Acute Toxicity and Behavioral Alterations as Biomarkers of Triclosan Exposure in Blue Tilapia *Oreochromis aureus* (Steindachner, 1864)

Iman M. Hameed[1], Nawras A. Al-Faiz[1], Rajaa N. Al-yassein[2]

1Department of Natural Marine Sciences, College of Marine Sciences, University of Basrah, Basrah, Iraq
2Department of Pathological analyses, College of Science, University of Basrah, Basrah, Iraq

*Corresponding Author E-mail: nawras.abdulzahra@uobasrah.edu.iq

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Abstract

Several behavioral changes were observed in blue tilapia upon exposure to triclosan for 96 hours. The severity of these behavioral changes increased with concentration. Fish exhibited rapid swimming, rapid fin movements, abnormal operculum movements, attempts to ascend, and vertical jumps towards the water surface. They also showed loss of balance, respiratory distress, and capsizing. Over 96 hours of exposure, triclosan exhibited concentration-dependent toxicity, resulting in a progressive, proportional increase in fish mortality. Neither the control treatment nor the 0.15 µg/L treatment showed any mortality. However, at higher concentrations, 20% of fish in the 0.5 µg/L treatment, 40% in the 1 µg/L treatment, and 65% in the 1.5 µg/L treatment died. Increasing the triclosan concentration to 2 µg/L resulted in 85% mortality. The highest mortality rate (100%) was recorded in the 3 µg/L treatment.

Keywords: Triclosan, *Oreochromis aureus*, toxicity, behavioral

Introduction

Triclosan is a broad-spectrum, synthetic antibacterial and antimicrobial compound. It is widely used in the formulation of many consumer products. These include personal care items like detergents and toothpaste, as well as medical, veterinary, and industrial products (Allmyr *et al*., 2008). The increasing global use of such products has led to a steady influx of triclosan into aquatic systems via sewage. This makes it a persistent organic pollutant with a high capacity for bioaccumulation in the tissues of living organisms (Capkin *et al*., 2017).

Studies indicate that triclosan in aquatic environments disrupts the biological balance of organisms, especially fish, which are key bioindicators of ecosystem health. Behavioral

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changes are particularly sensitive biomarkers, revealing early chemical stress. Documented effects of triclosan exposure include movement imbalances, irregular swimming, lethargy, and, at acute concentrations, even mass mortality, though specific responses vary across species (Drummond and Russom, 1990). Key findings from studies show that Oryzias latipes exhibited a significant decrease in swimming speed at 170 µg/L (Nassef *et al.*, 2010); Oncorhynchus mykiss and Danio rerio developed irregular swimming patterns at 71–500 µg/L (Orvos *et al.*, 2002, Oliveira *et al.*, 2009). Triclosan also disrupted overall performance in Pimephales promelas (Fritsch *et al.*, 2013), altered circadian activity and reduced predator avoidance in Gambusia holbrooki (Melvin *et al.*, 2016), and decreased aggressive behavior and nest defense in Pimephales promelas at very low concentrations (0.56–1.6 µg/L), threatening reproductive success (Schultz *et al.*, 2012).

Despite the clear nature of these behavioral risks, assessing acute toxicity by determining the sublethal concentration (LC_{50}) remains essential to understanding the toxic dynamics of this compound. The LC_{50} is the concentration that kills 50% of a population exposed within 24–96 hours and is a standard tool in environmental risk assessment (Rice *et al.*, 2000). This value depends on several factors, including exposure duration, concentration, the organism's biological traits (sex, size, physiological state), and environmental factors that affect absorption and excretion (Heras *et al.*, 1992; Little and Brewer, 2001). Previous studies found wide variation in fish sensitivity to triclosan. Key results show that Nile tilapia and trout had relatively high LC_{50} values 2.81 and 4.4 mg/L respectively (Orvos *et al.*, 2002; Nassef *et al.*, 2009; Adolfsson-Erici *et al.*, 2002), indicating greater resilience. In contrast, Oryzias latipes, Danio rerio, Pimephales promelas, and Lepomis macrochirus showed much lower LC_{50} values 1.7-0.37, 0.34, 0.26, and 0.37 mg/L, respectively (Vijitha *et al.*, 2017; Oliveira *et al.*, 2009; Orvos *et al.*, 2002). demonstrating higher sensitivity. These differences highlight the importance of species-specific data for environmental protection standards. The study aims to determine the acute toxicity values and the median lethal concentration (LC_{50}) of triclosan over 96 hours to determine the sensitivity of blue tilapia fish, and to monitor the subtle behavioral changes resulting from exposure to sub-lethal concentrations, in order to provide scientific reference data that will help in setting standards for protecting biodiversity in aquatic systems.

