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# Effects of deltamethrin on rainbow trout (*Oncorhynchus mykiss*)

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**Abstract**

The aim of this study was to assess the effect of deltamethrin on rainbow trout (*Oncorhynchus mykiss*). Control and experimental group of fish were exposed to Decis EW 50 pesticide preparation (active substance 50 g/l of deltamethrin). The acute semistatical toxicity test lasting 96 h was performed on rainbow trout juveniles. The 96h$LC_{50}$ value of Decis EW 50 was 0.02 mg/l. Examination of haematological and biochemical profile and histological tissue examination was performed on 1–2-year-old rainbow trout after 96 h of exposure to Decis EW 50 in a concentration of 0.02 mg/l. The experimental group showed significantly lower values ($p < 0.05$) of plasma glucose, alanine aminotransferase, cholinesterase and significantly higher ($p < 0.05$) values of erythrocyte count, haemoglobin content, haematocrit and plasma total protein, albumins, ammonia, aspartate aminotransferase, creatinekinase and calcium compared to the control group. The deltamethrin-based Decis EW 50 pesticide preparation was classified among substances strongly toxic for fish.


*Keywords:* Pyrethroids; Acute toxicity; Haematological profile; Biochemical profile of blood; Histological examination of tissues

## 1. Introduction

Synthetic analogues of the pyrethrins, extracts from the ornamental *Chrysanthenum cinerariaefolium*, have been developed to circumvent the rapid photodegradation problem encountered with the insecticidal natural pyrethrins. The widespread use of these pesticides consequently leads to the exposure of manufacturing workers, field applicators, the ecosystem and finally the public to the possible toxic effects of these pesticides (Smith and Stratton, 1986; Solomon et al., 2001).

During investigations to modify the chemical structures of natural pyrethrins, a certain number of synthetic pyrethroids were produced with improved physical and chemical properties and greater biological activity. Several of the earlier synthetic pyrethroids were successfully commercialised, mainly for the control of household insects. Other more recent pyrethroids have been introduced as agricultural insecticides because of their excellent activity against a wide range of insect pests and their non-persistence (Casida et al., 1983; Thomson, 1985). Toxic effects of pyrethroids on non-target organisms have been reviewed and reported to be in the parts per billion values of toxicity (Smith and Stratton, 1986).

In the environment, synthetic pyrethroids are fairly rapidly degraded in soil and in plants. Ester hydrolysis and oxidation at various sites on the molecule are the major degradation processes. The pyrethroids are strongly adsorbed on soil and sediments, and are hardly eluted with water. There is a little tendency for bioaccumulation in organisms (Haya, 1989).

Deltamethrin is a widely used pesticide based on pyrethroids. It is among the most effective pyrethroid preparations (Bradbury and Coast, 1989). Deltamethrin was synthesised in 1974, and first marketed in 1977 (Pham et al., 1984). It works by paralysing the insects' nervous system and therefore giving a quick knock-down effect after surface contact or digestion. It is used commonly to control caterpillars on apples, pears and hops, and for the control of aphids, mealy bugs, scale insects, and

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whiteflies on glasshouse cucumbers, tomatoes, potted plants, and ornamentals (Mueller-Beilschmidt, 1990).

The mechanism of its effectiveness in the case of fishes 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 Coast, 1989; Eshleman and Murray, 2001; Hayes, 1994).

