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Human and Ecological Risk Assessment: An International Journal

ISSN: 1080-7039 (Print) 1549-7860 (Online) Journal homepage: http://www.tandfonline.com/loi/bher20

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To cite this article: Necati Özok, Ahmet R. OĞuz, Ertuğrul Kankaya & Aslı Çilingir Yeltekin (2018): Hemato-biochemical responses of Van fish (Alburnus tarichi Guldenstadt, 1814) during sublethal exposure to cypermethrin, Human and Ecological Risk Assessment: An International Journal, DOI: 10.1080/10807039.2018.1443389

To link to this article: https://doi.org/10.1080/10807039.2018.1443389

Published online: 09 Mar 2018.

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HUMAN AND ECOLOGICAL RISK ASSESSMENT
https://doi.org/10.1080/10807039.2018.1443389

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Hemato-biochemical responses of Van fish (*Alburnus tarichi* Guldenstadt, 1814) during sublethal exposure to cypermethrin

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ABSTRACT

Cypermethrin is a highly active type II pyrethroid that is widely used to control harmful insects. The present study was conducted to investigate the possible effects of the sublethal concentration of cypermethrin (0.05 μL/L) on the biometric, hematological, and biochemical parameters of Van fish (*Alburnus tarichi* Guldenstadt 1814) for 24, 48, 72, and 96 h. Behavioral and physiological changes were observed in the fish treated with cypermethrin. Hematological parameters revealed a significant decrease in the erythrocyte (RBC), hemoglobin (Hb), and hematocrit (Hct) values ($p \leq .05$). Serum enzymes, aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2), and lactate dehydrogenase (LDH, EC 1.1.1.27) activities and serum cortisol level were significantly increased ($p \leq .05$). The results showed that changes in the biometric, biochemical, and hematological values of the Van fish exposed to cypermethrin can be used as biomarkers for monitoring toxicity.

ARTICLE HISTORY
Received 16 January 2018
Revised manuscript accepted 15 February 2018

KEYWORDS
acute toxicity; *Alburnus tarichi*; cypermethrin; hematological profile; Van fish

1. Introduction

The constant and rapid growth of the global population has led farmers to take measures to increase the yield of agricultural products per unit area. Pesticides employed for this purpose are used intensively by the farmers to increase the productivity and protect the agricultural areas against harmful organisms (Lamberth *et al.* 2013; Qureshi *et al.* 2016). Because of repeated and uncontrolled application of pesticides, target species not only develop resistance but also develop a high survival rate against these chemicals. However, nontarget species are also affected by the pesticides (Damalas and Eleftherohorinos 2011). These chemicals reach the water resources and the aquatic ecosystem in a variety of ways including rains and agricultural irrigation, even if used in confined areas, and can alter the physicochemical structure of water. Such chemical contamination can kill the aquatic organisms (Xiao *et al.* 2009; Murthy *et al.* 2013; Kohler and Triebskorn 2013; Gibbons *et al.* 2015).

Although the effects of the pesticides on the target organisms are clearly known, their effects on the nontarget organisms and their threat ratio are not fully understood (Kohler and Triebskorn 2013). Cypermethrin, a synthetic type II pyrethroid, is extensively used worldwide for public

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health and aquatic applications to control ectoparasites (Mugni *et al.* 2013). Because of its high toxicity to insects, it provides protection against the agricultural pests; however, it has adverse effects on the environment and animal health (Atamanalp and Cengiz 2002; Anadon *et al.* 2009; Palmquist *et al.* 2012; Yang and Suh 2015). Because of its lipophilic nature, cypermethrin has been reported to accumulate in the tissue, particularly in the central nervous system (Starr *et al.* 2012). A high concentration of cypermethrin in the brain has been reported to cause neurodevelopmental toxic symptoms (Ray and Fry 2006).

Hematological and biochemical markers in life are pathophysiological reflexes of the whole body (Adhikari *et al.* 2004). Alterations in the hematological and biochemical profiles resulting from the effects of various pollutants indicate changes in the metabolism and biochemical processes of the organism, and thus enable the investigation of the mechanisms on the effects of these pollutants (Luskova *et al.* 2002).

Van fish (*Alburnus tarichi* Guldenstadt, 1814) is an endemic species living in the Lake Van, the largest inland water lake of Turkey. In addition, this region is an important protein source for the aquatic organisms. The purpose of this study was to investigate the effect of the sublethal concentration of cypermethrin on the biometric, hematological, and biochemical parameters of Van fish.

2. Methods and materials

2.1. Chemical

Cypermethrin, ($C_{22}H_{19}C_{12}N0_3$) [α-cyano-(3-phenoxyphenyl)methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate, with the EC 25% concentration was purchased from an agricultural drug dealer (Agrofarm Chemical Industry and Trade Company in Istanbul/Turkey).

