Bull. Environ. Contam. Toxicol. (2004) 72:725–732

Bulletin of Environmental Contamination and Toxicology

Effect of Cadmium on Hematological Indices of Common Carp (*Cyprinus carpio* L.)

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Received: 6 May 2003/Accepted: 6 January 2004

Trace amounts of metals occur naturally in water, however, waste waters from mining and processing of ores, from metallurgical plants, rolling mills, metal surface finishing, from photographic-, textile-, leather- and chemical industries, from agriculture, etc. are the main sources of pollution. Additionally, rain contaminated by burning fossil fuels is another source of pollution (Svobodová et al. 1987, Cibulka et al. 1991, Pitter 1999).

Lead, cadmium and mercury are just some of the toxic metals occurring in waters. Environmental contamination with cadmium is mostly due to its increased use in industry. For example, most contamination of cadmium comes from the metal foundries, dye industry, production of plastics and of accumulators. Then, additional contamination comes from the combustion of fuels and oils, as well as use of natural phosphates and cadmium-containing pesticides in agriculture (Bencko et al. 1995).

Cadmium is not an essential element for organisms, but it is highly toxic for organisms that live in the aquatic environment. Harmful effects on zooplankton and on fish have been reported at concentrations of only a few μg/L to several tens of μg/L (Pitter 1999). Whereas, a source of acute cadmium contamination can occur from an accidental release of industrial wastewater, most significant cases of long-term contamination are recorded when cadmium is deposited in bottom sediments (Svobodová et al. 1996, Leontovičová 2003) and subsequently transferred into food chain in the aquatic environment (Šubrtová and Pavelka 1988).

There is variability in the mechanism of toxic effect of cadmium on fish. The majority of metals has an affinity to link with amino-acids and SH-groups of proteins and thus acts as enzymatic poisons. The toxic effects of cadmium are mainly similar to effects of other metals (mainly impairment of the central nervous system and of the parenchymatous organs). However, there are some specific effects of long-term exposure even of trace amounts of cadmium. The presence of trace amounts of cadmium concerns can invoke a negative effect on

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reproductive organs. For example, in rainbow trout (*Oncorhynchus mykiss*), effects on maturation, hatchability and larval development were manifested during a long-term exposure to a concentration of 0.002 mg/L (Svobodová et al. 1987, Cibulka et al. 1991). There is suspicion that cadmium is also a strongly suspected environmental endocrine disruptor and is listed by US Environmental Protection Agency, Centers of Diseases Control and Prevention, and World Wildlife Fund as a potential endocrine modifying chemical (Keith 1997).

Cadmium has an important accumulation capacity. It persists in the body for a very long time, and in contrast to mercury, it does not form biochemical volatile alkyl derivates. Detoxication is therefore slow and there is a danger of long-termed effects (Pitter 1999).

Data on toxicity of heavy metals and their effects on aquatic organisms are the basic ones for determination of ecotoxicological risks of heavy metals for the aquatic ecosystem. The present paper contributes to the assessment of toxicity and effects of cadmium. The aim was to assess the effect of cadmium on the oxidative blood capacity and the non-specific immune response by means of examination of erythrocyte and leukocyte profiles of carp.

MATERIALS AND METHODS

The toxic effect was assessed on the basis of results of acute toxicity tests and the results of hematological examination of common carp after exposure to this substance. Cadmium was tested in the form of cadmium chloride (ACS Reagent, f. Merck).

The acute toxicity test on common carp with cadmium chloride followed the OECD Direction No. 203 and Methodical Manual ISO 7346/2. Juveniles of common carp (*Cyprinus carpio* L.) with 2.63±0.59 g mean body weight and 47.73±3.45 mm mean standard length were used for the test. Seven concentrations (0.05, 0.5, 1, 5, 10, 20, 40 mg/L of cadmium chloride) and a control were used in the basic test. Ten fish were used for every concentration and also in the control. The test was performed semi statistically for 96 hr. The bath were changed every 24 hr. Basic physical and chemical indices of diluting water used in the acute toxicity test were as follows: pH 7.36, $ANC_{4.5}$ (alkalinity) 1.2 mmol/L, COD_{Mn} 1.6 mg/L, BOD_5 0.79 mg/L, NH_4^+ 0 mg/L, NO_3^- 11.51 mg/L, NO_2^- 0 mg/L, sum of Ca + Mg 14 mg/L. Water temperature in the test ranged from 19.2 to 21.6 °C, oxygen saturation of water ranged between 93 and 99%. The LC50 values in the respective time intervals were determined by probit analysis.

