Bull Environ Contam Toxicol (2007) 79:596–600
DOI 10.1007/s00128-007-9236-1

Avoidance Response of Rainbow Trout *Oncorhynchus mykiss* to Hexavalent Chromium Solutions

Gintaras Svecevičius

Received: 4 April 2007 / Accepted: 29 May 2007 / Published online: 17 July 2007
© Springer Science+Business Media, LLC 2007

Abstract Laboratory tests were conducted on one-year-old rainbow trout *Oncorhynchus mykiss* to evaluate their ability to detect and avoid hexavalent chromium (Cr^{6+}). Test fish were given a choice to discriminate between clean water and Cr^{6+} solutions of six sublethal concentrations ranging from 0.0015 to 0.3 mg Cr/L in a counter-current flow, steep gradient chamber. The intensity of avoidance response reached a significant level at test concentrations of 0.003 mg Cr/L and higher, and was directly proportional to the Cr^{6+} concentration logarithm. Avoidance threshold was estimated through regression analysis and to be 0.0017 mg Cr/L. This result is approximately sixfold lower than maximum-permitted concentration of 0.01 mg Cr/L accepted as the Lithuanian water-quality guideline for the protection of aquatic life.

Keywords Fish · Behavior · Avoidance · Hexavalent chromium

Hexavalent chromium (Cr^{6+}) is the dominant dissolved stable Cr species in aquatic ecosystems (Eisler 1986) usually produced from anthropogenic sources (Irwin et al. 1997). This pollutant is important because it is referred to selected water quality indicators (SCORECARD 2005) and is recommended for use as a reference toxicant in standard toxicity tests (ISO 7346-1:1996a(E); ISO 7346-2:1996b (E); ISO 7346-3:1996c (E); US EPA 2002a; US EPA 2002b) due to its ability to provide reproducible test results (Dorn et al. 1986).

G. Svecevičius (✉)
Laboratory of Hydrobiont Ecology and Physiology, Institute of Ecology of Vilnius University, Akademijos 2, 08412 Vilnius-21, Lithuania
e-mail: Gintaras.Svecevicius@takas.lt

Although the toxicity of chromium to aquatic life was intensively investigated during previous decades and a considerable amount of experimental data was compiled and reviewed (US EPA 1985; Eisler 1986; Irwin et al. 1997), studies into its sublethal effects in fish are still insufficient.

Meanwhile, alterations in fish behavioral responses are sensitive indicators of sublethal exposure to pollutants (Scherer 1992; Kane et al. 2005). These are no standardized procedures yet.

Avoidance of polluted water is one of the most ecologically significant sublethal responses of fish allowing them to survive in the altered environment (Sprague and Drury 1969). Fish avoidance studies are important because avoidance behavior represents the final integrated response of fish species to toxicant stress.

In the previous study it was established that rainbow trout was the most sensitive species to the acute toxic effect of Cr^{6+} among other four freshwater fishes tested (Svecevičius 2006).

The objectives of the present study were (1) to evaluate the ability of rainbow trout to detect and avoid Cr^{6+} solutions and (2) to compare behavioral test results with acute toxicity results under the same, controlled experimental conditions.

Materials and Methods

Rainbow trout adults (one-year-old) were obtained from Žeimena Hatchery (Švenčionys District, Lithuania). The test fish were acclimated to laboratory conditions for 1 week prior to testing. The fish were kept in flow-through 1,000 L holding tanks supplied with aerated deep-well water (minimum flow rate 1 L per 1 g of their wet body mass per day), under natural illumination and were fed commercial trout feed daily in the morning; the total amount was no less than 1% of their wet body mass per day. The day before and during the tests the fish were not fed. The average total length of test fish was 80 ± 0.5 mm and the total weight was 8.0 ± 0.5 g (mean ± SEM).

Reagent grade potassium dichromate ($K_2Cr_2O_7$) («REAKHIM» Company, Russia) was used as the toxicant. Stock solution was prepared by dissolving a necessary amount of potassium dichromate in distilled water, the final concentration being recalculated according to the amount of heavy metal ion.

