Effect of zeta-cypermethrin on common carp (*Cyprinus carpio* L.)

Alzbeta Stara [1], Christoph Steinbach [1], Teresa Wlasow [2], Piotr Gomulka [2], Elzbieta Ziemok [2], Jana Machova [1], Josef Velisek [1]

1 Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic
2 Faculty of Environmental Science and Fisheries, University of Warmia and Mazury, Olsztyn, Poland

Correspondence to: Dipl.-Ing. Alzbeta Stara
University of South Bohemia in Ceske Budejovice,
Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses,
Research Institute of Fish Culture and Hydrobiology,
Zatisi 728/II 389 25 Vodnany, Czech Republic
TEL: + 420 383 384 622; E-MAIL: staraa01@frov.jcu.cz

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Abstract

OBJECTIVES: The aim of this study was to assess the effect of pesticide Fury 10 EW, containing zeta-cypermethrin 100 g.l^{-1}, on common carp (*Cyprinus carpio* L.).
DESIGN: The toxicity tests were performed on common carp according to OECD 203 methodologies. The common carp were exposed to Fury 10 EW at concentrations, 5, 7, 10, 50 and 100 µg.l^{-1} for 96 h and compared to common carp in a non-treated control group. Acute toxicity tests were detected value 96hLC50=13.8 µg.l^{-1}. On the basis of the results was assessed the effect on the hematological profile, oxidative stress parameters, and antioxidants biomarkers in tissues, in another acute test.
RESULTS: The observed 96hLC50 value of Fury 10 EW was 13.8 µg.l^{-1}. A significantly lower large lymphocyte and monocyte count, and a significantly greater number of segmented eosinophil granulocyte and higher hematocrit was found in the pesticide-exposed common carp compared to controls. Oxidative damage was not detected in the experimental common carp, however there were significant differences from control in superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) in tissue after acute exposure to 13.8 µg.l^{-1} Fury 10 EW.
CONCLUSIONS: Zeta-cypermethrin as Fury 10 EW was classified as a substance highly toxic to fish. The hematological profile well oxidative stress parameters and antioxidant defensive systems provide important information about the internal environment of organisms. There is a lack of experimental results about the effects of zeta-cypermethrin on fish in the literature.

ORIGINAL ARTICLE

Abbreviations:

96hLC0	- concentration of test substance in water that kills 0% of fish tested within 96 h exposure
96hLC50	- concentration of test substance in water that kills 50% of fish tested within 96 h exposure
96hLC100	- concentration of test substance in water that kills 100% of fish tested within 96h exposure
CAT	- catalase
COD_{Mn}	- chemical oxygen demand
Er	- erythrocyte count
EW	- an emulsion, oil in water
GABA	- gamma-aminobutyric acid
GC/ECD	- gas chromatography with electron capture detector
GR	- glutathione reductase
GSH	- reduced glutathione
GSSG	- oxidized glutathione
Hb	- hemoglobin concentration
Ht	- hematocrit
Leuko	- leukocyte count
Leukogram	- differential leukocyte count
MCH	- erythrocyte hemoglobin
MCHC	- mean corpuscular hemoglobin concentration
MCV	- mean erythrocyte volume
NADPH	- nicotinamide adenine dinucleotide phosphate
NBT	- nitroblue tetrazolium
OECD	- Organization for economic cooperation and development
SD	- standard deviation
SOD	- superoxide dismutase
TBARS	- thiobarbituric acid reactive substances

INTRODUCTION

Zeta-cypermethrin is a pyrethroid compound. Pyrethroids are synthetic insecticides with chemical structure derived from natural pyrethrins produced by the ornamental *Chrysanthemum cinerariaefolium* and related species. They work by altering nerve function, causing paralysis in target insects and resulting in death (Kegley *et al.* 2010). Pyrethroids affect sodium channels of nerve axon membranes, lengthening their depolarization phase (Richterova & Svobodova, 2012), and causing neurological symptoms. They also affect GABA receptors in the nerve fibers (Hayes, 1994).

