© Environmental Toxicology and Ecology
2024, Vol. 4 (1)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

Histological Disruptive Effects of Tau-Fluvalinate in Zebrafish (*Danio rerio*) Testis

Cansu AKBULUT [1*], Ceyda ÖZTÜRK [1], Nazan Deniz YÖN ERTUĞ[1]

ABSTRACT

Due to the high use of pesticides, undesirable effects are observed in the environment and toxic effects occur in terrestrial and aquatic organisms via pesticide bioaccumulation. Although bioaccumulation levels are low, especially in aquatic ecosystems, toxicological effects on aquatic organisms have been observed. Synthetic pyrethroids are pesticides produced for domestic and agricultural pests and tau-fluvalinate is also a broad-spectrum synthetic pyrethroid. Our study investigated the histopathological effects of zebrafish exposed to tau-fluvalinate at two different doses (8µg/L,16µg/L) on testicular tissue. After 5 days of exposure, testicular tissues were dissected and routine histological methods were applied. Tissues were investigated under a light microscope after they were stained with hematoxylin & eosin. As a result of tau-fluvalinate exposure, deterioration in seminiferous tubule morphology, interstitial fibrosis, vascular congestion, karyorrhexis and hypertrophic spermatogenic cells were detected. Tau-fluvalinate exposure was found to induce apoptosis and cause germ cell deterioration.

Article Info

**Corresponding author*:

e-mail: cansua@sakarya.edu.tr

Institution: [1] Sakarya University

Article history

Received: 19//09/2023

Accepted: February 23/11/2023

Available online: 30/04/2023

Keywords:

Tau-fluvalinate, Pesticide, Histology, Testis, Zebrafish

How to Cite: C. Akbulut, C. Öztürk, ND. Yön Ertuğ " Histological Disruptive Effects of Tau-Fluvalinate in Zebrafish (*Danio rerio*) Testis", *Environmental Toxicology and Ecology, c. 4, sayı. 1, ss. 1-10, 2024.*

DOI: 10.59838/etoxec.1362835

1. INTRODUCTION

Environmental pollution due to toxic components is among the most critical problems globally [1]. In recent years, the use of pesticides has increased remarkably to protect plant products. The unconscious use of toxic components and pesticides has increased and undesirable adverse effects on organisms, depending on the exposure period. Depending on the increased use, pesticides contaminate the aquatic environment and negatively affect the organisms [2].

The use of synthetic pyrethroids, an important pesticide group, started in the 1970s. Although increases were observed in the number of agricultural products with the use of these substances, it was determined that they also caused damage to non-target organisms [3]. These substances, which have high insecticidal activities can act rapidly, and are neurotoxins that interact with sodium channels [4-5].

Tau-fluvalinate is a synthetic pyrethroid pesticide and environmental toxin used to control parasites and protect trees, plants, and vegetables against pests in large areas [6-7]. Like synthetic pyrethroids, it exerts an inhibitory

effect on sodium channels. Tau-fluvalinate acts on the nervous system by keeping sodium channels open in nerve cells in insects [8-9].

Zebrafish is a model organism species preferred by researchers in many fields such as biology, pharmacology, toxicology, genetics, neurophysiology, ecology and evolutionary biology since the 1930s [10]. One of the most critical reasons zebrafish are preferred in studies is that they are resistant to changing environmental conditions. Zebrafish frequently used in environmental toxicology studies to detect toxins in water samples or investigate the mechanisms of environmental toxins and related diseases [11].

Although tau-fluvalinate is an environmental hazard, reproductive toxicity in many aquatic organisms, including zebrafish, is still unknown. Gonads are the primary reproductive organs responsible for producing germ cells like sperms and oocytes. Histology is an important tool for toxicologists to detect adverse effects of environmental contaminants in the study of reproductive health of fishes [12-13]. In this study, it was aimed to investigate the histopathological effects of sublethal concentrations of tau-fluvalinate in testis tissue of adult zebrafish.

2. MATERIALS AND METHODS

2.1.Test Chemical

Tau-fluvalinate (CAS No: 102851-06-9) commercially named Mavrik® 2F, was obtained from Adama (Turkey).

