Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*)

J. Velisek[1,2], T. Wlasow[3], P. Gomulka[3], Z. Svobodova[1,4], R. Dobsikova[4], L. Novotny[4], M. Dudzik[3]

[1]Research Institute of Fish Culture and Hydrobiology, Vodnany, University of South Bohemia, Ceske Budejovice, Czech Republic
[2]Faculty of Agriculture, University of South Bohemia, Ceske Budejovice, Czech Republic
[3]Faculty of Environmental Science and Fisheries, University of Warmia and Mazury, Olsztyn, Poland
[4]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: The aim of this study was to assess the effect of cypermethrin [(R,S)-α-cyano-3-phenoxybenzyl (1RS)--cis,tra-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] on rainbow trout (*Oncorhynchus mykiss*). The effect was assessed on the basis of the results of acute toxicity tests and on the comparison of results of haematological, biochemical and histopathological tissue examinations of a control and experimental group exposed to Alimetrine 10 EM pesticide preparation (active substance 100 g/l of cypermethrin). The acute semistatical toxicity test lasting 96 h was performed on rainbow trout juveniles. The 96hLC50 value of Alimethrine 10 EM was 31.4 µg/l. Examination of erythrocyte, leukocyte and biochemical profile and histopathological tissue examination was performed on 15 control and 15 experimental specimens of one-to-two-year-old rainbow trout after 96 h of exposure to Alimetrine 10 EM in the concentration of 31.4 µg/l. The experimental group showed significantly higher values ($P < 0.01$) of plasma ammonia (NH_3), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), lactate (LACT) and significantly lower ($P < 0.01$) values of alkaline phosphatase (ALP) compared to the control group. Also, a significant decrease in count of developmental forms of myeloid sequence, and segmented neutrophile granulocytes in the experimental group were found. Teleangioectasiae of secondary gill lamellae and degeneration of hepatocytes were observed with histopathological examination. No histopathological changes were demonstrated in tissues (skin, spleen, cranial and caudal kidney) of rainbow trout following exposure to cypermethrine. The cypermethrine-based Alimethrinee 10 EM pesticide preparation was classified among substances strongly toxic for fish.

Keywords: pyrethroids; acute toxicity; haematological profile; biochemical profile of blood; histopathology

Cypermethrin is a widely used pesticide based on pyrethroids. It is among the most effective pyrethroid preparations (Bradbury and Coats, 1989a). The mechanism of its effectiveness in the case of fish is the same as that of other pyrethroids containing -cyano- 3-phenoxybenzyl groups. They block the sodium channels of nerve filaments, thereby lengthening their depolarisation phase; moreover, they affect the GABA receptors in the nerve filaments (Bradbury and Coats, 1989b; Hayes, 1994).

Cypermethrin is a synthetic pyrethroid used for the control of ectoparasites which infest cat-

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tle, sheep, poultry and some companion animals. Recently, the compound has been used as a chemotherapeutic agent for the control of ectoparasite infestations (*Lepeophtheirus salmonis* and *Caligus elongatus)* in marine cage culture of Atlantic salmon, *Salmo salar* (Richards, 1983; Roth et al., 1993; Hart et al., 1997; Boxaspen and Holm, 2001; Treasurer and Wadsworth, 2004).

Cypermethrin is very toxic for fish (in laboratory tests 96hLC50 were generally within the range of 0.4–2.8 µg/l), and aquatic invertebrates LC50 in the range of 0.01–5 µg/l (Sthephanson, 1982; Sarkar et al., 2005). Fish sensitivity to pyrethroids may be explained by their relatively slow metabolism and elimination of these compounds. The half-lives for elimination of several pyrethroids by rainbow trout are all longer than 48 h, while elimination half-lives for birds and mammals range from 6 to 12 h (Bradbury and Coats, 1989b).

