Antioxidant responses and plasma biochemical characteristics in the freshwater rainbow trout, *Oncorhynchus mykiss*, after acute exposure to the fungicide propiconazole

Z.-H. Li[1,2], V. Zlabek[1], J. Velíšek[1], R. Grabic[1], J. Machová[1], J. Kolařová[1], P. Li[1,2], T. Randák[1]

[1]University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Vodnany, Czech Republic

[2]Key field scientific observing and experimental station of fishery resources and environment of the middle and upper reaches of the Yangtze River of the Ministry of Agriculture, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Jingzhou, China

ABSTRACT: In this study, the toxic effects of PCZ, a triazole fungicide present in aquatic environment, were studied in rainbow trout, *Oncorhynchus mykiss*, by an acute toxicity test. Compared to the control group, fish exposed to PCZ (96-h-LC50, 5.04 mg/l) showed significantly higher ($P < 0.05$) plasma NH_3 and GLU concentration and the activities of plasma enzymes including CK, ALT, AST, LDH, but the TP content was not significantly different ($P > 0.05$). The oxidative stress indices (levels of LPO and CP) of brain and muscle in the experimental group were higher compared to the control group, especially for a significant change ($P < 0.05$) in the brain. SOD, CAT, GPx and GR activity in the brain of experimental groups was significantly lower ($P < 0.05$), however, an opposite tendency was found out in muscle. In addition, there are significant correlations between TBARS and CAT, TBARS and GPx, CP, and CAT, GR, and GPx in the fish brain. Thus, PCZ exposure changed the oxidative stress indices and plasma characteristics, and these changes may be used as potential bioindicators of the exposure and effect of PCZ in the controlled experiment. The use in monitoring of PCZ exposure under natural field conditions is possible, but it needs further investigations.

Keywords: triazole fungicide; fish; oxidative damage; blood parameters

List of abbreviations

PCZ = propiconazole; **ROS** = reactive oxygen species; **LPO** = lipid peroxidation; **CP** = carbonyl protein; **SOD** = superoxide dismutase; **CAT** = catalase; **GPx** = glutathione peroxidase; **GR** = glutathione reductase; **TBARS** = thiobarbituric acid-reactive substances; **DMSO** = dimethyl sulphoxide; **GSSG** = oxidized glutathione; **TCA** = trichloroacetic acid; **BHT** = butylated hydroxytoluene; **DNPH** = 2,4-di-nitrophenylhydrazine; **EDTA** = ethylenediamine-tetraacetic acid; **NADPH** = nicotinamide adenine dinucleotide phosphate; **HUFA** = highly unsaturated fatty acids; **NH_3** = ammonia; **GLU** = glucose; **TP** = total proteins; **CK** = creatine kinase; **LDH** = lactate dehydrogenase; **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase

Supported by the CENAQUA No. CZ.1.05/2.1.00/01.0024, Ministry of the Environment of the Czech Republic No. SP/2e7/229/07, the Czech Science Foundation – GACR No. P503/11/1130, the GAJU Nos. 047/2010/Z, 007/2010/Z and Special Fund for Agro-scientific Research in the Public Interest of P.R.China No. 200903048.

Propiconazole, 1-(2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl)-1H-1,2,4-triazole, is a triazole fungicide used to slow down or stop the growth of the fungus which infects agricultural fields. During its application to crops and plants it may contaminate both surface and ground waters, i.e. the aquatic environment (Li and Randak, 2009). Because of its peculiarities of non-target fungicide, many aquatic organisms (e.g. fish) can be affected (Sumpter, 2008).

Marked blood biochemical responses often occur after aquatic organisms have been exposed to environmental organic contaminants (Booth et al., 1988). Liver damage caused by these pollutants was correlated with the activities of certain plasma enzymes, such as CK, ALT, AST, ALP, LDH (Malbrouck et al., 2003), and meanwhile, the secondary toxic effect on basic metabolism was indicated by the alterations of levels of blood NH_3, GLU and TP (Zhang et al., 2008b). Therefore, the biochemical parameters of blood plasma are useful in monitoring the physiological status of fish and as indicators of the aquatic environmental health (Rehulka and Parova, 2000; Rehulka and Minarik, 2001; Dobšíková et al., 2009).

