100 ug Zn/L produced nearly a 5-fold increase in tolerance after 9 d, but further acclimation to 100, 300, and then 500 ug Zn/L for up to a total of 37 d produced no further increase. Tolerance to zinc was rapidly lost following return to control water, with almost complete reversion to control tolerance after only 7 d. Levels of liver MT again qualitatively followed the gain and loss of tolerance, but with no evidence of a strong quantitative relationship.

Table 4. Comparisons of zinc tolerance and liver MT in rainbow trout acclimated sequentially to 100, 300, and 500 ug Zn/L for up to 42 d and following return to control water for up to 7 d.

Acclimation		96-h LC50(95%C.L.) [ug/L]		Increased	
Period	[Zn]	Controls	Acclimated	Tolerance	MT
0d	-	246(191-315)			
(9d	100)	251(210-300)	1004(838-1204)*	4.0	1.26
(+5d	100				
+9d	300)	271(186-393)	1236(1043-1465)*	4.6	1.11
(+5d	300				
+9d	500)	191(148-245)	1017(870-1189)*	5.3	1.24
(+5d	500				
+3d	0)	204(153-272)	862(621-1198)*	4.2	1.30
(+4d	0)	226(187-273)	313(244-402)*	1.4	1.00

* Significantly different from controls (p=0.05)

The final series of tests compared the relative effect of Zn acclimation on tolerances to Zn, Cu, and Cd. Following a 17 day acclimation at 100 ug Zn/L, tests indicated similar increases in tolerances to Zn (3.6), Cu (4.7) and Cd (3.7) (Table 5). No statistical comparisons were conducted because either only one partial kill or all noncontrol responses in excess of 50% mortality precluded robust comparisons with logit, probit, or trimmed Spearman-Karber procedures. Listed LC50 values are from the logit analysis.

Table 5. Acute (120h) tolerance of rainbow trout to Zn, Cd, or Cu following 17-d acclimation to 100 ug Zn/L.

	Unacclimated			Acclimated		
Metal	[ug/L]	% Mort	LC50	[ug/L]	% Mort	LC50
Zn	Cont.	0		Cont.	0	
	210	70	170	190	0	
	330	90		330	0	
	655	100		640	60	618
	1500	100		1500	100	
	2400	100		2400	100	
Cd	Cont.	0		Cont.	0	
	0.5	0		0.5	0	
	1	30		1	0	
	2	100	1.1	2	0	
	4	100		4	70	4.1
	9	100		9	100	
	19	100		19	100	
Cu	Cont.	0		Cont.	0	
	5	40		5	0	
	14	80	7	11	30	
	21	70		18	10	
	40	80		32	30	33
	110	100		110	100	
	220	100		220	100	

Results of this study reveal both consistencies and inconsistencies with other reports of metal acclimation and fish. The rates of both development and loss of tolerance to zinc are very similar to those reported for copper and coho salmon (McCarter and Roch 1983), copper and rainbow trout (Dixon and Sprague 1981), and zinc and rainbow trout (Bradley et al. 1985). Increasing the acclimation concentration after the initial development of tolerance had little further effect on tolerance; increasing acclimation concentration also had no appreciable effect in further increasing liver MT levels. Acclimation to zinc produced parallel increases in tolerance to zinc, copper, and cadmium. This may be contrasted with the findings of Dixon and Sprague (1981a) where copper acclimation resulted in lower tolerance to zinc. Metal tolerance probably has several components, including influences on uptake, sequestering, and excretion; some aspects of tolerance may be more generic, others more ion specific, and each variously influenced by bonding strength, ionic radii, and other physical-chemical characteristics. Metal uptake and toxicity are influenced by such factors as pH, hardness, and alkalinity; these physiological and chemical factors probably influence the extent to which acclimation to one metal will influence tolerance to other metals.

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