Effects of subchronic exposure to Spartakus (prochloraz) on common carp *Cyprinus carpio*

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Abstract

OBJECTIVES: The aim of the study was to investigate effects of the fungicide formulation Spartakus (prochloraz 450 g.L^{-1}) on common carp *Cyprinus carpio* through biometric, biochemical, haematological and antioxidant indices, induction of xenobiotic metabolizing enzymes and histological examination of selected tissues.
DESIGN: The test was performed on juvenile fish, which was exposed to Spartakus (concentrations of prochloraz: 0.05; 0.15 and 0.38 mg.L^{-1}) for 28 days. Haematological indices were assessed using unified methods of haematological examination in fish. Plasma biochemical indices were determined by biochemical analyzer. Concentration of total cytochrome P450 (CYP), glutathione (GSH) content and glutathione-S-transferase (GST) activity were determined spectrophotometrically in hepatopancreas. Activity of liver ethoxyresorufin-O-deethylase (EROD) activity was measured spectrofluorimetrically. Ferric reducing ability of plasma (FRAP) and ceruloplasmin activity were assessed spectrophotometrically. Histological changes in samples of hepatopancreas, skin, gills, spleen, head kidney and caudal kidney were examined by light microscopy.
RESULTS: There was a significant rise in hepatosomatic index (HSI) (p<0.01), CYP and EROD (p<0.05) of fish exposed to prochloraz of 0.15 and 0.38 mg.L^{-1} whereas GST was induced by all concentrations tested and GSH by 0.38 mg.L^{-1} (p<0.05). Red blood cell count decreased significantly (p<0.05) in prochloraz of 0.05 and 0.15 mg.L^{-1}. Plasma potassium increased (p<0.01) in all Spartakus treated groups, a decline in total protein (p<0.05), ALT, Na^+ and Ca (p<0.01) was found in fish exposed to prochloraz of 0.38 mg.L^{-1}. Ceruloplasmin activity was elevated (p<0.05) in the highest concentration tested, FRAP declined (p<0.05) in the same group. Histopathological changes in gills were demonstrated in all pesticide treated groups, with a decreased activity of skin mucous cells in prochloraz of 0.38 mg.L^{-1}.
CONCLUSION: The subchronic exposure to Spartakus influenced HSI, induced xenobitic metabolizing enzymes, initiated a disorder of selected plasma indices and a decline in red blood cell count, caused minor histological impairment, and affected antioxidant activities of the test fish.

ORIGINAL ARTICLE

Abbreviations:

SL	- standard length
KI	- condition factor
HSI	- hepatosomatic index
b. w.	- body weight
RBC	- red blood cell count
WBC	- white blood cell count
PCV	- packed cell volume, haematocrit
Hb	- haemoglobin
MCV	- mean corpuscular volume
MCH	- mean corpuscular haemoglobin
MCHC	- mean corpuscular haemoglobin concentration
glc	- glucose
alb	- albumine
TP	- total protein
TAG	- triglycerides
LDH	- lactate dehydrogenase
AST	- aspartate aminotransferase
ALT	- alanine aminotransferase
XME	- xenobiotic metabolizing enzymes
CYP	- cytochrome P450
EROD	- ethoxyresorufin-O-deethylase
GST	- glutathione-S-transferase
GSH	- glutathione
FRAP	- ferric reducing ability of plasma
i. p.	- intraperitoneal

INTRODUCTION

The pollution of the aquatic environment with the pesticide discharge is still of a great concern. The attention has been recently focused on the effects caused by a long-term exposure to contaminants. Prochloraz (*N*-propyl-*N*-[2-(2,4,6-trichlorophenoxy)ethyl]imidazole-1-carboxamide) is a fungicide compound used as a seed treatment and foliar spray on a wide range of crops, e.g. cereals, oilseed rape, rice and ornamentals. It interferes with conversion of lanosterol to ergosterol (essential component of fungal cell membranes) by inhibition of the cytochrome P450-dependent 14α-demethylase (Zarn *et al.* 2003).

Prochloraz was reported to act as a weak tumour promoter of hepatocarcinogenesis in female rats, however, it did not initiate the process (Kato *et al.* 1998). The reproductive toxic effects of prochloraz have been widely studied both *in vitro* and *in vivo* (e.g. Vinggaard *et al.* 2002; Vinggaard *et al.* 2005; Andersen *et al.* 2002; Brande-Lavridsen *et al.* 2008). Prochloraz displays multiple endocrine activities which include inhibition of CYP enzymes involved in steroid synthesis and antagonism of the androgen receptor (Ankley *et al.* 2005; Kinnberg *et al.* 2007). Saglio *et al.* (2003) studied behavioral toxicity of prochloraz in goldfish *Carassius auratus. In vitro* and *in vivo* induction of xenobiotic metabolizing enzymes has been studied mostly in rainbow trout with a short exposure period (Bach & Snegaroff 1989; Snegaroff & Bach 1989).