Fish make intimate contact with the surrounding water through the gills. Due to their lipophilicity, pyrethroids have a high rate of gill absorption, which in turn would be a contributing factor in the sensitivity of the fish to aqueous pyrethroid exposures. Fish seem to be deficient in the enzyme system that hydrolyses pyrethroids. The main reaction involved in the metabolism of deltamethrin, cypermethrin, or cyhalothrin in mice and rats is ester cleavage mainly due to the action of carboxyesterase. Metabolism in fish is largely oxidative (Demoute, 1989; Kamalaveni et al., 2003). After short-term deltamethrin exposure, adult freshwater catfish (*Heteropneustes fossilis*) showed hypocalcaemia and the researchers attribute this condition to the possible impairment of either net electrolyte influx at the gill or renal function. Deltamethrin exposure also caused hypophosphataemia and was linked to the possible redistribution of electrolytes between intracellular or extracellular compartments and impairment of renal function. Deltamethrin may disturb the calcium and phosphate homeostasis and may lead to an effect on the reproductive state of the fish (Srivastav et al., 1997; Maund et al., 2002).

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 deltamethrin-based pesticide to fish.

## 2. Materials and methods

The goal was to assess the effect of deltamethrin [(*S*)-*a*-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromvinyl)-2,2-dimethylcyclo-propancarboxylate] on fish. It was tested in the form of Decis EW 50 pesticide, the active substance of which was deltamethrin in the amount of 50 g/l. The toxic effect was assessed by the results of acute toxicity tests and results of haematological, biochemical and histological examination of rainbow trout after exposure to this pesticide.

### 2.1. Acute toxicity

The acute toxicity test on rainbow trout with Decis EW 50 followed the OECD Direction No. 203 and Methodical Manual ISO 7346/2. Juveniles of rainbow trout (camloops) of 4.1 ± 0.39 g mean body weight and 65.1 ± 3.61 mm mean body length were used for the test. Six various concentrations and a control were used in the basic test. Seven fish specimens were used for every concentration and also in the control. The test was performed semistatically for 96 h. The bath was changed every 24 h. Basic physical and chemical indices of diluting water used in the acute toxicity test were as followed: acid neutralisation 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 temperatures in the test ranged from 14.5 to 16.5 °C, oxygen saturation of water ranged between 101 and 108%. The $LC_{50}$, $LC_0$ and $LC_{100}$ values in the respective time intervals were determined by probit analysis.

### 2.2. Haematological profile after exposure to deltamethrin

Haematological, biochemical and histological examination of rainbow trout (camloops) was performed at the end of 96 h acute toxicity test with Decis EW 50 in concentration of 0.02 mg/l. At the same time, the control group of trout was examined haematologically, biochemically and histologically. Rainbow trout (camloops) of 309.18 ± 64.80 g average weight and 307 ± 25 mm average body length were used. The test was performed semistatically with the bath exchanged every 24 h. Diluting water had the same physical and chemical parameters as described above. Water temperatures during the test ranged from 14.5 to 16.2 °C, oxygen saturation of water was above 60% (ranging from 70 to 83%), pH ranged from 7.40 to 7.82. The test was performed in two aquaria of 200 l volume. Each aquarium was stocked with 15 specimens of 1–2-year-old rainbow trout (one control aquarium, two aquaria with Decis EW 50 in the concentration 0.02 mg/l).

Heparinised injection needles were used to take samples of blood from hearts of fish stunned by a blow with a blunt object over the head. To stabilise blood samples, aqueous solution of heparin sodium salt at 0.01 ml per 1 ml blood was used (Svobodová et al., 1986). The indices used to evaluate the haematological profile included the erythrocyte count (Er), 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 (Svobodová et al., 1986).

Results of haematological examinations were tested by the variance analysis using the Statistica 6.0 (ANOVA—Tukey Test) software.

### 2.3. Biochemical blood plasma profile after exposure to deltamethrin

For biochemical profile of blood plasma tests, rainbow trout (camloops) of 309.18 ± 64.80 g average weight and 307 ± 25 mm average body length were used. Blood was sampled by means of cardiac puncture as mentioned above. Individual blood samples of all investigated fish were centrifuged (4 °C, 837 × *g*) to obtain blood plasma samples. 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), creatinekinase (CK), calcium ($Ca^{2+}$), lactate (LACT), cortisol, cholinesterase (ChE) and inorganic phosphate (PHOS). For the biochemical analysis of blood plasma, the VETTEST 8008 analyzer (IDEXX Laboratories Inc., USA) manufactured by Medisoft was used. The analyzer uses dry chemical and colorimetric analysis techniques. Selective test discs (multi-layer film slides, Kodak) were 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.