The pyrethroid zeta-cypermethrin is used for the control of insects such as beetles, aphids, and numerous Lepidoptera (*Ostrinia nubilalis*, *Sesamia nonagrioides*, *Cydia nigricana*, *Bruchus pisorum*, and *Thrips*) and is approved for use on barley, maize, peas, field beans, linseed, oats, oilseed rape, potatoes, sugar beet, vining peas, and wheat (Bastos *et al.* 2006; European Commission, 2010). The synthetic pyrethroid insecticides act on the central nervous system of vertebrates and show species-selective toxicity to fish, amphibians, mammals, birds, in that order (Edwards *et al.* 1986). Pyrethroid insecticides are extremely toxic to fish and aquatic invertebrates (Sarkar *et al.* 2005), with the 96hLC50 value determined up to 10 $mg.l^{-1}$ (Koprucu & Aydin 2004). Lethal concentration varies with species, size, and environmental (temperature, feeding) factors (Haya, 1989). Water pollution can be inferred by tissue damage, for example, in fish gills, which come into direct contact with the environment. Respiratory distress is one of the early symptoms of pesticide poisoning (Murty, 1986).

This study investigated the effects of zeta-cypermethrin on common carp (*Cyprinus carpio* L.) as reflected in hematological and antioxidant parameters and indicators of oxidative stress.

MATERIAL AND METHODS

Pesticide

The effect of zeta-cypermethrin [(S)-cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate] in the form of Fury 10 EW pesticide containing 100 $g.l^{-1}$ of active compound was assessed on common carp.

Acute toxicity test

An acute toxicity test followed the OECD Directive No. 203. Forty-eight days-old juvenile common carp of 37.63±19.53 mg (mean ± SD) mean body weight and 13.85±1.98 mm mean body length were used. Groups of ten fish each were exposed to Fury 10 EW at concentrations of 5, 7, 10, 50 and 100 $\mu g.l^{-1}$. A sixth group without pesticide exposure served as control. The physical and chemical indices of diluting water were used for the acute toxicity test were: total ammonia 0.02 $mg.l^{-1}$; NO_3^- 1.55 $mg.l^{-1}$; NO_2^- 0.006 $mg.l^{-1}$; PO_4^{3-} 0.09 $mg.l^{-1}$; chemical oxygen demand – COD_{Mn} 0.6 $mg.l^{-1}$. Water temperature ranged from 20.0–22.5 °C, oxygen saturation was 82–97%, pH 8.4–8.6, throughout the exposure. The test was performed semi-statically for 96 h with the bath was changed every 24 h. The LC0, LC50, and LC100 values at the respective time intervals were determined by probit analysis.

Acute toxicity test 96hLC50

On the based results acute toxicity test was determined 96hLC50 value of 13.8 $\mu g.l^{-1}$ Fury 10 EW for common carp. On the basis of this result was performed another test on hematological examination, oxidative stress and antioxidants parameters. For this acute toxicity test 96hLC50 was used common carp 12–18 month old (160.21±29.82 g mean weight and 22.14±1.41 cm mean body length). The trial was conducted in 300 l aquaria each stocked with 15 fish (2 control groups, 2 groups exposed to Fury 10 EW at 13.8 $\mu g.l^{-1}$). The test was performed semistatically with the bath exchange every 12 h. The tested substance was maintained at above 80% of the nominal concentration throughout the experimental period. The zeta-cypermethrin concentration in water was analyzed using gas chromatography with electron capture detection (GC/ECD). The water was extracted with ethyl acetate and the extract dried with sodium sulphate,, concentrated and then analyzed by GC/ECD. The reporting limit for this method is 0.01 $\mu g.l^{-1}$ (Mekebri *et al.* 2008). Water temperatures during the test ranged from 22.7–23.4 °C, oxygen saturation was 79.57±13.51% (mean±SD),

pH 7.76–8.35. From each group were randomly collected 15 fish for hematological examination of blood and tissues (muscle, liver, gills, brain, intestine) for the determination of oxidative stress parameters, antioxidant enzymes after the end of test.