Although prochloraz has been scrutinized in detail, the amount of information of its effects on fish in *vivo* is not complete, especially under chronic and subchronic conditions.

The aim of our study was to assess the subchronic effects of prochloraz exposure on common carp *Cyprinus carpio* through biometric, biochemical, haematological and antioxidant indices, biomarkers of xenobiotic metabolization and histological examination of the test fish. A commercial pesticide formulation Spartakus (prochloraz 450 g.L^{-1}) was used in the experiment.

MATERIAL AND METHODS

The common carp (218.4 ± 64.5 g, SL: 19.7 ± 2 cm) were obtained from a commercial fish farm. They were randomly distributed into ten 200L glass aquaria, 15 specimens per each. The fish were supplied twice a day with commercial feed (Coppens International bv) at a total rate of 1.5% body weight. After 2-week acclimation to laboratory conditions (water temperature 19–22 °C, photoperiod 12/12), the fish were exposed to Spartakus (prochloraz 450 g.L^{-1}; BASF SE, Germany) for 28 days. Each treated and control group was performed in duplicate. 96h LC_{50} of prochloraz was determined 1 mg.L^{-1} and 2.2 mg.L^{-1} for rainbow trout *Oncorhynchus mykiss* and bluegill sunfish *Lepomis macrochirus*, respectively (Gangolli 1999). Thus, proportional dosages 1/45, 1/15 and 1/5 of the latter value were chosen for the test. Test solutions were sampled and the concentrations of prochloraz were measured five times within the experiment. Actual concentrations were as follows; 0.05; 0.15 and 0.38 mg.L^{-1}, respectively. These are further used to refer to the test groups.

Prochloraz concentrations exceeded 80% of the nominal concentrations over the test period. The control groups were subjected to dechlorinated tap water. The producer states Spartakus contains these substances: prochloraz (40% W/W), solvent naphtha (30–40% W/W), N-methyl-2-pyrrolidon (1–5% W/W), calcium dodecylbenzene sulphonate (3–4% W/W), light solvent naphtha (1.2–1.8% W/W), mixture of emulsifiers (5.6–6.8% W/W) and isobutanol (1–1.4% W/W). The test was conducted in a flow-through system, the volume of test solutions was exchanged twice a day. The pH of the solutions varied from 7.1 to 7.9, oxygen saturation was ≥ 60%.

After the stipulated exposure period, individual blood samples were taken by cardiac puncture and stabilized with aqueous solution of heparin (50 IU per ml of blood). The fish were euthanized by cutting the vertebral column at the base of the skull, their body weight and standard lenght were recorded. Tissue samples and the whole liver (hepatopancreas) were removed. The sections of liver which were not used for histological examination were stored at –85 °C until further analyses were performed.

Biometric parameters

The condition factor of each fish was calculated using the following formula: KI = (b.w. [g]/SL [cm]^3)×100.

The hepatosomatic index was calculated according to the formula HSI = liver weight/b.w.×100.

Haematological parameters

RBC, WBC, PCV, Hb, MCV, MCH and MCHC were assessed according to Svobodova *et al.* (1991).

Biochemical profile

Plasma biochemical indices (glucose, albumin, total protein, triglycerides, total calcium, inorganic phosphorus, chloride, potassium, sodium, lactate, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase) were determined by the biochemical analyzer Konelab 20i using commercial test kits (BioVendor).

Xenobiotic metabolizing enzymes and biomarkers

Total CYP, activities of ethoxyresorufin-O-deethylase and glutathione-S-transferase and glutathione content were determined in the liver samples.

CYP and EROD activity

After homogenisation in buffer (pH 7.4), the liver samples were centrifuged (10,000 g, 20 min, 4 °C). The supernatant was re-centrifuged again (100,000 g, 1 h at 4 °C), the final supernatant was drained and the pellet was washed and resuspended in the buffer (pH 7.4). Each suspension was put into an Eppendorf tube and stored at –85 °C until enzymatic assays. Concentrations of microsomal protein were determined according to Lowry *et al.* (1951). Quantities of total CYP were determined spectrophotometrically at 400–490 nm, on the basis of the difference between readings at 450 and 490 nm. The activity of EROD was examined spectrofluorimetrically; in the presence of NADPH EROD converts the substrate ethoxyresorufin to the fluorescent product resorufin (Chang & Waxman 1998; Nilsen *et al.* 1998; Rutten *et al.* 1992). The excitation/emission wavelenghts setting used was 535/585 nm. For details see Siroka *et al.* (2005).

