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Environmental Contamination and Toxicology

Effect of Deltamethrin on the Biochemical Profile of Common Carp (*Cyprinus carpio* L.)

J. Velíšek,[1] R. Dobšíková,[2] Z. Svobodová,[2,3] H. Modrá,[2] V. Lusková[4]

[1] University of South Bohemia, Faculty of Agriculture, Studentská 13, 370 05 České Budějovice, Czech Republic
[2] University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1-3, 612 42 Brno, Czech Republic
[3] University of South Bohemia, Research Institute of Fish Culture and Hydrobiology, Zátiší 728/II, 398 25 Vodňany, Czech Republic
[4] Institute of Vertebrate Biology, AS CR Květná 8, 603 65 Brno, Czech Republic

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The contamination of surface waters by pesticides used in agriculture represents a problem of worldwide importance. The major negative impact of pesticide use upon fisheries is the accumulation of residues in fish tissues. Even though being relatively rapidly degradable, applied pesticides may be toxic for fish. Pyrethroids, synthetic analogues of pyrethrins, belong to the chemical group of non-systemic insecticides. They are very toxic to insects, amphibians and fish and are of a very low order of toxicity to birds and mammals (Bradbury and Coats 1989a). These chemicals can be divided into two major classes based on their structure, chemical and neurophysiological properties and toxicological actions. These classes are known as type I and type II pyrethroids. These compounds impact upon the central and peripheral nervous system. Their action is focused on sodium channels within the lipophilic component of the membranes at or close to the Na^+ gate proteins. They act by modulating opening and closing of the channels that can result in synaptic discharge, depolarisation and ultimately death (Roberts and Hudson 1998). Type II pyrethroids have also been found to interact with the GABA-ergic system.

Substances of this class are used to control wide-scale insect infestation in a wide range of crops, ornamentals and trees. They are also of importance to veterinary medicine for their use in ectoparasitics (Wardhaugh 2005; Bradbury and Coats 1989b). In aquaculture, pyrethroids are applied to control some parasitic diseases caused by, e.g. *Lepeophtherius salmonis* in salmon farming (Toovey and Lyndon 2000).

Deltamethrin is the first potent and photostable insecticide belonging to the type II pyrethroid group. After being used to control mosquito populations, the pesticide caused massive eel kills in Lake Balaton, Hungary, in the summer periods of 1991 and 1995. In 1995, presence of deltamethrin was demonstrated in several other fish species and sediment samples taken from the lake (Bálint et al. 1997). Acute toxicity and the effect of deltamethrin on haematological indices of the common carp were evaluated by Svobodová et al. (2003). Changes in the erythrocyte profile after exposure to deltamethrin may be referred to as possible disruption of haematopoiesis.

Correspondence to: R. Dobšíková

The present paper aims to contribute to the assessment of deltamethrin [(S)-a-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromvinyl)-2,2-dimethylcyclo-propan-carboxylate] effects by evaluation of its impact upon blood plasma biochemical indices of two-year-old common carp (*Cyprinus carpio* L.).

MATERIALS AND METHODS

The chemical formulation of the active substance deltamethrin, 25 g/L (Decis flow 2.5, Bayer CropScience GmbH, Germany) was used for testing. Blood plasma biochemical examination of two-year-old common carp (*Cyprinus carpio* L.) (M 72 polyhybrid strains of mirror carp) was performed at the end of a 96-hr acute toxicity test with 0.13 mg/L of Decis flow 2.5 (3.25 µg/L of deltamethrin). Simultaneously, the control group of carp was examined. The test was performed in 3 control aquaria and 3 aquaria with 0.13 mg/L of Decis flow 2.5. In each aquarium, five fish were kept in 200 L exposure volume, i.e. 15 experimental and 15 control fish were tested. The test was designed semistatically with bath renewal every 24 hr. Basic physical and chemical indices of the test diluting water were as follows: pH ranged from 7.8 to 7.9, $ANC_{4.5}$ (alkalinity) 1.15 mmol/L, COD_{Mn} 1.6 mg/L, BOD_5 0.79 mg/L, $NH_4^+ + NH_3$ 0.04 mg/L, $N0_3^-$ 11.5 mg/L, NO_2^- 0.005 mg/L, PO_4^{3-} 0.01 mg/L, a sum of Ca + Mg 14 mg/L. During the test, water temperature and oxygen saturation ranged from 20.0 to 21.2 °C and 84 to 99 %, respectively.

