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Avoidance Response of Rainbow Trout *Oncorhynchus mykiss* to Heavy Metal Model Mixtures: A Comparison with Acute Toxicity Tests

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Avoidance of polluted water is one of the most significant sublethal responses of fish (Sprague and Drury 1969). Fish populations can be affected by aquatic pollutants not only directly but their active retreat out of polluted areas can also result in disturbances of their migrations and distribution patterns (Saunders and Sprague 1967). Therefore, a reduction of their normal area of habitat, as well as their resources, can occur (Hansen et al. 1999a). From another point of view, the ability of fish to detect and avoid toxic substances is one of the forms of phenotypic adaptation allowing them to survive in altered environment (Flerov 1989).

At present it has already been established that fishes can detect and avoid many toxicants of different chemical origin (Beitinger and Freeman 1983; Giattina and Garton 1983). However, fish behavioral responses to toxicant mixtures are investigated insufficiently. Furthermore, the results of behavioral tests are significant only in the case when they are compared with particularly meaningful ecological indices for fish (survival, growth, reproduction success) and, in the first place, with the acute effects of toxicants.

The aim of the present study was to determine acute toxicity of 3 heavy metal model mixtures (HMMM) of different qualitative and quantitative composition to rainbow trout; to carry out behavioral tests in order to evaluate the capability of test fish to detect and avoid HMMM solutions; to compare the results of acute toxicity and behavioral tests.

MATERIALS AND METHODS

The tests were conducted on one-year-old rainbow trout obtained from the Žeimena Fish Farm. The test fish were acclimated to laboratory conditions at least for one week before starting the tests. They were maintained in a flow-through 1000-L holding tanks supplied with aerated artesian water, under natural illumination and were fed daily in the morning (on minced beef spleen). A day before and during the tests the fish were not fed. The average total length of test fish was 100±10 mm and the total weight was 10±2 g (mean±SEM).

The effects of 3 heavy metal model mixtures of the different composition were studied. The formation of model mixtures was carried out basing on: Maximum Permissible Discharges (MPD, accepted for Lithuania) of 5 common heavy metals into municipal sewage waste-water entering treatment plants (HMMM-1); available analytical data of average annual amounts of representative heavy metals in cooling waste-water discharging from the Ignalina Nuclear Power Plant into the Drūkšiai lake during 1996 (HMMM-2); the available analytical data of average annual amounts of representative heavy metals in waste-water after treatment discharged from Vilnius city into the Neris river during 1996-1997 (HMMM-3). The stock solutions of HMMM were prepared in acidified distilled water (concentrated H_2SO_4 was added to reach final pH=2) by use of the following chemically pure substances: $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $NiSO_4 \cdot 7H_2O$, $K_2Cr_2O_7$, $FeSO_4 \cdot 7H_2O$, $Pb(NO_3)_2$, $Cd(CH_3COO)_2 \cdot 2H_2O$, $MnSO_4 \cdot 5H_2O$ ("REAKHIM" Company, Russia), the final concentration being recalculated according to the amount of heavy metal ion. The concentrations of HMMM solutions which were considered to be equal to 100% are presented in Table 1.

Table 1. Composition of heavy metal model mixtures (HMMM). The concentration is equal to 100%.

Heavy metal model mixture	**Heavy metal and concentration (mg/L)**							
	Cu	**Zn**	**Ni**	**Cr**	**Fe**	**Pb**	**Cd**	**Mn**
HMMM-1		1.0	0.5	2.5	5.0	—	—	—
HMMM-2	0.75	6.4	0.21	0.28	—	1.42	0.018	0.99
HMMM-3	2.0	6.0	5.0	2.0	30.0	3.0	0.3	9.0

Note: heavy metal concentrations of HMMM-2 and HMMM-3 are artificially increased 100 times as compared with the real analytical data in order to approximate concentrations of different HMMM. Heavy metal concentrations in stock solutions were 1000 times higher.

All the tests were performed during September-November, 1997-1999.

Acute toxicity tests were conducted under semi-static conditions. The system included six 20-L continuously aerated and cooled test tanks. Artesian water was used for dilution. Its average physico-chemical characteristics were: hardness was 270- 300 mg/L as $CaCO_3$, alkalinity was 244 mg/L as HCO_3^-, pH=7.9-8.1, dissolved oxygen=10±1.2 mg/L and temperature=10±0.2^{O}C. The testing was started in pure water, 10 fish being placed into each tank for 24-hour acclimation period in order to accustom them to the altered environment. The water temperature in the test tanks was the same as in the holding tanks. After acclimation period desirable HMMM concentrations were formed in 5 tanks by adding necessary amounts (1-10 ml) of stock solutions into each tank. This procedure did not change any physico-chemical characteristic of dilution water in the tanks. The "dilution factor" was equal to 75-85% and the 6-th tank served as control. The test solutions were renewed every day, test fish were transferred into freshly prepared solutions and fish mortality observations were made at 24-hour intervals.

