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Acute Toxicity of Hexavalent Chromium to European Freshwater Fish

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Chromium is widely used in industry and is a common aquatic pollutant (Moore 1991). In natural waters chromium tends to speciate into two forms: trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) depending on water physico-chemical characteristics (Moore 1991). Under oxygenated conditions, Cr^{6+} is the dominant dissolved stable Cr species in aquatic systems (Eisler 1986). However, Cr^{6+} rarely occurs naturally, but is produced from anthropogenic sources (Irwin et al. 1997). The bioavailability of each chromium form is different mainly because of low solubility of Cr^{3+} (Gendusa et al. 1993).

Hexavalent chromium is referred to selected water quality indicators (SCORECARD 2005) and is recommended for use as a reference toxicant in standard toxicity tests (ASTM 1997; US EPA 2002) because of its ability to provide reproducible test results (Dorn et al. 1986).

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 1980; 1985; Eisler 1986; Irwin et al. 1997). Data on chromium toxicity to fish are scarce and most are from North American fishes often using a single species. Moreover, data on sensitivity of European fish species to chromium are almost totally lacking.

Toxicity testing with a single fish species is inadequate for evaluation pollutant hazard to the environment and does not allow for identification of pollutant selective toxicity. In order to obtain information on pollutant toxicity range, species with generally different susceptibilities or metabolic activities should be used, including those easily available and common in the area where the toxicant may occur. Therefore comparative toxicity studies should be developed to identify species which produce results suitable to the evaluation of ecotoxicity of the pollutant under study (Vittozi and De Angeles 1991). Further, the same fish species in distinct geographical regions may show different sensitivity to the same pollutant. That is why data obtained in different regions can hardly be extrapolated to local conditions.

Several physico-chemical factors of water are known that modifies Cr^{6+} toxicity to fish. Acute lethality of Cr^{6+} increases with decreasing water pH (Van der Putte et al. 1981; Stouthart et al. 1995) and decreases as hardness increases (US EPA 1985) water temperature having limited influence (Smith and Heath 1979).

The purpose of this account is by means of acute lethality tests to estimate the comparative sensitivity to hexavalent chromium of five common European freshwater fish species.

MATERIALS AND METHODS

Toxicity tests were conducted on the following fish species: rainbow trout *Oncorhynchus mykiss* (Walbaum), a species commonly used for aquatic toxicity testing; three-spined stickleback *Gasterosteus aculeatus* L.; roach *Rutilus rutilus* (L.); perch *Perca fluviatilis* L. and dace *Leuciscus leuciscus* (L.).

Species were chosen because they are common in the European region, and represent systematically and ecologically different fish groups.

Rainbow trout were obtained from Žeimena (Švenčionys District, Lithuania) Hatchery. The other species were collected in reference sites of the Neris River, using a drag-net of 6 mm mesh. Length and weight data of fishes used in toxicity tests are presented in Table 1.

Table 1. Length and weight of test fishes*

Fish species	Total length (mm)		Weight (g)	
	Range	Mean ± SEM	Range	Mean±SEM
Rainbow trout	90 - 100	94 ± 1.2	7.5 – 9.9	8.6 ± 0.3
Three-spined stickleback	47 - 50	49 ± 0.6	0.9 – 1.2	1.1 ± 0.03
Roach	83 - 88	86 ± 0.3	5.0 – 5.9	5.6 ± 0.1
Perch	67 - 70	68 ± 0.3	2.7 – 3.8	3.2 ± 0.1
Dace	68 - 70	69 ± 0.3	2.3 – 2.8	2.5 ± 0.06

*Number of fish for each species = 120.

The test fish were acclimated to laboratory conditions for one week prior to testing. The fish were kept in flow-through 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 live feed (*Chironomus* sp.) daily in the morning; the total amount was about 1 % of their wet body mass per day. The day before and during the tests the fish were not fed.

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 to 11.5°C, and oxygen concentration was maintained at the range from 8 to 10 mg/l.

