



Evaluation of Behavioral and Histopathological Alterations Induced by Acute Triclosan Toxicity in *Labeo rohita* (Hamilton, 1822)

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Abstract

Triclosan (TCS) is a man-made antimicrobial substance often found in self care products, household goods and industrial uses. Its widespread application and durability in the environment over the last twenty years have resulted in its designation as a significant emerging contaminant. Aquatic organism, especially fishes are known to have a vulnerable to lethal impact of TCS. In the present study, the *L. rohita* fish was exposed to acute toxicity doses of TCS, after which behavioural and histological alterations were assessed. Physico-chemical properties were also monitored throughout the experiment. The behavioral analysis conducted during the experiment revealed concentration-dependent alterations in fish behavior following TCS exposure, including increased erratic swimming, respiratory distress, air gulping, lethargic movement, excessive mucus secretion, *etc.* The behavioural irregularities, displayed a positive correlation with concentrations of TCS and were time linked. Histological finding include aneurysm, epithelial uplifting, curling and disruption of secondary gill lamella, necrosis, tissue vacuolization and degeneration in liver and congestion, degeneration of renal tubule and glomerulus, in kidney of *L. rohita*. Thus, the present study highlights the critical risks possessed by acute doses of TCS to aquatic organism at behavioural and organ level.

Keywords: Aquatic toxicology, Behavioral alterations, Degeneration, Histopathology, Necrosis, Triclosan

Introduction

TCS is a manmade, broad-spectrum antimicrobial agent that is extensively used in self care products like soaps and toothpaste, textiles, plastics, household disinfectants and various industrial formulations (Dann and Hontela, 2011). TCS predominantly enters aquatic environments as discharge from wastewater from treatment facilities as a result of their failure to completely degrade TCS, into harmless form. Its persistent character has resulted in extensive build-up of this compound in aquatic ecosystems, leading to indispensable concerns regarding its harmful impacts on aquatic life

(Chalew and Halden, 2009). It is commonly present in natural water bodies because of the failure of conventional wastewater treatment plants to fully remove (Dhillon *et al.*, 2015). TCS persistence has widely been studied in Indian sediments ($\mu\text{g kg}^{-1}$ dry wt) and waters ($\mu\text{g L}^{-1}$) across various locations, indicating severe risk to organisms dwelling in those ecosystems (Nag *et al.*, 2018; Ramaswamy *et al.*, 2011; Chakraborty *et al.*, 2019; Gopal *et al.*, 2021; Kumar *et al.*, 2024).

TCS has generated considerable environmental apprehension owing to its persistence and tendency to bioaccumulate

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within aquatic ecosystems. Numerous studies of acute and chronic toxicity of TCS in aquatic organisms particularly fishes have been reported and ascertained that TCS is a toxic compound. Previous studies reported 96 h LC₅₀ of TCS as 0.80 mg L⁻¹ in *C. carpio* (Wang et al., 2017), 1.1119 mg L⁻¹ in *C. auratus* (Wang et al., 2018), 0.36 mg L⁻¹ in *C. catla* (Hemalatha et al., 2019a), 0.05 mg L⁻¹ in *O. mykiss* (Capkin et al., 2017), 1.767 mg L⁻¹ in *A. testudineus* (Priyatha and Chitra, 2018), 1.403 mg L⁻¹ in *O. niloticus* (Thilakan et al., 2019), 0.398 mg L⁻¹ in *D. rerio* (Gyimah et al., 2020), 1.177 mg L⁻¹ and 1.458 mg L⁻¹ in *P. hypophthalmus* (Bera et al., 2020), 0.131 mg L⁻¹ in *C. mrigala* and 0.116 mg L⁻¹ in *C. idella* (Dar et al., 2020). The studies conducted by Dar et al. (2020) and Sharma et al. (2021) reported 96 h LC₅₀ TCS for *L. rohita* larvae and embryo, to be 0.126 mg L⁻¹ and 0.096 mg L⁻¹, respectively. Whereas, exposure of TCS in aquatic organisms over longer period causes oxidative damage (Dar et al., 2020), neurotoxicity (Gyimah et al., 2020), genotoxicity (Paul et al., 2019), behavioural changes (Orvos et al., 2002; Sahu et al., 2018), endocrine disruption (Raut and Angus, 2010) and histological abnormalities (Paul et al., 2019; Hemalatha et al., 2019b).