Many water contaminants can stimulate the production of ROS and result in oxidative damage to aquatic organisms (Sturve et al., 2008). Under normal conditions, ROS and other pro-oxidants are continually detoxified and removed in cells by antioxidant systems (Li et al., 2009). Some of the most important antioxidant enzymes are SOD, CAT, GPx, and GR (Li et al., 2010a,d). These systems can prevent the formation of ROS, which can react with susceptible biological macromolecules and produce LPO and CP, resulting in oxidative stress (Zhang et al., 2008a; Li et al., 2010b). Many antioxidant responses, including oxidative stress biomarkers and antioxidant enzymes activities, are used in environmental risk assessment (Song et al., 2006).

However, there are only a few reports about the ecotoxicological data on triazole fungicides (Egaas et al., 1999), and there is no information about effects of PCZ on fish oxidative stress biomarkers and plasma biochemical parameters. In this study, the rainbow trout, one of the widely used fish models in aquatic toxicology, was exposed to PCZ to determine its acute effects on physiological and biochemical responses. This was done by analysing the plasma parameters (including the enzyme activities of ALT, AST, ALP, and LDH, and the concentrations of blood NH_3, GLU and TP) as well as the antioxidant responses (SOD, CAT, GPx, GR activities) and oxidative stress indices (CP and TBARS levels) in the brain and white muscle.

MATERIAL AND METHODS

Chemicals

PCZ and other chemicals were obtained from Sigma-Aldrich Corp. (Saint Louis, USA). PCZ was dissolved in DMSO to make a stock solution at a concentration of 100 mg/ml.

Fish

Rainbow trout, weighing 69.22 ± 6.87 g (mean ± SD), were obtained from a local commercial hatchery (Husinec, Czech Republic). They were held in aquaria containing 250 l of freshwater continuously aerated to maintain dissolved oxygen values at 7.5–8.0 mg/l. The temperature was 15 ± 1°C and pH was 7.4 ± 0.2. The photoperiod was a 12:12 light-dark cycle. Fish were acclimatized for 14 days before the beginning of the experiment and were fed commercial fish food. The fish were starved for 24 h prior to exposure to avoid prandial effects during the assay and were not fed during the experimental period.

Determination of 96-h-LC50

The test was carried out in 20 l glass tanks. Seven fishes were exposed to each of the five experimental concentrations of PCZ (2, 4, 6, 8, 10 mg/l). Two other groups were used as contrast groups, one control group exposed to clean freshwater and one DMSO group to the volume of DMSO (v/v, 0.01%) used for the highest PCZ concentration. Each PCZ concentration group was duplicated. The tests were performed semi-statically for 96 h. Water was changed daily to maintain the concentrations of PCZ and DMSO and to maintain water quality. Basic physical and chemical indices of the water used in the acute toxicity test were maintained as described for the acclimation period. The tanks were checked twice a day and dead fish were removed.

Acute exposure to 96-h-LC50

The examinations were performed at the end of the acute toxicity test with PCZ at a concentration of 5.04 mg/l (96-h-LC50). Fish in the DMSO and non-treatment groups were held under conditions identical to those of the acute toxicity test and monitored concurrently. The test was performed in three 100 l tanks, each stocked with 10 fishes, i.e. one tank with 5.04 mg/l of PCZ, one control tank with clean freshwater, and a third containing the solvent (v/v, 0.01% DMSO). Each group was duplicated. The test was performed semi-statically for 96 h under the conditions described for the acute toxicity test. The medium was renewed daily to maintain the appropriate concentration of PCZ and DMSO and to maintain water quality.

Plasma biochemical characteristics

Blood samples were taken from each fish by caudal venipuncture using a syringe heparinized (Heparin inj., Léčiva, Czech Republic) at a concentration of 5000 IU heparin sodium salt in 1 ml. An aqueous solution of heparin sodium salt at 0.01 ml per 1 ml blood was used to stabilize the samples. Blood plasma obtained from cooled centrifuged blood samples (4°C, 837 × g) was stored at –80°C until use. Biochemical indices including GLU, TP, NH_3, AST, ALT, LDH, and CK were determined using a VETTEST 8008 analyzer (IDEXX Laboratories Inc., Maine, USA).

Antioxidant indices

Tissue samples and preparation of post-mitochondrial supernatant. After 96 h of exposure to PCZ, the fish of each experimental group were killed. Brain and muscle were quickly removed. The samples were immediately frozen and stored at –80°C until the biochemical determinations were carried out.

