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The Investigation of the Different Concentration of Dibrom on the Critical Swimming Speed and Hematology Parameters of *Oncorhynchus mykiss*

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ABSTRACT

Dibrom (Naled) is one of the organophosphate insecticides used to fight mosquitoes, mites and house flies. In this study, it was aimed to determine the behavioral changes and hematological parameters of rainbow trout *(Oncorhynchus mykiss*) altered by the chronic toxicity of dibrom. Fish were captured for a 14-day acclimation period then exposed to 2 different concentration of dibrom for 21 days. The experiment consisted of 3 groups; 2 of them applications and 1 control. The treatment concentrations (0.75 mg / L and 1 mg / L) were designed according to 96h LC_{50} values of dibrom.

Keywords: Dibrom, Rainbow trout, hematology, critical swimming performance

INTRODUCTION

Agrochemicals are used extensively in the agricultural areas of our country and all over the world to destroy the harmful organisms and produce much more products. Although quality products are grown, the chemicals are transported to different ecosystems directly or indirectly and affect non-target organisms (Parlak, 2018). Rain and irrigation waters, as well as wind factors, cause these chemicals to reach rivers, lakes, dams, seas and underground waters and adversely affect the aquatic ecosystem (Strmach and Braunbeck, 2000). Dibrom (naled), highly toxic in aquatic environments, is a rapid-acting, non-systemic organophosphate pesticide used to control aphids, mites, mosquitoes and nymphs in greenhouses, animal shelters, poultry, food processing plants and agricultural areas.

In order to maintain the sustainability of the aquatic ecosystem, it is important to know the biological state of the system. In this regard, the toxicology studies on aquatic organisms are important in determining the adverse effects of these chemicals and the alterations that will occur in the biological activities of the organism (Atamanalp, 2000). Fish are used as indicators of pollution in the aquatic ecosystem due to its place in the food chain and an important food source. It is therefore important for the future of the ecosystem to determine the physiological and biochemical changes that pollutants will cause in fish. Fish react to the effects of pollutants by altering their metabolic and physiological events as well as their behavior. In habitats where pollutants are involved, results such as deterioration in the behavioral and physiological functioning of fish and a decrease in survival rate.

It has been reported that the critical swimming speed determined by the swimming performance assessment system is one of the most important criteria in determining the effects of pollutants on fish (Hammer 1995). In the previous researches, it was determined that fish swimming performance was negatively affected exposed to sub lethal concentrations of organic and inorganic pollutants (Beamish, 1978; Nikl and Farrell, 1993; Heath, 1995; Beaumont *et al*., 1995; Jain *et al*., 1998; Baltz *et al.* 2005; McKenzie *et al*., 2007).

Recently, attention has focused on examining the physiological and biochemical mechanisms in fish to investigate the toxic effects of environmental pollutants on important biological systems (Ahmad *et al*., 1998). Physiological and biochemical parameters of fish blood are frequently used to identify changes in the presence of pollutants (Adhikari *et al*., 2004). Examining the alterations in behavior and hematologic parameters of economically important fish species play an important role in increasing the productivity of the aquaculture and decreasing the disease rate and determining the metabolic and physiological status of the organism under the influence of various environmental factors in natural conditions.

In this study, it was aimed to determine whether rainbow trout (*O. mykiss*) could be a useful biomarker in assessing hematology and response to pollutants by assessing critical swimming speed.

MATERIAL AND METHODS

60 pieces two-year-old rainbow trout (*Oncorhynchus mykiss*), weighing 100 ± 10 g obtained from Atatürk University Faculty of Fisheries Inland Fish Application and Research Center, were used. Fish were started to trial after a 14-day acclimation period and exposed to 2 different concentration of dibrom for 21 days. The experiment was designed with two repetitions 3 groups (2 applications and a control group). Application concentrations were determined according to the $LC_{50}96$ value, as 0.75 mg / L and 1 mg / L. According to the renewable environment tests procedure (Ünsal, 1998), the toxicant is given in every 12 hours as concentrations that will generate this desire. The care was taken to ensure that the fish did not enter stress and suffer damage during all acclimatization and testing procedures (Atamanalp, 2003).