GSH, GST

Liver samples were homogenised and extracted with phosphate buffer (pH 7.2). Homogenates were centrifuged (10,500 g, 20 min, at 4 °C) and the supernatants were used for determination. Protein concentration was quantified with the Bicinchoninic Acid Protein Assay Kit (Sigma–Aldrich) using bovine serum albumin as a standard. The activity of GST was determined spectrophotometrically at 340 nm using Habig's method (Habig *et al.* 1974). Glutathione was measured spectrophotometrically at 414 nm according to Ellman (1959). Concentrations (nmol GSH.mg^{-1} of protein) were calculated according to a standard calibration.

Ceruloplasmin

Ceruloplasmin activity was measured according to Ceron & Martinez-Subiela (2004) with slight modifications. The analysis was performed using Varioskan Flash spectral scanning multimode reader (Thermo Scientific). Plasma samples (15 µl) were mixed with 100 µl PPD solution (41 mg PPD/25 ml of sodium acetate buffer – pH 5.2) in a 96 well plate and incubated at 37 °C. The absorbance was recorded every 60 s for 30 min at 550 nm. At each point, the absorbance of blank (PPD solution in distilled water) was deducted from the obtained values. The results were expressed as the amount of the absorbance increase per minute × 10,000.

FRAP

The ferric reducing ability of plasma samples was determined according to Benzie & Strain (1996) including slight modifications. The antioxidants present in the plasma are evaluated through their ability to reduce ferric to ferrous ions at low pH, which causes a blue-coloured ferrous-tripyridyltriazine complex to form. We performed the procedure using a biochemical analyzer Konelab 20i. 5 µl of plasma were added to 150 µl of fresh FRAP reagent (pH 3.6) and the absorbance was measured at 600 nm. The monitoring period lasted for 280 s. Each sample was examined in quadriplicate. The influence of individual plasma absorbance was eliminated by measuring the absorbance of 5 µl plasma mixed with 150 µl of distilled water and substracting the values from the FRAP results. Final FRAP values were obtained by comparing the absorbances of test reaction mixtures with those containing ferrous ions in known concentrations.

Histological examination

Samples of liver, skin, gills, spleen, head kidney and caudal kidney of four fish from the control group and each concentration tested were fixed in buffered 10% neutral formalin and treated for histological examination (H&E).

Determination of prochloraz in water

Gas chromatography with ion trap mass spectrometry (GC/MS) was used for determination of the compound. Sample preparation was based on simple liquid-liquid extraction into hexane. Separation, identification and quantification of prochloraz were based on the GC/MS method described by Charlton and Jones (2007). Gas chromatograph Varian 450-GC with Varian 220-MS ion trap mass spectrometer and VF-5ms (30 m × 0.25 mm) column were used for the separation of prochloraz. Detection limit (3σ) was 1 µg.L^{-1}. Expanded uncertainty was 7% on condition that coefficient of expansion is k = 2.

Statistical analysis

Data were evaluated for analysis of variance (ANOVA–Tukey Test) using Unistat 5.1 software. Spearman's rank correlation test was used to find out correlation between CYP and EROD and between GSH and GST.

All data are reported as mean ± standard deviation obtained from the data of individual fish sampled.

RESULTS

Biometric indices

A significant increase in HSI (Figure 1) was found in fish exposed to prochloraz of 0.15 and 0.38 mg.L^{-1} (p<0.01). No significant changes in KI were revealed in pesticide treated fish when compared to the control.

Haematological profile

There was a significant decrease (Figure 2) in red blood cell count in fish exposed to prochloraz of 0.05 and 0.15 mg.L^{-1} (p<0.05), however, the decline in fish treated with concentration of 0.38 mg.L^{-1} was not significant. Other haematological indices (WBC, PCV, Hb, MCV, MCH and MCHC) were not significantly affected by the exposure.

Biochemical profile

A decline in plasma TP (p<0.05), ALT, Na^+ and Ca (p<0.01) was revealed in fish exposed to prochloraz of 0.38 mg.L^{-1} when compared to control fish whereas plasma potassium increased in all prochloraz treated groups (p<0.01) (Tab. 1). Other indices were not significantly influenced by the exposure.

XME

Cytochrome P450 (Figure 3) and ethoxyresorufin-O-deethylase activity (Figure 4) were induced (p<0.05) by prochloraz of 0.15 and 0.38 mg.L^{-1}, with a significant (p<0.01) Spearman's correlation (r = 0.49) between the indices. Activity of GST (Figure 5) increased significantly in all groups exposed to the formulation (p<0.05), GSH (Figure 6) content showed a significant rise (p<0.05) in fish treated with prochloraz of 0.38 mg.L^{-1}. Spearman's correlation (r = 0.38) between the indices was significant (p<0.01).

Ceruloplasmin activity

In comparison to the control, ceruloplasmin activity was significantly (p<0.05) increased in fish exposed to prochloraz of 0.38 mg.L^{-1} (Figure 7).