Frozen tissue samples were weighed and homogenized (1:10 w/v) in an Ultra Turrax homogenizer (Ika, Germany) using 50mM potassium phosphate buffer, pH 7.0, containing 0.5mM EDTA. The homogenate was divided into two portions, one portion for measuring TBARS and CP, and the other was centrifuged at 12 000 × g for 30 min at 4°C to obtain the post-mitochondrial supernatant for antioxidant enzyme analyses.

Indices of oxidative stress. A 500 µl aliquot of homogenate was mixed with 1 ml of 30% (w/v) TCA and centrifuged for 10 min at 5 000 × g. The supernatant was used for LPO assays and the pellet was used for CP assay.

The TBARS method described by Lushchak et al. (2005) was used to evaluate LPO in brain and white muscle. The supernatant was combined with an equal volume of TBA reagent containing a saturated solution of TBA and BHT dissolved in ethanol. The mixture was boiled for 30 min. After rapid cooling, butanol was added and mixed vigorously. Samples were centrifuged for 10 min at 5000 × g and the butanol phase was removed and used to measure TBARS. The TBARS concentration was calculated by the absorption at 535 nm and a molar extinction coefficient of 156mM/cm. The value was expressed as nanomoles of TBARS per g wet tissue weight.

Carbonyl derivatives of proteins were detected by reaction with DNPH according to the method described by Lenz (1989). The pellet from the TCA extract (above) was mixed with 1 ml of DNPH in 2M HCl. Control samples contained 1 ml of HCl instead of the DNPH solution. Samples were incubated for 1 h at 25°C and centrifuged for 10 min at 5000 × g. Supernatants were discarded and the pellets were washed three times with 1 ml of ethanol butylacetate (1:1 v/v) mixture. Pellets were dissolved in guanidine HCl and centrifuged to pellet insoluble particles. The amount of CP was measured spectrophotometrically at 370 nm using a molar extinction coefficient of 22mM/cm. The values were expressed as nanomoles of CP per g of wet tissue weight.

Assay of antioxidant enzyme activities. Total superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by the method of Marklund and Marklund (1974). This assay depends on the autoxidation of pyrogallol. SOD activity was assessed spectrophotometrically at 420 nm and expressed as the amount of enzyme per milligram of protein. The catalase (CAT; EC 1.11.1.6) activity assay, using the spectrophotometric measurement of H_2O_2 breakdown, measured at 240 nm, was performed following the method of Beers and Sizer (1952). Glutathione peroxidase (GPx; EC 1.11.1.9) activity was assayed following the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase. The specific activity was determined using the extinction coefficient of 6.22mM/cm (Lawrence and Burk, 1976). Glutathione reduct-

Table 1. Acute lethal test of PCZ toxicity to rainbow trout after 96-h exposure

Concentration of PCZ (mg/l)	log N	No. of tested fishes*	No. of dead fishes*	Mortality (%)
2	0.301	7	0	0.00
4	0.602	7	1	14.29
6	0.778	7	5	71.43
8	0.903	7	6	85.71
10	1.000	7	7	100.00

*Data are the average of two duplicate experiments; log N = logarithmic concentration of PCZ

ase (GR; EC 1.6.4.2) activity was determined spectrophotometrically, measuring NADPH oxidation at 340 nm (Carlberg and Mannervik, 1975). One unit of CAT, GPx, or GR activity is defined as the amount of the enzyme that consumes 1 μmol of substrate or generates 1 μmol of product per min; activity was expressed in international units (or milliunits) per mg of protein.

Protein estimation and statistical assays

Protein levels were estimated spectrophotometrically by the method of Bradford (1976) using bovine serum albumin as a standard. All values were expressed as mean ± SD and analyzed by SPSS for Win 13.0 software. One-way ANOVA following Tukey's test was carried out to determine whether treatments were significantly different from the control group ($P < 0.05$). Correlation analysis was performed using the Pearson correlation of SPSS for Win 13.0.

RESULTS

Acute toxicity test

As shown in Table 1, the acute toxicity of PCZ to rainbow trout was concentration-dependent. In 96-h exposure, 2 mg/l PCZ had no lethal toxicity to tested fish, and the corresponding lowest effect concentration was 4 mg/l, and the 100% lethal concentration was 10 mg/l. The 96-h-LC50 of PCZ to rainbow trout was 5.04 mg/l.