Swimming Performance Test Procedure

As per the trial procedure for defined volume retrials, the concentrations have been reapplied every 12 hours in order to recreate the dose (Esenbuğa *et al.*, 2017). At the end of the treatment period, fish were taken into swimming tunnel in the swimming performance unit (which has the same water quality as the environment of treatment), to measure critical swimming speeds. The swimming performance measurement system consists of a 14.65 cm perimeter rounded tank system with 1 m length and 40 cm diameter tunnels. The water temperature was measured directly by the system and the flow measurements were measured with a muline device (PemSantaş, 9001-54). This system is widely used to determine the environmental factors and the effects of pollutants on the fish's swimming performance (Hammer, 1995; Plaut, 2001; Özbilgin and Başaran, 2005).

Hematological Analyses

Blood was collected from the caudal vein of fish. Afterwards hemoglobin was measured according to the cyanmethemoglobin method. Later erythrocyte (RBC), leukocyte (WBC) and platelets (PLT) was measured and calculated (Blaxhall and Daisley, 1973; Uçar and Atamanalp, 2011).

Fresh blood is drawn up to 0.5 line with erythrocyte pipette. Then it was diluted to 1/200 with Dacie's solution up to 101 lines. The thoroughly agitated mixture was allowed to stain for 1-2 minutes. The un-homogenized first 4-5 drops of pipette were poured out the residue part poured into the Thoma chamber (Figure 1). The resulting value was calculated as 1/5 mm^2 on a microscope through a Thoma slice, calculated as 10^6 / mm^3 (Blaxhall and Daisley, 1973; Uçar and Atamanalp. 2011). For determination of leukocyte and platelet count, the method applied for erythrocyte count was used. The result is calculated as 10^3 / mm^3 (Blaxhall and Daisley, 1973).

Blood cell indices

Mean corpuscular volume (MCV) is the average volume of red cells in a species. MCV is elevated or decreased in accordance with average red cell size; ie, low MCV indicates microcytic (small average RBC size), normal MCV indicates normocytic (normal average RBC size), and high MCV indicates macrocytic (large average RBC size). These indices are calculated according to Morris and Davey (1996).

Mean cell volume (MCV)

$$\mathrm{MCV}\,(\mu\mathrm{m}^3) = \frac{\mathrm{Hct}\,(\%)\;\mathrm{x}\;10}{\mathrm{RBC}\,(10^6/\mathrm{mm}^3)}$$

Mean cell hemoglobin (MCH)

$$\mathrm{MCH}(\mu\mathrm{g}\ /\ \mathrm{hücre}) = \frac{\mathrm{Hb(g/100\ ml)\ x\ 10}}{\mathrm{RBC}(10^6/\mathrm{mm}^3)}$$

Mean cell hemoglobin concentration (MCHC)

$$\mathrm{MCHC}\,(\mathrm{g}\,/\,100\,\mathrm{ml}) = \frac{\mathrm{Hb}\,(\mathrm{g}\,/\,100\,\mathrm{ml})\,\mathrm{x}\,100}{\mathrm{Hct}\,(\%)}$$

Statistical Analyses

The data obtained from hematology indices, live weight gain and water quality parameters were expressed as the mean ± standard deviation (n=3). SPSS 20.0 software was used to perform all statistical analyses. One-way ANOVA with Duncan test was used to determine whether the results in treatment groups were significantly different from those of control. The data were expressed as the mean ± SEM. The level of significance was determined as $p<0.05$.

RESULTS

Swimming Speed

Rainbow trout critical swimming speed results were found to increase in group D2 (1 mg/L), although there was a decrease in D1 (0.75 mg/L) group when compared to the control group. This is thought to be caused by pollutants slowing down muscle movements by affecting the central nervous system in fish (Table 1).

