ORIGINAL ARTICLE

Effects of subchronic simazine exposure on some biometric, biochemical, hematological and histopathological parameters of common carp (*Cyprinus carpio L.*)

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Abstract

OBJECTIVES: This study assessed the subchronic effects of a triazine compound, simazine, on common carp (*Cyprinus carpio* L.) though, via by means of biometric, biochemical, hematological, and histological examination.
DESIGN: One-year-old fish were exposed to simazine at four concentrations, 0.06, (reported concentration in Czech rivers), 4, 20, and 50 μg L^{-1} for 28 days and compared to carp in a non-treated control group.
RESULTS: Exposure of fish to simazine at 0.06 μg L^{-1} had no effect on measured parameters. However, exposure to simazine at the concentrations of 4, 20, 50 μg L^{-1} showed significant differences in biochemical, hematological, and histopathological profiles of fish compared to controls.
CONCLUSION: Our data suggest that simazine in the recorded environmental concentration 0.06 μg L^{-1} had no effect on common carp. Subchronic exposure to 4, 20, and 50 μg L^{-1} of simazine was associated with alterations in biochemical and hematological indices and in fish organ tissues.

INTRODUCTION

Simazine was introduced by a Swiss company J. R. Geigy (Cremlyn, 1990) in 1956 and is a member of the triazine family compounds (a six-membered ring containing three carbon and three nitrogen atoms). It is a selective herbicide used for control of annual broadleaf and grass weeds in raspberries, loganberries, highbush blueberries, apples, asparagus, and ornamentals. Noncrop uses include total weed control in industrial areas, at airports, and along shelterbelts and rights-of-way, and aquatic weed control in ditches, farm ponds, fish hatcheries, aquaria, and fountains (Arufe *et al.* 2004). Its mode of action is the blocking of electron flow in photosystem II of photosynthesis (Strong *et al.* 2002).

Simazine has a low water solubility (3.5 mg L^{-1} at 20 °C) with a modest affinity to sorb to soil organic matter, as demonstrated by an organic carbon to water partition coefficient (Koc) of 135 mL g^{-1} (Bubb, 2001). Simazine can be persistent in aquatic systems, particularly in shallow, well-mixed lakes and ponds (Bester *et al.* 1995). Resi-

Abbreviations & Units:

RBC – red blood cell count
PCV – packed cell volume
Hb – hemoglobin
MCV – mean corpuscular volume
MCH – mean corpuscular hemoglobin
MCHC – mean corpuscular hemoglobin concentration
WBC – white blood cell count
Leucogram – differential leucocyte count
GLU – glucose
TP – total protein
ALB – albumin
GLOB – total globulins
NH3 – ammonia
TAG – triacylglycerols
AST – aspartate aminotransferase
ALT – alanine aminotransferase
LDH – lactate dehydrogenase
CK – creatine kinase
CREA – creatine
LACT – lactate
AMYL – amylase
ALP – alkaline phosphatase
Ca2+ – calcium
Mg – magnesium
PHOS – inorganic phosphate
BW – body weight
SL – standard length
LW – liver weight
CF – condition factor
HSI – hepatosomatic index

dues may persist up to 3 years in soil under aquatic field conditions. Dissipation of simazine in pond and lake water has been found to be variable, with half-life ranging from 50 to 176 days (Wauchope *et al.* 1992). Slow biodegradation of simazine may occur in water, similar to that observed in soil (Wackett *et al.* 2002).

Simazine and its degradation products are detected less frequently than atrazine in the aquatic environment. In the Czech Republic surface water, the highest concentration of simazine is reported 0.06 μg L^{-1} (Czech Hydrometeorological Institute, 2009). Simazine was the most frequently detected pesticide in 20 counties in California with concentrations ranging from 0.02 to 49.2 μg L^{-1} (U.S. EPA, 1994). In Europe simazine levels can reach values, up to 3 μg L^{-1} (Drevenkar *et al.* 2004; Belmonte *et al.* 2005).

Water pollution is a widespread problem in many aquatic environments (Blahova *et al.* 2008). Recently, an environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem (Modra *et al.* 2008). Due to their extensive use in agriculture and their persistence, many of these compounds are present in surface and ground waters and must be considered as a potential risk for aquatic organisms as well as for drinking water quality (Katsumata *et al.* 2005). Simazine and further seven s-triazines have been identified as relevant in a study on the prioritizing of substances dangerous to the aquatic environment in the member states of the European Community (European commission, 1999). Simazine is included in the EU Priority Pollutants List and the US Environmental Protection Agency's List. This study addresses whether, and how, low concentrations of simazine might affect physiology of common carp living in contaminated areas.