2.2.Animal Husbandary

The zebrafish used in this study were obtained from Sakarya University Aquaculture Lab. Esentepe, Sakarya, Turkey. Sexually mature zebrafish (approximately 3-4 cm in length, 1 year old) were used. They were raised in dechlorinated tap water and maintained as follows: a 12 h light/12 h dark photoperiod, 28 ± 1°C temperature, 7.0 ± 0.5 pH, and 6.0 mg/L dissolved oxygen. Zebrafish were fed with an artificial diet Tetramin Pro Energy®, (Tetra Werke, Germany) twice a day.

2.3.Exposure

The fish used in the study were randomly divided into 3 groups, one control and two experimental groups. Each group received 10 fish. 8 and 16 µg/L tau-fluvalinate was applied to the experimental groups by dilution from the prepared stock solution. After the 5-days of exposure, zebrafish were dissected.

2.4.Histopathology

Dissected testicular tissues were fixed in 10% neutral buffered formaldehyde solution for 24 hours. In order to remove excess water from the tissues, dehydration was carried out by ascending series of ethyl alcohol concentrations. Tissues were cleared with xylene and embedded in paraffin. Sections of approximately 5 µm thickness were taken from the tissues in the paraffin blocks with the help of a Leica RM2125RT brand rotary microtome. hemotoxylin & eosin staining method was used to examine the tissues under the light microscope (Leica DM500).

© Environmental Toxicology and Ecology
2024, Vol. 4 (1)
ISSN: 2757-9719

Research Article

2.5. Semi-quantitative Scoring

Semiquantitative scoring was performed according Mishra and Mohanty [14]. 10 slides of 3 randomly selected individuals from each group were examined. The histomorphological changes were categorized as none, mild (25% of sections), moderate (25–50% of sections), and severe (>50% of sections).

3. RESULTS

3.1.Semiquantitative scoring

The results of this study revealed that tau-fluvalinate exposure cause histopathological changes in testicular tissue of zebrafish. The histopathological lesions according to the exposure concentrations are showed in Table 1 by semiquantitative scoring as mild, moderate, and severe.

Tablo 1. Semiquantitative scoring of testicular tissue of zebrafish exposed to 8 and 16 µg/L of tau-fluvalinate

Histopathological Lesions	Control	8µg/L	16µg/L
Fusion of seminiferous tubules	-	++	+++
Testicular degeneration	–	+	+++
Hypertrophic spermatocytes	–	+	+++
Hypertrophic Leydig cells	–	+	+
Karyorrhexis and apoptosis	–	+	+++
Vascular congestion	–	++	+
Spermatogenic cells with pyknotic nuclei	–	+	+++
Interstitial fibrosis	–	+	++

Histopathological lesions were scored to the to their severity (-: none, +: mild, ++: moderate, +++: severe)

3.2.Histopathological Evaluation

3.2.1. Control Group

Histopathological alternations were not observed in control group. Seminiferous tubules and surrounding connective tissue were visualized in testicular tissue (Fig. 1a). Spermatogenetic cell groups located in the seminiferous tubule and Leydig cells responsible for hormone production between the seminiferous tubules were seen. In seminiferous tubules, spermatogonia, the largest spermatogenic cells, were distinguished with large and pale nuclei. Spermatocytes were observed to be smaller in size than spermatogonia. Primary spermatocytes were oval and with dense cytoplasm. On the other hand, secondary spermatocytes were similar to primary spermatocytes but were smaller in size and had a rounded structure. The spermatids in the seminiferous tubule were detected as round, small in size, and little cytoplasm. Sperms, the smallest spermatogenic cell, were observed in the central part of the seminiferous tubules (Fig. 1b).