The dead fish were weighed individually (after being lightly blotted dry) and measured (total length) at the time of mortality observation. The fish still alive at the end of the test were also weighed and measured at the same time. No mortality was observed among the control fish. Furthermore, the amount of oxygen in the tanks as well as temperature and pH were measured every 24 hours. Every test continued 96 hours and had two replications.

At the end of each test water samples were taken from tanks, and after being fixed with concentrated nitric acid the total amount of heavy metals was established by atomic absorption spectrophotometry on AAS Varian 250 PLUS. The analytical data obtained confirmed that the determined heavy metal concentrations coincided with the estimated data quite satisfactorily (the error did not exceed 10%).

LC50 values and their 95% confidence intervals were estimated by use of the trimmed Spearman-Karber method (Hamilton et al. 1977).

Avoidance tests were carried out by use of a flow-through U-shaped plastic steep gradient chamber of alternative preference with two parallel water streams in detail described in the previous research (Svecevičius 1998; 1999). The chamber was 1500 x 600 x 300 mm in size and the total flowing rate was 6 L/min. Fifteen minutes after the introduction of the test solution into one of the sections of the chamber two zones were formed: zone **a** (pure water) and zone **b** (solution).

The fish were tested in groups of ten individuals. Each group was placed in a gradient chamber and acclimated for 24 hours. The water temperature in the gradient chamber was the same as in the holding tanks. After acclimation period desirable HMMM The test procedure was conducted by means of multiple momentary recordings of fish in polluted and pure water zones during 2.5-hour control and test periods. After the control period the test solution of necessary concentration was introduced into one of the chamber sections and the fish were allowed to choose between pure and polluted water. The nature and intensity of behavioral response were estimated by the Response index through formula:

$$\text{Response index} = 50(2 - N_T/N_C)$$

were N_C is the average number of fish in polluted zone during the control period (water is pure) and N_T is the average number of fish in the same zone during the test period (pollutant is introduced).

Each group of fish was tested only once. Every test had ten replications. The value of index 100 denoted maximal avoidance, while 0 signified maximal preference and 50 stood for indifference. The control tests have shown that the test fish preferred both sections of gradient chamber with the same probability. Therefore the significance of test fish behavioral responses was determined by comparing their mean Response index value to theoretically neutral response, i.e. to the value of Response index equal to 50 by use of Student's *t*-test with $p \leq 0.05$.

At the end of each test water sample analysis was done in the same order as during the toxicity tests.

RESULTS AND DISCUSSION

The results of acute toxicity tests of HMMM to rainbow trout are presented in Table 2.

Table 2. Test concentrations of heavy metal model mixtures (HMMM) and rainbow trout mortality.

HMMM concentration (%)	Mortality(%)	96-hour LC50	95% confidence interval
HMMM-1			
0 (control)	0	144	137—152
100	0		
125	15		
150	60		
175	95		
200	95		
225	100		
HMMM-2			
0 (control)	0	29.3	26.5—32.4
24	20		
32	60		
42	100		
56	100		
75	100		
100	100		
HMMM-3			
0 (control)	0	26.8	24.6—29.2
17	0		
23	30		
30	60		
40	100		
53	100		

The results of rainbow trout avoidance response to HMMM solutions (Table 3) have shown that the intensity of avoidance response was directly proportional to

HMMM concentration logarithm (Figure 1). Therefore, the threshold avoidance concentrations were defined by use of regression analysis: the point of concentration scale at which logarithmic regression intersects Response index scale at the value being equal to 50, was considered as avoidance threshold (AT). The stimated heavy metal concentrations corresponding to threshold avoidance concentrations of HMMM are presented in Table 4.

Table 3. Response indices of rainbow trout (mean±SEM, N=10) to test concentrations of HMMM solutions.

Concentration (%)	HMMM-1	HMMM-2	HMMM-3
0.063	—	43±2.2	45±2.2
0.125	—	51±3.0	59±1.9*
0.25	—	78±1.7*	72±0.4*
0.5	52±7.8	91±1.9*	86±1.8*
1	64±6.8*	100±0.0*	100±0.0*
5	65±7.2*	—	—
10	90±3.4*	—	—
Avoidance threshold(AT) (%)	0.38	0.09	0.08
Ratio to 96-hour LC50	0.0026	0.0031	0.003

Note: asterisks (*) denote values significantly different from 50 (P≤0.05).

Table 4. Heavy metal concentrations in HMMM corresponding to threshold avoidance concentrations of rainbow trout to HMMM.

