Potassium Dichromate as a Reference Substance for Embryonic Tests of Toxicity in the Common Carp (*Cyprinus carpio* L.)

R. KREJČÍ[1], M. PALÍKOVÁ[2]

[1]Department of Zoology, Fisheries, Hydrobiology and Apiculture, Mendel's Agricultural and Forestry University in Brno, Czech Republic
[2]Department of Veterinary Ecology and Environmental Protection, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Received September 29, 2005
Accepted March 16, 2006

Abstract

Krejčí R., M. Palíková: Potassium Dichromate as a Reference Substance for Embryonic Tests of Toxicity in the Common Carp (*Cyprinus carpio* L.). Acta Vet. Brno, 75: 259-263.

Potassium dichromate ($K_2Cr_2O_7$) has already been used as a reference substance in tests of toxicity with aquatic animals. The aim of this study was to determine and compare values of LC_{50} for potassium dichromate during the whole period of embryonic development (i.e., 120 h) and 48 h after hatching of embryos in the common carp (*Cyprinus carpio* L.). Fish eggs and embryos were exposed to 5 different concentrations of potassium dichromate (i.e., 372, 409, 450, 495, 545 $mg \cdot l^{-1}$) during two experiments. Such characteristics as the cumulative mortality, the start and the end of hatching, the number of deformities, body length, and body mass of surviving individuals were studied during the tests. The highest mortality was found in the hatched embryos. Mortality and frequency of deformities increased with the growing concentration of potassium dichromate. The value of 120 LC_{50} for potassium dichromate was 464.91 ± 23.83 $mg \cdot l^{-1}$ and the value of 48 LC_{50} was 458.94 ± 4.14 $mg \cdot l^{-1}$ (mean ± SD). No statistically significant difference between values 120 LC_{50} a 48 LC_{50} was found. This is why reduction of the exposure period to only 48 h after hatching seems a reasonable method to study the control of susceptibility using potassium dichromate in embryonic tests of toxicity.

Lethal concentration, embryonic toxicity, mortality, deformations, hatching

The toxicity of chemical substances for aquatic organisms may be influenced by a variety of factors such as the physical and chemical characteristics of water (e.g., temperature, pH, oxygen concentration, concentrations of salts dissolved in water, etc.) and the state of health of the animals tested. Although it is possible to create standard laboratory conditions, maintaining indicators of the state of health as well as susceptibility may be more problematic. That is why toxicity tests employing reference substances are used in ecotoxicology. Results of these tests enable comparisons with those obtained by testing various substances on fish of different origin. Potassium dichromate ($K_2Cr_2O_7$) is the most commonly used and recommended reference substance for aquatic organisms (ČSN EN ISO 6341, 1996 and ČSN EN ISO 7346, 1996).

The effect of chromium on the fish organism has already been studied in detail. Studies were particularly focused on the hexavalent chromium that is more toxic to aquatic organisms than chromium with the valency of III (Van der Putte et al. 1981ab). Effects of chromium on fish are reported to include damage of gills, impairment of osmoregulation, blood count changes, etc. (Al-Akel et al. 1996; Nath et al. 1988; Van der Putte et al. 1982). Chromium Cr^{VI} is more toxic at a lower pH, like other metals (Stouthart et al.1996). Significantly higher levels of chromium were found in the epithelium of gills in the rainbow trout kept at the pH of 6.5 as compared to the pH of 7.8 (Van der Putte et al. 1981ab). In eggs and larvae of the common carp, Stouthart et al. (1995) also found the mortality as well as the accumulation of chromium to be the significantly highest at the pH of 6.3. These

Address for correspondence:
Ing. Roman Krejčí, Ph.D.
Department of Zoology, Fisheries, Hydrobiology and Apiculture
Mendel's Agricultural and Forestry University in Brno
Zemědělská 1, 613 00 Brno, Czech Republic

Phone: +420 545 133 267
E-mail: krejca@mendelu.cz
http://www.vfu.cz/acta-vet/actavet.htm

authors also found that the chorion and the perivitelline membrane of eggs acts like an effective barrier against Cr^{VI} and that after hatching the accumulation of Cr^{VI} in embryos and larvae starts to grow. In spite of that, there are no published data on the medium lethal dose for fish eggs and sac-fry of the common carp.