Hematological examination

Blood samples were drawn into heparinized needles directly from the heart of fish stunned by a blow to the head. An aqueous solution of heparin sodium salt at 0.01 ml per 1 ml blood was used to stabilize blood samples. The indices evaluated included hematocrit (Ht), hemoglobin concentration (Hb), erythrocyte count (Er), mean erythrocyte volume (MCV), erythrocyte hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocyte count (Leuko), and the differential leukocyte count (Leukogram). The procedures were based on standard methods for hematological examination of fish (Svobodova *et al.* 1991).

Indices of oxidative stress and antioxidant parameters

The TBARS method described by Lushchak *et al.* (2005) was used to evaluate lipid peroxidation. Total superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by the method of Marklund and Marklund (1974). Superoxide dismutase activity was assessed spectrophotometrically at 420 nm and expressed as the nmol of NBT per min per mg protein. The catalase (CAT; EC 1.11.1.6) activity assay, using the spectrophotometric measurement of H_2O_2 breakdown at 240 nm, was performed following the method of Beers and Sizer (1952). Glutathione reductase (GR) activity was determined spectrophotometrically, measuring NADPH oxidation at 340 nm (Carlberg & Mannervik 1975). Protein levels were estimated spectrophotometrically by the method of Bradford (1976) using bovine serum albumin as a standard.

Statistical analysis

The statistical software program STATISTICA (version 8.0 for Windows, StatSoft) was used to compare differences among the test groups. Prior to analysis, all measured variables were checked for normality (Kolmogorov-Smirnov test) and homoscedasticity of variance (Bartlett's test). If these conditions were satisfied, a one-way analysis of variance (ANOVA) was employed to determine differences in measured variables among experimental groups. When a significant difference was detected ($p<0.05$), Dunnett's multiple range test was applied. If the conditions for ANOVA were not satisfied, a non-parametric test (Kruskal-Wallis) was used.

RESULTS

Acute toxicity

The acute toxicity tests showed 96 hour lethal concentrations of Fury 10 EW to be 96hLC0 4.1 μg.l^{-1}, 96hLC50 13.8 μg.l^{-1}, and 96hLC100 47.3 μg.l^{-1}.

The 96hLC50 is the basic value in the acute toxicity test. For common carp, the 96hLC50 value of Fury 10EW was 13.8 μg.l^{-1}, which corresponded to 1.38 μg.l^{-1} zeta-cypermethrin. During exposure at toxic levels, the following clinical symptoms were observed in the highest tested concentrations to Fury 10 EW (50 and 100 μg.l^{-1}): accelerated respiration alternating with rest phases, loss of movement coordination, jerky movements, seizures alternating with lethargy. Fish were at the bottom aquarium of the resting phase, gasping for breath, and subsequently exhibited short periods of excitation (convulsions, jumping above the water surface), then reverting to a resting stage. Finally, fish lay on the tank bottom, moving mainly at the flank. Respiration slowed, and death followed.

Acute toxicity test 96hLC50

Fish behavior during the experiment

During the experiment, control and exposed (13.8 μg.l^{-1} Fury 10 EW) common carp to showed normal feeding behavior. There were no signs of respiratory distress, such as rapid ventilation, increased rate of gill opercular movements, or floating at the surface of water. There were no mortalities during the experiment.

Hematological profile

Compared to the control common carp, the acute exposure to Fury 10 EW at a concentration of 13.8 μg.l^{-1} showed significantly ($P < 0.05$) increased Ht (Table 1). A significant reduction was observed in counts of large lymphocytes, monocytes and significantly increased showed segmented eosinophil granulocytes in experimental groups compared to the control (Table 2).

Oxidative stress parameters

No significant differences were observed between the levels of TBARS in common carp exposed to Fury 10 EW and that in controls (Table 3).

Tab. 1. Hematological parameters in common carp affected by acute exposure to 13.8 μg.l^{-1} Fury 10 EW.

Hematological Index	Control group (n=15) *x*±SD	Experimental group (n=15) *x*±SD
Ht (l.l^{-1})	0.30±0.06	0.36±0.04*
Hb (G.l^{-1})	71.05±17.51	80.93±8.13
Er (T.l^{-1})	1.02±0.19	1.28±0.14
MCV (fl)	295.07±42.88	281.32±44.54
MCH (pg)	69.65±15.08	63.89±9.64
MCHC (G.l^{-1})	0.23±0.03	0.23±0.02

Experimental groups significantly different ($p<0.05$) from the control group.