The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on data on the toxicity and effects of pesticide preparations to non-target organisms. Fish are among the group of non-target aquatic organisms. The present paper is a contribution to the assessment of toxicity and effects of a cypermethrin-based pesticide to fish.

MATERIAL AND METHODS

Cypermethrin [(RS)-α-cyano-3-phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] was tested in the form of Alimethrine 10 EM pesticide, containing 100 g/l of active compound. The toxic effect was assessed through the results of acute toxicity tests and results of haematological, biochemical and histopathological examination of rainbow trout after exposure to this pesticide.

Acute toxicity

The acute toxicity test on rainbow trout with Alimethrine 10 EM followed the OEMD Direction No. 203 and Methodical Manual ISO 7346/2. Juveniles of rainbow trout (camloops) of 11.71 ± 1.06 g (mean ± SD) mean body weight and 88.9 ± 14.3 mm mean body length were used for the test. Six various concentrations and a control were used in the basic test. Ten fish specimens were used for every concentration and also in the control. The test was performed semistatically for 96 hours. The bath was changed every 24 hours. Basic physical and chemical indices of diluting water used in the acute toxicity test were as follows: acid neutralization capacity – $ANC_{4.5}$ 1.15 mmol/l; total ammonia 0.04 mg/l; NO_3^- 11.5 mg/l; NO_2^- 0.005 mg/l; PO_4^{3-} 0.01 mg/l; chemical oxygen demand – COD_{Mn} 1.6 mg/l. Water temperature in the test ranged from 15.1 to 16.6°C, oxygen saturation of water ranged between 94 and 98%. The LC50, LC0 and LC100 values in the respective time intervals were determined by probit analysis.

Haematological, biochemical and histopathological examination

Haematological, biochemical and histopathological examination of rainbow trout (camloops) was performed at the end of 96 h acute toxicity test with Alimethrine 10 EM in the concentration of 31.4 µg/l. At the same time, the control group of trout was examined. The test was performed semistatically with the bath exchange every 24 hours. Diluting water had the same physical and chemical parameters as described above. Water temperatures during the test ranged from 14.2 to 15.5°C, oxygen saturation of water was above 60% (ranging from 80 to 93%), pH ranged from 8.30 to 8.54. The test was performed in three 400 l aquaria. Each aquarium was stocked with 30 specimens of one- to two-year-old rainbow trout (1 control aquarium, 2 aquaria with Alimethrine 10 EM in the concentration 31.4 µg/l).

For the haematological, biochemical and histopathological examination, rainbow trout (camloops) of 144.50 ± 44.82 g average weight and 241.77 ± 26.0 mm average body length were used.

Haematological profile

Heparinised injection needles were used to take samples of blood from the hearts of fish stunned by a blow with a blunt object over the head. To stabilize blood samples, an aqueous solution of heparin sodium salt at 0.01 ml per 1 ml blood was used (Svobodova et al., 1991).

The indices used to evaluate the haematological profile included the erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean colour concentration (MCHC), erythrocyte haemoglobin

(MCH), leukocyte count (Leuko) and the differential leukocyte count (Leukogram). The procedures were based on Unified methods for haematological examination of fish (Svobodova et al., 1991).

The results of haematological examinations were tested by variance analysis (ANOVA – Tukey's test) using the Statistica 6.0 software.

Biochemical blood plasma profile

Blood plasma was obtained by the centrifugation of blood samples in a cooled centrifuge (4°C, 837 × g). Biochemical indices determined in blood plasma included glucose (GLU), total protein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), triacylglycerols (TRIG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline phosphatase (ALP), calcium (Ca^{2+}), lactate (LACT), cholinesterase (ChE) and inorganic phosphate (PHOS). For the biochemical analysis of blood plasma, the VETTEST 8008 analyser (IDEXX Laboratories Inc., U.S.A.) manufactured by Medisoft was used. The analyser uses dry chemical and colorimetric analysis techniques. Selective test discs (Multi-layer film slides, Kodak) are used for the evaluation by a laser reading bar codes. ChE and LACT were determined by a COBAS MIRA automatic analyser (Hoffman, La Roche, Co., Switzerland) using the BioVendor tests No. 12061 and 12351.