Materials And Methods

Fish collection and acclimatization

Blue tilapia (*O. aureus*) was collected from the aquaculture ponds of the Marine Science Centre station (Basrah, Iraq) between November 2024 and March 2025. A total of 90 fish, measuring a mean of 35–40 mm in length and 7.83–8.80 g in weight. Cast nets were used for collection, and the catch was immediately transported to the laboratory in polyethylene bags containing one-third pound of water and the remainder with air to minimize stress.

Upon arrival, the fish were placed in a 2% NaCl bath for 5min, or until signs of stress were observed. Afterward, they were distributed into six aquariums (30 cm × 30 cm × 60cm) with 15 fish per aquarium. Fish were acclimated to laboratory conditions for 7 days before experimentation. During acclimatization, environmental factors including temperature, salinity, pH were measured daily using multipara water quality meter, and dissolved oxygen was measured using Dissolved Oxygen Analyzer (model BLE-9100). Temperature was maintained at 18 ± 1°C, and a continuous aeration was supplied with dissolved oxygen via air stones. The fish were fed an artificial pelleted diet at 3% of body weight, with one meal per day. Aquarium water was renewed daily with chlorine-free water to remove waste and leftover food. Feeding was stopped for 24 hours prior to the exposure experiments. The fish were monitored throughout acclimatization to ensure no signs of stress or the absence of abnormal behaviors.

Preparation of triclosan stock and working solutions

High purity triclosan (5-Chloro-2-(2, 4-dichlorophenoxy) phenol; purity of 99.9%; Sigma 2025) was dissolved with 99.9% acetone to prepare a stock solution of 1.024 mg/ml. The stock was diluted with deionized water to obtain serially working exposure concentrations. A solvent control containing acetone at the same final concentration as the highest treatment. Purity for use in preparing standard solutions for acute toxicity tests (Table 1).

Table 1. Shows the chemical structure and important physicochemical properties of triclosan.

Chemical Formula	$C_{12}H_7Cl_3O_2$
Molar Mass	289.54 g·mol^{-1}
Color	White
Density	1.49 g/cm^3
Melting Point	55–57 °C (131–135 °F; 328–330 K)
Boiling Point	120 °C (248 °F; 393 K)

Acute toxicity tests and calculation of the sublethal concentration (LC_{50}) of triclosan

Blue tilapia was exposed for 96 hours to acute triclosan concentrations (0.15, 0.5, 1, 1.5, 2, 2.5, and 3 µg/L), prepared in deionized water. Fish were distributed into 2-liter aquariums, with three replicates per treatment and four fish per aquarium. Exposure aquaria were pre-cleaned with soap, thoroughly rinsed with tap and deionized water, and finally with acetone. Aquariums were filled with chlorine-free water, aerated by oxygen pumps, and covered with aluminum foil to prevent evaporation. A control was included, and water was changed daily to maintain a constant concentration. Each day, before water changes, temperature, salinity, pH, and dissolved oxygen were measured, and survival rates were observed. All tests were conducted under standardized conditions: temperature 18 ± 1°C, salinity 2 g/L, pH 7.10, and dissolved oxygen > 7 mg/L. Behavioral changes, including alterations in swimming patterns, feeding, and response to stimuli, were systematically observed and recorded throughout the experiment. Fish mortality was recorded for each dose and repetition at 96 hours of exposure. Dead fish were removed after each observation. All aquariums were aerated throughout the experiment.

Statistical analysis

The statistical program (XLSTAT) was used to analyse the research data, specifically the linear regression analysis, which was performed using the linear equation extracted from the linear regression and estimated the value of (LC_{50}).

$$\boldsymbol{Mortality}\ \% = \mathbf{2.46297705773621} + \mathbf{36.5032075676851} * \boldsymbol{Concentrations}\ (\boldsymbol{\mu g/L})$$

