Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT: The effect of the pyrethroid, bifenthrin, on rainbow trout (*Oncorhynchus mykiss*) was assessed based on biochemical, haematological and histopathological examination of fish exposed to Talstar 10 EC pesticide preparation (active substance 100 g/l bifenthrin) at a concentration of 14.7 μg/l. There was a significant ($P < 0.01$) decrease in plasma ammonia, and significant ($P < 0.01$) increase in glucose, creatine kinase, alkaline phosphatase and lactate dehydrogenase. Haematologically, fish showed a significant ($P < 0.01$) decrease in mean erythrocyte volume, erythrocyte haemoglobin, and band neutrophil granulocytes compared to controls. Degeneration of hepatocytes was observed histologically. The bifenthrin-based Talstar 10 EC pesticide preparation was therefore classified as a substance strongly toxic to fish.

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

Pyrethroids, synthetic analogues of natural pyrethrins produced by the ornamental plant *Chrysanthemum cinerariaefolium*, were developed in the 1970s to protect food grains and other agricultural products against pests and, later, began to be used to control animal ectoparasites. Their use has increased rapidly in the past two decades (Bradbury and Coats, 1989a; Wardhaugh, 2005). Despite having low toxicity for mammals and birds (Bradbury and Coats, 1989b), they present a risk for aquatic organisms. As their 96h LC50 for fish is below 30 μg/l, pyrethroids belong to a group of chemicals highly toxic to fish and other aquatic organisms (Dobsikova et al., 2006; Velisek et al., 2006a, 2007).

Bifenthrin [2-methylbiphenyl-3-ylmethyl (Z)-(1RS, 3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] is a third-generation synthetic pyrethroid insecticide characterized by strong environmental persistence and high insecticidal activity (Mokry and Hoagland, 1989). It is effective as a gut or contact insecticide that affects the nervous system of vertebrates and invertebrates. Bifenthrin acts on sodium channels at the nerve cell endings to depolarize the presynaptic terminals. It also affects cellular ATPase production (Roberts and Hutson, 1999).

Pesticides are one of the most potentially harmful chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are significant (John, 2007; Hazarika and Das, 1998). The contamination of surface waters by pesticides used in agriculture is a problem of worldwide importance (Hill, 1985; Sibley and Kaushik, 1991). Although the chance of pyrethroids entering aquatic environments is minimal, the risk of

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occasional contamination of water by pyrethroids should not be underestimated (Shan et al., 1997). Fish are sensitive aquatic organisms and show a wide range of responses to changes in their environment, as has been amply demonstrated by massive eel devastation in Lake Balaton in 1991 and 1995 (Balint et al., 1997). The sensitivity of fish to pyrethroids may be explained by their relatively slow metabolism and, therefore, elimination of the compounds (Bradbury and Coats, 1989b).

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

The aim was to assess the effects of bifenthrin, the active ingredient of Talstar EC 10 (100 g/l bifrenthin) on rainbow trout through acute toxicity tests and haematological, biochemical and histological evaluation.

MATERIAL AND METHODS

Experimental animals

For the acute test, rainbow trout, 26.50 ± 2.21 g mean body weight and 154.0 ± 12.34 mm mean total body length, were used.

Haematological, biochemical and histological examinations were conducted on one-to-two-year-old fish of the same strain of 275.60 ± 24.53 g mean body weight and 310.50 ± 14.87 mm mean total body length.

Acute toxicity test

Talstar 10 EC acute toxicity tests were performed in accordance with OECD guidelines on testing for chemicals (OECD 203 "Fish, acute toxicity test"). For determination of 96h LC50 values, ten juvenile rainbow trout were used with each of the seven concentrations (5, 10, 15, 20, 30, 50, 70 µg/l Talstar 10 EC) tested and with the control group. Fish mortalities were recorded at 24, 48, 72 and 96 h. Fish status and behaviour along with water temperature, pH, and oxygen saturation were monitored throughout the test. The test was performed semi-statically for 96 h, with water renewal every 12 h. Basic physical and chemical indices of diluting water were: acid neutralisation capacity – $ANC_{4.5}$ 1.10 mmol/l; sum of Ca and Mg 12 mg/l, total ammonia 0.03 mg/l; $N0_3^-$ 1.11 mg/l; NO_2^- 0.004 mg/l; PO_4^{3-} 0.02 mg/l; chemical oxygen demand – COD_{Mn} 1.4 mg/l. Water temperature and oxygen saturation ranged from 15.8–16.1°C and 94–99%, respectively.