Plasma biochemical characteristics

Biochemical blood plasma profiles of the control, DMSO and experimental group are documented in Table 2. Results showed a significantly ($P < 0.05$) higher concentration of CK, ALT, AST, LDH, NH_3 and GLU in the experimental fish compared to the control, but the content of TP was comparable in

Table 2. Biochemical indices of blood plasma in rainbow trout after acute exposure to PCZ. Data are means ± SD, $n = 10$

Indices	Unit	Control group	DMSO group	Experimental group
CK	μkat/l	13.68 ± 0.53	13.90 ± 0.46	19.77 ± 0.50*
ALT	μkat/l	0.56 ± 0.23	0.53 ± 0.14	0.80 ± 0.21*
AST	μkat/l	4.77 ± 1.48	5.25 ± 1.30	11.11 ± 0.96*
LDH	μkat/l	18.06 ± 0.46	18.68 ± 0.41	21.47 ± 0.42*
NH_3	μmol/l	359.10 ± 43.54	374.80 ± 40.28	729.60 ± 56.53*
GLU	mmol/l	3.11 ± 0.18	3.39 ± 0.24	7.46 ± 0.47*
TP	g/l	29.90 ± 1.37	30.70 ± 1.10	30.40 ± 1.11

*Denotes significant differences compared with the control value in Tukey's test ($P < 0.05$)

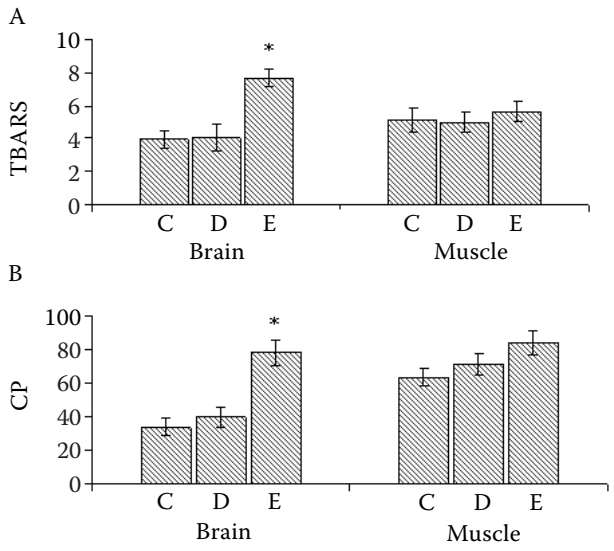

Figure 1. Effect of PCZ on level of thibarbituric acid reactive substances (TBARS, nmol/gww) and carbonyl proteins (CP, nmol/gww) in rainbow trout tissues. Control, DMSO and experimental groups' values are shown in column C, D, E, respectively. Data are mean ± SD, n=10

*Denotes significant differences compared with control value in the Tukey's test ($P < 0.05$)

all groups. There were no significant differences between the control and DMSO group.

Antioxidant indices

Levels of lipid peroxidation and carbonyl protein in the tissues of rainbow trout after acute exposure to PCZ are summarized in Figure 1. The levels of TBARS and carbonyl protein in the muscle of experimental group were little higher, but not significantly ($P > 0.05$) compared to the control group. However, significantly higher ($P < 0.05$) TBARS and carbonyl protein (1.95-fold and 2.33-fold, respectively) were observed in the brain. There was no significant difference ($P > 0.05$) in TBARS and CP between the control and DMSO group.

The activities of antioxidant enzymes in the rainbow trout tissues are shown in Figure 2. The acute exposure to PCZ caused a significantly lower ($P < 0.05$) level of the activity of SOD, CAT, GPx, and GR (0.47-fold, 0.68-fold, 0.46-fold and 0.64-fold, respectively) in the brain of the experimental group when compared to the control. However, in general,