Results

Lethal Concentration 50 (LC_{50}) and Behavioral study

The blue tilapia was exposed to different concentrations of triclosan at 18 ± 1°C, a dissolved oxygen (DO) of 6.95 ± 1 mg/L, and a pH of 7.0–7.6. Fish recorded various behavioral changes when exposed to triclosan, with the severity of effects depending on concentration. Careful visual observation showed that as triclosan concentration and exposure time increased, behavioral changes followed a clear sequence. Initially, fish became hyperexcitable, moving their pectoral and caudal fins more and swimming rapidly and erratically. Next, abnormal gill-cover movements appeared, and the fish surfaced, attempting to jump for air. As exposure intensified, especially near or above LC_{50} concentrations, fish struggled to breathe, swam in swirling, sideways, or unbalanced patterns, and could not maintain position or balance, eventually flipping onto their backs. This loss of equilibrium was followed by lethargy, reduced movement, and, finally, death. After 96 hours of exposure, there were differences in the toxicity of triclosan at all concentrations. The results in Table (2) show that exposing blue tilapia to different concentrations of triclosan led to a gradual, proportional increase in fish mortality. The control and 0.15 µg/L treatments showed no fish mortality, and the mortality rate remained at 0% throughout the experiment. However, when the concentration was increased to 0.5 µg/L, 20% of the fish in the treatment were affected, and mortality increased further with higher concentrations, reaching 40% at 1 µg/L and 65% at 1.5 µg/L. Increasing the triclosan concentration to 2 µg/L led to a steady increase in fish mortality, reaching 85% among exposed fish. Mortality then rose to 96% at a concentration of 2.5 µg/L. The highest mortality rate (100%) was observed in the 3 µg/L treatment group.

Table 2. Mortality % and LC_{50} for *O. aureus* exposure to different concentrations of triclosan (µg/L) after 96 hours of exposure.

Concentrations (µg/L)	Mortality %	LC_{50} (µg/L)
Control	0	***1.3022***
0.15	0	
0.5	20	
1	40	
1.3022	***50***	
1.5	65	
2	85	
2.5	96	
3	100	

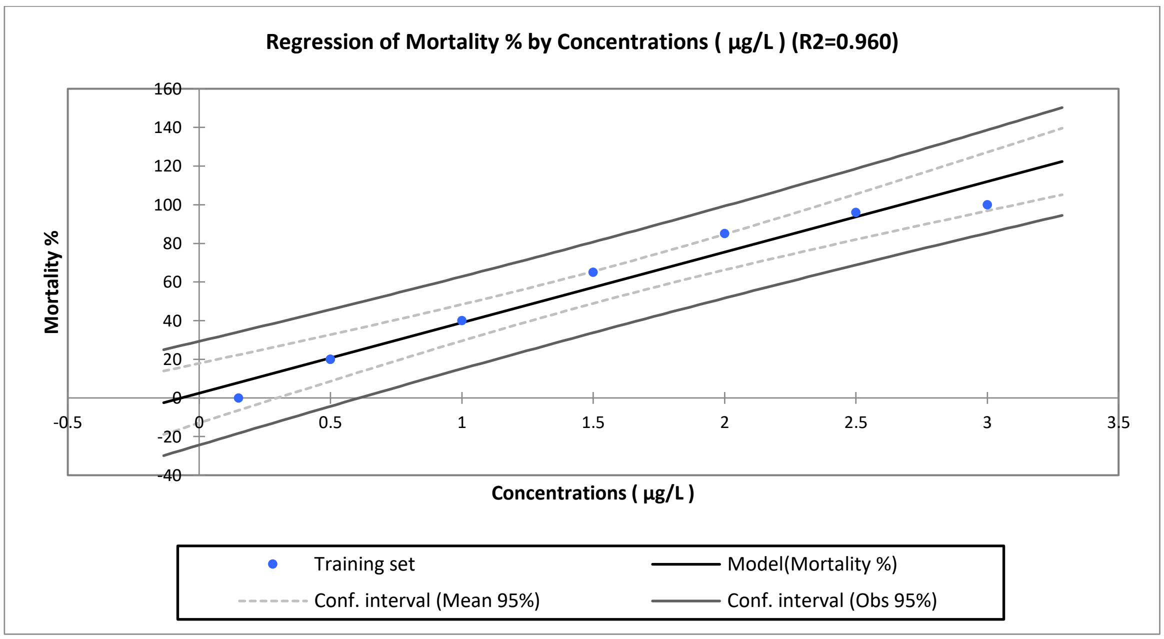

Fig. 1 Mortality percentage of *O. aureus* exposed to different concentrations of triclosan after 96 hours.