This paper is aimed at finding and comparing the LC_{50} values for potassium dichromate and the whole embryonic development (120 h) as well as the period of 48 h after hatching.

Materials and Methods

Two experiments were performed in order to evaluate the influence of different concentrations of potassium dichromate during the embryonic development in the common carp. Experiments were carried out to determine the influence of potassium dichromate during the whole embryonic development, i.e., from fertilization to the end of the embryonic period, and during 48 h after hatching, i.e., from hatching to the end of the embryonic development. Experiments were finished when control individuals had the gas bladders filled and were able to take feed. Fish eggs and embryos were subjected to the same concentrations of potassium dichromate. Values of LC_{50} (120 LC_{50} and 48 LC_{50}) were computed for individual periods of development. Both experiments were performed under the same laboratory conditions and in accordance with the OECD 212 (1998) guideline.

Eggs of the common carp were obtained using artificial spawning and unstuck for one hour by whole milk diluted with water in the ratio of 1:9. Production lines of the common carp were used for stripping (spawner female PL, spawner male M72). Fish eggs were used for the experiment 8 h after fertilization. Embryos were used just after hatching. Until hatching, fish eggs were incubated in Zugs vials in a re-circulating unit.

Fish eggs and embryos were stocked 15 individuals per 15 ml of water for dilution prepared according to the Czech State Norm ČSN EN ISO 7346 (1996). Water exchange was made using a semi-static method every 12 hours. Fish eggs and embryos were transferred using a fine sieve. Temperature of the medium during the experiment was kept at 23.5 ± 0.3 °C, pH values varied in the range of 6.0 ± 0.1, and the O_2 concentration ranged from 7.5 to 8.0 $mg \cdot l^{-1}$ (90 - 95% saturation). Photoperiod consisted of 14 h light and 10 h dark. The experiments included controls with water for dilution and 5 different concentrations of potassium dichromate (i.e., 372, 409, 450, 495, 545 $mg \cdot l^{-1}$) in three repeated measurements determined by calculation on the basis of LC_{50} found in preliminary tests.

Characteristics such as cumulative mortality, the onset and the end of hatching, the number of deformities, total body length (TL) and body mass (w) of surviving individuals were determined during the experiments. Body length and mass values of the surviving individuals were determined after 3 months of the specimens being fixed in 4% formalin.

A computer probit analysis program was used to determine concentrations of potassium dichromate in the experiments as well as the LC_{50} values. Values of LC_{50} after 120 and 48h were compared using *t*-test in STATplus (Matoušková et al. 1992). As the tests were performed in accordance with the OECD 212 guidelines and under identical conditions, we obtained statistical evaluation of six individual tests of toxicity.

Results

Cumulative mortality

Mortality during 120 h of exposure (Table 1)

Table 1. Cumulative mortality (mean ± SD)

Concentration of $K_2Cr_2O_7$ ($mg \cdot l^{-1}$)	120 LC_{50}			48 LC_{50}
	Mortality within 12 h (%)	Mortality until the start of hatching (%)	Mortality after 120 h (%)	Mortality after 48 h (%)
0	2.22 ± 3.14	3.34 ± 3.34	3.34 ± 3.34	0.00 ± 0.00
545	3.34 ± 3.34	3.34 ± 3.34	93.33 ± 9.43	100.00 ± 0.00
495	4.45 ± 4.97	4.45 ± 4.97	71.11 ± 19.88	84.44 ± 9.94
450	1.11 ± 2.49	1.11 ± 2.49	35.56 ± 12.57	33.33 ± 16.33
409	3.33 ± 5.09	3.33 ± 5.09	21.11 ± 4.58	5.56 ± 4.58
372	3.34 ± 3.34	3.34 ± 3.34	12.22 ± 4.59	3.34 ± 3.34

Mortality during the first 12 h of exposure to potassium dichromate varied from 1.1 to 4.4%. There was no mortality during the period from 12 h until hatching (except for the control). The highest mortality was found in the period after hatching. It was growing with

the increasing concentration of potassium dichromate. No increase in mortality was observed in control individuals after hatching.