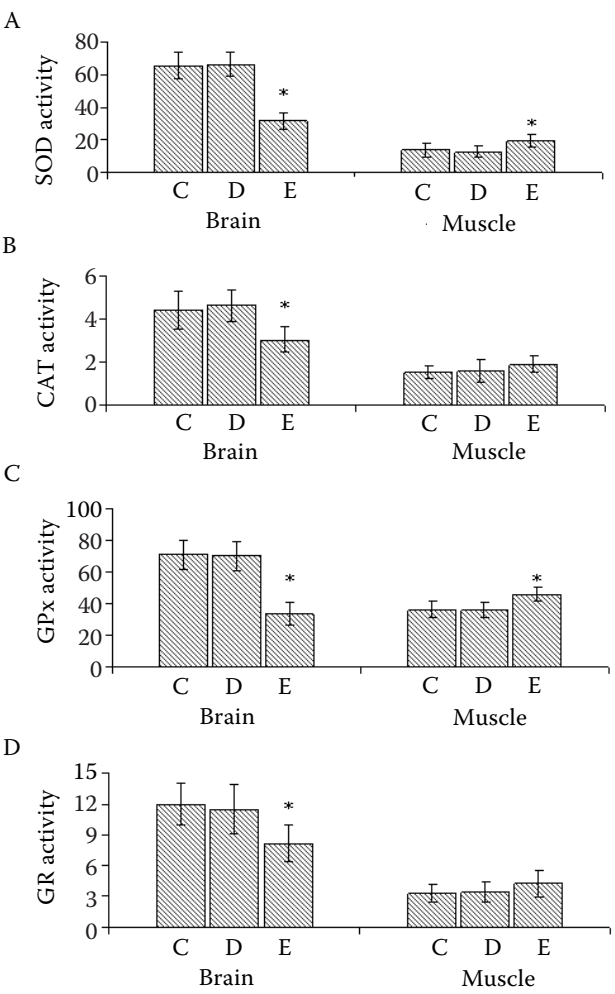

Figure 2. Effect of PCZ on superoxide dismutase (SOD, IU/mg protein) activities, catalase (CAT, IU/mg protein) activities, glutathione peroxidase (GPx, mU/mg protein) activities, glutathione reductase (GR, mU/mg protein) activities in rainbow trout tissues

the activities of antioxidant enzymes in the muscle were higher than those in the controls, but both the enzyme activities and the extent of changes were not more obvious than those in the brain. Furthermore, CAT and GR in the muscle showed no significant increase ($P > 0.05$). For the activities of all antioxidant enzymes, no significant differences were observed between the control and DMSO group.

Table 3 shows the correlation between the oxidative stress parameters and the activities of antioxidant enzymes in the brain of rainbow trout. A significant correlation ($P < 0.05$) between TBARS and CAT, TBARS and GPx, CP, and CAT, GR, and GPx was observed.

Table 3. The correlation (*r*) between the oxidative stress parameters and the activities of antioxidant enzymes in the brain of rainbow trout

		TBARS	CP	SOD	CAT	GR
CP	*r*	0.92				
	P	0.07				
SOD	*r*	–0.83	–0.75			
	P	0.06	0.12			
CAT	*r*	–0.91	–0.89	0.92		
	P	0.04	0.04	0.06		
GR	*r*	–0.72	–0.81	0.86	0.94	
	P	0.08	0.09	0.08	0.14	
GPx	*r*	–0.95	–0.86	0.82	0.90	0.96
	P	0.01	0.07	0.09	0.09	0.04

DISCUSSION

The worldwide occurrence of residual pesticides in aquatic environment makes it necessary to perform environmental risk assessment procedures to monitor the effects of pesticides on fish and other aquatic organisms. Oxidative stress biomarkers and blood parameters are valuable tools in this regard (Romero-Ruiz et al., 2003; Li et al., 2010b).

Plasma biochemical characteristics

Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Rehulka and Parova, 2000; Li et al., 2010b). Plasma enzymes, CK, AST, ALT and LDH, are frequently used to determine the toxic effects of various pollutants (Li et al., 2010b). CK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine and adenosine diphosphate (ADP), as a plasma marker of environmental stress (Li et al., 2010b). Increased release of ALT into the blood is indicative of damage to the integrity of hepatocyte membranes and the elevated AST activities are due to mitochondrial disruption as a consequence of heavy hepatitis (Qiu et al., 2009). LDH is a tetrameric enzyme recognized as a potential marker for assessing the toxicity of a chemical. The elevated levels of LDH in the haemolymph might be due to the release of isozymes from the destroyed tissues (Mishra and Shukla, 2003). In this study, after acute exposure to PCZ, the CK, LDH, AST and ALT levels were significantly higher than those in the control, which agreed with the previous results (El-Sayed et al., 2007). According to our results, PCZ caused a higher plasma ammonia concentration compared to the control, since detoxifying mechanisms were supposedly unable to convert the toxic ammonia to less harmful substances, which is in accordance with similar results of the same fish exposed to deltamethrin (Li et al., 2010b). Moreover, a significantly higher glucose concentration was observed, which demonstrated the response of exposed fish to metabolic stress.