Discussion

The sublethal concentration (LC_{50}) is the concentration that causes 50% mortality in exposed organisms over a specified period (Rice *et al.*, 2000). Acute toxicity refers to the toxic or harmful effects that occur following exposure to a single dose of a chemical or to a high concentration of it over a short period (Priyatha and Chitra, 2018). The results of the current study showed a decrease in survival rates in blue tilapia (*O. aureus*) with increasing concentrations when exposed to different concentrations of triclosan (0.15, 0.5, 1, 1.5, 2, 2.5, and 3 µg/L), reaching 0%, 20%, 40%, 65%, 85%, 96%, and 100%, respectively. The LC_{50} concentration reached 1.3022 µg/L after five days of exposure to triclosan. This is due to the toxicity of triclosan, a highly toxic substance to aquatic organisms. It has a high transmissibility, and fish are highly sensitive to it. Furthermore, it readily bioaccumulates in the fatty tissues of aquatic organisms, leading to its accumulation and causing long-term damage (Hemalatha *et al.*, 2019). Studies on the toxic and sublethal effects of triclosan have yielded varying results. Priyatha and Chitra (2018) determined the sublethal concentration to be 1.767 µg/L when Anabas testudineus fish were exposed to different concentrations of triclosan (1.0, 1.2, 1.4, 1.6, 1.8, 2, and 2.2 mg/L) for 96 hours. In contrast, the sublethal concentration was 740 µg/L when *O. mossambicus* fish were exposed to lethal concentrations of 131, 262, 523, and 1046 mg/L. Seenivasan *et al.* (2023) exposed *D. rerio* fish to concentrations of 34 µg/L (10% LC_{50}), 85 µg/L (25% LC_{50}), and 170 µg/L (50% LC_{50}) of triclosan (Arman, 2021). Song *et al.* (2021) demonstrated that mortality increased over time, with a triclosan LC_{50} of 1.399 mg/L in *G. affinis*. Variations in LC_{50} values are attributed to several factors, including fish size, strain differences, and environmental factors (Seenivasan *et al.*, 2023). Other factors include the organism's species, the purity of the triclosan used, species susceptibility and resistance, route of entry, animal adaptation, and detoxification mechanisms. (Vijitha *et al.*, 2017).

Studying the behavioral responses of organisms under chemical stress is a useful tool for assessing the environmental impacts of pollutants. Fish are an ideal model for understanding toxic dynamics in controlled environments (Sloman and Scott, 2004). The nature of the observed behavioral changes depends fundamentally on the target chemical's mechanism of action (Gruber and Munn, 1998). The results of the current study revealed that exposing blue tilapia (*O. aureus*) to concentrations of triclosan (TCS) (0.15, 0.5, 1, 1.5, 2, 2.5, and 3) µg/L resulted in a number of behavioral disturbances that increased in severity proportionally with increasing dose and duration of exposure. These responses ranged from hyperactivity, such as rapid swimming, increased fin and gill flail movements, to attempts to leap out, loss of balance, flipping, and ultimately, death. These behavioral changes are attributed to the toxic substances' pathway. Triclosan is absorbed through the gills and then transported via the bloodstream to the central nervous system (Sandahl *et al.*, 2005). Swim disturbances are a clear indicator of the neurological effects of TCS on the brain's motor centers (Hemalatha *et al.*, 2019). The fish's surfacing and air gulping represent an immediate avoidance response to prevent inhaling the toxic water and an attempt to compensate for tissue hypoxia resulting from gill damage (Priyatha and Chitra, 2018). These observations are consistent with those recorded by Orvos *et al.* (2002) in trout (*O. mykiss*) and other organisms such as *Daphnia magna* (Dann and Hontela, 2011). The study by Çelebi and Gök (2018) on zebrafish (*D. rerio*) was followed by a study by Fritsch *et al.* (2013) that revealed an interesting behavioral variation in *Labeo rohita* fish; low concentrations (0.131–0.262 µg/L) induced lethargy and benthic stability, while higher concentrations (1.046–2.092 µg/L) stimulated respiratory hyperactivity. This dose dependent variation confirms that triclosan may act through dual mechanisms, depending on exposure intensity. At high concentrations (mg/L), the severity of symptoms increases, including semicircular swimming, muscle twitching, and complete loss of neurological control (Priyatha and Chitra, 2018; Oliveira *et al.*, 2009).

Conclusions

Based on the above, we conclude that behavioral changes represent the most sensitive biomarker for assessing environmental toxicity, surpassing traditional mortality measurement criteria, as they reflect dysfunction in its early stages (Nassef *et al.*, 2010; Deepika *et al.*, 2021).

References

Allmyr, M.; Harden, F.; Toms, L.M.L.; Mueller, J.F.; McLachlan, M.S.; Adolfsson-Erici, M. and Sandborgh-Englund, G. (2008). The influence of age and gender on triclosan concentrations in Australian human blood serum. Sci. Total Environ., 393 (1): 162–167.