© Environmental Toxicology and Ecology
2024, Vol. 4 (1)
ISSN: 2757-9719

Research Article

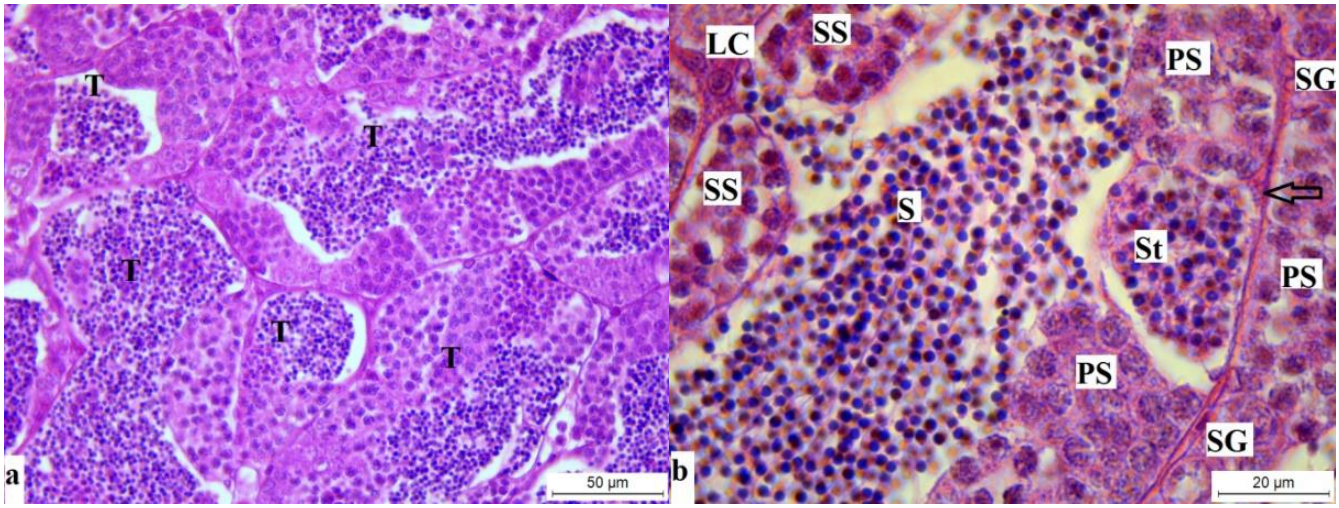

Figure 1 Testicular histology in the control group, a) general view of the seminiferous tubules, b) Spermatogenic cells in the seminiferous tubule, T: seminiferous tubule, LC: Leydig cells, SG: spermatogonia, PS: primary spermatocytes, SS: secondary spermatocytes, St: spermatides, S: sperms, arrow: Sertoli cell, H&E stain.

3.2.2. 8 µg/L Tau-Fluvalinate Exposed Group

In the 8 µg/L tau-fluvalinate exposed group, the integrity of the seminiferous tubule structures was impaired, the borders between the tubules were lost, the intercellular space was expanded and the fusion of the seminiferous tubules was detected compared to the control samples (Fig. 2a, 2b). A relative increase in the proportion of sperm cells to other spermatogenic cell types in the seminiferous tubule was observed (Fig. 2a, 2b, 2c). Hypertrophy of spermatocytes was apparent (Fig. 2a, 2b, 2c, 2d, 2e). Hyperplasia and hypertrophy were detected in Leydig cells (Fig. 2b, 2c) was observed. Vacuolization was seen among spermatogenetic cell clusters (Fig. 2a, 2b, 2d, 2e). Karyorrhexis was detected in some spermatogenic cells. The presence of karyorrhexis in a large number of spermatogenic cells suggested that these cells might be apoptotic (Fig. 2a, 2b, 2c, 2d). In addition, some spermatogenic cells with pyknotic nuclei were also visualized (Fig. 2d). In the interstitial area, vascular congestion was apparent (Fig. 2a, 2b, 2e). Interstitial fibrosis was observed between tubules (Fig. 2d, 2e).

© Environmental Toxicology and Ecology
2024, Vol. 4 (1)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