Biochemical analyses of plasma involved 14 control carp (C) (557 ± 48 g body weight) and 15 experimental carp (E) (573 ± 57 g body weight). Blood samples were obtained by cardiac puncture. Heparin in the amount of 50 IU sodium salt per 1 mL of blood was used for stabilization. Individual blood samples were centrifuged in a cooled centrifuge at 400 G for 15 min. Determined plasma biochemical indices included glucose (GLU), lactate (LACT), total proteins (TP), albumins (ALB), total globulins (GLOB), triacylglycerols (TAG), ammonia (NH_3), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkalic phosphatase (ALP), creatine kinase (CK), butyrylcholinesterase (BChE), calcium (Ca^{2+}) and inorganic phosphate (PHOS).

For plasma biochemical analysis, VETTEST 8008 analyser (IDEXX Laboratories Inc., USA; Medisoft Co.) was used. The apparatus is based upon dry chemical technology and colorimetric reaction. Sample analysis was carried out on selective testing discs (Multi-layer film slides, Kodak) by means of laser reading the bar codes. Detection limits of the methods were as follows: GLU (0.01 mmol/L), TP (1.0 g/L), ALB (1.0 g/L), GLOB (1.0 g/L), TAG (0.01 mmol/L), NH_3 (1.0 µmol/L), LDH (0.0167 µkat/L), AST (0.0835 µkat/L), ALT (0.0835 µkat/L), ALP (0.0167 µkat/L), CK (0.0167 µkat/L), Ca^{2+} (0.01 mmol/L) and PHOS (0.01 mmol/L). For the determination of CK activity, plasma was diluted 10 times with a physiological solution (0.6 g/L NaCl). BChE and LACT were determined by a COBAS MIRA automatic analyser (Hoffman, La Roche, Co., Switzerland) using the BioVendor tests No. 12061 and 12351. Detection limits of the methods were 1.35 µkat/L and 0.05 mmol/L for BChE and LACT, respectively.

QA/QC measures were consistently applied within the experiment. The measurements were carried out according to validated standard operation procedures.

The statistical analysis was conducted by basic descriptive and one-factor analysis of variance (STATISTICA, Version 6.0). The level of significance was calculated at the $p<0.01$ and $p<0.05$ levels. The program also performed categorized box and whiskers plots including box whiskers type mean, standard error of the mean (SE) and 1.96*SE.

Experiments on fish were approved by the Ethical Committee of the University of South Bohemia, Research Institute of Fish Culture and Hydrobiology Vodňany (approval No. 3/2004).

RESULTS AND DISCUSSION

During the course of deltamethrin poisoning in experimental carp the following clinical symptoms of choreoathetosis were observed: accelerated respiration, loss of movement and coordination, for example fish laying down at the bottom of the tank and moving on one spot was a common observation. Subsequently a short excitation stage (convulsions, movement in circles) was recorded. All two-year-old common carp survived at the exposure to 0.13 mg/L of Decis flow 2.5. The results of blood plasma biochemical indices in control and experimental carp are given in Table 1 and Figures 1 - 2. Exposure of carp to deltamethrin at 3.25 µg/L caused a significant increase in ammonia level ($p<0.01$) and aspartate aminotransferase ($p<0.05$) and alanine aminotransferase ($p<0.05$) activities (Figs. 1 - 2). The rest of the indices monitored were found to be at comparable levels in both groups under study, showing no significant differences between the groups.

An enhanced energy demand caused by short-term pyrethroid stress stimulates the activity of GDH (glutamate dehydrogenase) which induces glutamate fission into ammonia and α-ketoglutaric acid utilized in the TCA cycle (Philip and Rajasree 1996). In the study of Philip and Rajasree (1996), 5-d and 10-d exposure of common carp to 3.0 µg/L of cypermethrin caused decreased ammonia levels in gill, liver, brain and muscle tissues. By comparison of deltamethrin and cypermethrin acute toxicity to various fish species, deltamethrin can be supposed more toxic (Gangolli 1999). In our study, 3.25 µg/L of deltamethrin caused an increase in plasma ammonia concentration, since detoxifying mechanisms were supposedly unable to convert the toxic ammonia to less harmful substances.

The activities of plasma enzymes are also used as a relevant stress indicator. The enzymes used for that purpose are LDH, CK and the transaminases (ALT and AST). A significant increase in the activity of the above mentioned enzymes indicates stress-based tissue impairment (Svoboda 2001). After acute exposure to deltamethrin, a significant increase ($p<0.05$) in AST and ALT levels was found in treated carp in comparison to control specimens (Fig. 2). Increased activities of both transaminases indicated amplified transamination processes. An increase in transamination occurs due to amino acid input into the TCA cycle in order to cope with the energy crisis during pyrethroid-based stress (Philip et al. 1995).