2.2. Experimental animals and physicochemical parameters of water

A total of 48 (weight: 110.5 g; length: 21.33 cm) male and female Van fish (*Alburnus tarichi* Guldenstadt, 1814) were hunted with sprinkling nets from the Karasu stream that merged into the lake during the breeding season (May–June). The fish were then transferred to fiberglass tanks (300 L) and were kept in the stock pond for 7 d to acclimatize with the environmental conditions. The application was performed during the natural photoperiod using the static test method in the continuous aerated water. The mean values of the water quality parameters throughout the experiment were: temperature: 13.1 $\pm$ 2°C, pH: 8.57 $\pm$ 0.4, dissolved oxygen: 6.41 $\pm$ 0.14 mg/L, oxygen saturation: 61.1% L, conductivity: 731 μS/cm, and salinity: 0.47%. Experiments were carried out in accordance with the ethical rules of the Van Yüzüncü Yıl University, Animal Experiments Local Ethics Committee (YÜHADYEK (2018/2)).

2.3. Experimental design

The fish were divided into eight groups and six fish were randomly distributed in each tank. A total of 48 fish were used in this study. Group I fish were used as control group at 24, 48, 72, and 96-h periods, and were not treated at all. Group II fish were used as treatment group at 24, 48, 72, and 96-h periods, and treated with 0.05 μL/L cypermethrin. Cyp was changed

2 d to minimize decreases in the concentrations.Van fish has been assigned to the red list because it is an endangered species. Its hunting is prohibited during the breeding period, and thus, it is protected. For this reason, sublethal cypermethrin dose was determined according to the toxicity test on carp, a species closely related to Van fish (Stara *et al.* 2013). At the end of the administration period of each group, the Van fish was anesthetized using aminobenzoate methanesulfonate (MS222, 100 mg/L).

Blood from the control group and the cypermethrin group were drawn from the fish tail using the heparinized plastic disposable syringe. Hematological parameters measured in the study were erythrocyte count (RBC), hemoglobin (Hb), and hematocrit (Hct) values. For the biochemical analyses, blood samples were centrifuged at 3000 rpm for 5 min at 4°C. 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 cortisol analyzes were measured (Fırat *et al.* 2011).

2.4. Hematological and biochemical parameters

Erythrocytes were counted using a hemocytometer (Rusia *et al.* 1992). Hb concentrations were determined by the cyanmethemoglobin method (Drabkin *et al.* 1946), and Hct values were determined by the microhematocrit method (Nelson *et al.* 1989). Biochemical parameters in the serum samples were analyzed using biochemical analyzers (Architect ci-16200, Architect i-2000 SR, Abbott Laboratories, Diagnostic Division, Abbott Park, IL, USA). The following procedures were used to measure the serum parameters: UV assay technique for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activity; electrochemiluminometric technique was used for determining the cortisol level.

2.5. Statistical analysis

Statistical analysis of all data was performed using SPSS (Version 23.0 Inc, USA). The data are given as mean ± standard error of mean. Student t test was used to compare study groups. Statistically significant differences were considered significant at $p < .05$.

3. Results

3.1. Behavioral and physical observations

In our study, the control group showed normal behavior during the test period. Abnormal behaviors were observed in the fish exposed to cypermethrin. These behaviors were associated with irregular movement and hyper-excitability, loss of balance, inactivity on the surface, vertical position, spiral movement, rapid gills, openings in the mouth and opercula, and increased mucus secretion. No mortality was observed in cypermethrin-exposed fish at the end of the exposure periods.

3.2. Hematological parameters

The number of erythrocytes (10^6/mm^3), Hb content (g/dL), and Hct (%) decreased in the fish exposed to cypermethrin at 24, 48, 72, and 96 h according to the hematological data compared to the control group ($p \leq .05$) (Table 1).

Table 1. Changes in some hematological parameters of Van fish exposed to cypermethrin sublethal concentration.

Hours		RBC (10^6/mm^3) Mean ± SEM	Hb (g/dL) Mean ± SEM	HCT (%) Mean ± SEM
24	Control	2.48 ± 0.06	16.51 ± 0.06	46.61 ± 0.26
	Cypermethrin	1.70 ± 0.03*	12.40 ± 0.47*	34.92 ± 1.27*
48	Control	1.75 ± 0.06	13.36 ± 0.49	41.00 ± 0.77
	Cypermethrin	1.42 ± 0.09*	11.55 ± 0.58*	34.25 ± 0.97*
72	Control	1.71 ± 0.02	12.98 ± 0.32	40.79 ± 0.92
	Cypermethrin	1.39 ± 0.13*	10.55 ± 0.38*	31.46 ± 0.62*
96	Control	2.29 ± 0.09	14.06 ± 0.32	42.63 ± 1.09
	Cypermetrin	1.70 ± 0.09*	10.82 ± 0.26*	31.51 ± 0.95*

*The difference according to the control group was statistically significant ($p \leq .05$). (n = 6).

3.3. Biochemical parameters

Serum ALT, AST, and LDH enzyme activities significantly increased ($p \leq .05$) in the fish exposed to sublethal cypermethrin concentration compared to the control group (Table 2).

3.4. Serum cortisol

Significant increase in the serum cortisol levels ($p < .05$) was observed in the fish exposed to sublethal cypermethrin concentration compared to the control group (Table 3).