Results of biochemical examination were tested by the variance analysis using the Statistica 6.0 (ANOVA—Tukey Test) software.

### 2.4. Histological examination of tissues

For histological examination of tissues, rainbow trout (camloops) of 309.18 ± 64.80 g average weight and 307 ± 25 mm average body length were used. After blood sampling, samples of brain, gills, skin, liver, cranial and caudal kidney and spleen were taken for histological examinations. The samples taken were immediately fixed in 10% formaldehyde, drained and embedded in paraffin. Sections were made of the paraffin blocks and stained with haematoxylin–eosin.

## 3. Results

### 3.1. Acute toxicity

On the basis of tests of acute toxicity to rainbow trout, the 96-h lethal concentrations of Decis EW 50 were determined (96h$LC_{50}$, 0.02 mg/l; 96h$LC_0$, 0.01 mg/l; and 96h$LC_{100}$,

Table 1
Derived haematological parameters in rainbow trout affected by acute exposure to Decis EW 50

| Indices | Units | Control group ($x \pm$ S.D., $n = 15$) | Experimental group ($x \pm$ S.D., $n = 15$) |
|---|---|---|---|
| Er | T/l | 1.10 ± 0.22 | 1.20 ± 0.27* |
| Hb | g/l | 60.41 ± 10.79 | 63.33 ± 6.83* |
| PCV | l/l | 0.38 ± 0.06 | 0.41 ± 0.04* |
| MCV | fl | 362.15 ± 95.70 | 352.73 ± 71.98 |
| MCH | pg | 56.83 ± 14.22 | 55.29 ± 12.23 |
| MCHC | g/l | 158.11 ± 15.96 | 156.88 ± ± 15.79 |
| Leuko | G/l | 22.00 ± 9.62 | 25.87 ± 11.73 |

* Statistically significant $p < 0.05$ *t*-test

Table 2
Leukocyte differential count in rainbow trout affected by acute exposure to Decis EW 50

| Indices | Units | Control group ($x \pm$ S.D., $n = 15$) | Experimental group ($x \pm$ S.D., $n = 15$) |
|---|---|---|---|
| Lymphocytes | % | 83.11 ± 11.24 | 79.50 ± 13.95 |
| | G/l | 18.89 ± 7.32 | 20.67 ± 3.53 |
| Monocytes | % | 1.93 ± 1.62 | 1.80 ± 2.36 |
| | G/l | 0.42 ± 0.32 | 0.46 ± 0.61 |
| Neutrophile granulocytes segments | % | 9.87 ± 8.41 | 11.67 ± 8.61 |
| | G/l | 2.17 ± 1.85 | 3.01 ± 2.12 |
| Neutrophile granulocytes band | % | 0.08 ± 0.02 | 0.05 ± 0.03 |
| | G/l | 0.01 ± 0.004 | 0.01 ± 0.003 |
| Developmental phases—myeloid sequence | % | 4.99 ± 3.49 | 4.53 ± 3.39 |
| | G/l | 1.09 ± 0.77 | 1.17 ± 0.89 |

*Statistically significant $p < 0.05$ *t*-test

0.05 mg/l). The 96h$LC_{50}$ is the basic value in the acute toxicity test. For rainbow trout juveniles the 96h$LC_{50}$ value was 0.02 mg/l of Decis EW 50 preparation, which corresponded to 1 μg/l of deltamethrin. 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.