The tests were conducted under flow-through conditions. The system included 6 tanks of a 30 liters volume and the same number of proportional diluters according to the Mount and Brungs (1967) design. Test fishes were exposed to a series of concentrations ranging from 24.0 to 133.0 mg/L of Cr^{6+} with five treatments and one control. The factor of dilution was 0.75, and 400 mL of the solution was added to each tank every 3 minutes. The testing was started in clean water, 10 fish being placed into each tank for acclimation. In order not to stress the fish, the concentration of toxicant in tanks was increased gradually, 50 % test concentration being reached within 3.8 hours and full toxicant concentration within 18.8 hours. Each test had two replicates.

Mortality observations were made at 24-hour intervals. Dead fish were removed, weighed individually (after being lightly blotted dry), and measured (total length) at the time of mortality observation. Fish still living at the conclusion of a test were sacrificed and also weighed and measured at that time. No mortality was observed among control fish.

The amount of oxygen in the tanks as well as temperature and pH were measured every 24 hours with a hand held multi-meter (WTW Multi 340i/SET, Germany). Within increase in potassium dichromate concentration pH value decreased and averaged from 6.7 at 133.0 mg Cr/L to 7.5 at 24.0 mg Cr/L. Other of the analytical methods used are referenced in APHA (1995).

At the end of each test water samples were taken from the tanks and total amount of chromium was measured with a atomic absorption spectrophotometer (SHIMADZU AA-6800, Japan) either with the flame or graphite furnace techniques using proprietary software. Each sample was analyzed for 3 times. Mean measured concentrations were within 5 % of target.

Median-Lethal-Concentration (LC50) values and their 95% confidence intervals were estimated using the trimmed Spearman-Karber method (Hamilton et al. 1977).

RESULTS AND DISCUSSION

The data obtained (Table 2) showed that the calculated 96-hr LC50 values of the five fish species tested vary within the range of 28.5 – 71.7 mg Cr/L. No sharp boundary between lethal and non-lethal Cr^{6+} concentrations has been found. The lowest 96-hr LC50 was found for rainbow trout and the highest one for dace. The 95% confidence intervals of 96-hr LC50 for rainbow trout and for perch were close and overlapped. Rainbow trout was from 1.16 to 2.52 times more sensitive to Cr^{6+} than the other test species based on 96-hr LC50 data.

Table 2. Calculated LC50 as total chromium values for fish species tested in toxicity tests

Exposure duration (hours)	LC50 (mg Cr/L)	95% confidence interval (mg Cr/L)
	Rainbow trout	
24	51.6	45.0 — 59.1
48	49.0	43.7 — 54.8
72	41.0	34.4 — 48.8
96	28.5	24.2 — 33.5
	Perch	
24	71.4	63.2 — 80.7
48	55.7	47.3 — 65.6
72	34.3	26.9 — 43.5
96	33.1	29.0 — 37.8
	Three-spined stickleback	
24	> 100.0	—
48	64.8	59.6 — 70.5
72	43.4	40.2 — 46.9
96	38.3	35.5 — 41.4
	Roach	
24	> 133.0	—
48	88.1	77.8 — 99.7
72	64.1	56.2 — 73.1
96	49.3	45.3 — 53.6
	Dace	
24	> 133.0	—
48	115.3	104 — 127.9
72	87.7	79.3 — 96.9
96	71.7	64.5 — 79.6

According to the sensitivity to Cr^{6+} the species tested may be arranged in the following sensitivity order: rainbow trout > perch > three-spined stickleback > roach > dace.

Exposure duration evidently influenced the value of LC50. The greatest differences were observed between 24-hr and 96-hr values, while 72-hr and 96-hr values were quite close. Their ratio varied within a very small range, from 1.0 to 1.4, while the ratio between 24-hr LC50 and 96-hr LC50 was 1.8 for rainbow trout and 2.2 for perch.