The aim of the present study was to evaluate the effects of subchronic exposure of common carp (*Cyprinus carpio* L.) to the herbicide simazine, through biometric, biochemical, hematological, and histopathological examination.

MATERIAL AND METHODS

Experimental design. The experiment was performed as a semistatic assay conducted over a 28-day period. One hundred one-year-old common carp (mean body length 17.74 ± 0.79 cm, mean body weight 126.13 ± 16.96 g) were randomly allocated, in groups of 10 individuals , to one of four experimental regimes and to an untreated control group. The conditions were duplicated for a total of ten groups, each held in a glass tank containing 200 L of water. Water temperature was 15.5–17.2 °C and oxygen saturation 86–99%.

After a 14-day adaptation period, experimental fish were exposed to simazine (Sigma Aldrich, Czech Republic, chemical purity 99.5%) added to the tank water at the concentrations of:

Group 1: 0.06 μg L^{-1}simazine (a real environmental concentration reported in the Czech river)
Group 2: 4 μg L^{-1}simazine
Group 3: 20 μg L^{-1} simazine
Group 4: 50 μg L^{-1} simazine
Control: simazine-free tap water

Tanks for all the fish treated and the controls were replicated. Fish were transferred daily to the replicate tank, containing freshly diluted simazine at the appropriate concentration, or fresh water for the controls,. Fish were fed commercial fish pellets at about 1% of body weight day^{-1} in two feedings.

During the test, simazine concentrations were checked daily by a high performance liquid chromatography (HPLC). Water samples were assayed using the method of Katsumata *et al.* (2005). The values measured did not differ from the value stated for test purposes by more than 2%.

Hematological, biochemical and histopathological examination. After 28 days of exposure, eight fish from each replicate of each group were examined to determine their hematological, biochemical, and histopathological profile.

Blood was sampled from the *v. caudalis*, samples were stabilized with 40 IU of sodium heparin 1 mL blood. Measured indices included: RBC, PCV, Hb, MCV, MCH, MCHC, Leuko, and Leukogram. The procedures were based on unified methods for haematological examination of fish (Svobodova *et al.* 1991).

For biochemical analysis, plasma was separated by centrifugation (10 min at 12 000 x g) at 4 °C. Biochemical indices determined included GLU, TP, ALB, GLOB, NH_3, TAG, AST, ALT, LDH, CK, CREA, LACT, AMYL, ALP, Ca^{2+}, Mg, and PHOS. VETTEST 8008 analyzer (IDEXX Laboratories Inc. U.S.A.) manufactured by Medisoft was used for plasma analysis.

For histological studies, liver, spleen, cranial and caudal kidney were immediately fixed in formalin (10%

Table 1. Standard length (SL), body weight (BW), liver weight (LW), condition factor (CF) and hepatosomatic index (HSI) of common carp exposed to simazine.

Fish Group	Control	1	2	3	4
Simazine (μg L^{-1})	**-**	**0.06**	**4**	**20**	**50**
	M ± SD (n = 16)	M ± SD (n = 16)	M ± SD (n = 16)	M ± SD (n = 16)	M ± SD (n = 16)
SL (cm)	17.51 ± 0.42^a	17.98 ± 0.87^a	17.95 ± 0.88^a	17.31 ± 0.70^a	17.95 ± 0.73^a
BW (g)	117.69 ± 14.92^a	130.35 ± 16.03^a	129.56 ± 16.72^a	124.56 ± 16.35^a	128.48 ± 17.40^a
LW (g)	2.43 ± 0.44^a	2.81 ± 0.32^a	3.16 ± 0.65^a	3.15 ± 0.67^a	2.97 ± 0.64^a
CF	2.18 ± 0.16^a	2.24 ± 0.14^a	2.23 ± 0.17^a	2.39 ± 0.18^a	2.22 ± 0.19^a
HSI	2.07 ± 0.28^a	2.17 ± 0.25^a	2.43 ± 0.38^a	2.53 ± 0.44^a	2.29 ± 0.29^a

Groups with different alphabetical superscripts differ significantly at $p < 0.05$.

Table 2. Derived hematological parameters in common carp following subchronic exposure to simazine.