Hematological examination of one- to two-year-old common carp was performed at the end of a 96 hr acute toxicity test with cadmium chloride in a concentration of 12.5 mg/L (7.67 mg/L of cadmium). At the same time, the control group of common carp was also examined hematologically. The test was performed semi statistically with bath exchange every 24 hr. Basic physical and chemical indices of diluting water used in the acute toxicity test were as follows: pH 7.38, $ANC_{4.5}$

(alkalinity) 1.2 mmol/L, COD_{Mn} 4.5 mg/L, BOD_5 1.07 mg/L, NH_4^+ 0 mg/L, NO_3^- 6.77 mg/L, NO_2^- 0 mg/L, sum of Ca + Mg 14 mg/L. Water temperature during the test ranged from 18.9 to 20.5 °C, oxygen saturation of water was above 60% (ranging from 61.0 to 78.8%). The test was performed in 2 control aquaria and 3 aquaria with concentration of 12.5 mg/L cadmium chloride.

Examination of erythrocyte and leukocyte profiles was carried out on 20 controls (body weight 431.9±112.37 g) and on 20 experimental specimens (body weight 515.7±116.11 g) after 96 hr exposure to concentration of 12.5 mg/L cadmium chloride.

Blood was sampled by cardiac puncture immediately after catching and stabilized with 50 IU sodium heparin per 1 mL blood. Erythrocytes (RBC) and leukocytes (Leuko) were counted in Bűrker´s chamber using Haym´s solution. Hematocrit (PCV) was measured by using microhematocrit capillaries, hemoglobin (Hb) by cyanhemiglobine method. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was derived from basic hematological indices. Differential leukocyte count was determined by Pappenheim method. Methodical details of methods using in this study are taken from Svobodová et al. (1991).

QA/QC measures were consistently applied within all experiments. All the measurements described above were carried out according to validated standard operation procedures.

All statistics were counted by descriptive statistic basic and by one-factor analyses of variance (STATISTICA program 6.0 version). The signification of tests was calculated at the level $p<0.01$ and $p<0.05$. This program performed also categorized box and whiskers plots including box whiskers type mean, standard error of the mean (SE) and 1.96*SE.

Ethical Committee of the University of South Bohemia České Budějovice, Research Institute of Fish Culture and Hydrobiology Vodňany (approval No. 7/2002) approved the experiments on fish.

RESULTS AND DISCUSSION

The 96 h LC50 is the basic value in the acute toxicity test. For common carp juveniles the 96 h LC50 was 5–10 mg/L of cadmium chloride which was 3.07–6.14 mg/L of cadmium. During the test, we also determined values of 24hLC50 = 9.81 mg/L (6.02 mg/L of cadmium), 48hLC50 = 9.06 mg/L (5.56 mg/L of cadmium) and 72hLC50 = 5–10 mg/L (3.07–6.14 mg/L cadmium). Fish expressed accelerated respiration and they had reduced reaction to excitation even to loss of the escape reflex. During the later phase, fish expressed restlessness, jerky swimming movements, turning onto their sides and rotating on their longitudinal axis in a 360 °, circular motion. This phase was alternated with a quiescent phase. Fish died on their sides, apparently after periods of agony. Pathological and

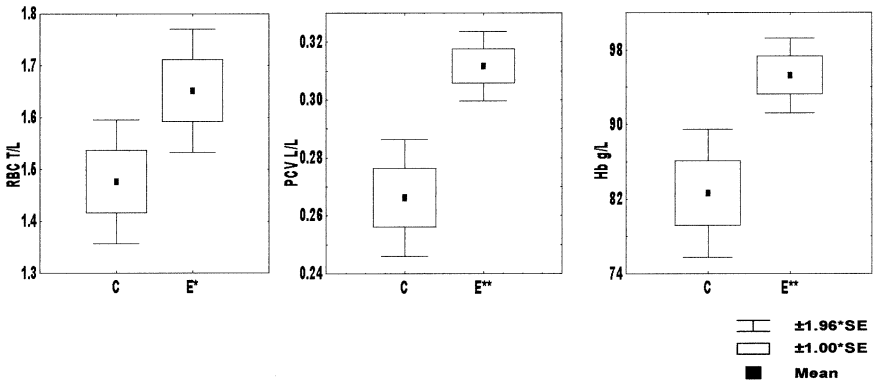

Figure 1. Hematological indices significantly differentiated in control and experimental groups of common carp affected by 96 hr exposure to cadmium chloride in concentration 12.5 mg/L (C – control group; E – experimental group; significance: **p<0.01, *p<0.05)

anatomical examination showed increased amount of mucus on the epidermis and on gills. Gills appeared anemic and were pale in colour, turgid and with showed frayed edges. The abdominal cavity was slightly moistened.