Behavioural bioassay is being extensively used in toxicity assessment studies. It is considered to be faster, more sensitive and ecologically relevant for the studies on risk assessment of emerging contaminants (Sharma, 2019). According to the previous reported studies, the behavioral changes observed in fish exposed to TCS includes breathing difficulties, clumsiness, incoherent swimming and slow movements (Sahu et al., 2018; Hemalatha et al., 2019a) and bulged hemorrhagic eyes (Priyatha and Chitra, 2018). On the other hand, histological changes in tissue of vital organs of an aquatic organism can considerably be used for studying the ecotoxicological impacts of numerous aquatic pollutants on the health of that organism, as well as, the overall health of the complete population residing in the ecosystem. Moreover, histopathological studies are complex tool for the analysis of direct and indirect lethal impacts of contaminants that affect the animal tissues (Yancheva et al., 2016). Previous chronic toxicity studies on histology showed that the fish exposed to TCS exhibited several histological alterations such as abnormally enlarged gill epithelium, lifting of gill epithelia and curling of secondary gill lamellae and arches. TCS toxicity also causes renal tubular degeneration, aneurysm at the tips of secondary gill lamella, edema in Bowman's capsule, glomerular shrinkage and pyknotic nuclei in kidney (Hemalatha et al., 2019a) and tissue vacuolization, anucleated hepatocytes, melanomacrophage aggregation and degenerated cytoplasm, in the liver tissue (Priyatha and Chitra, 2018).

Changes in the behavioral patterns are the external characteristic result of composite neurological and physiological processes happening internally inside the body of the fish. It depends on homeostasis of numerous organs or organ systems and their synchronized functioning; whereas, histopathology aids in evaluating the damage caused by pollutant at tissue level. These parameters can potentially

be used to study both the external and internal effects of emerging pollutants like TCS in aquatic organisms. However, there are limited reports on the histological analysis of vital organs of fish exposed to acute toxicity. Moreover, there is lack of data available on the toxicological impacts of TCS on fish behavior, especially on *Labeo rohita*. Thus, the aim of present study is to examine the acute toxicological impacts of TCS on the Indian major carp, *L. rohita* by evaluating the concentration dependent changes in fish behavior and organ specific histological alterations.

Materials and Methods

Experimental Animal

Labeo rohita was used for the present experimental study. About, two hundred fish fries weighing 2.0±1.0 g, were procured from the fish hatchery farm, Kanamia Aqua Pvt. Ltd., located in Navsari, Gujarat, India, for conducting acute toxicity experiment. The fries of *L. rohita* were packed in air tight packages with sufficient oxygen. On reaching the experimental station, the stock was transported into a circular fiberglass reinforced plastic (FRP) tanks of 1000 L capacity, having fresh and chlorine free water. The FRP tanks were continuously aerated throughout the acclimatization period, which was of about 3 weeks. Adequate water quality conditions (pH, temperature and dissolve oxygen) as per the culture conditions of rohu, were checked regularly. The fish were given commercial feed consisting of 30% crude protein during the acclimatization period, at the feeding rate of 3% body weight.

Test Chemical

A stock solution of TCS having concentration of 200 mg L⁻¹ was prepared using 0.01 M NaOH as solvent. For this, 0.020 g of TCS was dissolved in 25 mL of NaOH (0.01 M), followed by dilution with deionized water to a final volume of 100 mL (Lu et al., 2009). Appropriate dilutions using distilled water, were made to prepare test solutions of varying concentration.

