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# Investigation of Acute Toxicity of Cyfluthrin on Zebrafish (*Danio rerio*)

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**ABSTRACT**

Cyfluthrin is a synthetic pyrethroid that contaminates aquatic ecosystems and is also a potential toxic contaminant. This study, it was aimed to calculate the accumulation values of 0.5, 1, 5, 10, 20, and 50 µg/L dosing solutions of the chemical cyfluthrin in zebrafish (*Danio rerio*), a model organism, for 96 hours The experiments were performed in triplicate and the static test method was used in the acute toxicity test. At the same time, behavioral changes such as a decrease in swimming speed and swimming on the side and bottom were observed at all cyfluthrin concentrations in fish. Data obtained from acute toxicity tests of cyfluthrin were evaluated using the Probit Analysis Statistical Method. The 96-hour $LC_{50}$ value of zebrafish was calculated as 3.61 µg/L.

# Siflutrinin Zebra Balıkları (*Danio rerio*)'nda Akut Toksisitesinin Araştırılması

**Araştırma Makalesi**

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***Anahtar Kelimeler***
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$LC_{50}$
Piretroid

**ÖZ**

Siflutrin, su ekosistemlerini kirleten ve aynı zamanda potansiyel bir toksik kirletici olan sentetik bir piretroidtir. Bu çalışmada model bir organizma olan zebra balıklarında (*Danio rerio*) siflutrin kimyasalının 0,5, 1, 5, 10, 20 ve 50 µg/L olan dozlama çözeltilerinin 96 saat süreyle birikim değerlerinin hesaplanması amaçlanmıştır. Deneyler üç tekrarlı olarak yapılmış ve akut toksisite testinde statik test yöntemi kullanılmıştır. Aynı zamanda balıklarda tüm siflutrin konsantrasyonlarında yüzme hızında azalma, yan ve dip kısımda yüzme gibi davranış değişiklikleri gözlemlenmiştir. Siflutrinin akut toksisite testlerinden elde edilen veriler Probit Analizi İstatistik Yöntemi kullanılarak değerlendirilmiştir. Zebra balığının 96 saatlik $LC_{50}$ değeri 3,61 µg/L olarak hesaplanmıştır.

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## 1. Introduction

Pyrethroids, a class of pesticides with insecticidal activity, are widely used in agriculture to control ectoparasite infestation and also in animal husbandry. Unfortunately, many existing pyrethroids are toxic to humans, aquatic organisms, mammals, and other organisms. The use of these substances not only harms the ecosystem but also causes manufacturing workers and the public to be exposed to toxic

effects (Jokanovic, 2018; Tamagno et al., 2023). Pyrethroids act on neural sodium channels, which remain open for a long time, leading to insect death and hyper-excite the nervous system (Wang et al., 2010; Zhu et al., 2020). Also, their excessive persistence in the environment can cause pollution, such as polluting groundwater and disrupting agricultural production. Together with the risk reduction decisions taken by the US Environmental Protection Agency (EPA), it has resulted in increased use of pyrethroid insecticides and thus increased human exposure to pesticides. In order to evaluate the potential health risks, the endocrine-disrupting effects of pyrethroids, especially hormone receptor activities, should be studied (Du et al., 2010).