Haematological profile after acute exposure to bifenthrin

Examinations were performed at the end of a 96 h acute toxicity test with Talstar 10 EC at a concentration of 14.7 µg/l (96h LC50). Trout in the control group were monitored concurrently. The test was performed in three 300 l tanks. Each tank contained 20 rainbow trout, i.e., two tanks with 14.70 µg/l of Talstar 10 EC and one control tank. The presence of the tested substance (above 80% of the nominal concentration) was ensured by means of the exchange of the water in the testing bath every 12 h. Determination of bifenthrin concentration in water was measured using gas chromatography. The water was extracted with ethyl acetate and the extract dried with sodium sulphate, concentrated and then analyzed by gas chromatography with an electron capture detector (µECD). The reporting limit for this method is 0.01 µg/l (Mekebri et al., 2008). During the test, water temperature and pH were 15.8–8.43, respectively. Oxygen saturation of water was above 60% (86–95%).

Sixteen experimental (eight fish from each tank) and sixteen control rainbow trout were selected at random and used for haematological, biochemical, and histological examinations at the end of the test (96 h).

Blood was sampled from the *v. caudalis* using an 18G × 11/2" heparinised syringe (Heparin inj., Leciva, Czech Republic at a concentration of 5 000 IU heparin sodium salt in 1 ml). Indices measured included erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean erythrocyte haemoglobin (MCH), mean colour concentration (MCHC), leukocyte count (LEU), and differential leukocyte count. The procedures were based on unified methods for haematological examination of fish (Svobodova et al., 1991).

Biochemical blood plasma profile after acute exposure to bifenthrin

Blood was sampled from the *v. caudalis* as mentioned above. Blood plasma was obtained by centrifuging blood samples in a cooled centrifuge (4°C, 837× g). Biochemical indices included glucose (GLU), total proteins (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), tricylglycerols (TAG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gama-glutamyl-transpherase (GGT), creatine kinase (CK), lactate (LACT), alkaline phosphatase (ALP), calcium (Ca^{2+}), magnesium (Mg), and inorganic phosphate (PHOS). For the biochemical analysis of blood plasma, the VETTEST 8008 analyzer (IDEXX Laboratories Inc. U.S.A.) manufactured by Medisoft was used.

Histological examination of tissues

For histological studies, the gills, skin, liver, cranial and caudal kidney, and spleen were fixed in a solution containing ethanol, formalin, and acetic acid (ALFAC), then stored in 70% ethanol. Tissues were embedded in paraffin, sectioned (5 µm), and the slides stained with haematoxylin and eosin. The sections were examined by light microscopy, using as reference Takashima and Hibiya (1995), and photographed using a digital camera.

Statistical analysis

The presented values for data on the effects of bifenthrin on the haematological and biochemical profile are the mean ± standard deviation SEM (n = 16). Statistical assessment of results was carried out using Statistica software 8.0 for Windows (StatSoft). Data were first tested for normality (Kolmogorov-Smirnov test) and homoskedasticity of variance (Bartlett's test). If those conditions were satisfied, one-way analysis of variance (ANOVA) was employed to determine whether there were any significant differences in measured variables between control and experimental groups. When a difference was detected ($P < 0.05$), Tukey's multiple comparison test was applied to identify which treatments were significantly different. If the conditions for ANOVA were not satisfied, the non-parametric Kruskal-Wallis test was used (Zar, 1996).