FRAP

FRAP was observed to decrease significantly (p<0.05) in fish treated with prochloraz of 0.38 mg.L^{-1} (Figure 8).

Histological examination

The activity of skin mucous cells decreased in fish treated with prochloraz of 0.38 mg.L^{-1} whereas venostasis and desquamation of the respiratory epithelium was found in all pesticide exposed fish. No histological changes were detected in samples of hepatopancreas, spleen, head kidney and caudal kidney.

DISCUSSION

Biometric indices

Hepatosomatic index is used to indicate exposure of fish to pollutants. Its value is influenced by e.g. season, diseases, sex, and storing reserves (glycogen) (van der Oost *et al.* 2003; Svobodova 1977). An elevation of relative liver weights has been reported to be accompanied by hyperplasia and hypertrophy of liver cells in fish captured in polluted sites or exposed to pollutants (Slooff *et al.* 1983; Poels *et al.* 1980), as well as increased activities of xenobiotic biotransformation enzymes (Haluzova *et al.* 2009; Stephensen *et al.* 2000).

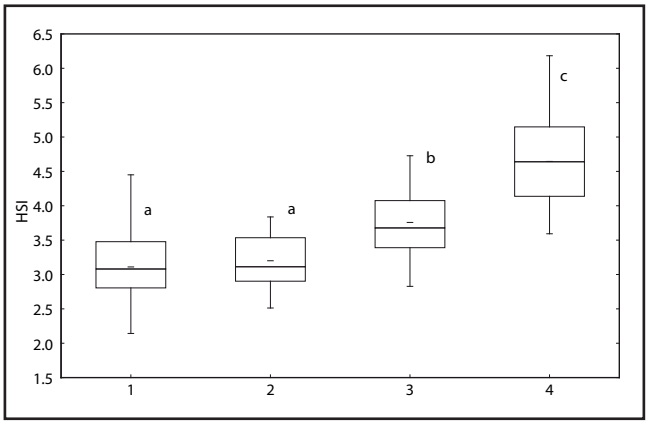

Fig. 1. Hepatosomatic index of the carps after 28-days exposure to Spartakus (prochloraz 450 g.L^{-1})
1 = control fish; 2–4 = fish exposed to prochloraz (2 = 0.05 mg.L^{-1}; 3 = 0.15 mg.L^{-1}; 4 = 0.38 mg.L^{-1}), n = 30
a, b, c = different alphabetic letters differ significantly (p<0.01)
Median = Middle line of the box; Lower (Upper) Quartile = Bottom (Top) line of the box; Lower (Upper) whisker = Lower (Upper) adjacent value

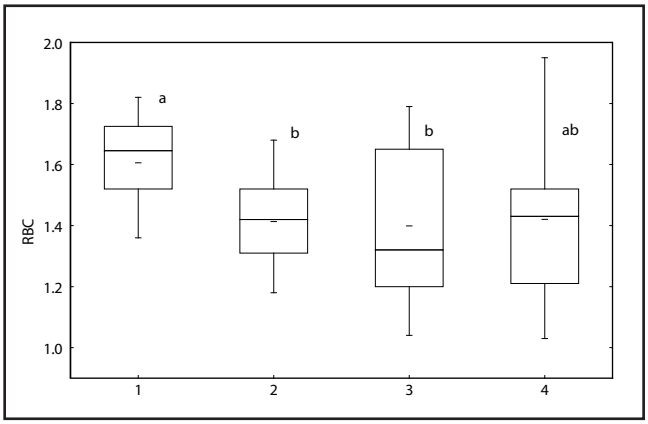

Fig. 2. Red blood cell count [T.L^{-1}] of the carps after 28-days exposure to Spartakus (prochloraz 450 g.L^{-1})
1 = control fish; 2–4 = fish exposed to prochloraz (2 = 0.05 mg.L^{-1}; 3 = 0.15 mg.L^{-1}; 4 = 0.38 mg.L^{-1}), n = 17–19
a, b = different alphabetic letters differ significantly (p<0.05)
Median = Middle line of the box; Lower (Upper) Quartile = Bottom (Top) line of the box; Lower (Upper) whisker = Lower (Upper) adjacent value