Lethal concentration 50 ($LC_{50}$), is an abbreviation for the exposure concentration of a toxic substance that was fatal to half of the test animals. Values for fish toxicity vary among species in the literature. Fish are very important for aquatic creatures and humans as an important component of the food chain (Swiacka et al., 2022). Zebrafish, as they have a short reproductive cycle, low cost, and transparent embryos, high productivity it is generally used in aquatic toxicity studies at larval, embryonic, and adult stages (Teame et al., 2019). Zebrafish are preferred in acute toxicity tests to determine the relationship between exposure to toxic compounds in vertebrates (Bambino and Chu, 2017; Putra et al., 2022). The externally fertilized embryo of zebrafish shows rapid and transparent development, allowing relatively easy collection and drug treatment at the early embryonic stage, as well as microscopic examination throughout embryonic development (Shi et al., 2018; Fan et al., 2021; Zhang et al., 2022). At the same time, they are easy to find, easy to maintain, and cost-effective, high fertility, short life cycles, and being more sensitive to chemicals are among the reasons why they are preferred. Especially in recent years, it is frequently encountered in the literature as an advantageous model organism used in the study of the effects of endocrine disruptors (Ankley and Johnson, 2004; Lawrence, 2007; Ni et al., 2019; Segner, 2009; Spence et al., 2008). Cyfluthrin is the active ingredient in nonsystemic insecticides used for the control of insects, in the garden, for pets and livestock, for mosquito control, and where necessary for public health. Cyfluthrin is the active ingredient used in important areas for the control of chewing and sucking insects, especially public health. Increased toxic effects of cyfluthrin on fish *Daphnia magna* and other aquatic invertebrates have been studied in previous years. For example, various fish species have been reported: 22 µg/L in carp (*Cyprinus carpio*); channel catfish (*Ictalurus punctatus*); 1.5 µg/L in bluegill (*Lepomis macrochirus*); sheepshead minnow (*Cyprinodon variegatus*) (Hill, 1989); 0.68 µg/L in rainbow trout (*Oncorhynchus mykiss*). Fathead min now (*Pimephales promelas*) 96-h LC50 2.49 µg/L (Poore et al., 2001), Rainbow trout (*Oncorhynchus mykiss*) 96-h LC50 0.3 µg/L (Powell, 2001) and Sheepshead minnow (*Cyprinodon variegatus*) 96-h LC50 4.05 µg/L. Wolansky and Harrill (2008) studied pyrethroid toxicology in mammals, amphibians, and birds as well as terrestrial and aquatic invertebrates (Wolansky and Harrill, 2008). In the studies conducted, synthetic pyrethroids; Have been shown to be highly toxic to fish, honeybees, and aquatic arthropods. Cyfluthrin affects the central nervous system and has a stimulating

effect on sodium ion channels, while also preventing neurotransmitter transmission through inhibition of calcium ion channels (Du et al., 2010; Kadiru, 2021). The aim of the study was to determine the 96-hour $LC_{50}$ value of cyfluthrin. When the studies were examined, no studies were reported regarding the acute toxicity test of cyfluthrin on the model organism zebrafish.

## 2. Material and Methods

Zebrafish with a mean length of 3.59±0.67 cm, obtained from a commercial company in Ankara, were used in the study. The study was completed at the Gazi University Faculty of Medicine Medical Biochemistry Research Laboratory. To ensure adaptation to the experimental environment, 40 experimental and control zebrafish (female/male equal distribution) were taken into the stock aquarium 15 days before the start of the experiment and were placed in 8 (5 fish in each) experimental aquariums with the size of 10x20x35 and one control aquarium. The fish were fed with flake fish food (Ahm Cichlid Flake Food), which was combined with vitamins and minerals and had high-quality protein content, rich in amino acids, carnivorous and plant-rich. Fish were dosed at the determined rates of 0.5, 1, 5, 10, 20, and 50 µg/L. It was dissolved in 0.052 g CYF/25 mL DMSO. Stock solutions were prepared by diluting the cyfluthrin weighed in determined amounts in dimethyl sulfoxide (DMSO). Two different control groups were used in the study, control and solvent control (DMSO). Control groups (Control and DMSO added control) were also conducted under the same conditions. It was observed that DMSO had no effect on the fish and there was no death in either control group. DMSO was dosed not to exceed 100 microliters per liter. The ambient temperature was kept constant at 23-24 ºC by providing a photoperiod of 12:12 in the research laboratory (Liu et al., 2016). The mean composition values of water used in the study; have a pH of 7.2, dissolved oxygen of 5.4 mg/L, and a conductivity of 75.7 µs/cm (21ºC). Stock solutions were prepared at concentrations of 0.5, 1, 5, 10, 20, and 50 µg/L for application for 96-h and using EPA Probit Analysis Program V 1.5. Five fish were placed in each of eight 10-litre aquariums and, excluding the control group, solutions concentrations were pipetted into the aquariums, respectively. After observation for 24, 48, 72, and 96-h, the dead fish were removed from the aquarium and the deaths were recorded. DMSO was used in the preparation of cyfluthrin (Cyfluthrin 95%) solution. Experimental aquariums were ventilated with the help of air motors and the subjects were not fed during the experiment. The 'probit analysis' was applied to the data obtained as a result of the experiment with the help of SPSS and the LC50 value was calculated.

