



# Responses of Fluvalinate Induced Stress in Hematological and Biochemical Parameters of Lake van Fish (*Alburnus tarichi guldenstadt 1814*)

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**Abstract.** *This research was conducted to determine the toxicity of fluvalinate to Lake Van fish (Alburnus tarichi) biometric, hematologic, and serum biochemicals. The fish were exposed to sub lethal fluvalinate concentration for 24, 48, 72 and 96 h for hematological and biochemical analyzes. Red blood cell, hemoglobin and hematocrit values decreased significantly in fish exposed to fluvalinate compared to control group. Significant increases in total leukocyte count were found. Compared with the control group, the serum enzyme aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase activity and serum glucose levels have increased significantly. This synthetic pyrethroid can be said to have a toxic effect on the Lake Van fish and can be used for controlling toxicity.*

**Keywords** *Alburnus tarichi, Biochemistry, Fluvalinate, Hematology*

## 1. Introduction

Synthetic pyrethroids are an insecticide used against ectoparasites in agricultural, aquatic, human, and animal health applications [1]. Aquatic invertebrates are highly susceptible to them as well as fish-synthetic pyrethroid insecticides [2]. Synthetic pyrethroids have been shown to be more toxic to fish than to mammals and birds [3, 4]. For these reasons, these pyrethroids are among the most commonly used pesticides [5-7]. As a result of exposure to these insecticides, organisms may show symptoms such as hyperexcitation, tremors, contraction, numbness, and paralysis [8, 9]. Fluvalinate is a member of the type-II pyrethroid group and contains an alpha-cyano group in their structure. These pesticides (such as fluvalinate, deltamethrin, esfenvalerate, cypermethrin, and bifentrin) may affect sodium channels in the central nervous system and ion channel targets, such as chloride and calcium [10]. In addition, pyrethroids can inhibit ATPase by modulating the release of acetylcholine esterase in the hippocampus region of the brain [11]. The use of biochemical and physiological biomarkers in aquatic toxicology is very common [12]. The low levels of fatal stress of organisms do not have to use their energies in their normal metabolic functions, so they can cause other problems, such as preventing growth or reducing reproductive ability [2]. Hematology is considered a useful biomarker for the evaluation of aquatic ecological toxicology. In fish exposed to type-II pyrethroids, different hematotoxic effects occur. These pesticides entering the fish body can cause destructive effects on hematopoietic tissues and blood cells [13]. Cypermethrin has been reported to have a toxic effect on hematological and other biochemical parameters in fish exposed to sublethal concentrations in Lake Van [14]. Lake Van fish (*Alburnus tarichi*) is an endemic species which live in Lake Van, one of the inland waters of Turkey. It is a significant source of protein to the region. This research aimed to examine the effect of fluvalinate at a sublethal concentration on the biometric, hematological, and biochemical parameters of fluvalinate.

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## 2. Materials and methods

### Experimental animals and water physico-chemical parameters

During the breeding season (May to June) a total of 56 (weight-90-120 g; length-18-22 cm) male and female Lake Van fish (*Alburnus tarichi*) were captured with Karasu stream sprinkling nets pouring into the lake. Fish were put in fiberglass tanks (300 L) and held for 7 days in stock ponds to adapt to the environmental conditions. The experiment was carried out in a natural photoperiod with continuously aerated water using the static test process. Average values of water quality during the experiment; temperature,  $13.1 \pm 2^\circ\text{C}$ , pH,  $8.57 \pm 0.4$ ; dissolved oxygen,  $6.41 \pm 0.14$  mg / L; oxygen saturation, 61.1% L; conductivity, 731  $\mu\text{S} / \text{cm}$ ; salinity, 0.47%. The experiments were carried out in compliance with the ethical legislation of the Central Ethics Committee of Yuzuncu Yıl University Animal Studies (YUHADYEK 2018/05).