RESULTS

Acute toxicity test

On the basis of acute toxicity tests on rainbow trout juveniles, 96h LCs of Talstar 10 EC were determined (96h LC50 14.7 µg/l, 96h LC0 10.4 µg/l, and 96h LC100 20.9 µg/l). The 96h LC50 value (14.7 µg/l) of Talstar 10 EC corresponded to 1.47 µg/l of bifenthrin. Clinical symptoms observed in the rainbow trout exposed to bifenthrin included accelerated respiration and loss of coordinated movement with fish lying on their side, followed by a short period of excitation with convulsions. This was followed by another resting stage and short period of excitation.

Haematological profiles after acute exposure to bifenthrin

Results of rainbow trout haematological profiling are given in Table 1 and Table 2. In the exposed fish, MCV, MCH, and band neutrophil granulocyte counts increased significantly ($P < 0.01$) but RBC, Hb, PCV, MCHC and LEU numbers were not different to the control group.

Biochemical blood plasma profiles after acute exposure to bifenthrin

Results of plasma biochemical profiling are given in Table 3. Experimental fish showed a significant ($P < 0.01$) decrease in NH_3 and a significant ($P < 0.01$) increase in GLU, CK, LDH and ALP. The remaining indices (TP, ALB, GLOB, TAG, AST, ALT, GGT, LACT, Ca^{2+}, Mg and PHOS) were similar to the control group.

Histopathological examination of tissues after acute exposure to bifenthrin

Degeneration of hepatocytes (Figure 1), especially in the periportal zones, was observed in 60% of individuals. Affected hepatocytes showed pycnotic nuclei and many small vacuoles or one large vacuole in the cytoplasm. Vacuole shape was typical of fatty degeneration of the liver. No changes were seen in the gills, skin, spleen, or cranial and caudal kidney.

Table 1. Derived haematological parameters in rainbow trout affected by acute exposure to Talstar EC 10

Indices	Control group $\bar{x}$ ± SEM (n = 16)	Experimental group $\bar{x}$ ± SEM (n = 16)
RBC (T/l)	1.18 ± 0.17[a]	1.29 ± 0.20[a]
Hb (g/l)	105.74 ± 11.77[a]	101.67 ± 17.95[a]
PCV (l/l)	0.38 ± 0.04[a]	0.37 ± 0.06[a]
MCV (fl)	327.08 ± 46.97[a]	285.41 ± 40.01[b]
MCH (pg)	90.86 ± 13.05[a]	79.28 ± 11.11[b]
MCHC (g/l)	276.34 ± 1.77[a]	277.73 ± 0.09[a]
Leuko (G/l)	35.80 ± 11.00[a]	38.13 ± 12.38[a]

Different superscript letters indicate significant ($P < 0.01$) differences between groups

Table 2. Leukocyte differential count in rainbow trout affected by acute exposure to Talstar EC 10

Indices	Units	Control group $\bar{x}$ ± SEM (n = 16)	Experimental group $\bar{x}$ ± SEM (n = 16)
Lymphocytes	%	75.67 ± 14.24[a]	83.92 ± 8.81[a]
	G/l	27.63 ± 5.19[a]	31.99 ± 6.37[a]
Monocytes	%	2.55 ± 2.57[a]	2.15 ± 2.18[a]
	G/l	0.91 ± 0.94[a]	0.82 ± 0.86[a]
Neutrophile granulocytes segments	%	9.15 ± 6.15[a]	7.91 ± 4.87[a]
	G/l	3.28 ± 2.24[a]	3.02 ± 1.87[a]
Neutrophile granulocytes Bands	%	12.07 ± 2.10[a]	6.02 ± 1.32[b]
	G/l	4.36 ± 0.98[a]	2.29 ± 0.36[b]
Developmental phases – myeloid sequence	%	0.69 ± 1.29[a]	0.01 ± 0.01[a]
	G/l	0.25 ± 0.48[a]	0.026 ± 0.004[a]

Different superscript letters indicate significant ($P < 0.01$) differences between groups