## 3.Results

In the bioassay system, the 96-h LC50 value of cyfluthrin was found to be 3.61 µg/L. The study was repeated three times and their behavior was monitored. Results are presented in Table 1 and Figure 1. It was observed that cyfluthrin was quite toxic to *Danio rerio.* The species used are recommended according to previously used reference/standard methods (Greally and Jacobs, 2013). Fish at concentrations of 0.5, 1, 5, and 10 µg/L showed normal behavior, similar to the behavior of the control group. Behavioral changes began 1 hour after dosing at 20 and 50 µg/L. At the end of the study, no deaths were recorded in the control groups. Irregular swimming, hanging, loss of balance, swimming vertically in the water, contractions, and lying down were observed at the bottom of the aquarium.

**Table 1.** 96-h toxicity of cyfluthrin to *Danio rerio.*

| Point | Concentration (µg/L) | 95% confidence limits | Intercept×S.E. | Slope×S.E. |
|---|---|---|---|---|
| **LC 1.00** | 0.09 | 0.00-0.47 | 2.66-0.66 | 4.17±0.40 |
| **LC 5.00** | 0.027 | 0.00-0.97 | | |
| **LC10.00** | 0.48 | 0.01-1.45 | | |
| **LC15.00** | 0.71 | 0.03-1.93 | | |
| **LC50.00** | 3.61 | 1.06-9.18 | | |
| **LC85.00** | 18.23 | 7.49-197.08 | | |
| **LC90.00** | 26.74 | 10.21-474.16 | | |
| **LC95.00** | 47.18 | 15.60-1802.02 | | |
| **LC99.00** | 136.82 | 32.69-23294.57 | | |

