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Accumulation of Zinc in Rainbow Trout *(Oncorhynchus mykiss)* After Waterborne and Dietary Exposure

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Zinc is a potential toxicant to fish with water hardness and pH constituting the principal modifying factors of Zn toxicity (Alabaster and Lloyd 1980; Bradley and Sprague 1985; Everall et al. 1989). Major toxic effects of elevated concentrations of waterborne Zn are disturbances of acid-base and ionoregulation (e.g., impairment of the branchial uptake of Ca^{2+}), disruption of gill tissue and hypoxia (Alabaster and Lloyd 1980; Hogstrand et al. 1994), Even at sublethal concentrations, chronic effects such as decreased reproduction, reduced growth and histopathological alterations in various tissues may occur (Spear 1981; Holcombe et al. 1979). Zinc can be accumulated via the gills and/or the digestive tract, However, the relative roles of water and food as sources of Zn uptake are not yet fully elucidated (Dallinger et al. 1987; Spry et al. 1988).

The aim of the experimental study presented in this paper was to find an explanation for the unexpected results of a field investigation of a Zn-polluted Austrian river, where despite very high total Zn concentrations in the water and in the sediment (water: mean value 1.6 mg•L^{-1}, extreme values up to 4.9 mg•L^{-1}; sediment: 30 gŽL^{-1}, dry weight), Zn levels in organs of rainbow trout were within the range of concentrations found in fish from a control site and similiar to those reported in the literature for fish from low-contaminated waters (Hofer et al. 1989). Furthermore, only minor histopathological changes in rainbow trout could be found. In consequence, we carried out a laboratory experiment to study Zn uptake from both Zn-contaminated water and diet at Zn concentrations found in the field study.

MATERIAL AND METHODS

The experiments were conducted with rainbow trout *(Oncorhynchus mykiss)* yearlings (40-60 g body weight) obtained from a local fish farm. Fish were maintained at 8°C in well aerated laboratory tap water with the following physiochemical characteristics: alkalinity 1700-1800 µeq•L^{-1}, hardness 140-180 mg•L^{-1} as $CaCO_3$, pH 7.8-8.2, conductivity 215-230 µSžcm^{-1}, sodium 0.2-0.3 mg•L^{-1}, potassium 0.15-0.20 mgŽL^{-1}, chloride 0.25-0.35 mgŽL^{-1}. Water hardness, pH and temperature corresponded to those found in the Zn-polluted river investigated in the field study.

One group (ZW) of 50 rainbow trouts was kept in each of two 200-L tanks (continous flow rate 1.5 LŽmin^{-1}). After acclimation, Zn (as $ZnSO_4$) was dosed by a LKB-peristaltic pump from a stock solution. Due to high mortality (up to 14%) after one day of exposure to the

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proposed experimental concentration of 1.6 mgŽL^{-1}, which corresponded to the Zn concentrations found in the field study, we decreased the exposure level to 0.5 mg ZnŽL^{-1}. Thereafter, we increased Zn concentrations within a period of few days to the final concentration of 1.6 ± 0.1 mgŽL^{-1}. Total Zn concentration in the water was measured twice a day by flame AAS. Zinc concentrations in the control group were below the detection limit of 0.01 mg•L^{-1}. Fish were fed a "basal diet" prepared by a formula similar to that used by Dabrowski and Köck (1989), with the exception that casein and gelatine were replaced by fish meal (TAGGER, Graz, Austria). Zinc content of the "basal diet" was 67.0 ± 3.0 mgžkg^{-1} (dry weight). Fish were fed 2% of their body weight per day. Duration of the experiment was 43 days.

A second group (ZD) of 50 fish was kept in each of two 200-L tanks (continuous flow rate 1.5 Lžmin^{-1}). After an acclimation period of four days, fish were fed an experimental diet consisting of the "basal diet" supplemented with Zn (as $ZnSO_4$), up to an experimental concentration of 3 gžkg$_1$ (dry weight). The experimental concentration used corresponded to the Zn concentrations found in the main food components (chironomids, tubificids) of fish from the Zn-polluted river. Duration of the experiment was 70 days,

A third group of 60 fish was kept as a control (for both treatment groups) in tap water in a 430-L tank (flow rate 1.5 l•min^{-1}). Fish were fed the "basal diet".