In the course of 96 hr toxicity test of cadmium chloride on common carp juveniles, there no mortalities in the controls. Oxygen saturation of water did not drop below 60% in any concentration tested, nor in the control group. Presence of the substance tested (above 80% of the nominal concentration) was provided by means of daily exchange of the testing bath. Fulfilling these conditions, the test may be considered valid. Based upon the registered 96 h LC50 value (5–10 mg/L), cadmium chloride can be classified into the group of substances toxic for fish (risk sentence R51). This sentence reports the 96 h LC50 values as 1–10 mg/L. The 96 h LC50 value 5–10 mg/L cadmium chloride refers to 3.07–6.14 mg/L cadmium. Svobodová et al. (1987) stated that the lethal concentration of cadmium for various fish species was within the range of 2–20 mg/L. Pitter (1999) reported acute lethal concentrations of cadmium (LC50) to range from 0.001 to 0.09 mg/L for salmonids and from 0.24 to 3.20 mg/L for cyprinids, depending on the physical and chemical properties of water. Sovenyi et al. (1993) reported 96 h LC50 value as 21.7 mg/L for common carp after acute exposure to cadmium at concentrations of 5 to 35 mg/L.

Results of the erythrocyte profile of both the control and experimental common carp under study are given in Figure 1 and in Table 1. Compared to the control specimens, fish after the acute exposure to cadmium had higher erythrocyte count ($p<0.05$), hemoglobin content ($p<0.01$) and hematocrit value ($p<0.01$). Values recorded for MCV, MCH and MCHC were comparable in both groups under study.

Table 1. Derived hematological parameters in common carp affected by 96 hr exposure to cadmium chloride in concentration 12.5 mg/L.

Indices	Units	Groups	N	Mean	SD	SE	Probability
MCV	fl	control	20	185,74	43,77	9,79	0,547
		experiment	20	193,50	36,58	8,18	
MCH	pg	control	20	57,23	12,00	2,68	0,611
		experiment	20	59,11	11,24	2,51	
MCHC	L/L	control	20	0,31	0,04	0,01	0,635
		experiment	20	0,31	0,01	0,00	

Regarding the erythrocyte profile of common carp, the main response to the acute exposure to a cadmium concentration of 12.5 mg/L was a significant increase in erythrocyte count ($p<0.05$), haemoglobin content ($p<0.01$) and haematocrit value ($p<0.01$) compared to the control group. Values of MCV, MCH and MCHC were comparable in both the control and experimental groups. Changes indicating to adaptation in the red blood cell system, including the increase in RBC, Hb and PCV were probably a response to impairment of gas exchange in cadmium-affected gills. Jezierska and Witeska (2001) reported that mucus secreted in large amounts by metal-affected gills reduced membrane transport of oxygen in the respiratory epithelium and that metals accumulated in this epithelium disturbed the oxygen diffusion. Ghazaly (1992) observed a significant ($p<0.05$) increase of RBC, Hb and MCHC and a significant decrease of PCV, MCV and MCH in *Tilapia zillii* after 24 hr exposure to cadmium at concentrations of 17.70 and 24.78 mg/L. Gill and Pant (1985) observed increase of MCH and MCV and morphological aberrations in mature erythrocytes in a cyprinid fish *Puntius conchonius* after long-termed exposure to 0.63 and 0.84 mg/L cadmium chloride. Koyama and Ozaki (1984) observed a significant decrease of Hb and PCV in carp after exposure to 0, 5, 10 and 50 μg/L cadmium chloride for 30, 50, 70 and 90 days.

Results of the leukocyte profile of both groups under study are given in Figures 2, 3 and Table 2. It was evident that the acute exposure to cadmium resulted in significantly lower leukocyte count ($p<0.01$), as well as both the relative and absolute lymphocyte count ($p<0.01$). On the contrary, there was a significant increase in the relative count of monocytes ($p<0.01$), metamyelocytes ($p<0.05$), band neutrophils ($p<0.05$), segmented neutrophils ($p<0.05$) and total neutrophils ($p<0.01$). Absolute count of monocytes, relative and absolute count of myelocytes, absolute counts of metamyelocytes, band- and segmented neutrophil granulocytes and total neutrophil granulocytes were comparable in both groups during the study.

Changes in leukocyte number are sensitive indicators of stress in fish. Although the response to environmental impacts varies with the type and severity of the stress, it often leads to a leukopenia associated with lymphopenia and sometimes