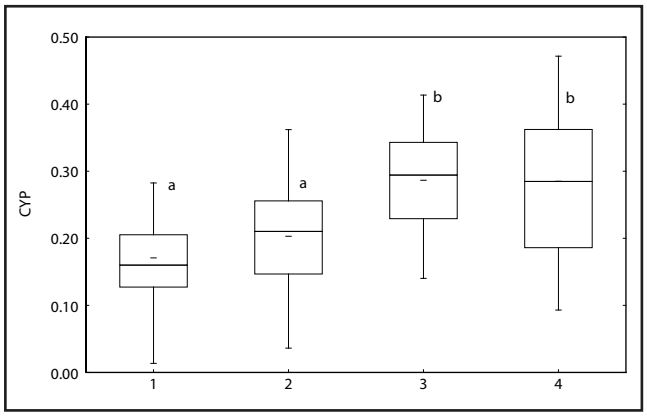

Fig. 3. CYP content [nmol.mg^{-1}micros. protein] in carp liver after 28-days exposure to Spartakus (prochloraz 450 g.L^{-1})
1 = control fish; 2–4 = fish exposed to prochloraz (2 = 0.05 mg.L^{-1}; 3 = 0.15 mg.L^{-1}; 4 = 0.38 mg.L^{-1}), n = 29–30
a, b = different alphabetic letters differ significantly (p<0.05)
Median = Middle line of the box; Lower (Upper) Quartile = Bottom (Top) line of the box; Lower (Upper) whisker = Lower (Upper) adjacent value

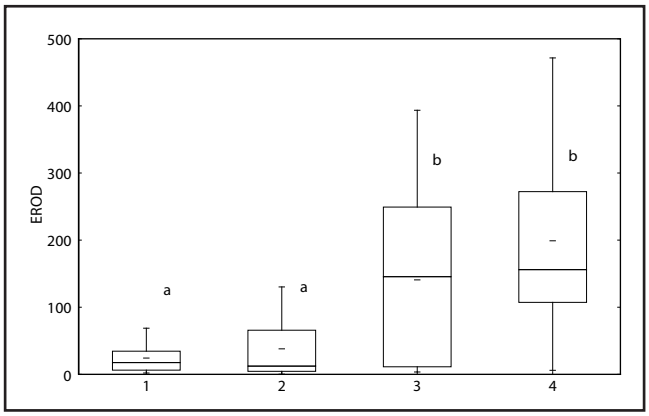

Fig. 4. EROD activity [pmol.min^{-1}.mg^{-1} micros. protein] in carp liver after 28-days exposure to Spartakus (prochloraz 450 g.L^{-1})
1 = control fish; 2–4 = fish exposed to prochloraz (2 = 0.05 mg.L^{-1}; 3 = 0.15 mg.L^{-1}; 4 = 0.38 mg.L^{-1}), n = 29–30
a, b = different alphabetic letters differ significantly (p<0.05)
Median = Middle line of the box; Lower (Upper) Quartile = Bottom (Top) line of the box; Lower (Upper) whisker = Lower (Upper) adjacent value

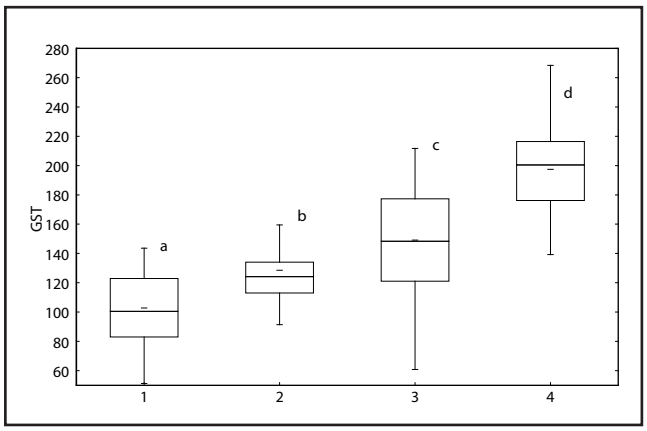

Fig. 5. GST activity [nmol.min^{-1}.mg^{-1} protein] in carp liver after 28-days exposure to Spartakus (prochloraz 450 g.L^{-1})
1 = control fish; 2–4 = fish exposed to prochloraz (2 = 0.05 mg.L^{-1}; 3 = 0.15 mg.L^{-1}; 4 = 0.38 mg.L^{-1}), n = 30
a, b, c, d = different alphabetic letters differ significantly (p<0.05)
Median = Middle line of the box; Lower (Upper) Quartile = Bottom (Top) line of the box; Lower (Upper) whisker = Lower (Upper) adjacent value