Experimental Design for Acute Toxicity

For conducting the acute toxicity test, clean, transparent rectangle shaped glass aquariums measuring 61 cm × 33 cm × 33 cm, having a volume of 40 liters, fitted with perforated lids, were used as experimental units. First, a toxicity range finding test was carried out (EPA, 2002), followed by definitive test, according to the prevailing procedures of US EPA (2002) and the United Nations Environment Programme (UNEP) in 1989, applying a static renewal system. The acute toxicity experiment, was done using a total of five experimental treatment groups, with three replicates each, having TCS concentration as 0.2, 0.4, 0.6, 0.8 and 1.0 mg L⁻¹ and labelled as T₁, T₂, T₃, T₄ and T₅. Along with these, a control group having 0.01 M NaOH (solvent used for TCS dissolution), without TCS, was employed for the experiment. Groups of seven fishes were chosen randomly from the acclimatization tank and transferred to each experimental unit. The experimental design for the acute toxicity study was made using the completely randomized design (CRD). The experimental fish was omitted with feeding for 48 hours before conducting the experiment and 96 hours during the

experiment. The entire experiment was done indoor under controlled environmental conditions. A natural photo-period of twelve hours of light followed by twelve hours of darkness, were also employed. The experiment was carried out by following the ethical guiding principle of animal care of ICAR-Central Institute of Fisheries Education, Mumbai, India. Varying degrees of abnormal behaviour were recorded from all five treatment groups at fixed intervals of time and compared with fish behaviour observed in the control group.

Physio-Chemical Properties of Water during the Experiment

The water quality parameters, temperature, pH, dissolved oxygen (DO), alkalinity, hardness, total ammoniacal nitrogen (TAN) and chloride were checked following procedures of APHA (2017). The temperature readings were taken using a dry bulb mercury-in-glass thermometer, which was submerged beneath the water surface for each experimental unit. The pH was determined using a digital pH meter (HANNA, USA) by dipping its probe into the tanks with test solutions. The readings were recorded from the display after a few minutes. DO was measured by using digital DO probe and the readings from the display screen, were recorded. The standard titrimetric method was used for estimation of total alkalinity using standard solution of 0.02 N H_2SO_4 as titrant and methyl orange indicator. The total hardness of the water sample was assessed using the ethylenediamine tetra-acetic acid (EDTA) titration method with Eriochrome Black-T indicator. The concentration of ammonia-nitrogen ($\text{NH}_4\text{-N}$) was measured using the phenate method using sodium nitroprusside solution, phenol solution and oxidizing solution (consisting of sodium hypochlorite and alkaline citrate). The sample reading were taken using UV-Visible Spectrophotometer (MONTRAS SCIENTIFIC), at a wavelength of 640 nm to quantify the concentration of ammonia-nitrogen. The absorbance was assessed utilizing deionized water as the reference blank. The concentration was measured in mg L^{-1} using the standard graph. The chloride level was estimated by the titration method using potassium chromate (K_2CrO_4) indicator and silver nitrate (AgNO_3) solution. The formula for calculation of alkalinity, total hardness and chloride are given below.

$$\text{Alkalinity (mg L}^{-1}\text{)} = \frac{\text{Acid added (ml)} \times \text{Normality of Acid} \times 5000}{\text{Volume of Sample (ml)}}$$

$$\text{Total hardness (mg L}^{-1}\text{ CaCO}_3\text{)} = \frac{0.001 \times \text{Volume of EDTA consumed} \times 10^6}{25}$$

$$\text{Chloride (mg L}^{-1}\text{)} = \frac{[\text{AgNO}_3 \text{ for sample (ml)} - \text{AgNO}_3 \text{ for blank (ml)}] \times \text{Normality of AgNO}_3 \times 35,450}{\text{Volume of Sample (ml)}}$$

Histological Investigations of Organ Section

The organs including gills, liver and kidney were evaluated from each treatment group, for histological examination. For those groups having records of mortality during the experiment, dead fish was dissected and organ specific

tissue were collected and preserved in 10% neutral buffered formalin (NBF) solution. For the experimental units without the record of any mortality, histological samples were collected at the end of the experiment and well-maintained in 10% NBF solution. The preservative was exchanged occasionally to increase the penetration of NBF into the tissue samples. The samples were dehydrated with alcohol solution with progressively increasing concentrations, such as 30%, 50%, 70%, 80%, 90% and 100%, for one hour duration. The tissues were cleared using xylene, followed by impregnation with paraffin wax at a temperature of 60 °C, which involved three successive changes of wax, each lasting 90 minutes. The tissue-wax blocks were created utilizing a histo-embedder. The blocks were sectioned using a rotary microtome and resulting ribbons of thickness of 3-5 μm were obtained, which were thereafter spread out on a clean glass slide. The slides were dewaxed using xylene, followed by dehydration using alcohol solutions with progressively lower concentrations such as 90%, 70% and 50% for a duration of 5 min. The slides were stained using a combination of haematoxylin and eosin stains (Roberts, 2012). To deparaffinize the stained sections xylene was used, followed by their mounting in distyrene plasticizer xylene. Triplicate slides were created for every tissue sample to ensure uniformity. Tissue sections on slides were subsequently scrutinized under an Olympus FSX 100 bright-field microscope.