The results of biochemical examination were tested by variance analysis (ANOVA – Tukey's test) using the Statistica 6.0 software.

Histopathological examination of tissues

After blood sampling, samples of gills, liver, skin, cranial and caudal kidney and spleen were taken for histopathological examinations. The taken samples were immediately fixed in 10% formaline, drained and embedded in paraffin. Sections were made of the paraffin blocks and stained with haematoxylin-eosin.

RESULTS

Acute toxicity

On the basis of the acute toxicity tests with rainbow trout, the 96-hour lethal concentrations of Alimethrine 10 EM were determined (96hLC50 31.4 µg/l, 96hLC0 19.8 µg/l and 96hLC100 49.6 µg/ l).

The 96hLC50 is the basic value in the acute toxicity test. For rainbow trout juveniles the 96hLC50 value was 31.4 µg/l of Alimethrine 10 EM preparation, which corresponded to 3.14 µg/l of cypermethrin. In the course of deltamethrin poisoning in rainbow trout, the following clinical symptoms were observed: accelerated respiration, loss of movement coordination, fish lay-down at their flank and are moving in this position. Subsequent short excitation stage (convulsions, jumps above the water surface, movement in circles) changes into a resting stage, and another short-time excitation follows again. In the end, fish fall into damp, move mainly at their flank. Respiration is slowed down, the damp phase and subsequent agony are very long.

Table 1. Haematological parameters in rainbow trout affected by acute exposure to Alimethrine 10 EM

Indices	Control group (n = 15) $\bar{x}$ ± SD	Experimental group (n = 15) $\bar{x}$ ± SD
RBC (T/l)	0.80 ± 0.15[a]	0.78 ± 0.24[a]
Hb (g/l)	41.71 ± 6.39[a]	42.69 ± 10.30[a]
PCV (l/l)	0.36 ± 0.04[a]	0.39 ± 0.05[a]
MCV (fl)	460.57 ± 88.82[a]	568.15 ± 291.13[a]
MCH (pg)	53.31 ± 10.87[a]	59.81 ± 23.85[a]
MCHC (g/l)	115.96 ± 12.36[a]	108.72 ± 17.68[a]

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)

Table 2. Leukocyte differencial count in rainbow trout affected by acute exposure to Alimethrine 10 EM

Indices	Control group (n = 15) $\bar{x}$ ± SD	Experimental group (n =15) $\bar{x}$ ± SD
Leuko (G/l)	13.15 ± 3.56[a]	10.22 ± 5.03[a]
Lymphocytes (G/l)	12.54 ± 3.11[a]	9.94 ± 4.08[a]
Monocyte (G/l)	0.01 ± 0.01[a]	0.01 ± 0.03[a]
Neutrophile granulocytes segments (G/l)	0.51 ± 0.19[a]	0.25 ± 0.21[b]
Neutrophile granulocytes bands (G/l)	0.05 ± 0.07[a]	0.02 ± 0.04[a]
Developmental phases – myeloid sequence (G/l)	0.03 ± 0.05[a]	0.01 ± 0.02[b]

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)

Haematological profile

The results of erythrocyte profile of the control and experimental rainbow trout under the study are given in Table 1. Compared to the control specimens, those after the acute exposure to cypermethrin at the concentration of 3.14 µg/l had no effect on the haematological indices studied (RBC, Hb, PCV, MCV, MCHC, MCH and Leuko).

It was evident that the acute exposure to cypermethrin resulted in a significant decrease in count of developmental forms of myeloid sequence and the segmented neutrophilic granulocytes in the experimental group. The results of examinations of the leukocyte profile of control and experimental rainbow trout, are given in Table 2.