Antioxidant indices

The toxicity of many contaminants in aquatic organisms is mediated through oxidative damage when reactive oxygen species (ROS) are formed. Under normal conditions, ROS are removed from the cell by the action of antioxidant defence systems. If the production of ROS is in excess, the balance between the formation and removal of ROS will be destroyed and it will produce the oxidative stress (Li et al., 2010c).

Lipid peroxidation (LPO) has been reported as a major contributor to the loss of the cell function under oxidative stress conditions and it is usually indicated by TBARS in fish (Oakes and Van der Kraak, 2003). Considering that the typical reaction during ROS-induced damage involves the peroxidation of unsaturated fatty acids, our results clearly

showed that the fish exposure to PCZ for 96 h led to oxidative stress, with higher LPO levels in both tissues, including significantly higher in the brain, when compared to the control group. Besides highly oxidizable lipids, ROS could directly attack protein and it could lead to the protein carbonyl formation (Bainy et al., 1996). The formation of carbonyl proteins is non-reversible, resulting in normal protein metabolism disrupted and accumulation of damaged molecules (Zhang et al., 2008a). We observed alterations in protein carbonylation in tissues similar to those seen with LPO.

To minimise the potential toxic effects of ROS, fish have evolved an enzymatic antioxidant defence system composed of SOD, CAT, GPx, GR and other molecules to inhibit the formation of oxygen radicals (Li et al., 2010b). SOD is a primary oxygen radical scavenger of tissues converting the superoxide anion radical to H_2O and H_2O_2 (Nordberg and Arner, 2001). CAT and GPx act cooperatively as scavengers of hydrogen peroxide (both enzymes) and other hydroperoxides (GPx) (Gate et al., 1999). GR plays an important role in cellular antioxidant protection and adjustment processes of metabolic pathways (Cazenave et al., 2006). In this study, after 96 h exposure to PCZ, all antioxidant enzyme activities were higher in the muscle of *O. mykiss*, and especially SOD and GPx activities were significantly higher when compared to the control. The responses of brain were opposite to those of muscle. We observed that the activities of all antioxidant enzymes were significantly inhibited. This finding, together with findings of previous studies, indicates that the brain has a relatively low antioxidant defence system (Mates, 2000), although the brain has a high mitochondrial oxidative metabolism to meet the high ATP demand for neural processing in fish (Li et al., 2010c,e). Tissue-specific differences in the effects of PCZ on the ROS handling suggest that there are different ROS-scavenging mechanisms in different tissues (Li et al., 2010f).

Correlation analysis suggested the connection of oxidative stress with the activities of antioxidant enzymes. The significant correlation between TBARS and CAT, GPx indicates that the activities of CAT and GPx are more sensitive to lipid peroxidation in the fish brain. Catalase is mainly located in peroxisomes and is responsible for the reduction of hydrogen peroxide produced by the metabolism of long-chain fatty acids in peroxisomes while GPx catalyses the reduction of both hydrogen peroxide and lipid peroxide (Winston and Digiulio, 1991). This result suggests that the significantly higher lipid peroxidation levels were mainly caused by the inhibition of CAT and GPx in the fish brain. The significant correlation between CP and CAT was observed in this study, which is in agreement with previous studies (Bagnyukova et al., 2005; Li et al., 2010e). It indicates that the disruption of CAT genes may result in increased protein carbonyl levels in the fish brain. The significant correlation between GR and GPx may be caused by the same glutathione-related enzymatic family.

CONCLUSION

In summary, this paper reported the effects of acute exposure to the fungicide propiconazole on plasma biochemical characteristics and antioxidant stress indices in the freshwater rainbow trout, *O. mykiss*. The changes of plasma biomarkers and antioxidant indices were the physiological responses of fish to the stress of PCZ exposure. In addition, it was found out from different changes of antioxidant defence systems in muscle and brain that the brain was a more sensitive target organ to oxidative damage. According to results of the present study, the plasma indices and antioxidant responses could provide useful parameters for evaluating the physiological effects of PCZ on rainbow trout, but a more detailed laboratory study will be carried out before these findings are applied to monitor the residual PCZ in aquatic environment. However, it is well known that acute toxicity in fish is unlikely to occur at the lower measured environmental concentrations, therefore, more long-term experiments at lower PCZ concentrations are needed to validate these parameters as biomarkers in large-scale environmental monitoring programmes.

REFERENCES

Bagnyukova T.V., Vasylkiv O.Y., Storey K.B., Lushchak V.I. (2005): Catalase inhibition by amino triazole induces oxidative stress in goldfish brain. Brain Research, 1052, 180–186.