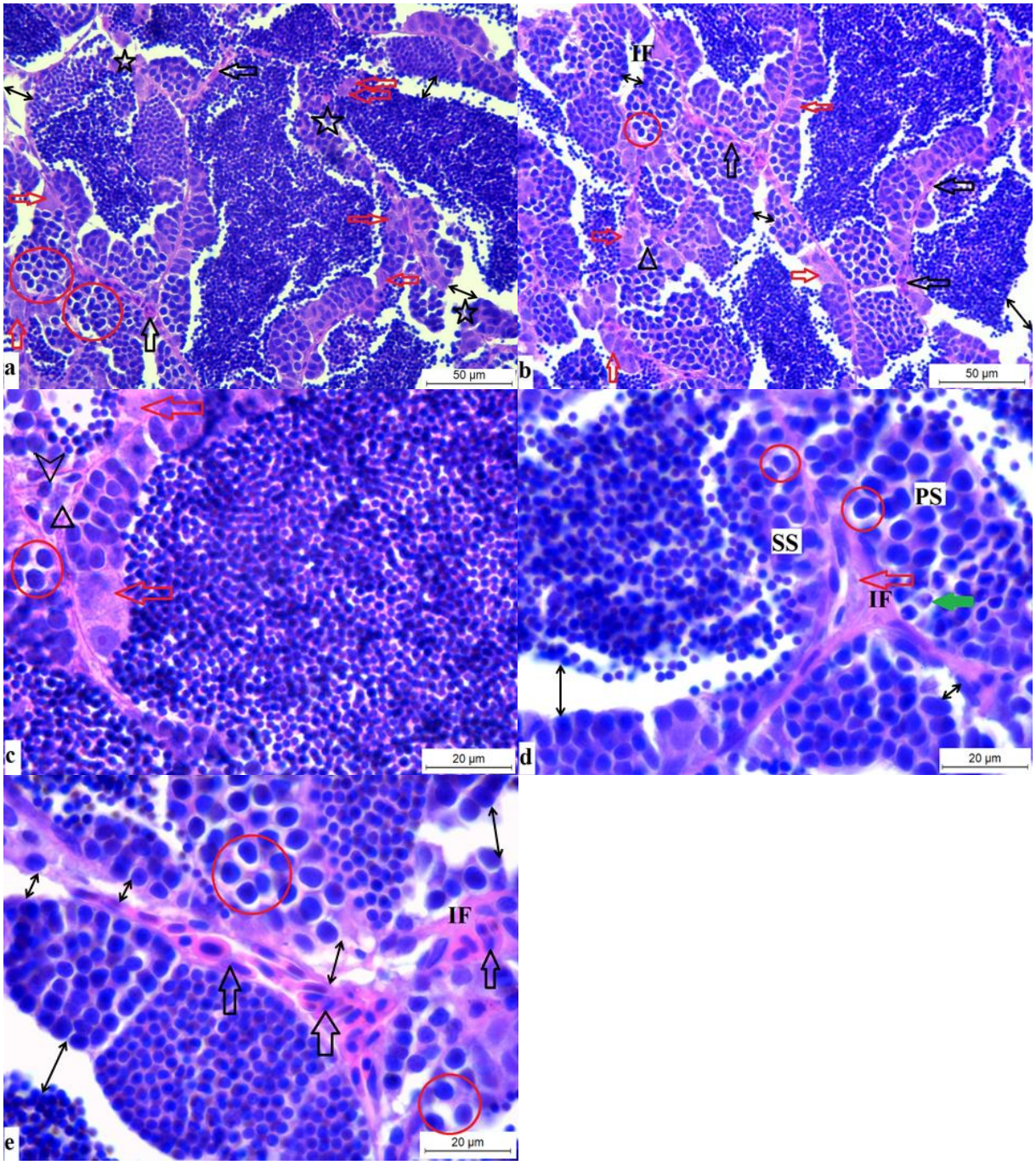

Figure 2 Testicular histology in the 8 µg/L tau-fluvalinate exposed group, a, b) general view of the seminiferous tubules, c, d, e) Spermatogenic cells in the seminiferous tubule, PS: primary spermatocyte, SS: secondary spermatocyte, IF: interstitial fibrosis, star: fusion of seminiferous tubules, black arrow: vascular congestion, red arrow: karyorrhexis, green arrow: spermatogenic cells with pyknotic nuclei, double headed arrow: vacuolization, red circle: hypertrophic spermatocytes, triangle: hypertrophic Leydig cells, arrow head: hyperplastic Leydig cells. H&E stain.

3.2.3. 16 µg/L Tau-fluvalinate Exposed Group

In the 16 µg/L tau-fluvalinate exposed group, severe histopathological changes were observed. Fusion of seminiferous tubules was displayed (Fig. 3a, 3b). Disintegration was seen in some seminiferous tubules (Fig. 3a, 3e). As in the 8 µg/L tau-fluvalinate exposed group, a decrease in the number of spermatogenic

© Environmental Toxicology and Ecology
2024, Vol. 4 (1)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

cells due to increase in sperm proportion was detected (Fig. 3a, 3b). Hypertrophic spermatocytes were remarkable (Fig. 3a, 3d, 3e). Syncytium was detected in primary spermatocytes (Fig. 3a, 3b, 3c, 3d). Karyorrhexis was observed in the spermatogenic cells in this group either (Fig. 3b, 3c). Some spermatogenic cells with pyknotic nuclei were detected (Fig. 3d, 3e). Apoptotic germ cells were identified. Vacuolization was apparent between spermatogenic cells in seminiferous tubule (Fig. 3a, 3b, 3c, 3d). Vascular congestion (Fig. 3a, 3b) and interstitial fibrosis (Fig. 3c, 3e) were observed between tubules.