Biochemical blood plasma profile

The results of biochemical blood plasma profile of the control and experimental rainbow trout under the study are given in Table 3 and Figures 1, 2 and 3. The experimental rainbow trout exposed to acute effects of the cypermethrin-based pesticide showed significantly ($P < 0.01$) decreased concentration of alkaline phosphatase and significantly ($P < 0.01$) increased concentration of ammonia,

Table 3. Biochemical indices of blood plasma in rainbow trout affected by acute exposure to Alimethrine 10 EM

Indices	Control group (n = 15) $\bar{x}$ ± SD	Experimental group (n = 15) $\bar{x}$ ± SD
GLU (mmol/l)	3.64 ± 0.75[a]	4.07 ± 1.84[a]
TP (g/l)	36.60 ± 5.14[a]	39.33 ± 4.30[a]
ALB (g/l)	6.80 ± 2.71[a]	8.60 ± 1.99[a]
GLOB (g/l)	29.80 ± 2.81[a]	30.87 ± 2.47[a]
TRIG (mmol/l)	0.97 ± 0.12[a]	0.86 ± 0.19[a]
ALT (µkat/l)	0.08 ± 0.02[a]	0.08 ± 0.01[a]
Ca^{2+} (mmol/l)	2.53 ± 0.18[a]	2.81 ± 0.38[a]
ChE (µkat/l)	2.03 ± 1.30[a]	2.52 ± 0.99[a]
PHOS (mmol/l)	1.46 ± 0.22[a]	1.39 ± 0.16[a]

Groups with different alphabetic superscripts differ significantly at $P < 0.01$ (ANOVA)

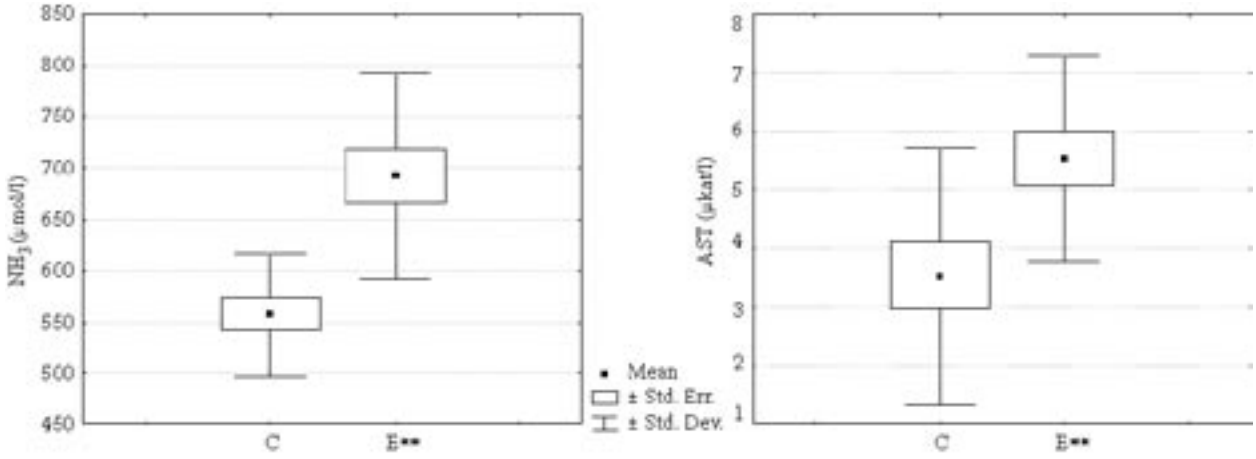

Figure 1. Effect of acute exposure to Alimethrine 10 EM (31.4 µg/l) on plasma NH_3 concentration and AST activity in rainbow trout. C – control group, E – experimental group; significance $^{**}P < 0.01$