4. Discussion

4.1. Behavioral and physical observations

In this study, we report that the behavioral changes in the fish exposed to cypermethrin (hyperexcitability, loss of balance, irregular swimming, vertical suspension position in water, rapid gill movement, mouth and operculum openings) were observed similar to as well as different from other type II pyrethroids and fish species (Adhikari *et al.* 2004; Saravanan *et al.* 2011).

Table 2. Changes in some biochemical parameters of Van fish exposed to cypermethrin sublethal concentration.

Parameters	Hours	Control	Cyp (0.05 μL/L)
AST (U/L)	24	260.50 ± 27.27	1655.83 ± 45.2*
	48	539.00 ± 36.97	1365.66 ± 60.36*
	72	634.33 ± 26.95	1180.33 ± 61.45*
	96	706.00 ± 15.61	1965.50 ± 67.38*
ALT (U/L)	24	8.33 ± 0.42	15.33 ± 0.84*
	48	7.00 ± 0.51	14.83 ± 0.70*
	72	7.33 ± 0.49	14.33 ± 0.42*
	96	12.33 ± 0.42	59.33 ± 3.82*
LDH (U/L)	24	1655.33 ± 15.52	2385.00 ± 23.64*
	48	1373.66 ± 15.69	1738.66 ± 16.42*
	72	1809.00 ± 21.26	2209.33 ± 53.00*
	96	719.16 ± 16.89	1429.16 ± 19.88*

*The difference according to the control group was statistically significant $p \leq .05$ (n = 6).

Table 3. Changes in serum cortisol levels of Van fish exposed to cypermethrin sublethal concentration.

Parameter	Hours	Control	Cyp (0.05 μL/L)
Cortisol (ng/dL)	24	66.53 ± 1.92	81.90 ± 2.34*
	48	46.73 ± 2.34	88.71 ± 2.41*
	72	54.68 ± 1.09	67.60 ± 0.64*
	96	56.48 ± 3.55	63.18 ± 2.97*

*The difference according to the control group was statistically significant $p \leq .05$ (n = 6).

Hematological parameters are commonly used as indicators for the physiological and biochemical changes in fish caused by stress (Brum *et al.* 2014). In the fish, pesticides are taken into the body through the gills and stomach-intestines, and then transported to the tissues and organs by blood. Their destructive effects are first exhibited by the blood cells and hematopoietic tissues, where they are produced (Witeska and Baka 2002). The cause of stress in fish is the alteration of endogenous and exogenous origin of the pesticides. Such alterations can be observed in the blood parameters instantaneously. The fish possess homeostatic mechanisms to restore the metabolism to normal levels during stress. However, the long-term effect of the stress factor is inadequate in response to the end-stage metabolism (Duran and Talas 2009). Decrease in the erythrocyte counts, Hb content, and Hct levels of the fish exposed to cypermethrin at 24, 48, 72, and 96-h period in our study might be because of erythropoiesis inhibition and erythrocyte destruction in the hematopoietic organs. It has been reported that significant changes in the blood parameters might occur because of the deterioration of the hematopoietic system after cypermethrin exposure in many studies conducted parallelly to our study (Dorucu and Girgin 2001; Svobodova *et al.* 2003; Gautam and Kumar 2008; Gupta *et al.* 2014).

Increase in the cellular enzyme levels in clinical enzymology are considered to be stress indicators. Serum ALT, AST, and LDH activities are commonly used to diagnose diseases and detect tissue damage caused by environmental pollution (Burtis *et al.* 2012). Serum ALT, AST, and ALP reported that the blood levels of serum LDH might increase because of the cellular damage in the liver, as these serum enzymes at high levels are usually necrosed in the liver of animals (Asztalos and Nemcsok 1985; Harvey *et al.* 1994; Velíšek *et al.* 2006). In our study, the increase in the levels of ALT, AST, and LDH might be because of the release of these enzymes into the bloodstream owing to the impaired cell membrane integrity, as a consequence of liver damage caused by type II pyrethroids. Stress is a process that requires energy. The organism activates energy substrates to cope with metabolic stress (Vijayan *et al.* 1997). The increase in the cortisol level in this study might be because of the increased energy demand during stress. It has been suggested that the increase in the cortisol levels in *Rhamdia quelen* and *Oreochromis niloticus* exposed to cypermethrin is because of the stress-dependent energy requirement (Borges *et al.* 2007; Fırat *et al.* 2011). Stress is a process that requires energy. The organism activates energy substrates to cope with metabolic stress (Vijayan *et al.* 1997).

5. Conclusion

This study shows that cypermethrin has a toxic effect on Van fish and its metabolism is affected at different levels. Decrease in the hematological parameters such as RBC, hemoglobin, and hematocrit values indicates the development of anemia. The increase in the activity

of serum enzymes ALT, AST, and LDH might be a result of liver necrosis. The increase in serum cortisol level might be because of the increased energy demand to cope with stress. The findings of this study could be further supported by histopathological and neurotoxic studies.

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