Bainy A.C.D., Saito E., Carvalho P.S.M., Junqueira V.B.C. (1996): Oxidative stress in gill, erythrocytes, liver and kidney of Nile tilapia (*Oreochromis niloticus*) from a polluted site. Aquatic Toxicology, 34, 151–162.

Beers R.F., Sizer I.W. (1952): A Spectrophotometric method for measuring the breakdown of hydrogen

peroxide by catalase. Journal of Biological Chemistry, 195, 133–140.

Booth C.E., Mcdonald D.G. Simons B.P., Wood C.M. (1988): Effects of aluminum and low pH on net ion fluxes and ion balance in the Brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences, 45, 1563–1574.

Bradford M.M. (1976): Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein dye binding. Analytical Biochemistry, 72, 248–254.

Carlberg I., Mannervik B. (1975): Purification and characterization of flavoenzyme glutathione reductase from rat liver. Journal of Biological Chemistry, 250, 5475–5480.

Cazenave J., Bistoni M.D.A., Pesce S.F., Wunderlin D.A. (2006): Differential detoxification and antioxidant response in diverse organs of *Corydoras paleatus* experimentally exposed to microcystin-RR. Aquatic Toxicology, 76, 1–12.

Dobšíková R., Svobodová Z., Bláhová J., Modrá H., Velíšek J. (2009): The effect of transport on biochemical and haematological indices of common carp (*Cyprinus carpio* L.). Czech Jornal of Animal Science, 54, 510–518.

Egaas E., Sandvik M., Fjeld E., Kallqvist T., Goksoyr A., Svensen A. (1999): Some effects of the fungicide propiconazole on cytochrome P450 and glutathione *S*-transferase in brown trout (*Salmo trutta*). Comparative Biochemistry and Physiology C, 122, 337–344.

El-Sayed Y.S., Saad T.T., El-Bahr S.M. (2007): Acute intoxication of deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical, biochemical and haematological effects. Environmental Toxicology and Pharmacology, 24, 212–217.

Gate L., Paul J., Ba G.N., Tew K.D., Tapiero H. (1999): Oxidative stress induced in pathologies: the role of antioxidants. Biomedicine and Pharmacotherapy, 53, 169–180.

Lawrence R.A., Burk R.F. (1976): Glutathione peroxidase-activity in selenium-deficient rat-liver. Biochemical and Biophysical Research Communications, 71, 952–958.

Lenz A.G., Costabel U., Shaltiel S., Levine R.L. (1989): Determination of carbonyl groups in oxidatively modified proteins by reduction with tritiated sodium borohydride. Analytical Biochemistry, 177, 419–425.

Li Z.H., Randak T. (2009): Residual pharmaceutically active compounds (PhACs) in aquatic environment – status, toxicity and kinetics: a review. Veterinarni Medicina, 52, 295–314.

Li Z.H., Zlabek V., Velisek J., Grabic R., Machova J., Randak T. (2009): Responses of antioxidant status and Na^+-K^+-ATPase activity in gill of rainbow trout, *Oncorhynchus mykiss*, chronically treated with carbamazepine. Chemosphere, 77, 1476–1481.

Li Z.H., Li P., Randak T. (2010a): Ecotoxocological effects of short-term exposure to a human pharmaceutical Verapamil in juvenile rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology C, 152, 385–391.

Li Z.H., Velisek J., Zlabek V., Grabic R., Machova J., Kolarova J., Randak T. (2010b): Hepatic antioxidant status and hematological parameters in rainbow trout, *Oncorhynchus mykiss*, after chronic exposure to carbamazepine. Chemico-Biological Interactions, 183, 98–104.

Li Z.H., Zlabek V., Grabic R., Li P., Machova J., Velisek J., Randak T. (2010c): Effects of exposure to sublethal propiconazole on the antioxidant defense system and Na^+-K^+-ATPase activity in brain of rainbow trout, *Oncorhynchus mykiss*. Aquatic Toxicology, 98, 297–303.

Li Z.H., Zlabek V., Grabic R., Li P., Randak T. (2010d): Modulation of glutathione-related antioxidant defense system of fish chronically treated by the fungicide propiconazole. Comparative Biochemistry and Physiology C, 152, 392–398.

Li Z.H., Zlabek V., Velisek J., Grabic R., Machova J., Randak T. (2010e): Modulation of antioxidant defence system in brain of rainbow trout (*Oncorhynchus mykiss*) after chronic carbamazepine treatment. Comparative Biochemistry and Physiology C, 151, 137–141.