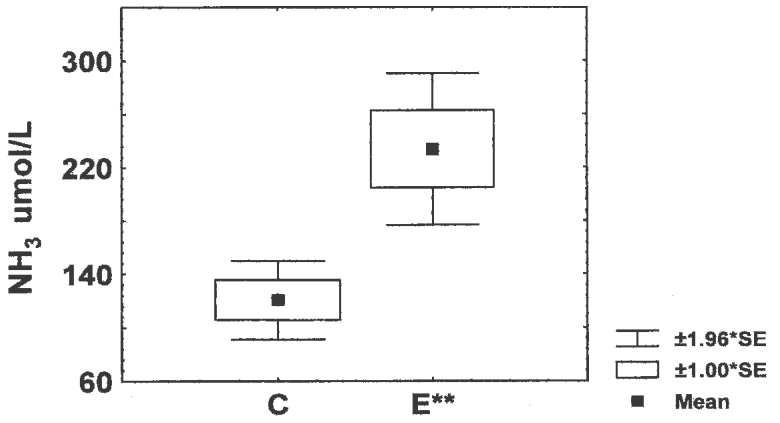

Figure 1. Effect of acute exposure to Decis flow (0.13 mg/L) on plasma ammonia concentration in carp (C - control group, E - experimental group; significance **p<0.01).

It has been suggested that the stress condition in general induces elevation of the transamination pathway (Natrajan 1985) and is likely to have contributed to toxic stress induced by deltamethrin and increased transaminase activities in the present study. Similar increases in ALT and AST activities were reported by Philip and Rajasree (1996) in gill, brain and liver of *Cyprinus carpio* during 5-d and 10-d exposure to 3.0 µg/L of the pyrethroid cypermethrin, and by Begum (2005) in gill and liver of *Clarias batrachus* after 10-d exposure to 0.07 mg/L of cypermethrin. In the study of Szegletes et al. (1995a), 96-hr exposure to 1.0 and 1.5 µg/L of deltamethrin induced AST activity in carp serum. In carp, the 2.5-fold increase in AST activity after 72-hr exposure to 2 µg/L of deltamethrin was also reported by Bálint et al. (1995).

Plasma cholinesterase (ChE) activity is composed of two distinct cholinesterases. Acetylcholinesterase (AChE) splits acetylcholine in cholinergic synapses and neuromuscular junctions. Only a small amount of AChE is found in plasma. The ChE of plasma is butyrylcholinesterase (BChE). The physiological role of BChE is still unclear. Both AChE and BChE have similar inhibitors and activators (drugs, organophosphate insecticides). Therefore, inhibition of BChE reflects inhibition of AChE (Kaneko 1989). Chuiko et al. (2003) compared the specific activities of brain and plasma AChE and BChE in 16 freshwater fish species. The correlation coefficient values between brain and plasma AChE, brain AChE and plasma BChE, plasma AChE and BChE were 0.67, 0.68 and 0.84, respectively.

In our study, we focused on measurement of BChE in plasma of common carp. In response to deltamethrin treatment, BChE activity remained almost the same as in the control fish. The difference between the groups was found to be 10.6 %.

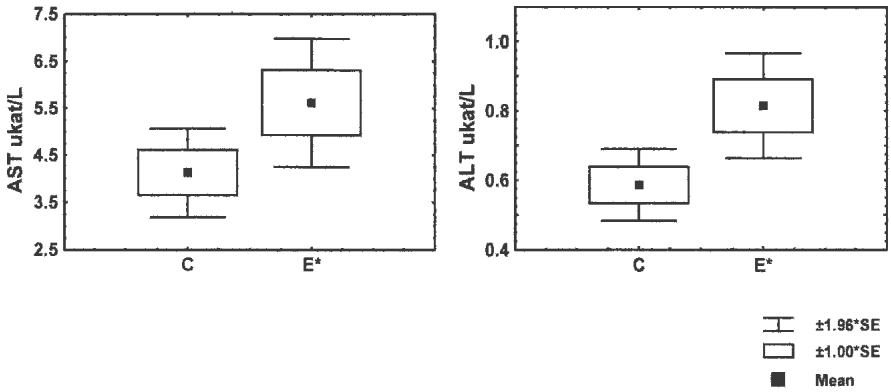

Figure 2. Effect of acute exposure to Decis flow (0.13 mg/L) on plasma AST and ALT concentrations in carp (C - control group, E - experimental group; significance *p<0.05).