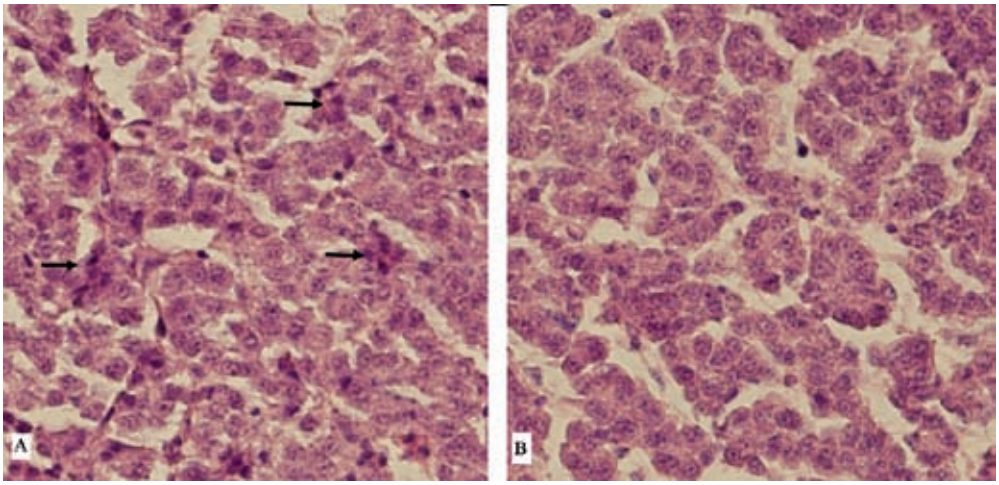

Figure 1. Liver of rainbow trout from experimental (A) and control (B) group; HE, 100×. Note degenerated hepatic cells with pycnotic nuclei (arrows)

Table 3. Derived biochemical indices of blood plasma in rainbow trout affected by acute exposure to Talstar EC 10

Indices	Control group $\bar{x}$ ± SEM (n = 16)	Experimental group $\bar{x}$ ± SEM (n = 16)
GLU (mmol/l)	4.13 ± 0.32[a]	7.89 ± 0.82[b]
TP (g/l)	47.50 ± 4.65[a]	48.83 ± 1.82[a]
ALB (g/l)	12.58 ± 2.40[a]	13.25 ± 2.20[a]
GLOB (g/l)	34.92 ± 2.33[a]	35.50 ± 1.76[a]
NH_3 (µmol/l)	805.58 ± 118.56[a]	494.83 ± 189.12[b]
TAG (mmol/l)	0.57 ± 0.20[a]	0.56 ± 0.11[a]
AST (µkat/l)	4.63 ± 1.37[a]	4.17 ± 1.86[a]
ALT (µkat/l)	0.15 ± 0.09[a]	0.14 ± 0.08[a]
LDH (µkat/l)	17.52 ± 1.53[a]	19.49 ± 1.43[b]
GGT (µkat/l)	0.11 ± 0.05[a]	0.13 ± 0.06[a]
CK (µkat/l)	13.93 ± 0.64[a]	15.96 ± 0.79[b]
LACT (mmol/l)	2.21 ± 1.07[a]	2.97 ± 1.49[a]
ALP (µkat/l)	0.36 ± 0.05[a]	0.62 ± 0.08[b]
Ca^{2+} (mmol/l)	2.86 ± 0.21[a]	2.99 ± 0.64[a]
Mg (mmol/l)	1.20 ± 0.10[a]	1.28 ± 0.24[a]
PHOS (mmol/l)	1.86 ± 0.59[a]	1.73 ± 0.61[a]

Different superscript letters indicate significant differences ($P < 0.01$)

DISCUSSION

In our study, the 96h LC50 of Talstar 10 EC was found to be 14.7 µg/l, which corresponds to 1.47 µg/l of bifenthrin. In view of this, Talstar 10 EC was included in the group of substances strongly toxic to fish. The values found in the study were in agreement with data reported by other authors who determined the toxicity of bifenthrin for various species of fish (Kidd and James, 1991). Liu et al. (2005) state the 96-h LC50 value to be 2.08 µg/l and 0.80 µg/l for common carp and tilapia (*Tilapia* spp.), respectively. Bifenthrin is more toxic at lower temperatures, and thus more toxic to cold- than warm-water fish, but the toxicity of pyrethroids is little affected by pH or water hardness (Mauck et al., 1976).