The 96-hr LC50 reported here are similar to previously published data on other fish species. US EPA (1985) reported Species Mean Acute Value (SMAV) of Cr^{6+} to fish ranging from 30.0 mg/L for guppy (*Poecilia reticulata*) to 139.9 mg/L for bluegill (*Lepomis macrochirus*). Rainbow trout in this study were shown to be more sensitive than those in previous studies. Benoit (1976) reported a 96-hr LC50 of 69.0 mg Cr/L. Water chemistry in these studies included hardness of 45.0 mg/L as $CaCO_3$ and a pH range of 7–8. Van der Putte et al. (1981) reported 96-hr LC50 values ranging from 20.2 mg Cr/L at pH = 6.5 to 65.5 mg Cr/L at pH = 7.8 in water with a hardness of 80.0 mg/L as $CaCO_3$.

The mechanism of the toxic effect of Cr^{6+} is only partly understood. Irwin et al. (1997) summarized data on the toxic mode of action of Cr^{6+} and concluded that it acts as a strong oxidizing agent, easily penetrates biological membranes and causes cellular damage. There are at least five ionic species of Cr^{6+}, of which two – the hydrochromate ion ($HCrO_4^{-1}$) and the chromate ion (CrO_4^{-2}) – are the predominant species. They vary with pH and probably are the main toxic agents to fish (Van der Putte et al. 1981). Overall, acute toxic concentrations of Cr^{6+} induced morphological changes in gills, kidney and stomach of rainbow trout as well as in plasma osmolality and hematocrit values of blood (Van der Putte et al. 1981). Similarly, the water pH was found to be crucial factor determining the toxicity of Cr^{6+} for embryonic development of fish (Stouthart et al. 1995). Recent investigations revealed hepatotoxicity and carcinogenicity in fish induced by Cr^{6+} (Nawaz et al. 2005).

Hexavalent chromium was selected as a reference toxicant because it meets the following criteria: it has universal toxicity; it is persistent and non-degradable; it is highly water soluble, and is readily quantified (Dorn et al. 1986). Hexavalent chromium exists in solution as a component of an anion, rather than a cation, and therefore, does not precipitate from alkaline solution (US EPA 1980). Apparently, this explains why static tests with unmeasured concentrations and flow-through tests with measured concentrations gave similar results in juvenile fathead minnow (*Pimephales promelas*). The LC50 values averaged 36.2 mg Cr/L and 36.9 mg Cr/L, respectively at pH = 7.7 in water with a hardness of 209 mg/L as $CaCO_3$. (Pickering 1980). Furthermore, the 96-hr LC50 value of 38.3 mg Cr/L for three-spined stickleback (*Gasterosteus aculeatus*) reported here duplicates static test data to this species of 35.0 mg Cr/L in water with a hardness ranging from 500 to 600 mg/L as $CaCO_3$ and a pH range of 8.0–8.1 (Jop et al. 1987).

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).

Recently a number of International Standards (ISO) for water quality determination using acute toxicity testing on zebrafish (*Brachydanio rerio*) proposed by the European Union were accepted for Lithuania (ISO 7346-1:1996(E); ISO 7346-2:1996(E); ISO 7346-3: 1996(E)). These documents support the use of potassium dichromate as a reference substance, whereas the recommendation to use the zebrafish does not preclude the use of other species with appropriate experimental condition modifications.

From this point perch are useful because they demonstrated almost the same sensitivity to Cr^{6+} as rainbow trout, a species commonly used for aquatic toxicity testing. Perch are native to Europe, a eurytopic species, widely distributed in rivers, lakes, and in brackish waters. They are easily available and maintained in the laboratory. Zebrafish is a warm-water species, not are native to Europe, and less sensitive than other species tested. For example, Bellavere and Gorbi (1981) reported the 96-hr LC50 value for adult zebrafish of 58.5 mg Cr/L at pH = 8.0 in water with a hardness of 350 mg/L as $CaCO_3$, i.e. almost 2-fold higher than for perch reported here. The perch (*Perca fluviatilis*) is a promising species. Further research into sensitivity of this species to pollutants is needed.

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