In the study of Bálint et al. (1995), the effect of deltamethrin on AChE activity in different tissues of adult common carp was demonstrated using *in vitro* and *in vivo* treatments. *In vitro* kinetic studies showed inhibition of brain AChE activity. *In vivo*, the carp exposure to 2 μg/L of deltamethrin for 3 days resulted in a 21.4% decrease in AChE activity in serum of tested carp. Szegletes et al. (1995b) demonstrated that exposure to 2 μg/L of deltamethrin caused AChE activity to decrease as much as 20 % in blood plasma of *Cyprinus carpio*. The same results were observed by Bálint et al. (1997) in dying eels in Lake Balaton due to contamination by deltamethrin.

Acute toxicity of pyrethroids to fish is temperature-dependent, i.e. the higher test water temperature the lower acute toxicity of pyrethroids (Bradbury and Coats 1989a). The results of the above mentioned studies are in contrast to the results of our study, in which the difference in plasma BChE activity between experimental and control groups was found to be non-significant. The contradiction of the results can be explained by different test water temperature since in our study fish were tested at mean water temperature of 20.5 °C, while Bálint et al. (1995) and Szegletes et al. (1995b) used a water temperature of 12 °C.

The increase in blood glucose concentration demonstrated the response of exposed fish to metabolic stress. The increase in LDH level indicated metabolic changes, i.e. glycogen catabolism and glucose shift towards the formation of lactate in stressed fish, primarily in the muscle tissue (Simon et al. 1983).

In the study of Bálint et al. (1995), blood plasma LDH and glucose levels of common carp were significantly increased after the 6-hr exposure to 2 μg/L of deltamethrin, with subsequent slight decreases in their levels. The activity of LDH increased 1.5- and 2.5-fold in 6- and 72-hr samples, respectively, and glucose level was 30 % higher (6-hr sample) as compared to LDH and glucose levels of

the control carp. Szegletes et al. (1995a) reported interesting changes in blood glucose content. After 24-hr exposure to 1 μg/L of deltamethrin, fish seemed to be stressed, although the increase in glucose was not significant. When the fish became adapted to deltamethrin, the glucose level decreased, especially after 72 hours. At the same time, control animals kept in similar conditions showed a small non-significant decrease. Meanwhile, fish in aquaria containing 1.5 μg/L of deltamethrin reacted to the treatment with increased glucose level after 48 hours, and this did not change until the end of the treatment.

Table 1. Effect of acute exposure to Decis flow (0.13 mg/L) on plasma biochemical indices in carp.

Indices	Control (C) mean ± SD	Experiment (E) mean ± SD
GLU (mmol/L)	5.15 ± 1.13 [a]	5.01 ± 0.41 [a]
LACT (mmol/L)	1.28 ± 0.57 [a]	1.47 ± 0.47 [a]
TP (g/L)	36.97 ± 5.96 [a]	38.60 ± 6.77 [a]
ALB (g/L)	6.42 ± 2.41 [a]	6.47 ± 1.09 [a]
GLOB (g/L)	30.50 ± 5.29 [a]	32.13 ± 3.91 [a]
TAG (mmol/L)	2.13 ± 0.25 [a]	2.18 ± 0.31 [a]
LDH (μkat/L)	6.78 ± 2.40 [a]	6.74 ± 1.88 [a]
ALP (μkat/L)	0.23 ± 0.11 [a]	0.30 ± 0.11 [a]
CK (μkat/L)	810.86 ± 3.86 [a]	810.27 ± 4.30 [a]
BChE (μkat/L)	5.10 ± 1.03 [a]	4.56 ± 1.15 [a]
Ca^{2+} (mmol/L)	2.56 ± 0.14 [a]	2.58 ± 0.13 [a]
PHOS (mmol/L)	1.84 ± 0.25 [a]	1.94 ± 0.32 [a]

[a] No statistical significance found.

The biochemical profile of blood can provide important information about the internal environment of the organism (Masopust 2000). In our study, ammonia, AST and ALT proved to be the most sensitive parameters in two-year-old common carp. Exposure of carp to Decis flow 2.5 at 0.13 mg/L (3.25 μg/L of deltamethrin) caused significant increases in the above mentioned indices. Other biochemical indices observed were considered to be less appropriate for the evaluation of pyrethroid-based stress response in common carp.

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