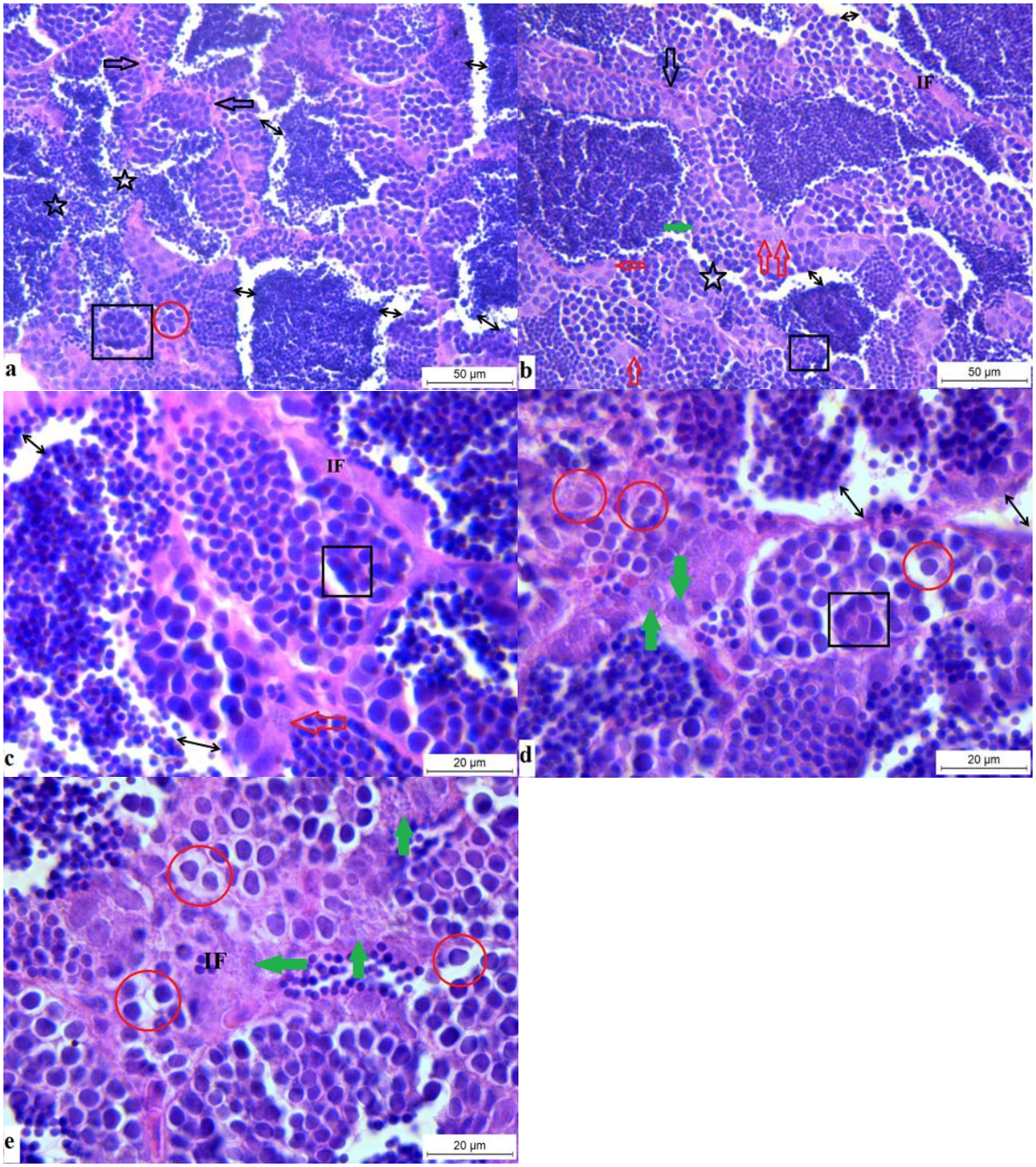

Figure 3 Testicular histology in the 16 µg/L tau-fluvalinate exposed group, a, b) general view of the seminiferous tubules, c, d, e) Spermatogenic cells in the seminiferous tubule, IF: interstitial fibrosis, star: fusion of seminiferous tubules, black arrow: vascular congestion, red arrow: karyorrhexis, green arrow: spermatogenic cells with pyknotic nuclei, double headed arrow: vacuolization, red circle: hypertrophic spermatocytes, square: syncytium, H&E stain.