Mortality during 48 h of exposure (Table 1)

There was no mortality in the control groups. Growing concentrations of potassium dichromate resulted in growing mortality of individuals.

Hatching

Table 2 presents the start and end of hatching in both experiments. In groups exposed to potassium dichromate hatching started from 57.3 to 61.5 h, and was finished from 69.2 to 74.2 h. Hatching started at 62.8 h, and ended at 73 h in the control group.

Deformities

The number of deformities varied from 6.7 to 6.8 % in control animals (Table 2). A control animal without deformities is presented in Fig.1 (Plate IX). Deformities of the vertebral

Table 2. Hatching and deformities (mean ± SD)

Concentration of $K_2Cr_2O_7$ ($mg \cdot l^{-1}$)	120 LC_{50}			48 LC_{50}
	Start of hatching (h)	End of hatching (h)	Deformities after 120 h (%)	Deformities after 48 h (%)
0	62.83 ± 1.95	73.00 ± 2.31	6.83 ± 5.65	6.67 ± 5.44
545	57.33 ± 2.67	69.67 ± 4.31	91.67 ± 11.79	-
495	57.33 ± 2.67	70.50 ± 5.65	88.29 ± 12.23	90.28 ± 13.96
450	57.67 ± 3.59	69.17 ± 3.24	63.60 ± 22.94	52.89 ± 23.37
409	59.83 ± 2.79	71.83 ± 4.14	45.67 ± 13.93	33.16 ± 18.14
372	61.50 ± 3.04	74.17 ± 3.02	33.87 ± 15.78	18.48 ± 9.48

column and yolk sac were most frequent in control groups. The number of deformities was growing with the increasing concentration of potassium dichromate. Deformities of embryos exposed for 120 h to potassium dichromate included bowing of the vertebral column (Fig. 2), deformities of the head, missing fin margins (Fig. 2), strangulation and enlargement of the yolk sac (Fig. 2 and 3). Swelling of the heart area was very frequent at higher concentrations of potassium dichromate (Fig. 3, Plate X, Fig. 4, 5). Individuals exposed to potassium dichromate for 48 h showed bowing of the vertebral column less frequently. This duration of exposure resulted most frequently in deformities of the head, yolk sac and swelling of the heart area.

Body length and mass

Table 3 presents such characteristics as the total body length (TL) and body mass (w) of individuals surviving until the end of the experiment. The total body length and body mass in individuals exposed to different concentrations of potassium dichromate were even lower than in the control animals.

Table 3. Total body length (TL) and mass (w) (mean ± SD)

Concentration of $K_2Cr_2O_7$ ($mg \cdot l^{-1}$)	120 LC_{50}		48 LC_{50}	
	TL (mm)	w (mg)	TL (mm)	w (mg)
0	6.16 ± 0.29	1.63 ± 0.21	6.38 ± 0.22	1.60 ± 0.23
545	5.31 ± 0.11	1.12 ± 0.25	-	-
495	5.51 ± 0.34	1.36 ± 0.22	6.03 ± 0.32	1.35 ± 0.33
450	5.59 ± 0.24	1.27 ± 0.06	6.04 ± 0.32	1.52 ± 0.27
409	5.79 ± 0.14	1.33 ± 0.09	6.07 ± 0.21	1.47 ± 0.11
372	5.80 ± 0.15	1.31 ± 0.10	6.12 ± 0.28	1.54 ± 0.16

LC_{50} estimate for potassium dichromate

The value of 120 LC_{50} for potassium dichromate was 464.91 ± 23.83 mg·l^{-1} (mean ± SD) and the value of 48 LC_{50} was 458.94 ± 4.14 mg·l^{-1}. No significant difference was found between these values.

Discussion

Mortality during the first 12 h of the experiment was probably caused by unfertilized fish eggs or damage through handling during transport.