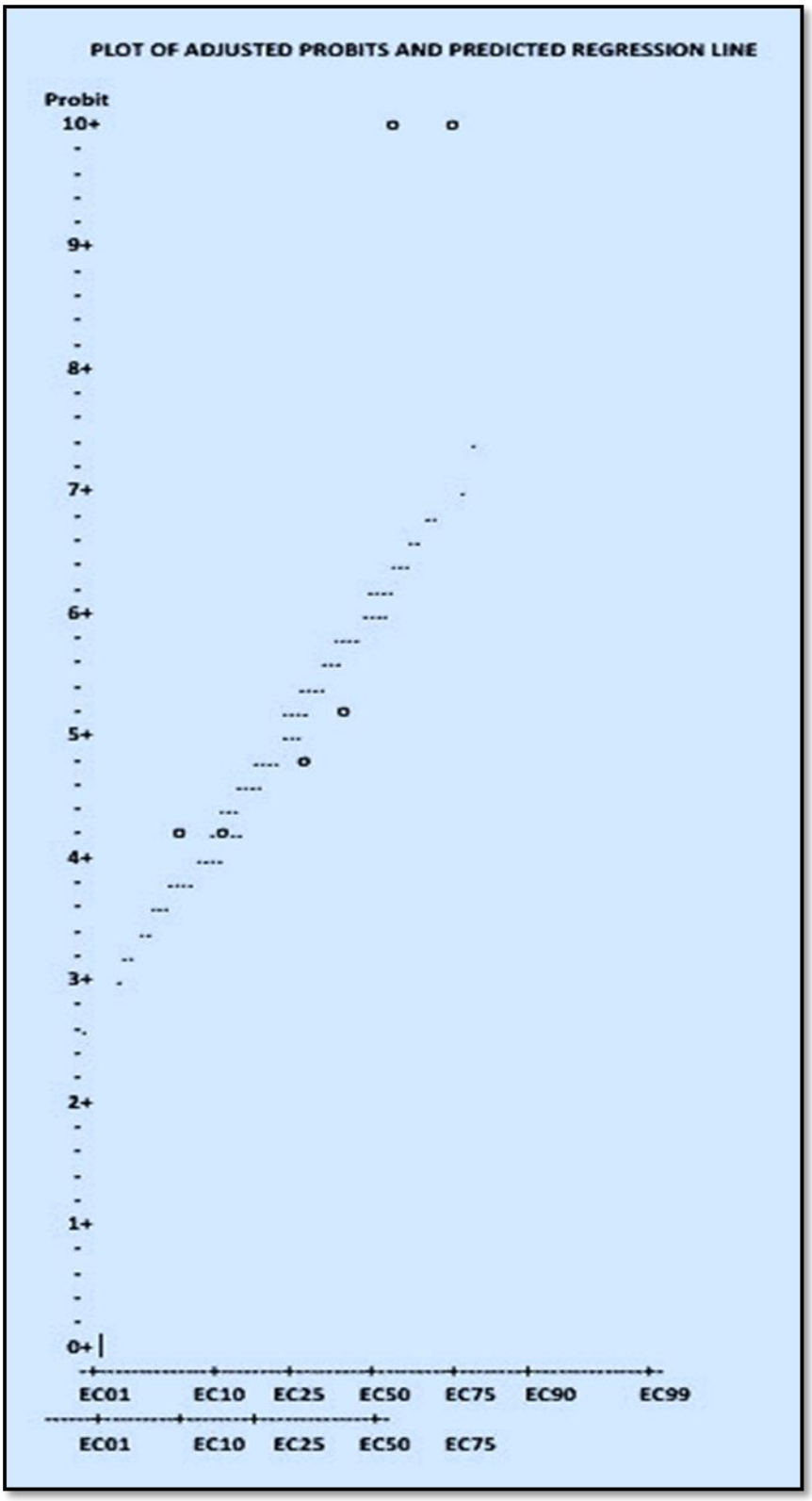


**Figure 1.** Regression line and probits estimated at 96-h for cyfluthrin of *Danio rerio*.

## 4.Discussion

Cyfluthrin is a potent pyrethroid insecticide with generally low toxicity. Due to its long degradation cycle, it can accumulate in the environment for a long time. Cyfluthrin poses a threat to both aquatic and other organisms, and ecotoxicological data are very limited and they do not dissolve easily in water (Niell et al., 2010; Lanteigne et al., 2015). It can also cause potential harm to living things due to their exposure to water, air, and soil. In such studies, zebrafish offer many advantages as model organisms, such as their genetic structure, high reproductive rate, rapid development, small size, and high reproductive structure. Cyfluthrin, a synthetic pyrethroid, damages nerve signals by negatively affecting membrane sodium channel systems in target organisms. Synthetic pyrethroids have been shown in laboratory studies to be toxic to fishies, honeybees, and aquatic arthropods (Antwi and Reddy, 2015; Hill, 1989; Weston et al., 2005; Zhan et al., 2020). There are different studies showing that it negatively affects AChE activity, and with the decrease in AChE activity, anxiety-like behaviors occur and impaired motor activity (Sarasamma et al., 2020; Zhang et al., 2022). AChE plays a role in decreased swimming distance, acute dysfunction, and disruptions in motor development, as well as regulating the development of the central nervous system in zebrafish larvae. (Irons et al., 2013; Shi et al., 2018). The cumulative mortality rate continues to increase depending on time and dose compared to the control group at every stage of the experiment, indicating that this substance is toxic. One researcher reported an $LC_{50}$ value of 96-hour 21.07 µg/L for cyfluthrin in Nile tilapia fingerlings (*Oreochromis niloticus*). 48-hour $LC_{50}$ value for cyfluthrin in guppy fish was estimated at 8.07 µg/L17 and 0.68 µg/L in rainbow trout (Selvi et al., 2008; Singh et al., 2022). The toxicity of a chemical depends on the environment, the physiological conditions of the species exposed, and the purity of the chemicals used. Acute toxicity, as well as developmental effects of cyfluthrin, were analyzed in zebrafish embryos and larvae at 24, 48, 72, and 96-h. This study highlighted that short-term exposure to cyfluthrin could cause death in the early life stages of zebrafish besides severe developmental deformities.