Antioxidant response

Significantly higher (p<0.05) SOD activity compared to control common carp was observed in the intestine of experimental fish after 96 hours of exposure to Fury 10 EW (Table 4). Glutathione reductase activity was significantly higher (p<0.01) in gill, and lower in muscle, in the experimental group compared to the control group (Table 5). Catalase activity was significantly decreased (p<0.05) in the experimental group compared with control in brain and intestine, but significantly higher in liver (p<0.01) (Table 6).

DISCUSSION

The observed 96hLC50 value indicates that the preparation Fury 10 EW is highly toxic to fish. The risk phrase R50 states the value of 96hLC50 less than 1 mg. l^{-1}. The observed Fury 10 EW 96hLC50 value in common carp of 13.8 µg.l^{-1} corresponds to 1.38 µg.l^{-1} zeta-cypermethrin. The European Food Safety Authority (2008) reported 96hLC50 of zeta-cypermethrin as 0.69 µg.l^{-1} in rainbow trout (*Oncorhynchus mykiss*) and 2.37 µg.l^{-1} in sheepshead minnow (*Cyprinodon variegatus*). Caliskan *et al.* (2003) determined 96hLC50 of 21.35 µg.l^{-1} in common guppy (*Lebistes reticulatus*). Pyrethroids are highly toxic to fish compared to other animals, due partly to species specific differences in pyrethroid metabolism, but principally to the increased sensitivity of the fish nervous system to these pesticides (Moore

Tab. 2. Leukocyte differential count in common carp following acute exposure to 13.8 µg.l^{-1} Fury 10 EW.

Hematological Index	Control group (n=15) *x*±SD	Experimental group (n=15) *x*±SD
Leuko (G.l^{-1})	3.31±0.95	3.52±1.15
Lymphocytes big (G.l^{-1})	0.63±0.29	0.42±0.20*
Lymphocyte small (G.l^{-1})	2.19±0.70	2.39±0.80
Monocyte (G.l^{-1})	0.13±0.07	0.07±0.07*
Myelocyte (G.l^{-1})	0.02±0.02	0.05±0.05
Neutrophil granulocytes segments (G.l^{-1})	0.02±0.05	0.06±0.05
Neutrophil granulocytes bands (G.l^{-1})	0.02±0.03	0.03±0.03
Eosinophil granulocytes segments (G.l^{-1})	0.14±0.06	0.28±0.15*
Eosinophil granulocytes bands (G.l^{-1})	0.14±0.10	0.23±0.13
Basophil granulocytes (G.l^{-1})	0.00±0.00	0.00±0.01
Plasmocytes (G.l^{-1})	0.01±0.01	0.00±0.01

Experimental groups significantly different (p<0.05) from the control group.

Tab. 3. TBARS (nmol.mg^{-1} protein) content in tissues of common carp following acute exposure to 13.8 µg.l^{-1} Fury 10 EW.

Tissue	Control group (n=15) *x*±SD	Experimental group (n=15) *x*±SD
Brain	0.452±0.03	0.461±0.04
Gill	0.438±0.02	0.409±0.09
Intestine	0.552±0.12	0.478±0.07
Liver	0.987±0.17	1.019±0.27
Muscle	0.905±0.16	0.902±0.12

Tab. 4. SOD activity (nmol NBT.min^{-1}.mg^{-1} protein) in tissues of common carp following acute exposure to 13.8 µg.l^{-1} Fury 10 EW.

Tissue	Control group (n=15) *x*±SD	Experimental group (n=15) *x*±SD
Brain	0.288±0.02	0.282±0.04
Gill	0.339±0.02	0.426±0.13
Intestine	0.306±0.08	0.437±0.09*
Liver	0.251±0.04	0.284±0.04
Muscle	0.174±0.02	0.176±0.02

Significant differences compared with control value (*p<0.05).

Tab. 5. GR activity (nmol NADPH.min^{-1}.mg^{-1} protein) in tissues of common carp following acute exposure to 13.8 µg.l^{-1} Fury 10 EW.