Clinical symptoms observed during bifenthrin acute exposure of rainbow trout correspond to observations by other authors reporting on the toxicity of pyrethroid pesticides (Prashanth et al., 2005; Dobsikova et al., 2006; Velisek et al. 2006a,b, 2007). Bradbury and Coats (1989a) reported signs of fenvalerate poisoning in fish, which included loss of schooling behaviour, swimming near the water surface, hyperactivity, erratic swimming, seizures, loss of buoyancy, increased cough rate, increased gill mucus secretions, flaring of the gill arches, head shaking and listlessness before death.

The principal haematological responses of rainbow trout in the study differed slightly from the findings of some other authors studying responses of rainbow trout and common carp to synthetic pyrethroid exposure (Dorucu and Girgin, 2001; Svobodova et al., 2003). Significantly lower values ($P < 0.01$) of RBC, Hb, and PVC were reported as a result of possible disruption of haematopoiesis, but there were no changes in white blood cell profiles in carp after acute exposure to deltamethrin (Svobodova et al., 2003). A decrease in the levels of PVC, Hb, LEU, and RBC was reported in carp after poisoning with cypermethrin (Dorucu and Girgin, 2001), and a decrease in total leucocyte count and neutrophil granulocyte count was observed in carp following acute poisoning with permethrin (Sopinska and Guz, 1998).

The main biochemical responses of rainbow trout to Talstar EC 10 were significantly ($P < 0.01$)

increased levels of plasma glucose, creatine kinase, lactate dehydrogenase, alkaline phosphatase and significant decreased ammonia compared with the control group. 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. Velisek et al. (2006b) found an increase in activity of ALP and CK in rainbow trout after exposure to cypermethrin (3.14 µg/l). Atamanalp et al. (2002) reported changes in the concentration of calcium and phosphorus in rainbow trout following cypermethrin exposure.

We observed degeneration of hepatocytes in periportal zones which may suggest the influence of toxic compounds in the digestive tract. The biochemical changes in liver profile may also be related to hepatocyte damage. Significant changes such as hyperplasia, disintegration of hepatic mass, and focal coagulative necrosis were found in *Labeo rohita* exposed to cypermethrin (Jee et al., 2005). Velisek et al. (2007) failed to observe histopathological changes in tissues of rainbow trout after acute exposure to delthamethrin (2.0 µg/l). Cengiz (2006) observed histopathological effects of deltamethrin on the gill (desquamation, necrosis, aneurysm in secondary lamellae, lifting of the lamellar epithelium, oedema, epithelial hyperplasia and fusion of the secondary lamellae) and kidney (degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the haematopoietic tissue, dilatation of glomerular capillaries, degeneration of glomeruli, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen) of common carp after acute exposure at a concentration of 0.029 and 0.041 mg/l. Sarkar et al. (2005) found significant changes such as hyperplasia, disintegration of hepatic mass, and focal coagulative necrosis in rohu, *Labeo rohita* exposed to cypermethrin. The sublethal effects of pyrethroids on fish include gill damage and behavioural changes. Because they are highly lipophilic, pyrethroids are likely to be strongly absorbed by the gills, even from water containing low levels of pyrethroids (Smith and Stratton, 1986).

To conclude, the exposure of rainbow trout to 14.7 µg/l of Talstar 10 EC caused alterations to haematological and biochemical indices as well as to tissue enzymes, all of which resulted in stress to the organism. The bifenthrin-based pesticide Talstar 10 EC was, therefore classified as belonging to substances strongly toxic for fish.

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