It is mostly reported that the LC_{50} value for the hexavalent chromium amounts to 96 h in fish. According to Benout (1976), the 96 LC_{50} values for the rainbow trout and the brook trout equal to 69 and 59 mg Cr·l^{-1}, respectively. Al-Akel (1996) mentions the 96 LC_{50} of hexavalent chromium in the common carp to be 93.6 mg·l^{-1}. These authors, however, were not experimenting with embryos, but older age categories. Buhl (1997) reports the 96 LC_{50} value of hexavalent chromium of three species of fish (i.e., colorado squawfish – *Ptychocheilus lucius*, bonytail – *Gila elegans* and razorback sucker – *Xyrauchen texanus*) in the range of 32 – 123 mg·l^{-1}. This author reports the same or higher susceptibility of larvae than juveniles of these species of fish. All these values are many times lower than those found in our study, but they concern a longer time period, i.e., 96 h as compared to 48 h of our experiments. It should hold good that the longer the test period, the lower the lethal concentration of the substance. Such results are reported, e.g., by Murti et al. (1983) for chromium and the freshwater prawn – *Macrobrachium lamarreia*. The LC_{50} values are 24, 48, 72, and 96 h and show a decreasing tendency of 5.44, 3.69, 2.47, and 1.84 mg·l^{-1}, respectively. Comparing the 96 LC_{50} and 48 LC_{50} values, we see that the first one is twice as high as the second. Výkusová and Svobodová (1990) report the range of the 48 LC_{50} values for chromium in the common carp to be 100 – 350 mg·l^{-1}. Again, these authors were not engaged in embryonic tests, but tests using small fish.

From our results it is clear that the highest mortality in all concentrations of potassium dichromate can be found after hatching, i.e. in the life period when embryos are away from the egg envelopes. This statement has also been confirmed by Stouthart (1995) finding the highest mortality in hatched embryos. The content of Cr^{VI} in fish eggs drops nearly to zero levels during hatching and starts to grow in the embryos again after hatching. The higher mortality of the period after hatching can be explained by a barrier formed by the perivitelline membrane of fish eggs, preventing the accumulation of Cr^{VI} in embryos. This fact is reflected in values of LC_{50} determined after 120 and 48 h, which are very similar. Considering the fact that chromium accumulates in the embryos only after hatching, the duration of action of potassium dichromate lasts *de facto* 48 h from the total of 120 h of exposure. The difference between 120 LC_{50} and 48 LC_{50} values amounted to 4.43 % (A) and 6.42 % (B). The LC_{50} values differing by up to 10 %, however, are considered to be rather the same.

The 120 LC_{50} value may be, due to the susceptibility of embryos to potassium dichromate only after hatching, influenced by this process. The time of hatching may influence the mortality of individuals due to differences in the action of potassium dichromate on hatched embryos. An unusually early or late start of hatching, on the other hand, may also influence mortality and thus the LC_{50} value.

No statistically significant deference between values 120 LC_{50} and 48 LC_{50} was found. That is why the reduction of the exposure period to only 48 h after hatching seems a reasonable method to study the control of susceptibility using potassium dichromate in embryonic tests of toxicity. A similar reduction of the exposure to a reference substance (from 96 to 24 h) has already been accepted in the Czech State Norm ČSN EN ISO 7346.

Využití dichromanu draselného jako referenční látky při provádění embryonálních testů toxicity s kaprem obecným (*Cyprinus carpio* L.)

Dichroman draselný $K_2Cr_2O_7$ se používá jako referenční látka v testech toxicity s vodními živočichy. Cílem této studie bylo zjistit a porovnat LC_{50} pro $K_2Cr_2O_7$ za období celého embryonálního vývoje (120 h) a za období 48 h po vykulení embryí u kapra obecného (*Cyprinus carpio* L.).