Tissue	Control group (n=15) *x*±SD	Experimental group (n=15) *x*±SD
Brain	0.233±0.11	0.367±0.31
Gill	0.389±0.10	0.642±0.17**
Intestine	0.155±0.03	0.168±0.03
Liver	0.242±0.11	0.258±0.13
Muscle	1.526±0.70	0.611±0.38**

Significant differences compared with control value (**p<0.01).

Tab. 6. CAT activity (µmol H_2O_2.min^{-1}.mg^{-1} protein) in tissues of common carp following acute exposure to 13.8 µg.l^{-1} Fury 10 EW.

Tissue	Control group (n=15) *x*±SD	Experimental group (n=15) *x*±SD
Brain	0.097±0.02	0.068±0.02*
Gill	0.121±0.02	0.102±0.03
Intestine	0.576±0.16	0.339±0.11*
Liver	2.874±0.57	3.550±0.38**
Muscle	0.096±0.03	0.076±0.02

Significant differences compared with control value (*p<0.05, **p<0.01).

and Waring 2001). Fish have been shown to be deficient in enzymes that hydrolyze these insecticides (Aydin *et al.* 2005).

In our experiments with common carp, a significant decrease in large lymphocyte and monocyte counts and significant increase in segmented eosinophil granulocyte and hematocrit was observed in the experimental group. These blood parameters can provide important information about changes in the organism (Masopust, 2000). Velisek *et al.* (2011) detected changes in Er, Hb, MCV, MCHC, lymphocytes, and neutrophils, including segmentation, in rainbow trout and common carp after acute exposure to pyrethroids. They attributed these changes to organ damage and changes in hematological parameters, which can be interpreted as a compensatory response to increase or decrease in the O_2 carrying capacity of the blood, also indicating change in the water-blood barrier for gas exchange in gill lamellae. Dobsikova *et al.* (2006) observed increased Er, segmented neutrophil granulocyte and eosinophil counts, and decreased values of MCV, MCH, and lymphocyte count in common carp after acute exposure to deltamethrin. Velisek *et al.* (2009) reported increased monocytes in common carp after acute exposure to bifenthrin.

Oxidative stress, along with antioxidant systems, may be a good indicator of pesticide accumulation in fish tissue (Stara *et al.* 2012a,b, 2013). In this study, we did not observe the association of acute exposure to zeta-cypermethrin with oxidative tissue damage. However, we found statistically significant changes in antioxidant systems of SOD, CAT, and GR in common carp tissue. Pesticides, as other xenobiotics, can induce oxidative stress in organisms. Oxidative stress results in polyunsaturated fatty acid oxidation through the process known as lipid peroxidation (Hostovsky *et al.* 2012). Antioxidant enzymes such as CAT, SOD, and GR counteract the toxicity of reactive oxidative species, protect against oxidative damage to organisms, and maintain balance in the cell (Monteiro *et al.* 2006; Stara *et al.* 2012a). Superoxide dismutase catalyzes the conversion of superoxide radicals to hydrogen peroxide, while CAT converts hydrogen peroxide to water (Mansour & Mossa, 2009). Glutathione reductase catalyzes NADPH-dependent conversion of GSSG (oxidized glutathione) to GSH (reduced glutathione), and probably plays a role in preventing alteration in GSH status upon exposure to xenobiotics (Dinu *et al.* 2010). Yonar and Sakin (2011) studied the effect deltamethrin at concentrations of 0.018 and 0.036 $\mu g.l^{-1}$ on common carp for 14 days. They observed decrease SOD, CAT, and GSH activity and significantly increased levels of malondialdehyde in liver and gill. A decrease in antioxidant enzyme activity could be explained by oxidative stress caused by exposure to xenobiotics and could be a component of xenobiotic toxicity. This may be the result of excessive superoxide radical production or direct action of the pesticide on the synthesis of the enzyme. Amin and Hashem (2012) evaluated the effect of deltamethrin on oxidative stress levels in catfish (*Clarias gariepinus*). They reported that 48 h exposure to 0.75 $\mu g.l^{-1}$ deltamethrin significantly increased lipid peroxidation in liver, kidney, and gills, while catalase activity was significantly decreased in the same tissues. Dinu *et al.* (2010) exposed *Carassius auratus gibelio* to deltamethrin at a concentration of 2 $\mu g.l^{-1}$ over 14 days. Reduced activity of SOD and CAT in liver was observed after the first day of exposure. Significantly higher GR activity and lipid peroxidation was seen by day 2. In intestine, increased CAT activity was observed after 2 days and GR activity at 14 days, together with a higher level of lipid peroxidation. These differences indicate tissue specificity, which is a consequence of metabolic and anti-oxidative differences (Ognjanivic *et al.* 2008). Oxidative damage to cells and the balance of antioxidants is differs with respect to species, habitat, feeding behavior, dose, and exposure time in tested organisms (Dobsikova *et al.* 2006; Stara *et al.* 2012b).