### Experimental design

The 56 fish were divided into eight groups, with 7 fish distributed randomly into each tank. The 8 groups were then divided into 2 groups: Group I was used as the control group at 24, 48, 72 and 96 h. This group was untreated. Group II was used as a 24, 48, 72 and 96 h treatment group. In the case of Lake Van fish, the LC50 value for 96 h is  $0.333 \mu\text{g}/\text{L}$  for fluvalinate [15]. The concentration chosen for this study was  $0.112 \mu\text{g}/\text{L}$  which is 1/3 of the LC50 value at 96 h. The fish were anesthetized with aminobenzo-nate methanesulfonate (MS222, 100mg / L), at the end of each group administration period. The blood of the control group and fluvalinate group with the previously used heparinized plastic disposable syringe were taken from the fish tail. Total white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb) and hematocrit (Ht) values were calculated using hematological parameters. Blood samples were centrifuged at 3000 rpm for 5 min at  $+4^\circ\text{C}$  for biochemical analyses. Serum enzymes such as aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2), lactate dehydrogenase (LDH, EC 1.1.1.27) and serum glucose analyses were performed.

### Hematological and biochemical parameters

The RBC and WBC were counted via the haemocytometer method [16]. The Hb and Ht concentrations were determined using the cyanomethemoglobin and microhematocrit methods, respectively [17]. Biochemical parameters were analyzed using biochemical analysers in serum samples (Architect ci-16200, Architect i-2000 SR, Abbott Laboratories, Diagnostic Division, Abbott Park, IL, USA). The UV assay technique was used to measure the ALT, AST, LDH activities and glucose levels.

### Statistical analysis

Statistical analysis of all of the data was performed using SPSS (Version 23.0 Inc, USA). The data are given as the mean  $\pm$  the standard error of the mean. The Student t test was used to compare the groups of study. Statistical significance was accepted as  $p \leq 0.05$ .

## 3. Results and discussions

### Behavioral and physical observations

Both of groups were observed carefully at regular intervals. No abnormal behavior was observed in the control group during the experiment. In cases of fishes exposed to fluvalinate we observed uncontrolled movements, loss of balance, hypo and hyperactivity, suppression of the water surface and base, vertical posture, spiral movement, rapid gill activity, mouth and opening operation and actions, and increased mucus secretion. None of them died during the study.

### Hematological parameters

The hematological parameters RBC ( $\text{cell} \times 10^6/\text{mm}^3$ ), WBC ( $\text{cell} \times 10^3/\text{mm}^3$ ), Hb (g/dL) and Htc (%) are shown in Table 1. After exposure to fluvalinate for 24, 48, 72 and 96 h, the RBC, Hb and Ht



values were significantly decreased when compared to the control. Besides that, we observed an increase in WBC count that was also significant ( $P \leq 0.05$ ).

**Table 1.** Changes in some hematological parameters due to sublethal exposure of fluvalinate

Exposure period (hours)	Hematological parameters	Control Mean + SEM	Experiment Mean + SEM
24 h	RBC (cell $\times 10^6/\text{mm}^3$ )	2.39 $\pm$ 0.02	2.06 $\pm$ 0.03*
	WBC (cell $\times 10^3/\text{mm}^3$ )	2.28 $\pm$ 0.18	3.85 $\pm$ 0.34*
	Hb (g/dL)	16.90 $\pm$ 0.32	12.38 $\pm$ 0.34*
	Ht (%)	44.01 $\pm$ 0.45	36.25 $\pm$ 0.46*
48 h	RBC (cell $\times 10^6/\text{mm}^3$ )	2.38 $\pm$ 0.05	2.01 $\pm$ 0.03*
	WBC (cell $\times 10^3/\text{mm}^3$ )	2.04 $\pm$ 0.30	3.70 $\pm$ 0.29*
	Hb (g/dL)	16.7 $\pm$ 0.29	12.13 $\pm$ 0.46*
	Ht (%)	30.37 $\pm$ 0.61	31.67 $\pm$ 0.61*
72 h	RBC (cell $\times 10^6/\text{mm}^3$ )	2.44 $\pm$ 0.04	2.01 $\pm$ 0.04*
	WBC (cell $\times 10^3/\text{mm}^3$ )	3.42 $\pm$ 0.20	1.85 $\pm$ 0.20*
	Hb (g/dL)	15.67 $\pm$ 0.20	11.94 $\pm$ 0.36*
	Ht (%)	37.44 $\pm$ 0.21	33.13 $\pm$ 0.46*
96 h	RBC (cell $\times 10^6/\text{mm}^3$ )	2.40 $\pm$ 0.04	2.07 $\pm$ 0.02*
	WBC (cell $\times 10^3/\text{mm}^3$ )	1.57 $\pm$ 0.20	2.71 $\pm$ 0.28*
	Hb (g/dL)	15.42 $\pm$ 0.24	10.91 $\pm$ 0.18*
	Ht (%)	38.93 $\pm$ 0.33	33.60 $\pm$ 0.40*