Jikry a embrya byly ve dvou pokusech exponovány v 5 koncentracích $K_2Cr_2O_7$ (372, 409, 450, 495, 545 mg•l^{-1}). Během testů byla sledována kumulativní mortalita, začátek a konec kulení, počet deformací, celková délka těla a hmotnost přežívajících jedinců. Nejvyšší mortality bylo dosaženo až u vykulených embryí. Mortalita a počet deformací se zvyšovaly se stoupající koncentrací $K_2Cr_2O_7$. Hodnota 120 LC_{50} pro dichroman draselný byla 464.91 ± 23.83 mg•l^{-1} (průměr ± SD) a hodnota 48 LC50 byla 458.94 ± 4.14 mg•l^{-1}. Statistickým hodnocením nebyl shledán žádný statisticky významný rozdíl mezi hodnotami 120 LC_{50} a 48 LC_{50}. Proto se jako vhodná metoda pro kontrolu citlivosti s využitím dichromanu draselného při provádění embryonálních testů toxicity jeví zkrácení expoziční doby na období 48 h po vykulení.

Acknowledgement

This study was supported by the Research Project of the Ministry of Education, Youth and Sports of the Czech Republic MSM 62 15712402 "Veterinary aspects of food safety and quality".

References

AL-AKEL AS, SHAMSI MJK 1996: Hexavalent chromium: Toxicity and impact on carbohydrate metabolism and haematological parameters of carp (*Cyprinus carpio* L.) from Saudi Arabia. Aquat Sci **58**: 24-30

BUHL KJ 1997: Relative Sensitivity of Three Endangered Fishes, Colorado Squawfish, Bonylail, and Razborback Sucker, to Selected Metal Pollutans. Ecotox and Env Safety **37**: 186-192

BENOIT DA 1976: Toxic effects of hexavalent chromium on brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). Water Res **10**: 497-500

ČSN EN ISO 6341 1996: Determination of the inhibition of the mobility of *Daphnia magna* Straus (*Cladocera, Crustacea* – Acute toxicity test. (in Czech) ČNI Praha, 16 p.

ČSN EN ISO 7346-2: 1996. Determination of the lethal toxicity of substances to a freshwater fish (*Brachidanio rerio* Hamilton- Buchanan (*Teleostei, Cyprinidae*) – Part 2: Semi-static method. (In Czech) ČNI Praha, 16 pp

MURTI RO, SHUKLA GS 1983: Chromium toxicity to a freshwater prawn *Macrobrachium Lamarrei* (h.m.edwards). Tox Letters **18**: 257-261

MATOUŠKOVÁ O, CHALUPA J, CÍGLER M, HRUŠKA K 1992: Statistic system STAT PLUS, version 1,01. Book of reference. Veterinary Research Institute, Brno, 168 p.

NATH K, KUMAR N 1988: Hexavalent chromium: Toxicity and its impact on certain aspects of carbohydrate metabolism of the freshwater teleost, *Colisa fasciatus*. Sci of the Total Environ **72**: 175-181

OECD guideline for the testing of chemicals 212: Fish, short-term toxicity test on embryo and sac-fry stages, 1998, 20 p.

STOUTHART AJHX, SPANINGS FAT, LOCK RAC, BONGA SEW 1995: Effect of water pH on chromium toxicity to early life stages of the common carp (*Cyprinus carpio*). Aquat Tox **32**: 31-42

STOUTHART AJHX, HAANS JLM, LOCK RAC, BONGA SEW 1996: Effect of water pH on copper toxicity to early life stages of the common carp (*Cyprinus carpio*). Envir Tox. and Chem **15**: 376-383

VAN DER PUTTE I, BRINKHORST MA, KOEMAN JH 1981a: Effect of pH on the acute toxicity of hexavalent chromium to rainbow trout (*Salmo gairdneri*). Aquat Toxicol **1**: 129-142

VAN DER PUTTE I, LUBBERS J 1981b: Effect of pH on uptake, tissue distribution and retention of hexavalent chromium in rainbow trout (*Salmo gairdneri*). Aquat Toxicol **1**: 3-18

VAN DER PUTTE I, LAURIER MBHM, VAN EIJK GJM 1982: Respiration and osmoregulation in rainbow trout (*Salmo gairdneri*) exposed to hexavalent chromium at different pH values. Aquat Toxicol **2**: 99-112

VYKUSOVÁ B, SVOBODOVÁ Z 1990: Acute Toxicity of Standards to Fish. Buletin VÚRH Vodňany (In Czech) **26**: 15-20

Fig. 1. Control embryo 120 h old. Magnification × 20; fixed in 4% formaldehyde.