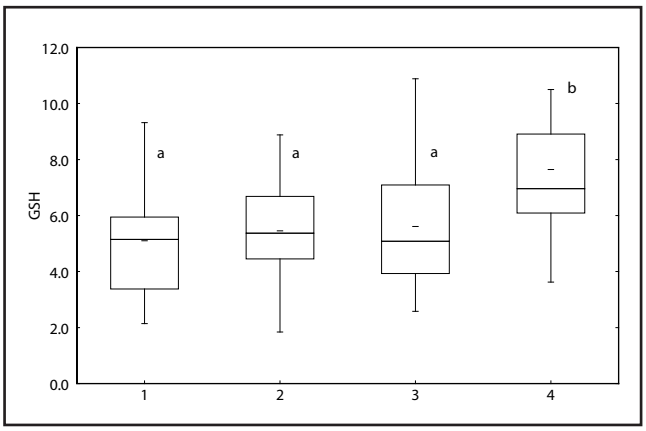

Fig. 6. GSH content [nmol.mg^{-1} protein] in carp liver after 28-days exposure to Spartakus (prochloraz)
1 = control fish; 2–4 = fish exposed to prochloraz (2 = 0.05 mg.L^{-1}; 3 = 0.15 mg.L^{-1}; 4 = 0.38 mg.L^{-1}), n = 29–30
a, b = different alphabetic letters differ significantly (p<0.05)
Median = Middle line of the box; Lower (Upper) Quartile = Bottom (Top) line of the box; Lower (Upper) whisker = Lower (Upper) adjacent value

Haematological profile, biochemical profile

The decline in red blood cell count in fish has been shown after exposure to several pesticides under subchronic conditions (Jee *et al.* 2005; Mikula *et al.* 2008). It can be caused by either a decline in erythropoiesis or increased erythocyte destruction induced by the pesticide or both. The effect of the exposure did not manifest statistically significantly in the highest concentration tested owing to high variability among test specimens. However, the significant increase (p<0.01) in plasma potassium in all exposed groups indicates that the changes originated from cell destruction, as potassium is a major intracellular ion. It is possible that the gill impairment releaved in prochloraz treated fish contributed to a disorder in selected extracellular ions (statistically significant decline in sodium and calcium) and the rise in potassium acted partially as a compensation mechanism. Transitory releases of potassium from intracellular fluid can occur also in an attempt to maintain blood osmolarity (Folmar 1993). The level of total proteins has a tendency to decrease in fish after a long-term exposure to pollutants (Svobodova *et al.* 1994). The decline in ALT may originate from depletion of the enzyme. However, no histological signs of hepatic damage were detected, as well as other indices of hepatic function were not significantly affected by

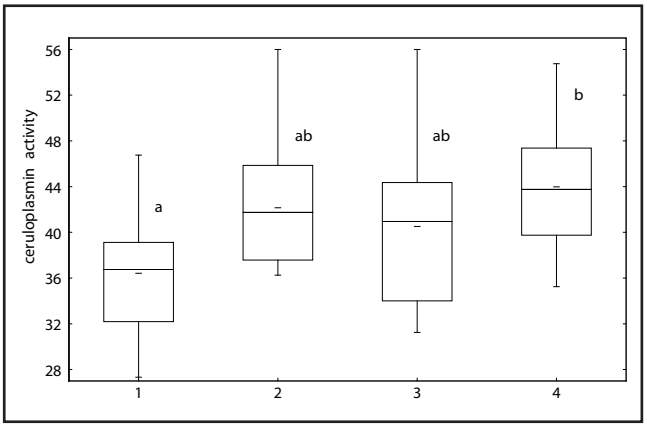

Fig. 7. Ceruloplasmin activity [increase in absorbance per min ×10,000] in carp liver after 28-days exposure to Spartakus (prochloraz 450 g.L^{-1})
1 = control fish; 2–4 = fish exposed to prochloraz (2 = 0.05 mg.L^{-1}; 3 = 0.15 mg.L^{-1}; 4 = 0.38 mg.L^{-1}), n = 12–15
a, b = different alphabetic letters differ significantly (p<0.05)
Median = Middle line of the box; Lower (Upper) Quartile = Bottom (Top) line of the box; Lower (Upper) whisker = Lower (Upper) adjacent value

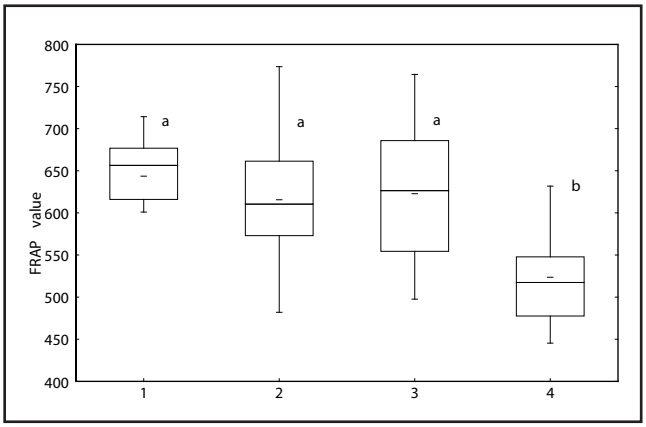

Fig. 8. FRAP value [Fe^{2+} equivalent μmol.L^{-1}] in carp plasma after 28-days exposure to Spartakus (prochloraz 450 g.L^{-1})
1 = control fish; 2–4 = fish exposed to prochloraz (2 = 0.05 mg.L^{-1}; 3 = 0.15 mg.L^{-1}; 4 = 0.38 mg.L^{-1}), n = 14
a, b = different alphabetic letters differ significantly (p<0.05)
Median = Middle line of the box; Lower (Upper) Quartile = Bottom (Top) line of the box; Lower (Upper) whisker = Lower (Upper) adjacent value