Fish Group	Control	1	2	3	4
Simazine (μg L^{-1})	**-**	**0.06**	**4**	**20**	**50**
	M ± SD (n = 16)	M ± SD (n = 16)	M ± SD (n = 16)	M ± SD (n = 16)	M ± SD (n = 16)
PCV (L L^{-1})	0.282 ± 0.024^a	0.270 ± 0.045^a	0.381 ± 0.039^b	0.372 ± 0.032^b	0.376 ± 0.019^b
MCHC (g L^{-1})	276.55 ± 6.07^a	279.72 ± 9.68^a	249.30 ± 10.89^b	244.73 ± 11.31^b	243.39 ± 10.39^b
Lymphocytes (%)	30.57 ± 11.28^a	23.25 ± 5.82^a	83.42 ± 12.39^b	79.20 ± 20.98^b	73.50 ± 22.03^b
Neutrophil granulocytes bands (%)	30.01 ± 9.25^a	46.08 ± 10.84^a	12.67 ± 8.67^b	14.90 ± 8.22^b	12.20 ± 5.68^b
Developmental phases – myeloid sequence (%)	13.97 ± 8.02^a	14.75 ± 9.26^a	0.37 ± 0.17^b	0.19 ± 0.10^b	0.20 ± 0.10^b

Groups with different alphabetical superscripts differ significantly at $p < 0.05$.

Table 3. Derived biochemical indices of blood plasma in common carp following subchronic exposure to simazine.

Fish Group	Control	1	2	3	4
Simazine (μg L^{-1})	**-**	**0.06**	**4**	**20**	**50**
	M ± SD (n = 16)	M ± SD (n = 16)	M ± SD (n = 16)	M ± SD (n = 16)	M ± SD (n = 16)
GLU (mM L^{-1})	3.73 ± 0.93^a	3.54 ± 0.79^a	6.80 ± 0.85^b	6.76 ± 1.16^b	7.87 ± 1.38^b
NH_3 (μM L^{-1})	62.80 ± 27.87^a	61.40 ± 25.66^a	266.40 ± 42.82^b	252.20 ± 25.30^b	265.40 ± 20.03^b
AST (μkat L^{-1})	5.56 ± 0.61^a	5.61 ± 0.89^a	2.17 ± 0.55^b	2.04 ± 0.50^b	2.08 ± 0.49^b
LDH (μkat L^{-1})	18.29 ± 1.35^a	18.61 ± 0.49^a	23.27 ± 1.70^b	24.80 ± 0.51^b	24.96 ± 1.35^b
CK (μkat L^{-1})	13.98 ± 0.24^a	13.80 ± 0.25^a	19.14 ± 1.24^b	20.36 ± 0.63^b	19.84 ± 0.91^b
CREA (mM L^{-1})	14.20 ± 2.50^a	14.05 ± 0.50^a	40.50 ± 4.51^b	44.00 ± 0.82^b	44.33 ± 2.05^b

Groups with different alphabetical superscripts differ significantly at $p < 0.01$.

formaldehyde), dehydrated, and embedded in paraffin. Sections (5 μm) were stained with heamatoxylin and eosin, examined by a light microscopy and photographed using a digital camera.

Determination of biometric parameters. After blood sampling, fish were killed by severing the spinal cord, and BW and SL were recorded. For each fish tested, a condition factor was calculated according to the formula $CF = BW\ (g)/SL\ (cm)^3 \times 100$. Livers were removed and weighed, and a HSI ($HSI = LW/BW \times 100$) was calculated for each fish.

Statistical analysis of the data. The Shapiro–Wilk test was used to assess the normal distribution of parameters studied. Since non-normal distributions of parameters ($P<0.05$) were identified, non-parametric tests were used. To compare values of parameters among groups, the Kruskal–Wallis test was used. This test was followed by a multiple comparison when significant differences were found among groups.

RESULTS

Biometric parameters. Results of biometric parameters are given in ***Table 1***. No differences in parameters (SL, BW, LW, CF, and HSI) investigated were found among any groups (Kruskal–Wallis test in all comparisons $p > 0.05$).

Hematological profile after exposure to simazine. Results of hematological profiling are given in ***Table 2***. Subchronic exposure to simazine at the recorded envi-