Li Z.H., Zlabek V., Velisek J., Grabic R., Machova J., Randak T. (2010f): Physiological condition status and muscle-based biomarkers in rainbow trout (*Oncorhynchus mykiss*), after long-term exposure to carbamazepine Journal of Applied Toxicology, 30, 197–203.

Lushchak V.I., Bagnyukova T.V., Husak V.V., Luzhna L.I., Lushchak O.V., Storey K.B. (2005): Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in goldfish tissues. International Journal of Biochemistry and Cell Biology, 37, 1670–1680.

Malbrouck C., Trausch G., Devos P., Kestemont P. (2003): Hepatic accumulation and effects of microcystin-LR on juvenile goldfish *Carassius auratus* L. Comparative Biochemistry and Physiology C, 135, 39–48.

Marklund S., Marklund G. (1974): Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, 47, 469–474.

Mates J.M. (2000): Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology, 153, 83–104.

Mishra R., Shukla S.P. (2003): Endosulfan effects on muscle malate dehydrogenase of the freshwater catfish *Clarias batrachus*. Ecotoxicology and Environmental Safety, 56, 425–433.

Nordberg J., Arner E.S.J. (2001): Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radical Research Biology and Medicine, 31, 1287–1312.

Oakes K.D., Van der Kraak G.J. (2003): Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. Aquatic Toxicology, 63, 447–463.

Qiu T., Xie P., Guo L., Zhang D. (2009): Plasma biochemical responses of the planktivorous filter-feeding silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) to prolonged toxic cyanobacterial blooms in natural waters. Environmenta Toxicology and Pharmacology, 27, 350–356.

Řehulka J., Minařík B. (2001): Effect of some physical and chemical characteristics of water on the blood indices of rainbow trout, *Oncorhynchus mykiss*, fed an astaxanthin-containing diet. Czech Journal of Animal Science, 46, 413–420.

Řehulka J., Párová J. (2000): Effect of diets with different lipid and protein contents on some blood and condition indices of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Czech Journal of Animal Science, 45, 263–269.

Romero-Ruiz A., Amezcua O., Rodriguez-Ortega M.J., Munoz J.L., Alhama J., Rodriguez-Ariza A., Gomez-Ariza J.L., Lopez-Barea J., Alhama J., Rodriguez-Ariza A., Gomez-Ariza J.L., Lopez-Barea J. (2003): Oxidative stress biomarkers in bivalves transplanted to the Guadalquivir estuary after Aznalcollar spill. Environmental Toxicology and Chemistry, 22, 92–100.

Song S.B., Xu Y., Zhou B.S. (2006): Effects of hexachlorobenzene on antioxidant status of liver and brain of common carp (*Cyprinus carpio*). Chemosphere, 65, 699–706.

Sturve J., Almroth B.C., Forlin L. (2008): Oxidative stress in rainbow trout (*Oncorhynchus mykiss*) exposed to sewage treatment plant effluent. Ecotoxicology and Environmental Safety, 70, 446–452.

Sumpter J.P. (2008): The ecotoxicology of hormonally active micropollutants. Water Science and Technology, 57, 125–130.

Winsto, G.W., Digiulio R.T. (1991): Prooxidant and antioxidant mechanisms in aquatic organisms. Aquatic Toxicology, 19, 137–161.

Zhang X., Yang F., Zhang X., Xu Y., Liao T., Song S., Wang H. (2008a): Induction of hepatic enzymes and oxidative stress in Chinese rare minnow (*Gobiocypris rarus*) exposed to waterborne hexabromocyclododecane (HBCDD). Aquatic Toxicology, 86, 4–11.

Zhang X.Z., Xie P., Wang W.M., Li, D.P., Shi Z.C. (2008b): Plasma biochemical responses of the omnivorous crucian carp (*Carassius auratus*) to crude cyanobacterial extracts. Fish Physiology and Biochemistry, 34, 323–329.

Received: 2010–01–20
Accepted after corrections: 2010–09–10

Corresponding Author

Zhi-Hua Li, University of South Bohemia Ceske Budejovice, Research Institute of Fish Culture and Hydrobiology, Departement Aquatic Toxicology and Fish Diseases, Zatisi 728/II, 389 25 Vodnany, Czech Republic
Tel. +420 383 382 402, Fax +420 383 382 396, E-mail: lizhih00@vurh.jcu.cz