Tab 1. Biochemical indices in common carp after 28-days exposure to Spartakus (prochloraz 450 g.L^{-1})

Indice	Unit	Control	Prochloraz concentration [mg.L^{-1}]		
			0.05	0.15	0.38
glc	[mmol.L^{-1}]	4.85±1.19	5.26±1.62	4.28±1.79	4.33±0.90
TP	[g.L^{-1}]	29.19±2.40^{a}	29.59±2.33^{a}	28.44±1.96^{a}	24.29±5.60^{b}
alb	[g.L^{-1}]	9.12±0.86	9.47±1.50	8.47±2.10	8.02±2.39
ALT	[μkat.L^{-1}]	1.32±0.30^{ab}	1.34±0.34^{a}	0.96±0.24^{bc}	0.78±0.42^{c}
AST	[μkat.L^{-1}]	1.67±0.71	1.38±0.32	1.60±1.06	1.97±1.15
TAG	[mmol.L^{-1}]	2.93±0.62	2.99±0.62	2.65±0.65	2.54±0.84
LDH	[μkat.L^{-1}]	2.16±1.93	1.42±0.70	1.63±2.12	2.56±2.48
lactate	[mmol.L^{-1}]	2.37±2.31	3.62±2.33	2.27±1.39	1.58±1.08
Na^{+}	[mmol.L^{-1}]	142.58±1.73^{a}	142.38±2.96^{a}	140.25±2.38^{ab}	137.8±2.85^{b}
K^{+}	[mmol.L^{-1}]	2.91±0.35^{a}	3.62±0.41^{b}	3.43±0.26^{b}	3.53±0.31^{b}
Ca	[mmol.L^{-1}]	2.32±0.12^{a}	2.34±0.18^{a}	2.21±0.09^{a}	2.00±0.17^{b}
Cl^{-}	[mmol.L^{-1}]	108.27±1.91	110.38±1.79	106.71±2.56	108.12±2.64
P	[mmol.L^{-1}]	2.60±0.68	2.26±0.46	2.30±0.53	1.94±0.42

a, b, c = different alphabetic letters differ significantly at p<0.01, excluding TP (p<0.05); n = 12–13

prochloraz, although some changes occured (a slight decline in albumin, a minor increase in AST).

XME

Prochloraz was found to induce cytochrome P4501A (CYP1A) protein and the catalytic activity of EROD *in vitro* after 24-h exposure of rainbow trout hepatocyte culture to 1.0 μM prochloraz. Higher prochloraz concentrations induced only CYP1A above control levels, but not EROD activity. *In vivo* exposure of juvenile rainbow trout *O. mykiss* to 0.27 μM prochloraz resulted in an induction of CYP1A and EROD after 7 and 14 days (Sturm *et al.* 2001). Babin *et al.* (2005) reported that prochloraz activated CYP1A determined as EROD activity in a concentration-dependent way, as well as enhanced expression of CYP1A mRNA using the rainbow trout liver cell line, RTL-W1. Snegaroff & Bach (1989) added prochloraz to the incubation medium of rainbow trout liver microsomal suspension and reported inhibited EROD activity *in vitro*. Ethoxyresorufin-O-deethylase

activity was induced in a transient manner after 7-day exposure of *Gasterosteus aculeatus* to 50 and 100 μg.L^{-1} prochloraz. Fish exposed to 500 μg.L^{-1} exhibited significant induction of EROD after 7 and 14 days. After 7 days of depuration, following 2 weeks exposure, no significant difference was observed between exposed and unexposed groups (Sanchez *et al.* 2008). In our experiment, CYP concentration and EROD activity were found to rise after 28-day exposure to prochloraz of 150 and 380 μg.L^{-1} but not in 50 μg.L^{-1}.

Bach & Snegaroff (1989) studied *in vivo* induction of XME in rainbow trout using various experimental designs. When the fish were killed 24 hours after i. p. administration of prochloraz, cytochrome P450 increased with increasing dose of prochloraz to a maximum around 100 mg.kg^{-1}. EROD activity was significantly risen in exposed fish, too. Hepatic glutathione-S-transferase was not significantly affected. In fish fed with prochloraz-sprayed pellets for 7 days, with euthanasia 24 hours after the last feeding, liver concentration of CYP and GST activity were unchanged, EROD activity significantly declined. In a time course study, the liver CYP of trouts injected with prochloraz increased significantly during 8 days of observation and EROD activity was first significantly inhibited (1 and 2 days after administration) and then returned to control levels, GST was unchanged. The authors explained variations in the activities of XME partially by different experimental temperatures of water (18; 7; 2 °C).