Table1. The effect of different doses of dibrom on the swimming performance of *Oncorhynchus mykiss*

Group	Swimming Speed (U_{crit} (bl/sn))
Control	8.12 ± 0.36^{b}
D1(0.75 mg/L)	7.84 ± 0.93^{b}
D2(1 mg/L)	8.77 ± 0.68^{a}

Mean value± Standard deviation. Different letters in averages means statistical difference ($p<0.05$).

Hematology Parameters

The basic functions of blood are oxygen transport to tissues, feeding of tissues, and maintenance of acid-base balance and removal of metabolic waste products from tissues. For this reason, any disorder that occurs in a calming manner has a significant effect on the physiological activities of the whole body. It is used as a useful biomarker in determining changes in the cellular structure and chemical effects.

Hematological indices including hemoglobin, hematocrit, erythrocyte sedimentation rate, erythrocyte, thrombocyte, mean erythrocyte volume, mean hemoglobin amount per erythrocyte and average hemoglobin concentration per erythrocyte were determined at different doses of dibrom exposition to rainbow trout (*O. mykiss*) (Table 2).

Table 2. The effect of different doses of dibrom on hematology indices of Oncorhynchus mykiss

Parameters	Control	D1(0.75 mg/L)	D2(1 mg/L)
RBC (10^6/mm^3)	0.94±0.12 ^{b}	0.79±0.13 ^{c}	1.10±0.03 ^{a}
WBC (10^4/mm^3)	11.9±1.93 ^{a}	9.42±1.81 ^{b}	13.12±0.9 ^{a}
PLT (103^3/mm^3)	1.94±0.23 ^{b}	1.95±0.27 ^{b}	2.50±0.18 ^{a}
Hb (g/dl)	8,78±0.97^{ab}	7.72±1.36^b	9.74±0.54^a
ESR (mm/h)	0.32±0.3 ^{a}	0.13±0.04 ^{a}	0.18±0.04 ^{a}
HTC (%)	7.26±1.0 ^{b}	6.06±1.0 ^{c}	8.94±0.3 ^{a}
MCV (µm^3)	76.68±1.89 ^{a}	76.69±5.76 ^{a}	81.18±4.17 ^{a}
MCH (pg)	93.27±9.24 ^{a}	97.70±10.88 ^{a}	88.48±6.29 ^{a}
MCHC (g/100ml)	121,60±11,13 ^{a}	128,63±23,81 ^{a}	108,93±3,87 ^{a}

Mean value± Standard deviation. Different letters in averages means statistical difference ($p<0.05$)

DISCUSSION

The behavioral response of the rainbow trout under the effect of dibrom is thought to be due to the increase in oxygen requirement in the stress conditions caused by the effect of the pollutant on the reservoir instead of the nutrients instead for the vital events because the pollutant directly affected the central nervous system and slowed spontaneous muscle movements. In this study, it has been determined that swimming performance in rainbow trout varies significantly depending on the effect of the pollutant. The difference in responses to both activation and immune responses in fish is due to the variety of factors involved in the exposure time, pollutants, the toxicity of the substance, and the species of fish (Cuesta e*t al*., 2011).

Kori-Siakpere *et al*., (2009) stated that reduction of blood parameters after exposure to toxicants leads to anemia and reduce the physical activity of the fish because the oxygen provided to the tissues is insufficient. The knowledge of fish blood cell physiology has significantly increased in recent years and the body of literature on blood cell morphology, blood cell functions, and even reference intervals for some species continues to grow (Harms *et al*., 2002).

When the results of hemoglobin value were examined, it was determined that D1 group decreased and D2 group increased according to the control group. The level of low hemoglobin indicates that the mechanism of iron synthesis in fish is impaired. This is reported to be due to the anemic state of the fish and the hemoglobin level due to the low level of hemolysis and to the restriction of aerobic glycolysis which interrupts the synthesis of hemoglobin (Parlak, 2016). Hematocrit percentage is a parameter of the erythrocyte index as well as the number of erythrocytes and hemoglobin level, expressed as the percentage of erythrocyte volume that is present in blood. In the present study, the value of hematocrit was lower in the D1 group compared to the control group, but increased in the D2 group due to the toxicant dose. Changes in dehydration, nutrition, erythrocyte synthesis, and membrane permeability cause changes in hematocrit levels. Hemodilution (increase in the proportion of water present) is defined as a mechanism to reduce the concentration of foreign substances in circulatory systems.