HMMM	Heavy metals and their concentrations (mg/L)							
	Cu	Zn	Ni	Cr	Fe	Pb	Cd	Mn
HMMM-1	0.0019	0.0038	0.0019	0.0095	0.019	—	—	—
HMMM-2	0.0007	0.0058	0.0002	0.0003	—	0.0013	0.00002	0.0009
HMMM-3	0.0016	0.0048	0.004	0.0016	0.024	0.0024	0.0002	0.0072
MPC	0.001	0.01	0.01	0.005	0.1	0.1	0.005	0.01

It is evident that avoidance response of rainbow trout to HMMM is behavioral response of high sensitivity. The concentrations of heavy metals corresponding to threshold avoidance concentrations are very low. Such heavy metal concentrations can be found only in clean unpolluted water. Furthermore, many of them are even lower than their Maximum Permissible Concentration (MPC) accepted to the inland waters of Lithuania. From this point of view the avoidance response of rainbow trout to HMMM can be successfully used in solving practical problems of

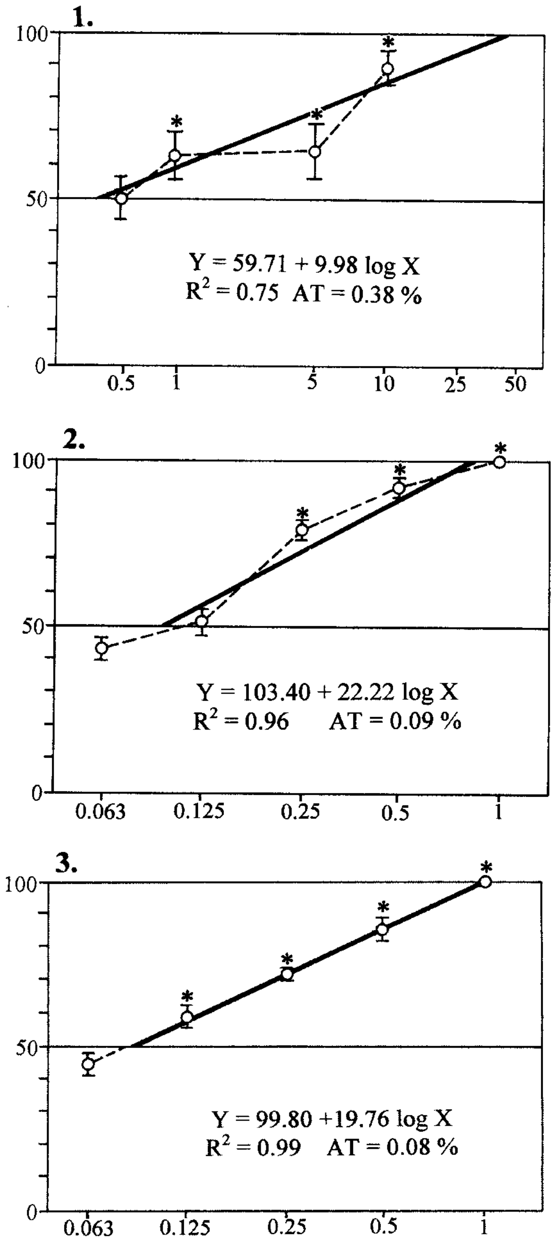

Figure 1. Rainbow trout response diagrams: to HMMM-1 (1), to HMMM-2 (2), to HMMM-3 (3). X axis corresponds to HMMM concentration in % (logarithmic scale), Y axis corresponds to Response index, circles (O) indicate mean index values, vertical lines refers to ±SEM, asterisks (*) denote values significantly different from 50 ($P \leq 0.05$), AT stands for avoidance threshold.

aquatic toxicology, such as quality hazard assessment of natural or waste-water containing complexes of heavy metals.

Another very important result of the present research is the fact that the ratio between HMMM avoidance threshold (AT) concentrations and acutely lethal concentrations (96-hour LC50) remains practically stable (about 0.003), independently from the HMMM composition. This indicates that the behavioral response of rainbow trout to HMMM is adequate enough and directly depends on HMMM toxicity.

The data obtained contradict suggestions of many authors that the intensity of fish avoidance response does not depend on the toxicity of the test substance (Sprague and Drury 1969; Beitinger and Freeman 1983; Giattina and Garton 1983; Svecevičius 1994). This is observed in the case when one specific substance is studied. However, the toxic effects of pollutants in mixture and their interactions are investigated incompletely. It is established that fish olfactory system is involved in forming avoidance response to individual heavy metals (Brown et al. 1982; Svecevičius 1991). Moreover, heavy metals can affect fish olfactory system directly inducing structural and functional alterations (Rhenberg and Schreck 1986; Baatrup 1991; Blaxter and Ten Hallers-Tjabbes, 1992; Julliard et al. 1993; Saucier and Astic, 1995; Hansen et al. 1999b). The complex effect, both toxic and irritant, of even low concentrations of heavy metals can be synergetic with respect to fish olfactory system and that can cause such fish behavioral responses. Thus, more conplete experimental studies on the given problem are needed.

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