Deep-well water was used for dilution. Average hardness of the water was approximately 284 mg/l as $CaCO_3$, alkalinity was 244 mg/l as HCO_3^-, pH was from 7.9 to 8.1, temperature was maintained at 10.5–11.5°C, and oxygen concentration was maintained at the range from 8 to 10 mg/l.

Avoidance tests were carried out by use of a counter-current flow plastic steep gradient chamber of alternative preference similar to ones used by Sprague (1964), Giattina and co-workers (1982), and Woodward and co-workers (1995) under even laboratory illumination of ~250 lux. Water entered each end of the chamber at equal flow rates of 3 L/min and drained at the center (Fig. 1). The chamber was 127 × 15 × 15 cm in size and water depth was 11 cm. The baffles with numerous small holes were fixed at 13.5 cm from each end and the chamber was 100 cm long between these baffles.

Both sides of the chamber were fed by gravity flows incoming from flow-through head tank of 200-L volume, which was constantly refilled by aerated dilution water. Toxicant supply was performed from 1-L volume graduated plastic bottle (Compat®, SandozNutrition) with a constant flow rate of 30 mL/min. The control tests with dye (methylene blue) showed that 10 min after the introduction of the test solution into one end of the chamber a stable, steep gradient with a sharp boundary between clean water and test solution was established.

Each trial used one group of ten fish and consisted of three periods; an acclimation period, a control period, and a test period. Each group was placed in a gradient chamber and acclimated for 1 h. The density of fish in the test area was similar to that in the holding tanks in order to eliminate aggressive interactions. After that they swam freely exploring the chamber. The next 10 min was the control period during which fish position in the chamber was recorded with a video camera recorder (SONY video Hi8 Model No CCD-TR713E, Japan) connected to TV set (in total 60 momentary recordings every 10 s). Immediately after the control period, a test solution was introduced into one end of the chamber through toxicant inlet (this procedure did not change any physico-chemical characteristic of the dilution water). The fish were allowed to choose between clean water and solution for 20 min and their position was recorded during the last 10 min of this (test period).

A series of ten trials was conducted for each concentration of the test solutions. Fish were tested only once. Chromium solutions were added at one end or at the other of the chamber, in random order to prevent the possibility of error resulting from one end preference of the test fish.

The nature and intensity of behavioral response were estimated by the Response index through formula:

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

N_C is equal to the average number of fish in polluted end during the control period (water is clean), and N_T is equal to the average number of fish in the same end during the test period (toxicant is introduced).

The value of index 100 denoted maximal avoidance, while 0 signified maximal preference and 50 stood for indifference. The control tests showed that the test fish preferred both ends of gradient chamber with the same probability. The results of these blank tests confirmed that data were normally distributed and had homogeneous variance. 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 at $p \leq 0.05$. The data obtained were analyzed statistically through STATISTICA (Version 6.0) and GraphPad Instat™ (Version 2.04) softwares.

The amount of oxygen in the chamber as well as temperature and pH were measured routinely with a hand held multi-meter (WTW Multi 340i/SET, Germany). At the end of the tests water samples were taken from the gradient

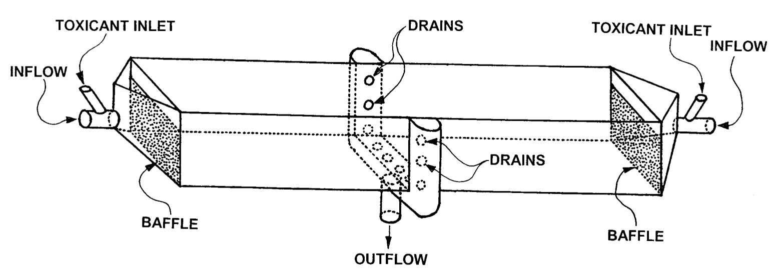

Fig. 1 Diagram of the gradient chamber

chamber and total amount of chromium was measured with an atomic absorption spectrophotometer (SHIMADZU AA-6800, Japan) with the graphite furnace techniques using proprietary software. Each sample was analyzed for three times. Mean measured concentrations were within 10% of target.