Fig. 2. Embryo (120 h old) exposed to the action of $K_2Cr_2O_7$ at the concentration of 495 mg·l^{-1}. Deformities of the vertebral column (bowing), long yolk sac, absence of fin margins. Magnification × 20; fixed in 4% formaldehyde

Fig. 3. Embryo (120 h old) exposed to the action of $K_2Cr_2O_7$ at the concentration of 409 mg·l^{-1}. Deformities of yolk sac, head, vertebral column, moderate heart area oedema. Magnification × 20; fixed in 4% formaldehyde

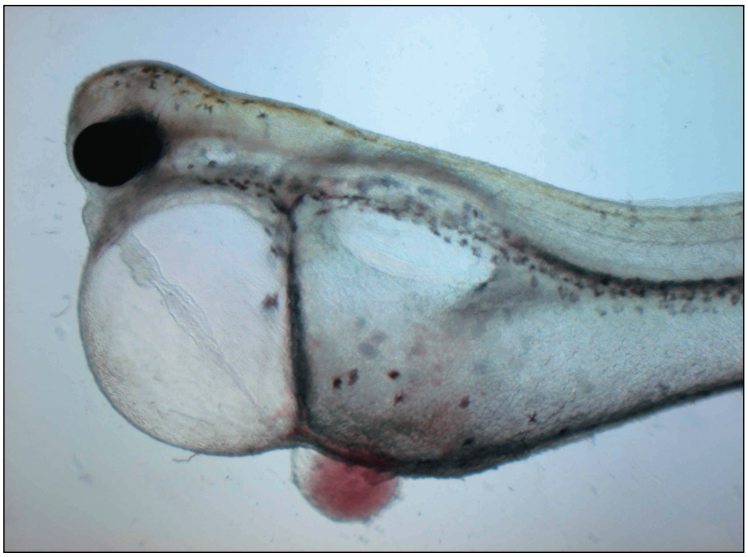

Fig. 4. Embryo (120 h old) exposed to the action of $K_2Cr_2O_7$ at the concentration of 495 mg·l^{-1}. Deformities of yolk sac and head, heart area oedema. Magnification × 20

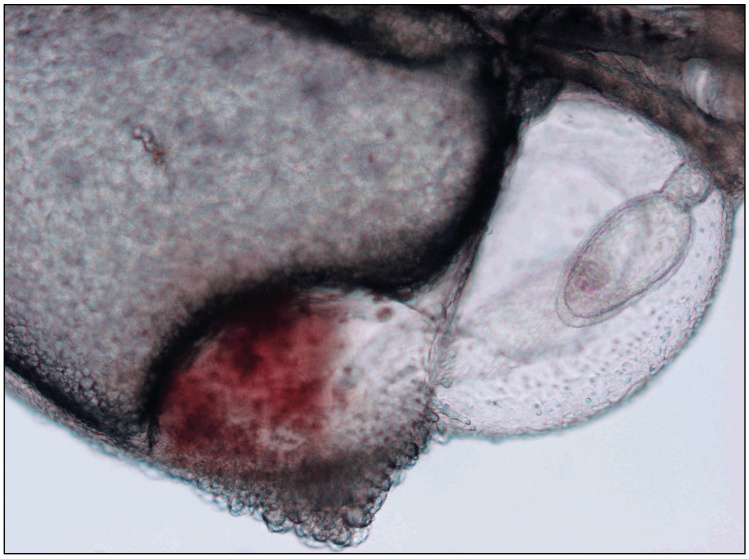

Fig. 5. Embryo (120 h old) exposed to the action of $K_2Cr_2O_7$ at the concentration of 545 mg·l^{-1}. Heart area in detail. Magnification × 40