CONCLUSION

The present study demonstrated changes in hematological profile, oxidative stress, and antioxidant defense systems in tissues of common carp after acute exposure to zeta-cypermethrin. The 96hLC50 of Fury 10 EW was found to be 13.8 $\mu g.l^{-1}$. Antioxidants and oxidative stress parameters are potential biomarkers of pollution in fish and other aquatic animals. The measured parameters can provide useful information for evaluating the toxicological effects of pyrethroids on fish.

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REFERENCES

1 Amin KA, Hashem KS (2012). Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (*Clarias gariepinus*): antioxidant defense and role of alpha-tocopherol. Vet Res. **8**: 45.
2 Aydin R, Koprucu K, Dorucu M, Koprucu SS, Pala M (2005). Acute toxicity of synthetic pyrethroid cypermethrin on the common carp (*Cyprinus carpio* L.) embryos and larvae. Aquacul Int. **13**: 451–458.
3 Bastos CS, de Almeida RP, Suinaga FA (2006). Selectivity of pesticides used on cotton (*Gossypium hirsutum*) to *Trichogramma pretiosum* reared on two laboratory-reared hosts. Pest Manag Sci. **62**: 91–98.
4 Beers RF, Sizer IW (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. **195**: 133–140.

5 Bradford MM (1976). Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein dye binding. Anal Biochem. **72**: 248–254.
6 Caliskan M, Erkmen B, Yerli SV (2003). The effects of zeta cypermethrin on the gills of common guppy *Lebistes reticulatus*. Environ Toxicol Pharmacol. **14**: 117–120.
7 Carlberg I, Mannervik B (1975). Purification and characterization of flavoenzyme glutathione reductase from rat liver. J Biol Chem **250**: 5475–5480.
8 Dinu D, Marinescu D, Munteanu MC, Staicu AC, Costache M, Dinischiotu A (2010). Modulatory effects of deltamethrin on antioxidant defense mechanisms and lipid peroxidation in *Carassius auratus gibelio* liver and intestine. Arch Environ Contam Toxicol. **58**: 757–764.
9 Dobsikova R, Velisek J, Wlasow T, Gomulka P, Svobodova Z, Novotny L (2006). Effects of cypermethrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). Neuroendocrinol Lett. **27**: 91–95.
10 Edwards R, Millburn P, Hudson HD (1986). Comparative toxicity of cis-cypermethrin in rainbow trout, frog, mouse, and quail. Toxicol Appl Pharmacol. **84**: 512–522.
11 European Commission (2010). Review report for the active substance zeta-cypermethrin finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 23 January 2009 in view of the inclusion of zeta-cypermethrin in Annex I of Directive 91/414/EEC. Health and consumers directorate-general, 8 p.
12 European Food Safety Authority (EFSA) 2008. Conclusion regarding the peer review of the pesticide risk assessment of the active substance zeta-cypermethrin. EFSA **196**: 1–119.
13 Haya K (1989). Toxicity of pyrethroid insecticides to fish. Environ Toxicol Chem. **8**: 381–391.
14 Hayes AW (1994). Principles and Methods of Toxicology. New York, Raven Press, 1468 pp.
15 Hostovsky M, Blahova J, Plhalova L, Stepanova S, Praskova E, Marsalek P, Svobodova Z (2012). Oxidative stress parameters in early developmental stages of common carp (*Cyprinus carpio* L.) after subchronic exposure to terbuthylazine and metribuzin. Neuroendocrinol Lett. **33**: 124–129.
16 Kegley SE, Hill BR, Orme S, Choi AH (2010). PAN Pesticide Database, Pesticide Action Network, North America. http:www.pesticideinfo.org.