## 5.Conclusion

This study revealed that cyfluthrin, widely used in agriculture, is a highly toxic synthetic pyrethroid pesticide. The increasing use of Cyfluthrin in recent years poses a threat to aquatic ecosystems. Therefore, caution should be exercised in agricultural use and awareness should also be exercised in domestic use. The use of environmentally friendly, effective, and appropriate pesticides is important for the health of many living organisms as well as aquatic ecosystems.

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**References**


Ankley GT., Johnson RD. Small fish models for identifying and assessing the effects of endocrine-disrupting chemicals. ILAR Journal/National Research Council, Institute of Laboratory Animal Resources 2004; 45(4): 469–483.

Antwi FB., Reddy GV. Toxicological effects of pyrethroids on non-target aquatic ınsects. Environmental Toxicology and Pharmacology 2015; 40: 915–923.

Bambino K., Chu J. Zebrafish in toxicology and environmental health. Current Topics in Developmental Biology 2017; 124: 331–367.

Du G., Shen O., Sun H., Fei J., Lu C., Song L., Xia Y., Wang S., Wang X. Assessing hormone receptor activities of pyrethroid ınsecticides and their metabolites in reporter gene assays. Toxicol Science 2010; 116(1): 58–66.

Fan R., Zhang W., Jia L., Li L., Zhao J., Zhao Z., Peng S., Chen Y., Yuan X. Combined developmental toxicity of the pesticides difenoconazole and dimethomorph on embryonic zebrafish. Toxins 2021; 13(12): 854.

Greally JM., Jacobs MN. In vitro and in vivo testing methods of epigenomic endpoints for evaluating endocrine disruptors. Altex 2013; 30(4): 445–471.

Hill IR. Aquatic organisms and pyrethroids. Pesticide Science 1989; 27(4): 429–457.

Irons TD., Kelly PE., Hunter DL., Macphail RC., Padilla S. Acute administration of dopaminergic drugs has differential effects on locomotion in larval zebrafish. Pharmacology Biochemistry and Behavior 2013; 103(4): 792-813.

Jokanovic M. Neurotoxic effects of organophosphorus pesticides and possible association with neurodegenerative diseases in man: A review. Toxicology 2018; 410, 125-131.

Kadiru S. Developmental toxicity of cyfluthrin in embryo-larval stages of zebrafish. International Journal of Advanced Scientific Research and Management 2021; 3-12

Lanteigne M., Whiting SA., Lydy MJ. Mixture toxicity of imidacloprid and cyfluthrin to two non-target species, the fathead minnow pimephales promelas and the amphipod hyalella azteca. Archives of Environmental Contamination and Toxicology 2015; 68, 354-361.

Lawrence C. The husbandry of zebrafish (Danio rerio): a review. Aquaculture 2007; 269(1–4): 1–20.

Liu Y., Zhang Y., Tao S., Guan Y., Zhang T., Wang Z. Global dna methylation in gonads of adult zebrafish danio rerio under bisphenol a exposure. Ecotoxicology and Environmental Safety 2016; 130: 124–132.

Poore LM., King G., Stefanik K. Toxicology information resources at the environmental protection agency. Toxicology 2001; 157(1–2): 11–23.

Ni H., Peng L., Gao X., Ji H., Ma J., Li Y., Jiang S. Effects of maduramicin on adult zebrafish (Danio rerio): acute toxicity, tissue damage and oxidative stress. Ecotoxicology and Environmental Safety 2019; 168: 249-259.

Niell S., Pareja L., Geis Asteggiante L., Cesio MV., Heinzen H. Comparison of extraction solvents and conditions for herbicide residues in milled rice with liquid chromatography-diode array detection analysis (lc-dad). Food Additives and Contaminants 2010; 27(2): 206-211.