Statistical Analysis

The IBM Statistical package for Social Sciences (SPSS) software, version 25.0 was used for statistical examination of the obtained results. The statistics of numerous parameters were verified for their normality by means of Shapiro-Wilk test followed by application of one-way analysis of variance (ANOVA). The study applied the Duncan's test as a post-hoc analysis to recognize significant differences amongst the means of different treatment groups and the control at a significance level ($p < 0.05$) of 5%. The outcomes are represented as means $\pm$ standard error.

Results and Discussion

Behavioral Responses of *L. rohita* to TCS

L. rohita subjected to the acute toxicity experiment displayed significant clinical signs of toxicity. The observed behavioural disarrays in the treatment groups include erratic swimming, respiratory distress, air gulping, lethargic movement and excessive secretion of mucus on the surface of fish body. These detected changes in the behaviour of fish were intensified as the concentrations of TCS increased across the treatment groups from T_1 to T_5 , as compared to the fishes in control group showing no such changes in behaviour (Table 1). The abnormal behavioural patterns were also studied to increase in range with exposure time and therefore are recommended to be time linked. Maximum behavioural alternation along with death occurred in the experimental tank with highest concentration of TCS.

Behavioural changes in organism are activities linked with the central and peripheral nervous system. Aquatic organisms

Table 1: The behavioural responses of *L. rohita* observed at various concentrations of TCS over a duration of 96 hours

Observed behavioural irregularities	TCS concentration (mg L ⁻¹)					
	Control	0.2	0.4	0.6	0.8	1.0
Air gulping	-	-	+	++	+++	+++
Respiratory distress	-	+	+	++	+++	+++
Erratic swimming patterns	-	+	+	++	+++	+++
Lethargic movement	-	+	+	++	+++	+++
Mucus secretion	-	+	++	++	+++	+++

-None; +Moderate; ++Strong; +++Extremely strong

like fish are able to uptake or accumulate contaminants dissolved in water. Therefore, the presence of pollutant, or any chemical contaminant in the water bodies may cause modification in the behaviour of fish dwelling in it (Çelebi and Gök, 2018). In the present study, the acute exposure of TCS led to changes in swimming pattern of the fish to a greater extent. The erratic swimming patterns of the *L. rohita* are likely to be due to imbalance caused by impairment of neurological system. According to Sahu et al. (2018) the observed behavioural alterations, could be an avoidance syndrome established by fishes in response to toxic compounds. Difficulty in breathing observed in fish exposed to TCS, could be because of neurological dysfunction of gill epithelia or by precipitation of excessive mucous on the skin surface clogging the gills and thereby reducing gaseous exchange. The inhibition of acetylcholinesterase activity in brain and other neural enzymes could also be possible reasons of such behavioral changes studied in the present study (Çelebi and Gök, 2018; Bhattacharjee et al., 2022). Additionally, Mushigeri and David (2005), Sahu et al. (2018) and Arias-Cavieres et al. (2018) have informed in their studies that there was a significant inhibition of AChE activity in brain with increase in TCS toxicity, which possibly points out towards significant operative association of this enzyme with behavioural changes in fish. The present observations are similar to those reported by Çelebi and Gök (2018), for *Poecilia reticulata* and *Danio rerio* and Sahu et al. (2018) for *Pangasianodon hypophthalmus*, exposed to TCS. The detected behavioural abnormalities in the current

study are also constant with those of Orvos et al. (2002) in *Oncorhynchus mykiss*, Oliveira et al. (2009) in *Danio rerio* and Priyatha et al. (2018) in *Anabas testudineus* exposed to TCS. In contrast to the present observation, Karmakar et al. (2021), Majumder (2024) and Maurya et al. (2024) observed hyperexcitability, erratic swimming and loss of equilibrium/jumping tendencies in *L. rohita* subjected to acute pesticide toxicity.