In the vast majority of fish species, erythrocytes, which are predominant cell types, have biconvex structure (Parkash, 2016). Under the influence of environmental pollutants, changes occur in the blood components. These changes are thought to be caused by the disorder in the erythropoietic system. After administration, erythrocyte count decrease in the D1 group is accepted as the erythropoietic tissues affected, the condition of the organism has deteriorated and the anemia developed. On the other side, the sudden increase in erythrocytes in the D2 group can be explained by the induction of stress-related catecholamine and contraction of the spleen and circulation of new erythrocytes. In the case of hypoxia, since acid release occurs at a very high rate in the blood, the blood pH, and the hemoglobin's oxygen binding capacity decreases. With this decrease, the distribution of oxygen in the tissues may be reduced (Parlak and Atamanalp, 2017).

Leukocytes are important components of the defense system of organisms, and species which have a large number of leukocyte cells are able to fight pollutants more effectively. The number of leukocyte cells is influenced by physiological and environmental factors. In the present study, the number of leukocyte counts decreased in D1 group while the increase in the high dose D2 group was observed. Changes in the number of leukocytes in the fish treated can be explained by the introduction of the hormesis mechanism. Hormesis is a concept that has been proposed to explain the adaptive nature of toxic substances, which can sustain life at low concentrations and develop against these substances. This concept implies that toxic substances and environmental conditions have two opposite effects, both life supporting and destructive. Activated hormesis mechanism with low doses of toxic agents gives a vital adaptive response (Kısım and Uzunoğlu, 2012).

In fish, the platelet cells have the phagocytic ability and participate in the defense mechanism. These cells represent the link between innate and subsequently acquired immunity and represent intracellular and extracellular molecules, including immunological functions. Platelets are blood phagocytes that form the protective wall, and plays an important role in the blood clotting process. As a result of the study, the platelet count increased in both doses compared to the control group. In the case of stress, the blood coagulation system is more active in fish, which can increase the number of platelets (Parlak, 2016).

Cell indices of erythrocytes such as MCV and MCH are used to diagnose the classification of anemic situations. It is also used to determine cell activities of erythrocytes (Şahan and Cengizler, 2002). Erythrocyte indices (MCV, MCH and MCHC) are related to hematocrit, erythrocyte and hemoglobin concentration and indicate the size or diameter of erythrocytes and the amount of hemoglobin. The present study revealed a significant increase in MCV, MCH and decrease of MCHC. This may suggest that the anemia was of the macrocytic type and may be described as a defensive reaction against pollutants or due to the decrease of RBCs, Hb and Hct values following the disturbances of the metabolic and hemopoietic activities after exposure to pollutants (Vaseem and Banerjee, 2012).

Increasing environmental pollution from day to day and more intense effects make a positive contribution to the development of environmental awareness. Aquatic toxicology addresses the adverse effects of exposure of aquatic organisms to pollutants and improves the accuracy and reliability of results by adding novelty to test methods that have been in use for a long time. Biomarkers, especially in the aquatic environment, have broadened the scope of investigations in the field of aquatic toxicology (Alak *et al*., 2007).

It is very important not mixing foreign materials to fish cultured water (fresh water, salt water, stream and stagnant water) from the point of view of human health, economically and health of the organisms which are housed in water

In this study, it was determined that rainbow trout (*O. mykiss*) may be useful biomarkers in determining the responses to pollutants and susceptibility to pesticides in hematology and critical swimming speed assessment. It will be possible to share proposals for taking necessary measures in line with the results of these studies.

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