Hemalatha, D.; Nataraj, B.; Rangasamy, B.; Shobana, C. and Ramesh, M. (2019). DNA damage and physiological responses in an Indian major carp *Labeo rohita* exposed to an antimicrobial agent triclosan. Fish Physiol. Biochem., 45(4): 1463-1484.

Schultz, M.M.; Bartell, S.E. and Schoenfuss, H.L. (2012). Effects of triclosan and triclocarban, two ubiquitous environmental contaminants, on anatomy, physiology, and behavior of the fathead minnow (*Pimephales promelas*).Arch. Environ. Contam. Toxicol., 63(1): 114-124

Adolfsson-Erici, M.; Pettersson, M.; Parkkonen, J. and Sturve, J. (2002). Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. Chemosphere., 46(9-10): 1485-1489.

Arman, S. (2021). Effects of acute triclosan exposure on gill and liver tissues of zebrafish (*Danio rerio*). Ann Limnol-int. J. Limnol.,(Vol. 57: p. 6). EDP Sciences.

Capkin, E.; Ozcelep, T.; Kayis, S. and Altinok, I. (2017). Antimicrobial agents, triclosan, chloroxylenol, methylisothiazolinone and borax, used in cleaning had genotoxic and histopathologic effects on rainbow trout. Chemosphere., 182: 720-729.

Celebi, H. and Gok, O. (2018). Effect of triclosan exposure on mortality and behavioral changes of Poecilia reticulata and Danio rerio. Hum. Ecolo.l Risk Assess: Inter. J., 24(5): 1327-1341.

Dann, A. B. and Hontela, A. (2011). Triclosan: environmental exposure, toxicity and mechanisms of action. J. App.toxicol., 31(4): 285-311.

Deepika, S.; Padmavathy, P.; Srinivasan, A.; Sugumar, G. and Jawahar, P. (2021). Effect of triclosan (TCS) on the protein content and associated histological changes on tilapia, *Oreochromis mossambicus* (Peters, 1852). Enviro. Sci. Pollu. Res., 28(42): 59899-59907.

Drummond, R.A. and Russom, C.L. (1990). Behavioral toxicity syndromes: a promising tool for assessing toxicity mechanisms in juvenile fathead minnows. Enviro.Toxicol. chem., 9 (1): 37–46.

Fritsch, E. B.; Connon, R. E.; Werner, I.; Davies, R. E.; Beggel, S.; Feng, W. and Pessah, I. N. (2013). Triclosan impairs swimming behavior and alters expression of excitation-contraction coupling proteins in fathead minnow (*Pimephales promelas*). Enviro.sci. technol., 47(4): 2008-2017.

Gruber, S.J. and Munn, M.D. (1998). Organophosphate and carbamate insecticides in agricultural waters and cholinesterase (ChE) inhibition in common carp (*Cyprinus carpio*). Arch. Environ.Contam. Toxicol., 35(3): 391-396.

Heras, H.; Ackman, R.G. and Macpherson, E.J. (1992). Tainting of Atlantic salmon (*Salmo salar*) by petroleum hydrocarbons during a short-term exposure. Mar. Pollut. Bull., 24(6): 310-315.

Little, E.E. and Brewer, S.K. (2001). Neurobehavioral toxicity in fish. Target organ toxicity in marine and freshwater teleosts, 141-176.

Melvin, S.D.; Buck, D.R. and Fabbro, L.D. (2016). Diurnal activity patterns as a sensitive behavioural outcome in fish: effect of short-term exposure to treated sewage and a sub-lethal PPCP mixture. J. App.Toxico., 36(9): 1173-1182.

Nassef, M.; Matsumoto, S.; Seki, M.; Kang, I. J.; Moroishi, J.; Shimasaki, Y. and Oshima, Y. (2009). Pharmaceuticals and personal care products toxicity to Japanese medaka fish (*Oryzias latipes*). J. Fac. Agr., Kyushu Univ., 54(2): 407–411

Nassef, M.; Matsumoto, S.; Seki, M.; Khalil, F.; Kang, I. J.; Shimasaki, Y.; Oshima, Y.and Honjo, T. (2010). Acute effects of triclosan, diclofenac and carbamazepine on feeding performance of Japanese medaka fish (*Oryzias latipes*). Chemosphere, 80(9): 1095-1100.