Results and Discussion

The data obtained are presented in Table 1. At lowest Cr^{6+} concentration of 0.0015 mg/L test fish demonstrated complete indifference, while at the next test concentration of 0.003 mg Cr/L the intensity of avoidance response reached a significant level and gradually increased within increase in Cr^{6+} concentration. The test concentration of 0.3 mg Cr/L was avoided by rainbow trout with almost maximal intensity.

In all cases avoidance response in test fish occurred at low sublethal levels of Cr^{6+} (Table 1). Furthermore, the intensity of avoidance response was directly proportional to Cr^{6+} concentration logarithm (Fig. 2). Therefore, the threshold avoidance concentration was defined by use of regression analysis: a method suggested by a number of researchers (Höglund 1961; Fava and Tsai 1976; Anestis and Neufeld 1986). The point of concentration scale at which logarithmic regression intersects Response index scale at the value being equal to 50 was assumed as avoidance threshold. Estimated Cr^{6+} concentration corresponding to threshold avoidance concentration and its 95% confidence interval was equal to 0.0017 (0.0008–0.0039) mg Cr/L.

Comparisons of changes in behavioral responses with classical toxicity test endpoints are needed to evaluate the sensitivity of behavioral toxicity tests (Atchison et al. 1987).

The ratio between threshold avoidance concentration and 96-h LC50 of 28.5 mg Cr/L derived from acute toxicity tests under the same experimental conditions (Svecevičius 2006) amounted 0.00006. Similar results were obtained previously on the same fish species for other heavy metals using different type of test apparatus. These ratios were equal to 0.0015 for copper and to 0.00003 for zinc, respectively (Svecevičius 1999). Obviously, there is no correlation between heavy metal toxicity and its avoidance intensity, and avoidance response is a very sensitive indicator of presence of these heavy metals at sublethal levels in water.

Table 1 Avoidance response of rainbow trout to test concentrations of Cr^{6+} solutions

Chromium concentration (mg/L)	Chromium concentration as a part of 96-h LC50	Response indices (mean ± SEM, N = 10)
0.0015	0.00005	48 ± 1.8
0.003	0.0001	57 ± 2.7*
0.015	0.0005	65 ± 4.8*
0.03	0.001	77 ± 4.7*
0.15	0.005	85 ± 2.5*
0.3	0.01	95 ± 1.9*

Asterisks (*) denote values significantly different from 50 ($p \le 0.05$)

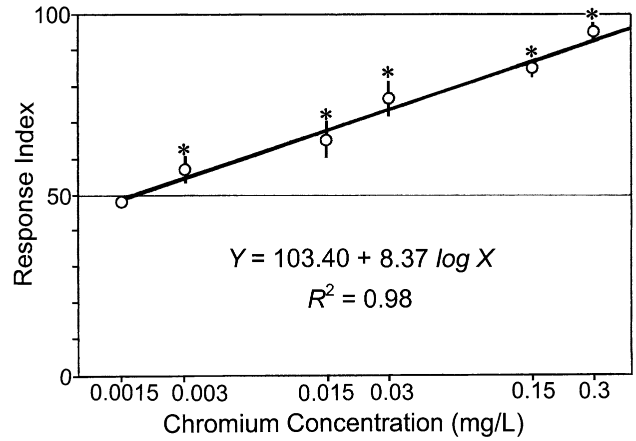

Fig. 2 Diagram of rainbow trout avoidance response to Cr^{6+} test solutions. *X*-axis corresponds to Cr^{6+} concentration in mg/L (logarithmic scale), *Y*-axis corresponds to Response index, *circles* indicate mean index values, *vertical lines* refers to ±SEM, *asterisks* denote values significantly different from 50 ($p \le 0.05$)

Such great differences in the results from behavioral and mortality categories probably can be explained by their reliance to the different forms of phenotypic adaptation (behavioral and physiologo-biochemical or non-behavioral) as described by Flerov (1989). Avoidance response can be initiated through chemosensory irritation, since it was established that the fish olfactory system is involved in the formation of an avoidance response to heavy metals (Brown et al. 1982; Svecevičius 1991). In the second case a complex of non-specific defensive physiological and biochemical responses of fish organism is started-up to withstand the toxic effect of the given heavy metal.