Six fish were randomly sampled from the two treatment tanks on days 1, 2, 3, 4, 6, 21 and 43 of the exposure period from the ZW group, and on days 9, 21, 43 and 70 from the ZD group. On each sampling date subsamples (six fish) were also taken from the control group. Fish were killed by a blow on the head and dissected immediately. Samples of gill, liver, kidney and gut tissue were rinsed thoroughly in distilled water to avoid contamination by Zn-polluted water, food particles or gut contents. Tissue samples were transferred into pre-washed and pre-weighed 10-mL polypropylene tubes. After drying (at 60° C to constant weight) and determination of dry weight, the samples were digested by addition of HNO_3 and H_2O_2 in a microwave oven. After complete digestion the samples were diluted with double-deionized water to the final volume of 10 mL. Metal analysis was performed by flame atomic absorption spectrophotometry (Perkin Elmer 2380, deuterium background correction). National Research Council Canada standard reference material (TORT-1 lobster hepatopancreas, DOLT-1 dogfish liver) analyses were within 4% of the certified values, with a coefficient of variation of < 3%. In all cases, Zn concentrations in procedural blanks were below detection limits.

One-way ANOVA was used to test the significance of treatment effects between the sampling dates and differences between the subsamples of the control group. Since no significant differences were found between the subsamples of the controls, a subsample at day 1 was used for comparison of treatment groups versus the control group (Bonferroni's test). Statistical significance was assigned at $p < 0.05$.

RESULTS AND DISCUSSION

After one day of Zn exposure, fish of the ZW group behaved in an extremely nervous manner, showing decreased swimming ability and disorientation, mortality was high (4-14% per day). After a few days mortality dropped to zero, but the behavior of fish changed only slowly to a normal level. Behavioral abnormalities observed also in other studies (Ellgaard 1978; Alabaster and Lloyd 1980) may be attributed to negative effects of Zn on the sensory and nervous system. Although it is well known that acute Zn stress may lead to severe gill damage (Skidmore and Tovell 1972), histological investigation revealed no pathological changes of gill tissue during the first days of the experiment. The concentration of 1.6 mg Zn•L^{-1} used in the experiment was lower than the LC_{50} value (1.9-2.3 mg•L^{-1}) as calculated from the correlation between LC_{50} values for rainbow and brown trout reported in the literature and hardness of the water used in this investigation (Köck

Table 1. Zinc concentrations in tissues of rainbow trout (means, standard deviation in brackets, N = 6; dry weight) after exposure to waterborne(ZW) and dietary (ZD) Zn. * denotes significant difference from control group.

	ZW-Group			
Day	**Gills**	**Liver**	**Kidney**	**Gut**
Control group	371.1 (207.1)	97.5 (14.5)	117.6 (10.1)	828.2 (334.8)
1	450.2 (304.7)	118.1 (4.9)	123.9 (12.9)	
2	503.8 (281.0)	117.3 (10.1)	126.4 (10.8)	880.2 (279.0)
3	408.8 (220.4)	129.2 * (10.7)	128.7 (9.9)	
4	532.7 (203.2)	124.4 * (12.3)	133.5 (15.9)	
6	654.1 (209.9)	123.4 * (38.4)	146.9 * (16.3)	
21	589.7 (249.9)	120.7 * (25.6)	183.5 * (37.5)	995.7 (525.0)
43	1173.4 * (709.5)	106.2 (14.6)	172.6 * (10.0)	1192.2 (104.3)
	ZD-Group			
9	523.7 (228.4)	99.7 (11.9)	146.9 * (11.3)	780.8 (102.7)
21	293.2 (176.8)	106.0 (21.4)	121.1 (13.6)	804.8 (175.9)
43	(310.3) 197.9	105.6 (9.3)	112.1 (19.2)	826.1 (214.4)
70	(488.6) (318.0)	116.0 * (9.3)	161.8 * (30.2)	1287.3 * (519.7)

et al. 1991). Furthermore, when fish were kept in aquaria made of flexible PVC foil and at reduced light, Zn concentrations up to 3.7 mg•L^{-1} could be applied with no mortality. Thus, we assume that the observed mortality during the first days of the experiment was attributed to heavy stress, death being caused by collisions with the walls of the aquarium and not to direct toxic action by Zn. In the second half of the experiment mortality rose again. This coincided with histopathological changes (fusion of secondary gill lamellae, proliferation of chloride- and mucus cells, hyperplasia of gill epithelia cells, increased hyaline droplet degeneration of the first proximal segment of the kidney tubules) observed in these fish. This is in contrast to our findings from the field study, where we observed only minor histopathological effects of Zn on fish (Hofer et al. 1989). Similary, a 59-day experiment in which rainbow trout were exposed to water from the Zn-polluted river (1 .5-1.7 mg•L^{-1} Zn) revealed no histopathological changes in fish tissues (Köck et al. 1991).