4. DISCUSSION

Pesticide derivatives, which are preferred for chemical control against harmful species due to the increase in their populations, have had negative effects on different organisms over time. Chemical structure and formulation are important in the effect of pesticides on the organism. These harmful chemicals, which contaminate the aquatic environment in different ways, show a wide variety of toxic effects on aquatic organisms.

Studies have been conducted on the negative effects of environmental and industrial pollutants on the reproductive system. In these studies, it has been shown that environmental pollutants cause a decrease in sperm count, abnormal sperm formation, and deterioration in the morphology of testicular tissue, especially in male individuals [15-19]. In a study, clustering of sperms and deterioration of seminiferous tubules were observed in testicular tissue of *Lepomis macrochirus* exposed to diazinon, an organophosphate insecticide. Adverse effects were observed in testicular tissue from the beginning of spermatogenesis and diazinon application was reported to prevent sperm formation [20]. Similarly, disruption in the structure of seminiferous tubules and aggregation of sperms occurred in the testicular tissue of zebrafish exposed with tau-fluvalinate.

As a result of the exposure of endosulfan, a polychlorinated hydrocarbon insecticide, deterioration in the structure of the seminiferous tubules, decrease in the number of spermatogonia and irregularity in the structure of the testicular tissue in Lepomis macrochirus was stated [21]. These histopathological changes observed in the seminiferous tubule and spermatogonia were similar to our study.

As a result of cypermethrin exposure in the testicular tissue of *Channa punctatus*, clustering and vacuolization among spermatogenic cells in seminiferous tubule and deteriorated spermatids occurred [22]. Staicu et al. [23] determined that there was a decrease in sperm count in *Corassius auratus gibelio* exposed to deltamethrin for 14 days. In a study conducted by Bayar et al. [24], it was stated that deltamethrin had adverse effects on the testicular tissue in *Oreochromis niloticus*, such as a decrease in the number of spermatogenic cells and structural deterioration in spermatogonia. It was observed that the adverse effects of deltamethrin on testicular tissue was more than tau-fluvalinate.

In Rocha et al. [25]'s study, histopathological effects in testis tissue of zebrafish were investigated after exposure to carbamazepine, fenofibric acid, propranolol, sulfamethoxazole and trimethoprim for 21 days. Compared to the control group, a decrease in sperm count and an increase in the number of spermatocytes were observed in exposure groups. It has been determined that these chemicals cause testicular damage during the maturation period of the fish. In our study, unlike other chemicals, it was determined that the tau-fluvalinate exposure caused an increase in sperm proportion, while it caused a decrease in the number of other spermatogenic cells.

The histopathological effects of etofenprox, a synthetic pyrethroid, were investigated in zebrafish testicular tissue. While no histopathological effect was observed in tissue samples dissected after 48 hours, sperm cells decreased and the number of Leydig cells increased in testicular tissues dissected after 96 hours. It has

been showed that the tissue structure deteriorated and became unable to perform its normal function [26]. The histopathological effects of etofenprox were much more than the tau-fluvalinate was concluded.

In a study examining the effects of fenvalerate on the *Barbus carnaticus* testis structure, disruption of the seminiferous tubules and aggregation of the spermatogonia, spermatids and sperms was reported [27]. Chen et al. [28] investigated the reproductive toxicity of dibutyl phthalate and diisobutyl phthalate in male zebrafish. It has been stated that these chemicals disrupt the spermatogenesis process in the testis, has affect testicular morphology adversely, and cause an enlargement in the intercellular area. Considering these results, it can be said that tau-fluvalinate has similar effects with dibutyl phthalate and diisobutyl phthalate in testicular tissue.