Perhaps Cr^{6+} avoidance by fish was thoroughly documented only in one study by Anestis and Neufeld (1986). The authors reported threshold avoidance concentration in rainbow trout of 0.028 mg Cr/L (~17-fold higher than such reported here). The test fish in this study were pre-exposed to sublethal concentrations of chromium for 7–20 weeks. After that they demonstrated much higher avoidance thresholds of chromium than the control fish. Water chemistry in these studies included hardness of 100 mg/L as $CaCO_3$, alkalinity of 50 mg/L as HCO_3^-, and pH of 7.2, i.e. quite different from that used here. Moreover, the test apparatus used included combined steep and shallow gradients characteristics.

It seems quite difficult to explain such great differences in these two final results but there is no doubt that the absence of standardized test procedure can lead to disagreements of such kind. The main lack of mentioned-above investigation was that the authors did not conduct acute toxicity tests. The fact that the same fish species in distinct geographical regions may show different sensitivity to the same pollutant is also universally recognized.

It should be noted that threshold avoidance concentration of Cr^{6+} is quite low and corresponds to the background level of this metal in unpolluted inland waters. This result is approximately sixfold lower than maximum-permitted concentration (MPC) of 0.01 mg/L of total chromium in receiving waters proposed by the European Union and newly-accepted (in 2002) as Lithuanian water-quality guideline for the protection of aquatic biota.

Determination of water-quality criteria is based on conventional standard acute and chronic testing procedures. Unfortunately, fish behavioral toxicity tests have not been standardized yet although even Atchison and co-workers (1987) noted that avoidance tests seem sensitive, rapid, ecologically significant, and they could be easily standardized.

Fish avoidance response is of very high ecological significance because their active retreat from polluted areas can 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. 1999).

It is evident that this newly accepted MPC for chromium is too high and could be revised based on fish behavioral response parameters.

For example, Giattina and co-workers (1982) reported avoidance threshold concentrations of 0.0064 mg/L for copper and of 0.024 mg/L for nickel in rainbow trout and these concentrations were very close to ambient water-quality criteria proposed by US Environmental Protection Agency of 0.0056 and 0.021 mg/L, respectively. Later Giattina and Garton (1983) pointed out that whole-organism behavioral responses should be included in considerations for water quality criteria and environmental impact, since such responses cannot be predicted from physiological and histological studies.

Consequently, fish avoidance response could be a powerful tool in determining or revising water-quality guidelines for the protection of aquatic biota.

In every standard test procedure reference substances must be used because reference toxicant tests indicate the reliability of the analytical procedure used for verifying toxicant concentrations, and allow one to control and detect the variability associated with test organisms and their relative health (Dorn et al. 1986). Since Cr^{6+} at the present is one of the most widely used reference toxicants, the data obtained in this study could be useful in further studies.