Physico-Chemical Properties of Water

The physico-chemical properties of water significantly influence the fish physiology and their response to chemical contaminants. As water quality worsens, the survival and growth rates of aquatic animals in cultured environments diminish (Boyd, 2017). During the acute toxicity study, the water quality parameters including temperature, pH, DO, alkalinity, hardness and TAN (Table 2), across all conditions were within the ideal range that is suitable for rearing *L. rohita*; whereas, the chloride levels were higher in TCS treated groups. This progressive presence of chloride across the experimental tanks with increasing concentration of TCS indicates active dichlorination of TCS (Wu et al., 2021). However, the safe level of chloride, at normal conditions in the water bodies, is recorded to be higher than the obtained level. Therefore, it can be considered that in present study, chloride does not play a significant role in inducing toxicity to fish and the detected toxicity is exclusively because of TCS alone (Roseboom and Richey, 1977).

Table 2: The physicochemical parameters of the water monitored throughout the acute experiment

Parameters	Treatments					
	Control	T ₁	T ₂	T ₃	T ₄	T ₅
Temperature (°C)	26.19±0.11	26.36±0.09	26.14±0.32	26.46±0.12	27.22±0.45	26.15±0.34
pH	8.1-8.2	8.0-8.1	8.0-8.1	8.0-8.1	7.9-8.0	7.9-8.1
Dissolved Oxygen (mg L ⁻¹)	6.4-6.9	6.3-6.7	6.5-6.9	6.5-6.8	6.4-6.7	6.3-6.8
Alkalinity (mg L ⁻¹)	168.31±0.42	155.52±0.36	161.73±0.51	164.12±0.65	157.17±0.35	167.81±0.87
Hardness (mg L ⁻¹)	175.64±0.42	173.32±0.16	170.14±0.59	171.34±0.72	171.58±0.26	175.18±0.35
Ammonia (mg L ⁻¹)	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01
Chloride (mg L ⁻¹)	7.60±0.34	8.50±0.46	8.8±0.05	8.2±0.78	8.5±0.35	8.5±0.56

Histology

TCS toxicity caused various histological variations in gill, liver and kidney tissue, especially in T_5 and T_4 , followed by T_3 and T_2 (Table 3). The gills exhibited hyperplastic conditions, aneurysm, disruption of secondary gill lamella, epithelial uplifting, curling of secondary gill lamella and telangiectasis (Figure 1). Severe lesions were detected in T_5 and T_4 with telangiectasis observed exclusively in T_5 as compared with other treatment groups and control. Severe to moderate lesions were observed in T_3 and T_2 , and moderate lesions were observed in T_1 , as compared with control group. The gills are regarded as the first line of defense against unfavorable environmental conditions and are typically the primary site where the toxic effects of chemicals become evident (Benli *et al.*, 2008; Parvathy *et al.*, 2023). Histopathological examination of gill tissues revealed several abnormalities demonstrating the lethal effect of TCS. Gill is as a very important organ because it facilitates various respiratory as well as excretory processes,

in the fish's body. The fusion of gill lamella observed in T_5 and T_4 could be a protective mechanism to avoid the entrance of toxic compounds into the body (Karmakar *et al.*, 2021). Lamellar fusion and epithelial uplifting may decrease the surface area for gaseous exchange. Furthermore, conditions of hyperplasia may lead to narrowing of the interlamellar spaces and make branchial ventilation more difficult for the fish. Additionally, the mucous generated in the gills and skin of fish serves as a natural barrier against toxic chemicals present in water bodies and also the level of its secretion indicates the harmfulness of these contaminants. On the other hand, excess mucous accumulation in the gills as a result of higher TCS concentrations may disrupts gaseous exchange, leading to a state similar to asphyxia and thereby causing gill lesions (Capkin *et al.*, 2017). The present results are consistent with Hemalatha *et al.* (2019b) and Paul *et al.* (2019), who reported similar gill lesions in *L. rohita* and *P. hypophthalmus*, respectively, exposed to sub lethal concentrations of TCS.