Powell S. New challenges: residential pesticide exposure assessment in the california department of pesticide regulation, usa. The Annals of Occupational Hygiene 2001; 45: 119-123.

Putra RB., Hertika A., Fadjar M., Wicaksono S., Hakim GA., Saputra F. Acute toxicity of cinnamaldehyde in profile hematology and gill histology of zebrafish. Egyptian Journal of Aquatic Biology & Fisheries 2022; 26(4): 623-635.

Sarasamma S., Audira G., Siregar P., Malhotra N., Lai YH., Liang ST., Chen JR., Chen KHC., Hsiao CD. Nanoplastics cause neurobehavioral impairments, reproductive and oxidative damages, and biomarker responses in zebrafish: throwing up alarms of widespread health risk of exposure. International Journal of Molecular Sciences 2020; 21(4): 1410.

Segner H. Zebrafish (Danio rerio) as a model organism for ınvestigating endocrine disruption. Comparative Biochemistry and Physiology-C Toxicology and Pharmacology 2009; 149(2): 187–195.

Selvi M., Sarıkaya R., Erkoç F., Koçak O. Acute toxicity of the cyfluthrin pesticide on guppy fish. Environmental Chemistry Letters 2008; DOI, 10.

Shi Q., Wang M., Shi F., Yang L., Guo Y., Feng C., Liu J., Zhou B. Developmental neurotoxicity of triphenyl phosphate in zebrafish larvae. Aquatic Toxicology 2018; 203: 80–87.

Singh AK., Kumar A., Chandra R. Environmental pollutants of paper industry wastewater and their toxic effects on human health and ecosystem. Bioresource Technology Reports 2022; 20, 101250.

Spence R., Gerlach G., Lawrence C., Smith C. The behavior and ecology of the zebrafish, danio rerio. Biological Reviews. 2008; 83(1): 13–34.

Swiacka K., Maculewicz J., Kowalska D., Caban M., Smolarz K., Świeżak J. Presence of pharmaceuticals and their metabolites in wild-living aquatic organisms–current state of knowledge. Journal of Hazardous Materials 2022; 424, 127350.

Tamagno WA., Alves C., Pompermaier A., Veneral AL., Lampugnani JAD., Reolon GK., Barcellos LJG. Transfluthrin-and prallethrin-based insecticides elicit specific enzymatic antioxidant responses in different tissues of zebrafish. Environmental Pollution 2023; 327, 121530.

Teame T., Zhang Z., Ran C., Zhang H., Yang Y., Ding Q., Xie M., Gao C., Ye Y., Duan M., Zhou Z. The use of zebrafish (Danio rerio) as biomedical models. Animal Frontiers 2019; 9(3): 68–77.

Wang X., Zhou S., Ding X., Zhu G., Guo J. Effect of triazophos, fipronil and their mixture on miRNA

expression in adult zebrafish. Journal of Environmental Science and Health Part B 2010; 45(7): 648-657.

Weston DP., Holmes RW., You J., Lydy MJ., Aquatic toxicity due to residential use of pyrethroid ınsecticides. Environmental Science and Technology 2005; 39(24): 9778–9784.

Wolansky MJ., Harrill JA. Neurobehavioral toxicology of pyrethroid ınsecticides in adult animals: a critical review. Neurotoxicology and Teratology 2008; 30(2): 55–78.

Zhan H., Huang Y., Lin Z., Bhatt P., Chen S. New ınsights into the microbial degradation and catalytic mechanism of synthetic pyrethroids. Environmental Research 2020; 182: 109138.

Zhang W., Fan R., Luo S., Liu Y., Jin Y., Li Y., Li B., Chen Y., Jia L., Yuan X. Combined effects of chlorpyrifos and cyfluthrin on neurobehavior and neurotransmitter levels in larval zebrafish. Journal of Applied Toxicology 2022; 42(10): 1662-1670.

Zhu Q., Yang Y., Zhong Y., Lao Z., O'Neill P., Hong D., Zhang K., Zhao S. Synthesis, ınsecticidal activity, resistance, photodegradation and toxicity of pyrethroids (a review). Chemosphere 2020; 254: 126779.