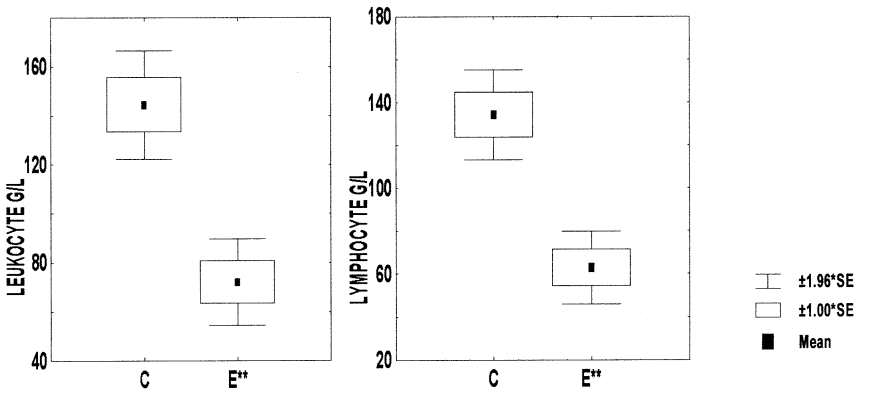

Figure 2. Leukocyte and lymphocyte counts significantly differentiated in control and experimental groups of common carp affected by acute exposure to cadmium chloride (C – control group; E – experimental group; significance: ** $p<0.01$, * $p<0.05$).

neutrophilia, which is similar to the classic leukocytic response to stress in mammals (Feldman et al. 2000). We observed a significant decrease ($p<0.01$) of leukocyte count and significant relative and absolute lymphopenia in common carp after the acute exposure to cadmium. These changes provided an evidence for a decrease of non-specific immunity of common carp. Murad and Houston (1988) observed a significant decrease of leukocytes and lymphocytes and increase of granulocytes in goldfish exposed for 3 wk to 90, 270 and 445 μg Cd^{2+}/L. Ghalazy (1992) observed a decrease of leukocyte count and lymphocytes in *Tilapia zillii* after a 48 hr exposure to cadmium at concentrations of 17.70 and 24.78 mg/L. Witeska (1998, 2003) observed lower white blood cell count after 48 and 96 hr from the end of 3 hr exposure to cadmium in a concentration of 10 mg/L. Sjöbeck et al. (1984) observed 45-100 % higher number of lymphocytes in perch (*Perca fluviatilis*) from the cadmium contaminated river (0.1-0.2 μg/L). Jezierska and Witeska (2001) showed that poisoning of fish with metals might cause not only alteration in count or share of particular white blood cell types, but it might also result in impairment of their function causing suppression of non-specific and specific immune response.

Cadmium in the form of cadmium chloride was classified among toxic substances for fish. Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to gill damage. Nature of the changes shows a release of erythrocytes from the blood depots. The decrease of leukocyte count and lymphopenia indicate a decrease of non-specific immunity in carp.

Acknowledgments. This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic (FRVŠ Project No. 360/2002 and MSM Project No. 122200003).

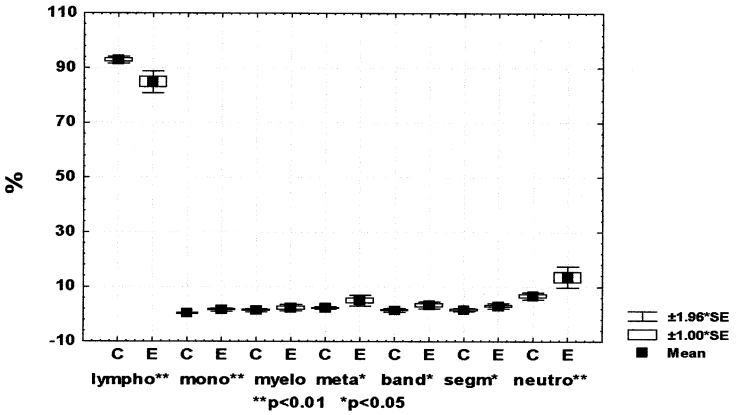

Figure 3. Leukocyte differential count (%) in control (C) and experimental (E) groups of common carp affected by 96 hr exposure to cadmium chloride in concentration 12.5 mg/L.

Table 2. Leukocyte differential count (G/L) in common carp affected by 96 hr toxicity test with cadmium chloride in concentration 12.5 mg/L.
(significance: ** p<0.01)

Indices	Groups	N	Mean	SD	SE	Probability
Lymphocytes	control experiment	20 20	134,12 62,80	47,77 38,57	10,68 8,63	0.000**
Monocytes	control experiment	20 20	0,54 1	0,71 0,82	0,16 0,18	0,069
Myelocytes	control experiment	20 20	2,25 1,22	2,41 1,29	0,54 0,29	0,098
Metamyelocytes	control experiment	20 20	3,28 2,77	2,20 2,04	0,49 0,46	0,455
Band neutrophils	control experiment	20 20	1,94 2,10	2,13 1,97	0,48 0,44	0,814
Segmented neutrophils	control experiment	20 20	2,21 2,12	2,16 2,15	0,48 0,48	0,888
Neutrophils	control experiment	20 20	9,69 8,15	5,21 4,34	1,16 0,97	0,316

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