The decreased toxicity of Zn in the polluted river could be attributed to diminished bioavailability of the metal, possibly by adsorption onto organic or inorganic suspended solids and/or complexation by dissolved organic material (Alabaster and Lloyd 1980; Spear 1981). No mortality occurred in the ZD and control groups. The *Costia* infection observed during the second half of the experiment in the ZD group, and to a lesser extent in the ZW group, might have been a consequence of chronic Zn stress since decreased resistance to diseases and parasites has been observed in Zn-exposed fish (Sarot and Perlmutter 1976).

Fish of the ZW group responded quickly to Zn concentrations in the water (Table 1). Zn concentration in the liver of the ZW group increased to a significantly higher level compared to the controls during the first few days, but decreased during the following weeks and reached nearly the level of the controls at the end of the experiment, indicating the induction of regulatory processes. In other studies no correlation between Zn level in the liver and Zn concentration in the water could be found (Roth et al. 1982; Hofer et al. 1989). Zinc is an essential element which can be regulated by fish over a wide range of concentrations (Spry et al. 1988). Hogstrand et al. (1994) suggested an adaptation to waterborne Zn by a change of K_m of a mutual Ca^{2+}/ Zn^{2+} carrier which may have reduced Zn influx in hard water. Zinc concentration in the kidneys of the ZW group increased during the first 3 weeks, but leveled and remained constant until the end of the experiment. Our results indicate the kidney as a target organ for Zn storage in rainbow trout. This corresponds to the field study where only the Zn content of the kidney was found to be positively correlated to the Zn contamination of the river (Hofer et al. 1989), indicating the kidney to be a suitable indicator of Zn contamination in rainbow trout. Zinc concentration in the gills of the ZW group increased during the experimental period, but was significantly different from the controls only at the end of the experiment. This could have been a consequence of accumulation of Zn in gill tissues or mucus layer, or by excretion of the metal via the branchial route (Hardy et al. 1987; Handy and Eddy 1990). No significant Zn accumulation could be found in gut tissue of the ZW group.

Zinc concentration in tissues of the ZD group was significantly influenced by dietary Zn only at the end of the feeding trial. Zinc concentration in the kidneys of ZD group was significantly higher than that of the control group after 9 days of the feeding trial, but decreased again during the following weeks. Only after 70 days did the Zn content of the kidneys increase again to a significantly higher level compared to the controls. In the liver of the ZD group, Zn concentration increased constantly during the experimental period but reached a significantly higher level only at the end of the feeding trial. In the gut, Zn levels were significantly higher only at the end of the experiment. No consensus exists in the literature on the relative importance of Zn uptake from dietary or aqueous sources in fish (Dallinger et al. 1987; Spry et al. 1988). In our study the accumulation of Zn was much higher from the water than from the diet (Table 1). This corresponds to other studies where fish fed Zn-enriched diets revealed no or only minor accumulation of the metal in liver and kidney (Jeng and Sun 1981; Knox et al. 1984; Overnell et al. 1988). The efficiency of Zn absorption efficiency has been found to vary between contaminated artificial diets and natural food (Pentreath 1973). Several studies reported the availability of Zn in the diet to be related to the contents of phytic acid and calcium or to the presence of soy bean protein or whitefish meal (Knox et al. 1984, Gatlin and Phillips 1989). Thus, the low efficiency of Zn uptake in the fish investigated might be attributed to the composition of the diet offered in our feeding experiment.

In summary, concentrations of 1.6 mg Zn$\check{z}L^{-1}$ lead to severe histopathological changes in rainbow trout. The apparent difference to the field study, where only minor histopathological alterations were observed at similar concentrations of Zn, is likely to be due to decreased bioavailabiliy of the metal in the river. In our study Zn accumulation from the water was higher than from the food, indicating the gills to be the major route for

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