### 3.2. Haematological profile

Results of erythrocyte profile and leukocyte profile of the control and experimental rainbow trout under study are given in Tables 1 and 2. Compared to the control specimens, those after the acute exposure to deltamethrin had significantly higher ($p < 0.05$) erythrocyte count, haemoglobin content and

Table 3
Derived biochemical indices of blood plasma in rainbow trout affected by acute exposure to Decis EW 50

| Indices | Units | Control group ($x \pm$ S.D., $n = 15$) | Experimental group ($x \pm$ S.D., $n = 15$) |
|---|---|---|---|
| GLU | mmol/l | 5.27 ± 1.42 | 4.87 ± 0.59* |
| TP | g/l | 43.80 ± 4.18 | 49.87 ± 2.45* |
| ALB | g/l | 6.07 ± 2.91 | 11.27 ± 1.34* |
| GLOB | g/l | 37.80 ± 2.37 | 37.93 ± 2.67 |
| $NH_3$ | μmol/l | 946.73 ± 195.78 | 1122.0 ± 235.24* |
| TRIG | mmol/l | 0.54 ± 0.21 | 0.89 ± 1.11 |
| AST | μkat/l | 4.20 ± 0.73 | 5.57 ± 0.84* |
| ALT | μkat/l | 0.18 ± 0.09 | 0.12 ± 0.04* |
| LDH | μkat/l | 32.59 ± 5.24 | 32.79 ± 2.01 |
| CK | μkat/l | 18.56 ± 5.07 | 25.66 ± 4.03* |
| $Ca^{2+}$ | mmol/l | 2.61 ± 0.24 | 2.98 ± 0.19* |
| LACT | mmol/l | 2.47 ± 0.95 | 3.51 ± 2.20 |
| Cortisol | nmol/l | 203.96 ± 125.79 | 193.68 ± 108.06 |
| ChE | μkat/l | 3.46 ± 1.35 | 2.14 ± 1.06* |
| PHOS | mmol/l | 3.84 ± 0.16 | 3.93 ± 0.35 |

*Note:* For the determination of $NH_3$, LHD and CK, blood plasma at 1:2 dilution with physiological saline was used.
* Statistically significant $p < 0.05$ *t*-test.

haematocrit. Values recorded for MCV, MCH, MCHC, Leuko and leukogram were comparable in both groups under study.

### 3.3. Biochemical blood plasma profile

Results of biochemical blood plasma profile of the control and experimental rainbow trout under study are given in Table 3. The experimental rainbow trout exposed to acute effects of the deltamethrin-based pesticide showed a significantly ($p < 0.05$) decreased concentration of glucose, alanine aminotransferase, cholinesterase and significantly ($p < 0.05$) increased total protein, albumins, ammonia, aspartate aminotransferase, creatinekinase and calcium in blood plasma. The rest of the indices (GLOB, TRIG, LDH, LACT, cortisol and PHOS) were comparable in the two groups during the study.

### 3.4. Histological examination of tissues

No histopathological changes were demonstrated in tissues (brain, gills, skin, liver, spleen, cranial and caudal kidney) of rainbow trout following after exposure to deltamethrin.

## 4. Discussion

In the course of 96 h toxicity test of deltamethrin-based pyrethroid preparation Decis EW 50 on rainbow trout juveniles, there was no mortality of fish in the control aquarium. Oxygen saturation of water did neither drop below 60% in any concentration tested, nor in the control group. Presence of the substance tested (above 80% of the nominal concentration) was provided by means of daily exchange of the testing bath. Fulfilling these conditions, the test may be considered valid. On the basis of the observed value of 96h$LC_{50}$ (0.02 mg/l), the preparation Decis EW 50 can be included in a group of substances that are highly toxic for fish: the risk sentence R50 states the value of 96h$LC_{50}$ less than 1 mg/l (Act No. 356/2003 Coll. in the Czech code of laws). The value of 96h$LC_{50}$ for Decis EW 50, 0.02 mg/l essentially corresponds to 1 μg/l deltamethrin. The values observed by us were in agreement with those reported by other authors who have determined the toxicity of deltamethrin for various species of fish. Bradbury and Coast (1989), Haug and Hofman (1990), Viran et al. (2003) report the mean lethal toxicity for various fish species in laboratory conditions as varying between $LC_{50}$ 0.001 and 0.01 mg/l, Golow and Godzi (1994) state the value of $LC_{50}$ 0.0037 mg/l for gibel carp. Žlábek (1999) state the value of $LC_{50}$ 0.099 mg/l Decis flow 2.5 for rainbow trout. Gangolli (1999) reports the values of 96h$LC_{50}$ for common carp and rainbow trout as varying between 0.0005 and 0.0018 mg/l.