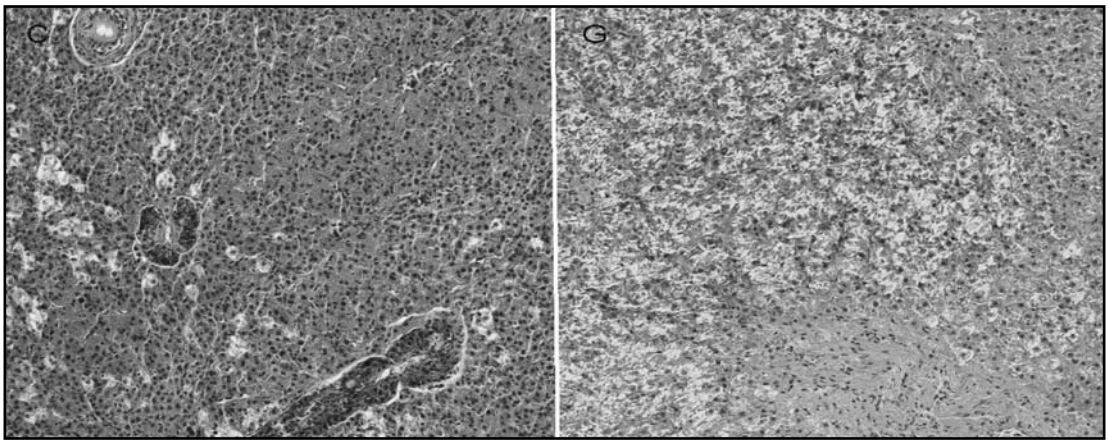

Figure 1. Liver of common carp following subchronic exposure to simazine in a 28-day trial. C – control; G – group 4 (concentration 50 μg L^{-1} of simazine). Haematoxylin and eosin, × 400.

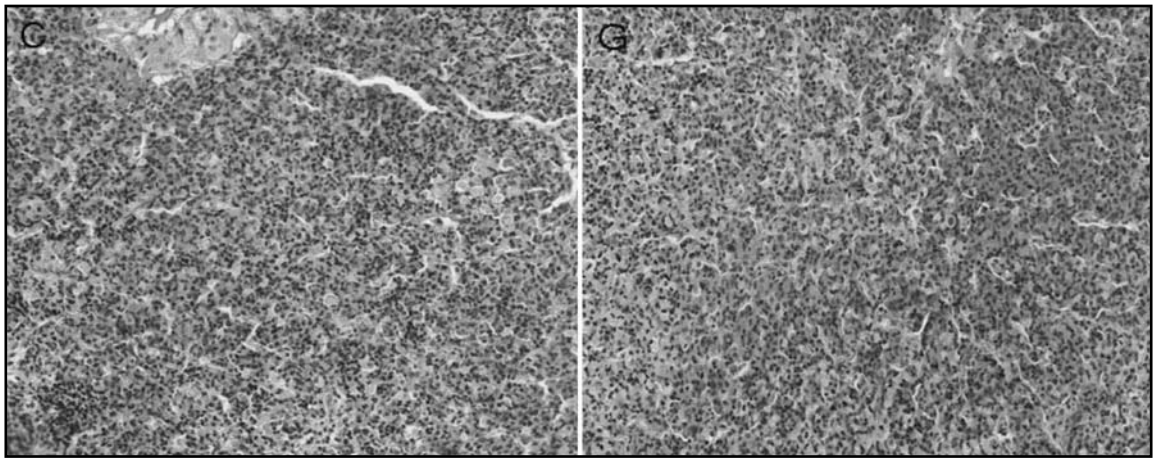

Figure 2. Cranial kidney of common carp following subchronic exposure to simazine in a 28-day trial. C – control; G – group 4 (concentration 50 μg L^{-1} of simazine). Haematoxylin and eosin, x 400.

ronmental concentration of 0.06 μg L^{-1} had no effect on a hematological profile of common carp treated.

In the fish exposed to 4 μg L^{-1}, 20 μg L^{-1}, and 50 μg L^{-1} of simazine, increased ($p < 0.05$) PCV and lymphocytes and decreased ($p < 0.05$) MCHC, neutrophile granulocyte bands, and development phases myeloid sequence were found. The remaining indices were comparable within all groups.

Biochemical plasma profile after exposure to simazine. Results of carp plasma biochemical profiling are given in ***Table 3***. Biochemical profile of carp exposed to simazine at the recorded environmental concentration of 0.06 μg L^{-1} showed no differences when compared to the control.

In the fish exposed to 4 μg L^{-1}, 20 μg L^{-1}, and 50 μg L^{-1} of simazine, a significant ($p < 0.01$) decrease in the activity of AST and a significant ($p < 0.01$) increase in GLU, NH_3, LDH, CK, and CREA levels in blood plasma were observed. The remaining indices were comparable within all groups.

Histological examination of tissues. Differences were found in the histology of liver in experimental groups 2, 3, and 4 (i.e. 4 μg L^{-1}, 20 μg L^{-1}, and 50 μg L^{-1} of simazine) compared to control and group 1 (environmental concentration 0.06 μg L^{-1}), with more pronounced effects with increasing simazine concentration. In the control group and group 1 histology was normal but a slight focal steatosis in liver cells. Greater histological changes, comprising steatosis, hyperaemia, and necrosis developed in group 2 and 3. The most pronounced pathologies were found in a group 4, where an extensive fatty dystrophy with a massive coagulation necrosis, and a degradation of physiological cell structure were observed in liver (Figure 1).