After a single i. p. injection of prochloraz 100 mg. kg^{-1} b.w., CYP increased for 10 days and maintained at a higher level till the end of observation period (21 days). The difference from control was statistically significant on day 10 and 21. EROD increased 4 days after dosing with prochloraz and then returned to control level (Snegaroff & Bach,1989).

Biotransformation of prochloraz was reported in rainbow trout *O. mykiss* with mainly glucuronide conjugates (Debrauwer *et al.* 2001). The presence of glucuronide conjugates in rainbow trout was detected also by Cravedi *et al.* (2001) both *in vivo* and *in vitro*. For gill, fat, brain and carcasses, residue levels of prochloraz were significantly higher in males than in females. Sanchez *et al.* (2008) observed an increase in GST activity in *G. aculeatus* after 7 and 14-day exposure to prochloraz of 100 and 500 μg.L^{-1}, and after 14 days the increase was found also in the concentration of 10 μg.L^{-1}. The induction persisted also after 1 week depuration in clean water when GST activity rose also in fish previously exposed to 50 μg.L^{-1}. In our experiment, the activity of GST increased significantly in all pesticide treated fish, however, GSH content was enhanced in the highest concentration tested. In the above mentioned study, GSH declined in prochloraz of 100 and 500 μg.L^{-1} after 1-week exposure. The enhanced activity of hepatic glutathione-S-transferase in our fish relates to intensive metabolization of prochloraz. There is also glutathione-S-transferase exhibiting activity similar to glutathione peroxidases (Slatinska *et al.* 2008). The different results in GSH content could be caused by different length of the exposure period, the longer period could enable adaptation of the fish to the substance and synthesis of new glutathione molecules.

Ceruloplasmin activity

Many pesticides have been shown to induce oxidative stress in fish (Slaninova *et al.* 2009). Mechanisms of antioxidant defences include enzyme systems that act to remove ROS, low-molecular-weight compounds that directly scavenge ROS, and proteins that act to sequester pro-oxidants, particularly iron and copper (Di Giulio & Meyer 2008). Ceruloplasmin, which binds 95% of circulating copper, has multiple functions which include transport of copper, ferroxidase activity, modulation of coagulation, angiogenesis, inactivation of biogenic amines and defence against oxidative stress. However, also prooxidative activities of ceruloplasmin have been reported (Shukla *et al.* 2006). Ceruloplasmin is an "acute-phase reactant" participating in inflammatory responses (Bielli & Calabrese 2002). The increase of ceruloplasmin activity was probably connected with tissue impairment revealed by the histological examination. Ceruloplasmin activity was found to increase in *O. mykiss* after a single i.p. injection of lindan (Dunier *et al.* 1994). The activity was increased after 7, 14 and 21 days, and was observed to decline after 28 days. *Perca flavescens* caught in metal and PCB contaminated areas showed a significant rise in head kidney ceruloplasmin activity (Dautremepuits *et al.* 2009). Although ceruloplasmin exhibits antioxidant activities, antioxidant capacity of plasma represented by its reducing ability significantly declined in the prochloraz concentration which caused a significant enhancement of the ceruloplasmin activity in carp.

FRAP

Ferric reducing ability of plasma is a non-specific test, which measures the ferric-to-ferrous iron reduction in the presence of antioxidants. The antioxidant compounds contributing to the ferric reducing ability of plasma are uric acid (60%), ascorbate (15%), proteins (10%), alpha-tocopherol (5%), bilirubin (5%), and other unknown antioxidants (5 %) (Regano *et al.* 2008). The FRAP assay does not measure the SH-group-containing antioxidants (Cao & Prior 1998). Dabrowski *et al.* (2004) reported a significant decline of FRAP in *O. mykiss* in sequel to a decrease in water oxygen saturation level, the decline was not affected by either enhanced or lowered dietary supplementation of vitamin C.

Histological examination

An increased activity of skin mucous cells is usually observed in fish owing to chemical irritation. The mucus serves a protective role and reduces the hydrodynamic resistance to swimming (Kleinow *et al.* 2008). Thus, depression of the mucus production is a consid-

erable disadvantage for the specimen. Histopathological founding in gills is a non-specific sign of the polluted aquatic environment. The effects of toxicants manifest as interaction with the ion-transporting mechanism of the gills and increased permeability of the branchial epithelia to water and ions (Wendelaar Bonga & Lock 2008).

In conclusion, the subchronic exposure of common carp to a prochloraz based formulation influenced many parameters tested, including HSI, xenobitic metabolizing enzymes, selected plasma indices and red blood cell count, affected antioxidant activities and caused minor histological impairment of the test fish.

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