\* Statistically significant at  $p \leq 0.05$  (n=7).

### Biochemical parameters

The serum values of ALT, AST, LDH and glucose are shown in Table 2. When values were compared, a significant increase of them was observed in the fish exposed to a sublethal concentration of fluvalinate [(0.122  $\mu\text{g/L}$ ) for 24, 48, 72 and 96 h] ( $P \leq 0.05$ ) (Table 2).

**Table 2.** Changes in some biochemical parameters due to sublethal exposure of fluvalinate

Biochemical parameters	Hours	Control Mean + SEM	Experiment Mean + SEM
AST (U/L)	24 h	573.91 $\pm$ 15.09	1163.50 $\pm$ 15.09*
	48 h	574.34 $\pm$ 13.91	1150.84 $\pm$ 12.64*
	72 h	540.14 $\pm$ 15.39	1199.35 $\pm$ 25.26*
	96 h	622.12 $\pm$ 8.72	1300.18 $\pm$ 11.13*
ALT (U/L)	24 h	73.44 $\pm$ 0.35	88.17 $\pm$ 2.56*
	48 h	72.74 $\pm$ 2.26	90.31 $\pm$ 3.72*
	72 h	74.45 $\pm$ 1.12	126.65 $\pm$ 2.92*
	96 h	79.19 $\pm$ 1.92	94.39 $\pm$ 3.97*
LDH (U/L)	24 h	820.14 $\pm$ 19.49	1081.14 $\pm$ 6.54*
	48 h	765.86 $\pm$ 8.75	1106.43 $\pm$ 20.95*
	72 h	625.00 $\pm$ 18.09	1530.01 $\pm$ 27.54*
	96 h	824.14 $\pm$ 14.10	1248.57 $\pm$ 15.68*
Glucose (mg/dL)	24 h	351.83 $\pm$ 5.03	394.33 $\pm$ 10.11*
	48 h	259.53 $\pm$ 2.90	376.44 $\pm$ 5.41*
	72 h	263.58 $\pm$ 4.35	426.10 $\pm$ 10.71*
	96 h	333.51 $\pm$ 2.94	415.50 $\pm$ 10.22*

\* Statistically significant at  $p \leq 0.05$  (n=7).



Pesticides have adverse effects on the aquatic ecosystem. Although fish and other vertebrates in the aquatic environment are not the target of such pesticides, their widespread and excessive use can lead to serious problems in the water biota. Hematological and biochemical indicators show different sensitivity to environmental factors and chemicals [16, 18]. Researchers have demonstrated that water quality will be affected by toxic substances and that will be reflected in the hematological values of aquatic organisms [14, 17, 19].