Oliveira, R.; Domingues, I.; Koppe Grisolia, C. and Soares, A.M. (2009). Effects of triclosan on zebrafish early-life stages and adults. Enviro. Sci. Pollut. Res., 16(6): 679-688.

Orvos, D.R.; Versteeg, D.J.; Inauen, J.; Capdevielle, M.; Rothenstein, A. and Cunningham, V. (2002). Aquatic toxicity of triclosan. Enviro. Toxicol. Chem., 21(7): 1338-1349.

Priyatha, C.V. and Chitra, K.C. (2018). Acute toxicity of triclosan on the native freshwater fish, *Anabas testudineus* (Bloch, 1792): behavioral alterations and histopathological lesions. Int. J. Life Sci., 6(1): 166-172.

Rice, S.D.; Short, J.W.; Heintz, R.A.; Carls, M.G. and Moles, A. (2000). Life-history consequences of oil pollution in fish natal habitat. Energy, 1210-1215.

Sandahl, J.F.; Baldwin, D.H.; Jenkins, J.J. and Scholz, N.L. (2005). Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. Enviro. Toxicol. Chem., 24(1): 136-145.

Scott, G.R. and Sloman, K.A. (2004). The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat.toxicol., 68(4): 369-392.

Seenivasan, D.; Pandurengan, P.; Srinivasan, A.; Gopalrajan, S. and Paulraj, J. (2023). Short term effects of antimicrobial agent triclosan on *Oreochromis mossambicus*

(Peters, 1852): Biochemical and genetic alterations. Indian. J. Anim. Res., 57(6): 788-794.

Song, X.; Wang, X.; Li, X.; Yan, X.; Liang, Y.; Huang, Y.; Huang, L. and Zeng, H. (2021). Histopathology and transcriptome reveals the tissue-specific hepatotoxicity and gills injury in mosquitofish (*Gambusia affinis*) induced by sublethal concentration of triclosan. Ecotoxicol. Nviron. Saf., 220: 112325.

Vijitha, C.K.; Asifa, K.P. and Chitra, K.C. (2017). Assessment of genotoxic and haematological consequence of triclosan in the fish, *Oreochromis niloticus* (Linnaeus, 1758). Int.J. Appl. Res., 3(2): 101-109.

التأثيرات السمية والسلوكية كمؤشرات حيوية لتعرض للتريكلوسان في اسماك البلطي الأزرق (*Oreochromis aureus*)

ايمان مجيد حميد[1]، **نورس عبدالغني الفائز**[1]، **رجاء نوري مكي**[2]

1 قسم علوم البحار الطبيعية، كلية علوم البحار، جامعة البصرة، البصرة، العراق
2 قسم التحاليل المرضية، كلية العلوم، جامعة البصرة، البصرة، العراق

*Corresponding Author E-mail: mhaisenft@yahoo.co.uk

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المستخلص

لوحظت عدة تغيرات سلوكية في أسماك البلطي الأزرق عند تعرضها لتراكيز مختلفة من التريكلوسان (0.15، 0.5، 1، 1.5، 2، 2.5، و3 ميكروغرام/لتر) لمدة 96 ساعة. وقد ازدادت حدة هذه التغيرات السلوكية مع زيادة تركيز المادة. أظهرت الأسماك سباحة سريعة، وحركات زعانف سريعة، وحركات غير طبيعية لغطاء الغلاصم، ومحاولات للصعود لسطح الماء، وقفزات عمودية نحو سطح الماء. كما عانت من فقدان التوازن، وصعوبة التنفس، والانقلاب على الظهر. خلال 96 ساعة من التعرض، أظهر التريكلوسان سمية تعتمد على التركيز، مما أدى إلى زيادة تدريجية ومتناسبة في معدل نفوق الأسماك. لم تُظهر معاملة السيطرة أو المعاملة بتركيز 0.15 ميكروغرام/لتر أي هلاكات. ومع ذلك، عند التراكيز الأعلى، نفقت 20% من الأسماك في المعاملة 0.5 ميكروغرام/لتر، و40% في المعاملة 1 ميكروغرام/لتر، و65% في المعاملة 1.5 ميكروغرام/لتر. أما عند زيادة تركيز التريكلوسان إلى 2 ميكروغرام/لتر فقد أدت إلى نفوق 85% من الأسماك. سُجِّل أعلى معدل وفيات (100%) في المعاملة التي تعرضت لتركيز 3 ميكروغرام ترايكلوسان/لتر.

الكلمات المفتاحية: تريكلوسان، اسماك البلطي الازرق، السمية، السلوك، *Oreochromis aureus*