References

Anestis I, Neufeld J (1986) Avoidance–preference reactions of rainbow trout (*Salmo gairdneri)* after prolonged exposure to chromium (VI). Water Res 20:1233–1241

Atchison GJ, Henry MG, Sandheinrich MB (1987) Effects of metals on fish behavior: a review. Environ Biol Fish 18:11–25

Brown SB, Evans RE, Thompson BE, Hara TJ (1982) Chemoreception and aquatic pollutants. In: Hara TJ (ed) Chemoreception in Fishes. Dev Aquacult Fish Sci 8:362–393

Dorn PB, Rodgers JH Jr, Jop KM, Raia JC, Dickson KL (1986) Hexavalent chromium as a reference toxicant in effluent toxicity tests. Environ Toxicol Chem 6:435–444

Eisler [s1]R (1986) Chromium hazards to fish, wildlife and invertebrates: a synoptic review. US Fish and Wildlife Service Biological Report 85, Laurel, MD

Fava J, Tsai C (1976) Immediate behavioral reactions of blacknose dace (*Rhinichthys atratulus*) to domestic sewage and its toxic constituents. Trans Amer Fish Soc 105:430–441

Flerov BA (1989) Ecological and physiological aspects of toxicology in fresh-water animals (in Russian). Nauka, Leningrad

Giattina JD, Garton RR, Stevens DG (1982) Avoidance of copper and nickel by rainbow trout as monitored by a computer-based data acquisition system. Trans Amer Fish Soc 111:491–504

Giattina JD, Garton RR (1983) A review of the preference–avoidance responses of fish to contaminants. Res Rev 87:43–90

Hansen JA, Woodward DF, Little EE, DeLonay AJ, Bergman HL (1999) Behavioral avoidance: a possible mechanism for explaining abundance and distribution of trout species in a metals impacted river. Environ Toxicol Chem 18:313–317

Höglund LB (1961) The reactions of fishes in concentration gradients. Fisheries Board, Swedish Institute of Freshwater Research, Drottningholm, Report 43:1–147

Irwin RJ, Van Mouwerik M, Stevens L, Seese MD, Basham W (1997) Chromium VI (Hexavalent chromium). Environmental Contaminants Encyclopedia. National Park Service,Water Resources Division, Fort Collins, Colorado

ISO 7346-1 (1996a) Water quality—determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] Part 1: Static method. ISO, Switzerland

ISO 7346-2 (1996b) Water quality—Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] Part 2: Semi-static method. ISO, Switzerland

ISO 7346-3 (1996c) Water quality—Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] Part 1: Flow-through method. ISO, Switzerland

Kane AS, Salierno JD, Brewer SK (2005) Fish models in behavioral toxicology: automated techniques, updates and perspectives. In: Ostrander GK (ed) Methods in aquatic toxicology. Vol 2. Lewis Publishers, Boca Raton, FL, p 559–590

Saunders RL, Sprague JB (1967) Effects of copper–zinc mining pollution on a spawning migration of Atlantic salmon. Water Res 1:419–432

Sprague JB (1964) Avoidance of copper–zinc solutions by young salmon in the laboratory. J Water Pollut Control Fed 36:990–1004

Sprague JB, Drury DE (1969) Avoidance reactions of salmonid fish to representative pollutants. In: Jekins SH (ed) Advances in water pollution research. Pergamon Press, London, p 169–179

Scherer E (1992) Behavioural responses as indicators of environmental alterations: approches, results, developments. J Appl Ichthyol 8:122–131

SCORECARD (2005) Water quality indicators http://www.scorecard.org

Svecevičius G (1991) The role of olfaction in avoidance reactions to pollutants by vimba *Vimba vimba* (L.). Ekologija 4:3–8

Svecevičius G (1999) Fish avoidance response to heavy metals and their mixtures. Acta Zoologica Lithuanica 9:103–113

Svecevičius G (2006) Acute toxicity of hexavalent chromium to European freshwater fish. Bull Environ Contam Toxicol 77:741–747

Woodward DF, Hansen JA Bergman HL, Little EE, DeLonay AJ (1995) Brown trout avoidance of metals in water characteristic of the Clark Fork River, Montana. Can J Fish Aquat Sci 52:2031–2037

US EPA (1985) Ambient water quality criteria for chromium-1984. EPA-440/5-84-029. Office of water regulations and standards. Washington

US EPA (2002a) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-821-R-02-012, 5th edn. Office of Water, Washington

US EPA (2002b) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA-821-R-02-013, 4th edn. Office of Water, Washington