### **Behavioral and physical observations**

Pyrethroids cause sodium channels to remain open, resulting in toxic effects on the fish nervous system by breaking the ion balance and disrupting the continuity of neural transmission [13]. Synthetic pyrethroids have been shown to be affected in voltage sensitive channels such as calcium and chloride channels [16, 20, 21]. Behavioral changes caused by synthetic pyrethroids in fish are associated with the inhibition of AChE active in both neuromotor and nerve connections in muscle tissues [19]. In Lake Van fish exposed to fluvalinate, behavioral inconsistencies, such as hyperactivity, loss of balance, increased swimming speed and body twitching, hyperexcitability, rapid gill movement, mouth and operculum openings, etc. may be due to the inhibition of neuromuscular AChE and increased ACh (acetylcholine) at the nerve endings.

### **Hematological parameters**

In ecological, toxicological, and chemical risk assessments in fish, hematology is used as the primary biological determinant of stress [22]. Pesticides enter fish through their gills and then pass into the stomach and small intestine. Following this, they are transported via the blood to other organs and tissues within the fish. We can observe the destructive and toxic effects of these chemicals on the hematological parameters (WBCs, RBCs, Hb, Ht, etc.) and then, on the entire hematopoietic system. Changes in the hematologic parameters include the inhibition of erythropoiesis, destruction of RBCs (such as anemia), RBC production due to hypoxia, abnormal production of hemoglobin levels. Ineffective hematopoiesis and osmoregulatory dysfunctions may cause changes in the WBCs [23]. According to a study, significant changes in some blood parameters (RBC, Hb, Hg, etc.) have been reported in Korean rock fish (*Sebastes schlegeli*) exposed to cypermethrin. In the present study, hematological changes in Lake Van fish exposed to fluvalinate (RBC, WBC, Hb, Ht) may have been due to the inhibition of erythropoiesis, an inadequate osmoregulation system, or gill damage [24]. In this study, the significant increase in the WBC count may be a protective response to fluvalinate stress. Under stress condition [25], like fatal and chronic doses of fluvalinate, the bone marrow is stimulate to release lymphocytes; this effect is known as leukocytosis [26].

### **Biochemical parameters**

Determination of serum enzyme levels of aquatic organisms (such as fish and aquatic vertebrates) can be used as an important biological marker in ecotoxicological studies [27]. The rise in the levels of cellular enzymes is considered a stress measure in clinical enzymology. Serum ALT, AST, and LDH are widely used to detect tissue damage from environmental pollution [28]. All of the biochemical parameters recorded in this study are presented in Table 2. High levels of serum ALT, AST and LDH enzymes indicate liver damage. These enzymes have generally been reported to increase in the blood with liver damage [29]. In the present study, the increase in serum ALT, AST and LDH enzyme levels, could be the result of releasing them into the bloodstream due to the degradation of the cell membrane integrity, because of the fluvalinate-induced oxidative stress. In cases of Lake Van fish exposed to sublethal concentrations of cypermethrin, significant increases in ALT, AST, and LDH have been reported to be associated with liver cell damage [14]. The blood glucose level is one of the biomarkers commonly used in toxicological and chemical risk assessment. After exposure of fluvalinate, an increase in the serum glucose level, indicates the hyperglycemic profile of the fish. The increased metabolic demand, due to exposure, may increase the blood glucose level, depending on



gluconeogenesis or glycogenolysis. In addition, hyperglycemia occurs because of the disruption in the balance of glycogen and glucose metabolism by releasing of hyperglycemic hormones during stress (glucocorticoids and catecholamines) [30, 31]. In this study, a significant increase in the serum glucose level may have been due to gluconeogenesis or glycogenolysis, in order to meet the energy needs in line with the increased metabolic demands, due to fluvalinate toxicity.

#### 4. Conclusions

The results of this study showed that exposure to a sublethal concentration of fluvalinate caused significant changes in the behavioral, hematological, and biochemical parameters of Lake Van fish (*Alburnus tarichi*). Significant changes in these parameters may generate early warning signals in determining the acute and sublethal toxic levels of pesticides and their effects in the aquatic environment. In addition, this and similar studies reveal the effects of water pollutants on nontarget organisms and provide more controlled use and protection of water habitats. The histopathological and neurotoxic studies can support this research.

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