In another study, it has been determined that adverse effects occur on spermatogonium, spermatid and sperm cells in zebrafish exposed to pyriproxyfen. It has been reported that spermatids and sperm are significantly reduced and spermatocyte and spermatogonia are increased [29]. As a result of exposure to pyriproxyfen, an increase was observed in the size and number of spermatogonia in the testicular tissue, while no change was observed in the number and size of the other spermatogenetic cells [30]. The application of tebucosanol and diphenicosanol causes a significant increase in the percentage of spermatogonia in the early spermatogenesis stage and a decrease in the percentage of spermatocyte was reported in zebrafish testis [31].

In this study, it has been showed that tau-fluvalinate exposure cause several histopathological changes and induced apoptosis in testicular tissue of zebrafish. As a result of excessive and unconscious use of pesticides, the aquatic ecosystem has been polluted, which harms the reproductive potential of organisms. Therefore, biological control should be preferred in agriculture or the use of biopesticides should be encouraged.

Funding

The author did not receive any financial support for the research, authorship, or publication of this study.

The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors

Author's Contribution

The first author contributed 60%, the second author 20%, the third author 20%.

REFERENCES

[1] R. Chadran, A.A. Sivakumar, S. Mohandass, M. Aruchami, "Effect of Cadmium and Zinc on Antioxidant Enzyme Activity in the Gastropod, *Achatina fulica*", Comp. Biochem Phsiol., 140C: 422-426, 2005.
[2] N. Delen, "Fungisitler", Nobel Yayın Dağıtım, 552 s. 2008.
[3] N. Vural, "Toksikoloji", A.Ü. Basımevi, 221 s. 2000.
[4] L.B. Smith, Kasai S., J.G. Scott, "Pyrethroid resistance in Aedes aegyptiand Aedes albopictus: Important mosquito vectors of human diseases. Pestic. Biochem. Physiol., 133: 1-12, 2016.
[5] O. Güntay, H. Çay, B. Durusel, Y. Terzi, "Senteik Piretroidlere Genel Bakış." Atatürk Univ. J. of Agricultural Faculty, 52 (2): 201-223, 2021.
[6] T.R. Roberts, D.H. Hutson, "Metabolic Pathways of Agrochemicals", Royal Society of Chemistry, 670, 1999.
[7] EPA, United States Environmental Protection Agency https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID7024110&abbreviation=IRIS#exec_sum 01.10.2021
[8] EPA, Office of Pesticide Programs, What is pesticide? http://www.epa.gov/pesticides/about, 2005
[9] M. Yalçınkaya "Fluvalinate'ın lebistes balıkları (*Poecilia reticulata*) üzerine akut etkisinin belirlenmesi", Hacettepe Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, 2013.
[10] F. Kayhan, G. Kaymak, H. E. Esmer Duruel and Ş. Tartar Kızılkaya, "Biyolojik Araştırmalarda Zebra Balığının (*Danio rerio* Hamilton, 1822) Kullanılması ve Önemi", Gaziosmanpaşa Bilimsel Araştırma Dergisi, 7(2): 38-45, 2018.
[11] K. Bambino, J. Chu, "Zebrafish in Toxicology and Environmental Health", Curr Top Dev Biol., 124:331-367, 2017.
[12] V. Blazer, "Histopathological assessment of gonadal tissue in wild fishes", Fish Physiology and Biochemistry, 26: 85–101, 2002.
[13] P.M. Costa, "The Handbook of Histopathological Practices in Aquatic Environments: Guide to Histology for Environmental Toxicology", Academic Press, p277, 2018.
[14] A.K. Mishra, B. Mohanty, "Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch)", Environ Toxicol Pharmacol., (26): 136-41, 2008.
[15] S.A. Sakr, A.E. Azab, "Effect of pyrethroid inhalation on the testis of albino rat", Pakistan Journal of Biological Sciences, 4(4): 498-500. 2001.
[16] Ö.F. Tekbaş, "Kimyasallar ve Üreme Sağlığı", TSK Koruyucu Hekimlik Bülteni, 5 (1), 2006.
[17] P. Sharma, R. Singh, M. Jan, "Dose-Dependent Effect of Deltamethrin in Testis, Liver, and Kidney of Wistar Rats", Toxicol Int., 21(2): 131–139, 2014.
[18] U. Habeeba, M. David, "Histological Respoense In a Common Carp *Cyprinus carpio* Induced by Sublethal Concentration of Syntetic Pypretroid Lambda Cyhalothrin", International Journal of Pharmaceutical Chemical and Biological Science, 10(3): 58-64, 2020.
[19] A.M.A. Hameed, H.S. Mahmoud, "Cypermethrin induced apoptosis and testicular toxicity by upregulation of p53 in the brain and testis of male rats is alleviated by Sesame oil", Journal of Taibah University for Science, 14(1), 2020.
[20] H.M. Dutta, H.J.M. Meijer, "Sublethal effects of diazinon on the structure of the testis of bluegill, *Lepomis macrochirus*: a microscopic analysis", Environmental Pollution, 125: 335-360, 2003.
[21] H.M. Dutta, D. Misquitta, K. Sanaullah, "The effects of Endosulfan on the testes of Bluegill fish, *Lepomis macrochirus*: a histopathological study", Archives of Environmental Contamination and Toxicology, 51:149-156, 2006.