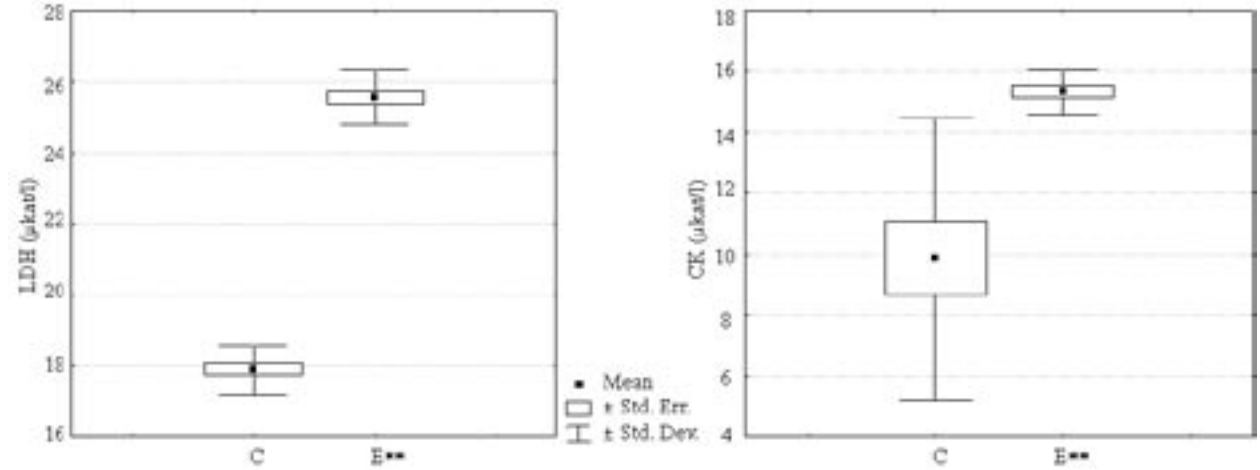

Figure 2. Effect of acute exposure to Alimethrine 10 EM (31.4 µg/l) on plasma LDH and CK activity in rainbow trout. C – control group, E – experimental group; significance $^{**}P < 0.01$

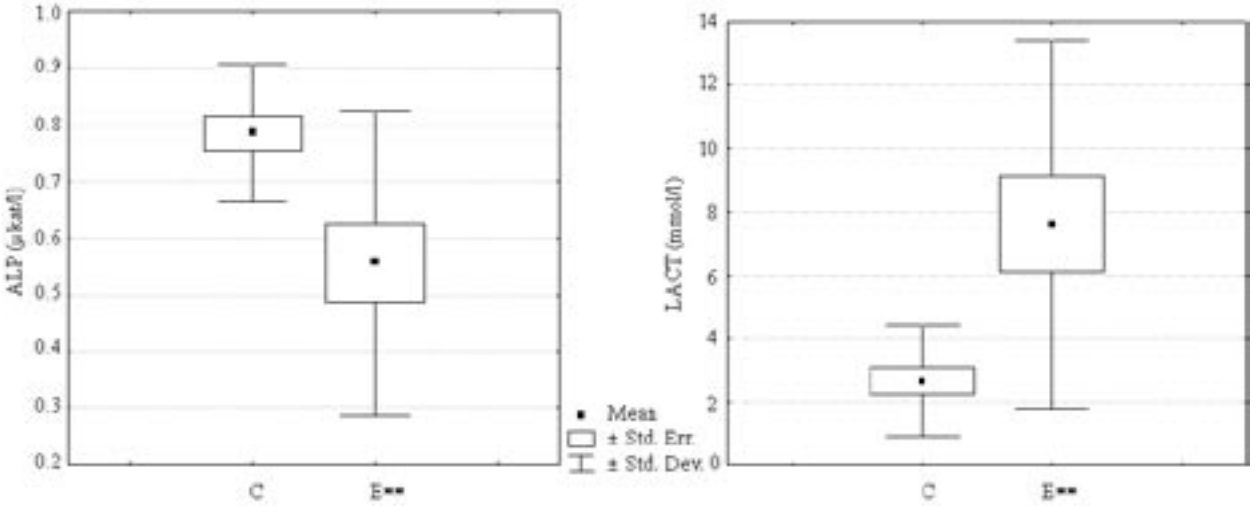

Figure 3. Effect of acute exposure to Alimethrine 10 EM (31.4 µg/l) on plasma ALP and LACT activity in rainbow trout. C – control group, E – experimental group; significance $^{**}P < 0.01$