The histological changes in a cranial kidney were represented by a decline in hematopoietic tissue corresponding to an increasing simazine concentration. In the control group and group 1, an unbroken hematopoietic tissue was observed, while only residual hematopoietic tissue in isolated trabeculae was observed in simazine exposed fish (Figure 2). In other organs tissues tested, no pathological changes were found.

DISCUSSION

During the laboratory toxicity test, both control and exposed carp showed normal feeding habits and exhibited no abnormal behaviour. In addition, no signs of

respiratory distress, such as rapid respiration, increased rate of gill cover movements, or floating at the water surface were detected. This was similar to observations by Oropesa *et al.* (2009) of simazine exposed common carp to 45 μg L^{-1} at the temperature 20.0 ± 2.0 °C. Movement imbalance in freshwater fish (*Labeo rohita, Mystus vittatus*, and *Cirrhinus mrigala)* acutely exposed to simazine and cyanazine has been reported by Dad & Tripathi (1980).

Biometric parameters are regarded as a general indicator of fish health and the quality of the aquatic environment. In our experiment no differences in biometric parameters investigated were found among any groups.

The main hematological responses of carp exposed to simazine at the concentration of 4, 20, and 50 μg L^{-1} were a significant ($p < 0.05$) increase in PCV and lymphocytes and decreased ($P<0.05$) MCHC, neutrophil granulocyte bands, and development phases of myeloid sequences levels. The change in PCV can be brought about by the release of catecholamines during the primary stages of stress, which can mobilize red blood cells from the spleen or induce red blood cell swelling as a result of fluid shift into the intracellular compartment (Lebelo *et al.* 2001). Change in leukocyte number are sensitive indicator of stress in fish (Feldman *et al.* 2000). An increase in lymphocytes number may be the compensatory response of lymphoid tissues to the destruction of circulating lymphocytes. Oropesa *et al.* (2009) reported no effects of simazine on hematological profiles of common carp at 45 μg L^{-1}. On the other hand, a significant decrease in PCV has been reported by Horn & Hanke (1980) in common carp exposed to atrazine at the temperature 19.4 °C. Similar changes in packed cell volume, lymphocytes, erythrocytes, and neutrophil granulocytes were also reported by Svobodova & Pecena (1988) in common carp following acute poisoning with atrazine at the temperature 18.2 °C. The results of hematological examination indicated a decrease in nonspecific immunity.

In our experiment, the exposure to simazine at 4, 20, and 50 μg L^{-1} resulted in a significant increase in plasma GLU, NH_3, LDH, CK and CREA levels, and a significant decrease in AST activity. The elevated blood glucose concentration in exposed fish indicated metabolic stress (Simon *et al.* 1983). Increased ammonia concentration indicated the organism's inability to convert the toxic ammonia to less harmful substances. The activities of plasma enzymes (ALT, AST, LDH, and CK) were also found relevant stress indicators (Svoboda, 2001). LDH is the terminal enzyme of anaerobic glycolysis, therefore being of crucial importance to the muscular physiology, particularly in conditions of chemical stress when high levels of energy may be required in a short period of time (Monteiro *et al.* 2007). The increase in LDH level could indicate tissue damage, hypoxia conditions and switch over to anaerobic metabolism. Mekkawy *et al.* (1996) observed an increase in GLU and a decrease in TP levels in *Oreochromis niloticus* and *Chrysichthyes auratus* after atrazine exposure to 3 mg L^{-1} at the temperature 26.3 °C. Oropesa *et al.* (2009) reported no effect of simazine on TP levels and LDH activity in common carp.

Histopathological tissue changes, in liver and cranial kidney especially, were similar to the changes found in common carp and sea bream by other authors (Arufe *et al.* 2004; Oropesa *et al.* 2009). Liver and cranial kidney pathological changes results in an altered fat metabolism, and a degradation of the hematopoietic system, thereby in generalized stress of the fish exposed. Found histopathological tissue alterations corresponded with the changes in the measured hematological (MCHC) and biochemical parametres (GLU, AST, LDH, CK).

Our data suggest that simazine in the recorded environmental concentration 0.06 μg L^{-1} had no effect on the biometric, biochemical, hematological, and histopathological profile of common carp. Subchronic exposure to simazine at the concentrations of 4, 20, and 50 μg L^{-1}were associated with alterations in biochemical, and hematological indices and in fish organ tissues.

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