[22] R.K. Srivastava, K.K. Yadav, S.P. Trivedi, "Devicyprin induced gonadal impairment in a freshwater food fish, *Channa punctatus* (Bloch)", Journal of Environmental Biology, 29(2):187-191, 2008.

[23] A.C. Staicu, M.C. Munteanu, D. Costin, M. Costache, A. Dinischiotu, "Histological changes in deltamethrin- induced intoxication in Carassius auratus gibelio (Pisces- Cyprinidae)", Biotechnol Anim. Husb., 23: 619-626, 2007.

[24] A.S. Bayar, S. Bilici, E.İ. Cengiz, A. Satar, M. Yanar, "The effects of Vitamin E supplemantation on ovary and testis histopathology in *Oreochromis niloticus* exposed to deltamethrin", Toxicological & Environmental Chemistry, 96:1, 114-135, 2014.

[25] R.E. Madureira, C. Cruzeiro, C., M.J. Rocha, E. Rocha, "The toxicity potential of pharmaceuticals found in the Douro River estuary (Portugal)—Experimental assessment using a zebrafish embryo test", Environmental Toxicology and Pharmacology, 32(2): 212-217, 2011.

[26] N. Ağırbaşlı, "Çevresel Kirleticileri Etofenpoksun Zebra Balığı (*Danio rerio*)'da Genotoksik ve Histopatolojik Etkilerinin Belirlenmesi", Gazi Üniversitesi. Doktora tezi. 2016.

[27] A.M. Vibhandik, R.B. Gaikwad, G.P. Wani, "Fenvalerate Induced Changes in Histological Structure of Gonads of Freshwater Teleost Fish *Barbus carnaticus*", International Journal of Scientific Research in Science and Technology, 6(2): 138-142, 2019.

[28] H. Chen, K. Chen, X. Qiu, H. Xu, G. Mao, T. Zhao, W. Feng, E.S. Okeke, X. Wu, L. Yang, "The reproductive toxicity and potential mechanisms of combined exposure to dibutyl phthalate and diisobutyl phthalate in male zebrafish (*Danio rerio*)", Chemosphere, 258: 127238, 2020.

[29] K. Maharajan, S. Muthulakshmi, C. Karthik, B. Nataraj, K. Nambirajan, D. Hemalatha, S. Jiji, K. Kadirvelu, K. Liu, M. Ramesh, "Pyriproxyfen induced impairment of reproductive endocrine homeostasis and gonadal histopathology in zebrafish (*Danio rerio*) by altered expression of hypothalamus-pituitary-gonadal (HPG) axis genes" Science of The Total Environment, 735: 139496, 2020.

[30] V.S. Oliveria, A.J.G. Castro, K. Marinsi, K.B. Mendes, G.A.A. Leite, A. Zamoner, G.D. Kraak, F.R.M.B. Silva, "Pyriproxyfen induces intracellular calcium overload and alters antioxidant defenses in *Danio rerio* testis that may influence ongoing spermatogenesis", Environmental Pollution, 270: 116055, 2021.

[31] J. Jiang, L. Chen, X. Liu, L. Wang, S. Wu, X. Zhao, "Histology and multi-omic profiling reveal the mixture toxicity of tebuconazole and difenoconazole in adult zebrafish", Science of The Total Environment, 795(15): 148777, 2021.