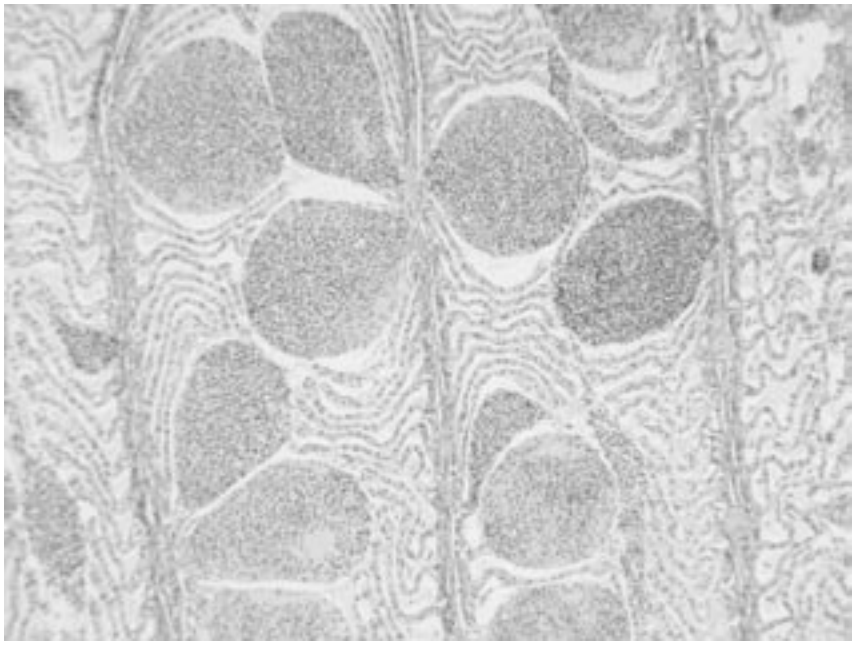

Figure 4. Gills of rainbow trout from the experimental group with the teleangioectasiae in the secondary lamellae; HE, 100×

aspartate aminotransferase, lactate dehydrogenase, creatine kinase and lactate in blood plasma. The rest of the indices (GLU, TP, ALB, GLOB, TRIG, ALT, ChE, Ca^{2+}, PHOS) were comparable in the two groups during the study.

Histopathological examination of tissues

Histopathological examination revealed severe teleagioectasiae in the secondary lamellae of gills with the rupture of pillar cells (Figure 4) in the 60 % individuals of experimental group (at the concentration of 31.4 µg/l Alimethrine 10 EM). Degeneration of hepatocytes, especially in the periportal zones, was observed in the 40 % of individuals. Affected hepatocytes showed pycnotic nuclei and many small or one big vacuole in the cytoplasm. The shape of vacuoles was typical for fatty degeneration of liver. No changes were seen in other examined organs.

DISCUSSION

On the basis of the observed 96hLC50 value, the preparation Alimethrine 10 EM can be included in a group of substances that are highly toxic for fish: the risk sentence R50 states the values of 96hLC50 less than 1 mg/l. The value of 96hLC50 for Alimethrine 10, 31.4 µg/l, essentially corresponds to 3.14 µg/l cypermethrin. Bradbury and Coats (1989b); Davis et al. (1993); Polat et al. (2002) report a mean lethal toxicity of cypermethrin to various fish species in laboratory conditions as values below 10 µg/l. Bradbury and Coasts (1989b) state LC50 0.50 µg/l and 1.2 µg/l the value for rainbow trout and brown trout (*Salmo trout*), respectively. Shires (1985) reported the value of 96hLC50 for rainbow trout to be 2.57 µg/l. Whalon et al. (1990) state the value of 24hLC50 4.50 µg/l and 20 µg/l for common carp (*Cyprinus carpio* L.) and silver carp (*Ctenopharyngodon idea*), respectively. Pyrethroids are more toxic to fish at lower temperatures and appear to be more toxic to smaller fish than larger ones (Mauck et al., 1976; Hill, 1985; Baser et al., 2003).

Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Masopust, 2000).

In our experiments with rainbow trout, a significant decrease in count of developmental forms of myeloid sequence and the segmented neutrophilic granulocytes in the experimental group was observed. No significant differences were observed in the levels of RBC, Hb, PCV, MCV, MCHC, MCH and Leuko. On the other hand, Atamanalp et al. (2002a) and Atamanalp and Yanik (2003) found a significant increase ($P < 0.05$) in the levels of RBC and a significant decrease ($P < 0.05$) in the Hb, MCH, MCHC, thrombocyte count and erythrocyte sedimentation rate in rainbow trout (*Oncorhynchus*

mykiss) following cypermethrin and mancozeb acute exposure.

The main biochemical blood profile response of rainbow trout to the acute effect of 31.4 µg/l of Alimethrine 10 EM was a significantly ($P < 0.01$) decreased concentration of alkaline phosphatase and significantly ($P < 0.01$) increased concentration of NH_3, LDH, AST, CK and LACT in blood plasma.

Cypermethrin caused an increase in plasma ammonia level supposedly due to an increase in amino acids catabolism and a failure of ammonia excretion mechanisms (Svoboda, 2001).

The activities of enzymes in blood plasma can be also used as a relevant stress indicator. The enzymes used for the purpose are above all LDH, CK and transaminases (ALT and AST). A significant increase in the activity of the above mentioned plasma enzymes indicates stress-based tissue impairment (Svoboda, 2001). After acute exposure to cypermethrin, a significant increase ($P < 0.01$) in AST level was found in experimental trout in comparison to control specimens. 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).

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

On the other hand, Atamanalp et al. (2002b) found changes in the concentration of calcium and phosphor in rainbow trout following cypermethrin exposure. Jee et al. (2005) found an increase in levels of serum glutamic-acid-oxylacetic-acid-transaminase, glutamic-acid-pyruvic-acid-transaminase, glucose and alkaline phosphatase and a decrease in the concentration of plasma total protein, albumin, cholesterol and lysozyme in Korean rockfish (*Sebastes schlegeli*) exposed to cypermethrin.

We observed teleangioectasiae of secondary lamellae of the gills and degeneration of hepatocytes in periportal zones in our experiment. Teleangioectasiae comps acute respiratory distress. Degeneration of hepatocytes in periportal zones can imply the influence of toxic compounds in the digestive tract. The biochemical changes in liver profile can relate to hepatocytes damage. Sarkar et al. (2005) found significant changes as hyperplasia, disintegration of hepatic mass, focal coagulative necrosis in *Labeo rohita* exposed to cypermethrin. Sublethal effects of pyrethroids on fish include damage of gills and behavioral changes. Because they are highly lipophilic (attracted to the non-water soluble components of cells), pyrethroids are likely to be strongly absorbed by the gills, even from water containing low levels of pyrethroids (Smith and Stratton, 1986). Edwards et al. (1986) reported acute toxicity symptoms of cypermethrin in rainbow trout such as, gill flailing, hyperactivity, loss of buoyancy and inability to remain upright.

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Corresponding Autor:

Ing. Josef Velisek, University of South Bohemia, Ceske Budejovice, Research Institute of Fish Culture and Hydrobiology, Zatisi 728/II, 389 25 Vodnany, Czech Republic

Tel. +420 383 382 402, fax +420 383 382 396, e-mail: velisek.j@centrum.cz