17 Koprucu K, Aydin R (2004). The toxic effects of pyrethroid deltamethrin on the common carp (*Cyprinus carpio* L.) embryos and larvae. Pest Biochem Physiol. **80**: 47–53.
18 Lushchak VI, Bagnyukova TV, Husak VV, Luzhna LI, Lushchak OV, Storey KB (2005). Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in goldfish tissues. Int J Biochem Cell Biol. **37**: 1670–1680.
19 Mansour SA, Mossa AH (2009). Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pest Biochem Physiol. **93**: 34–39.
20 Marklund S, Marklund G (1974). Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. **47**: 469–474.
21 Masopust J (2000). Clinical Biochemistry . Prague, Karolinum, 832 pp. (in Czech)
22 Mekebri A, Crane DB, Blondina GJ, Oros DR, Rocca JL (2008). Extraction and analysis methods for the determination of pyrethroid insecticides in surface water, sediments and biological tissues at environmentally relevant concentrations. Bull Environ Contam Toxicol. **80**: 455–460.
23 Monteiro DA, Almeida JA, Rantin FT, Kalinin AL (2006). Oxidative stress biomarkers in the freshwater characid fish *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (Methyl parathion). Comp Biochem Physiol C. **143**: 141–149.
24 Moore A, Waring CP (2001). The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in atlantic salmon (*Salmo salar* L.). Aquatic Toxicol. **52**: 1–12.
25 Murty AS (1986). Toxicity of Pesticides to Fish. Boca Raton, CRC Press, 160 pp.
26 Ognjanivic BI, Milovanovic JG, Dordevic NZ, Markovic SD, Zikic RV, Stajn AS, et al (2008). Parameters of oxidative stress in liver and white muscle of hake (*Merluccius merluccius* L.) from the Adriatic Sea. Kragujevac J Sci. **30**: 137–144.
27 Richterova Z, Svobodova Z (2012). Pyrethroids influence on fish. Slov Vet Res. **49**: 63–72.
28 Sarkar B, Chatterjee A, Adhikari S, Ayyappan S (2005). Carbofuran- and cypermethrin-induced histopathological alterations in the liver of *Labeo rohita* (Hamilton) and its recovery. J Appl Ichthyol.**21**: 131–135.
29 Stara A, Machova J, Velisek J (2012a). Effect of chronic exposure to prometryne on oxidative stress and antioxidant response in early life stages of common carp (*Cyprinus carpio* L.). Neuroendocrinol Lett. **33**: 130–135.
30 Stara A, Machova J, Velisek J (2012b). Effect of chronic exposure to simazine on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.). Environ Toxicol Pharm. **33**: 334–343.
31 Stara A, Kristan J, Zuskova E, Velisek J (2013). Effect of chronic exposure to prometryne on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.). Pesticide Biochem Physiol. **105**: 18–23.
32 Svobodova Z, Pravda D, Palackova J (1991). Unified methods of haematological examination of fish (in Czech). Methods No. 22, Research Institute of Fish Culture and Hydrobiology, Vodnany, 31 pp.
33 Velisek J, Stara A, Svobodova Z (2011). The effects of pyrethroid and trazine pesticides on fish physiology. In: Pesticides in the Modern World – Pests Control and Pesticides Exposure and Toxicity Assessment, Stoytcheva, M., (Ed.). InTech Open Access Publisher. pp. 377–402.
34 Velisek J, Svobodova Z, Machova J (2009). Effects of bifenthrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). Fish Physiol Biochem. **35**: 583–590.
35 Yonar ME, Sakin F (2011). Ameliorative effect of lycopene on antioxidant status in *Cyprinus carpio* during pyrethroid deltamethrin exposure. Pest Biochem Physiol. **99**: 226–231.