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. Similar changes in the white blood picture are also reported by Svobodová et al. (2003) in carp following acute poisoning with permethrin. Bradbury and Coast (1989) 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, elevated cough rate, increased gill mucus secretions, flaring of the gill arches, head shaking, and listlessness before death.

The main haematological response of rainbow trout to the acute effect of deltamethrin-based preparation was a significant ($p < 0.05$) increase in erythrocyte count, haemoglobin content and haematocrit compared to the control group. Svobodová et al. (2003) reported opposite changes in the red blood picture in carp after acute exposure to deltamethrin. In this study, there were no changes in the white blood picture of rainbow trout. Sopinska and Guz (1998) observed decrease in total leucocyte count and neutrophil granulocyte count in carp following acute poisoning with permethrin.

The main biochemical response of rainbow trout to the acute effect of deltamethrin-based preparation was a significant ($p < 0.05$) decrease in plasma glucose, alanine aminotransferase, cholinesterase and significant ($p < 0.05$) increase in plasma total protein, albumins, ammonia, aspartate aminotransferase, creatine kinase and calcium compared to the control group.

The activities of plasma enzymes (ALT, AST, LDH and CK) are also used as a relevant stress indicator. Velíšek et al. (2006) observed a significant increase ($p < 0.05$) in AST and ALT levels in carp after acute exposure to deltamethrin in concentration of 3.25 μg/l. Balint et al. (1995) observed an increase of lactate dehydrogenase and glucose in common carp (*Cyprinus carpio*) after exposure to deltamethrin.

AChE is used as an indicator for organophosphorus pollution in natural waters (Galgani and Bocquene, 1989). In general, $LC_{50}$ values of organophosphates are associated with approximately an 80% reduction in AChE activity (Habig et al., 1986). Balint et al. (1995) found a 70–90% decrease in acetylcholinesterase activity of brain, heart, blood, liver and skeletal muscle of carp after 5 days exposure to deltamethrin in concentration of 2 mg/l.

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 utilised in the TCA cycle (Philip and Rajasree, 1996). Increased ammonia concentration indicates organism inability to convert the toxic ammonia to less harmful substances. The same results were observed by Velíšek et al. (2006) in carp after acute exposure to deltamethrin in concentration of 3.25 μg/l.

No histopathological changes were demonstrated in tissues of rainbow trout after acute exposure to deltamethrin. 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, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial

cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen) tissues of the common carp after acute exposure in concentration of 0.029 and 0.041 mg/l. Cengiz and Unlu (2006) determined the histopathological effects of deltamethrin on the gill (desquamation, necrosis, epithelial hypertrophy, lifting of the lamellar epithelium, oedema, dilatation of the capillaries primary lamellae, aneurysm, epithelial hyperplasia and fusion of the secondary lamellae), liver (hypertrophy of hepatocytes, significant increase of kupffer cells, circulatory disturbances, focal necrosis, fatty degeneration, nuclear pycnosis and narrowing of sinusoids) and gut (infiltration of mononuclear leucocyte and eosinophils towards lamina propria, necrosis tissues) of the mosquitofish, *Gambusia affinis*, exposed to two sublethal concentrations of deltamethrin (0.25–0.50 μg/l) for periods of 10, 20 and 30 days.

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