Table 3: Histopathological lesion scoring based on the severity of lesions observed in different tissues of *L. rohita* subjected to acute concentrations of TCS

Histological lesions	Treatments					
	C	T_1	T_2	T_3	T_4	T_5
<u>Gill</u>						
Hyperplasia (H)	-	+	-	+	-	-
Disruption of secondary gill lamella (DSGL)	-	-	+	++	+++	+++
Aneurysm (A)	-	+	++	++	+++	+++
Epithelial uplifting (EU)	-	-	++	++	+++	+++
Curling of secondary gill lamella (CSGL)	-	-	+	++	-	-
Telangiectasis (T)	-	-	-	-	-	+++
<u>Liver</u>						
Tissue degeneration (TD)	-	+	++	+++	+++	+++
Necrosis (N)	-	-	++	+++	+++	+++
Vacuolization (V)	-	-	++	++	+++	+++
Edema (E)	-	-	-	-	-	+
<u>Kidney</u>						
Congestion (Cong.)	-	+	++	++	+++	+++
Necrosis (N)	-	-	+	-	+	+
Degeneration of renal tubule (DRT)	-	+	++	++	+++	+++
Glomerular degeneration (GD)	-	+	++	++	+++	+++

*Classification of histological alterations was categorized as severe (+++), moderate (++) , mild (+) and none (-)

Histological alterations in liver of *L. rohita* studied in the present study include tissue degeneration, necrosis, vacuolization and edema (Figure 2). Severe histological alterations were observed in T_5 , along with T_4 and T_3 ; moderate lesions were detected in T_2 , as compared to control. Liver is the main organ accountable for decontamination and purification and is also regarded as,

the organ with predominant impact of toxic substances. Hepatocytes primarily play a role in breakdown, detoxification and the elimination of xenobiotics and their metabolites. In the present study, the lesion in liver tissue were observed to become more pronounced, in treatments with higher concentration of TCS. The possible pathways for this characteristic increase in damage of liver tissue across

the treatment groups mainly includes generation of reactive oxygen species, leading to inflammation of hepatocytes, necrosis of epithelial and other membranous parts (Singh et al., 2019). According to Capkin et al. (2017), vacuolation in liver tissue may be because of excessive build-up of fat within the cytoplasm, reflecting considerable impairment of liver function and cellular integrity. Moreover, being the primary metabolic centre, the liver becomes extremely vulnerable to toxicant, in conditions of acute toxicity. Due to its several attempts to biotransform toxic compound, the numerous metabolic pathway assumptively becomes hyper activated and exhausted, failing to regulate the over production of ROS. Furthermore, TCS is known for upregulation of the TLR4-Myd88-TRAF6 signaling pathway, which is responsible for activation of the NF- κ B cascade, leading to initiation of NLRP3 inflammasome pathway, thus inducing uncontrolled degeneration of liver tissue (Peng et al., 2024). Similar observations were made for chronic exposure study of TCS by Capkin et al. (2017) and Priyatha et al. (2018). TCS is reported to cause damage of mitochondrial functioning and hepatocytic inflammation, resulting in necrosis, tissue degeneration and congestion (Paul et al., 2020; Chen et al., 2023).

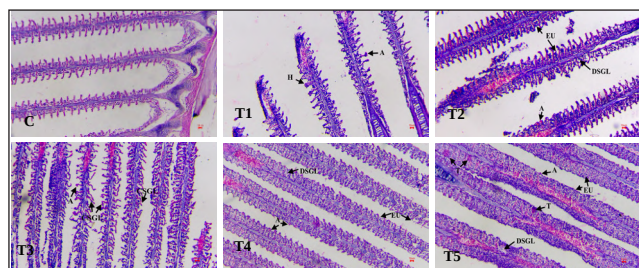


Figure 2: Photomicrographs showing histological sections of liver at various acute concentrations of TCS. H&E stain, 10X magnification. TD: Tissue degeneration, N: Necrosis, V: Vacuolization, E: Edema. [C: control, T1: 0.2, T2: 0.4, T3: 0.6, T4: 0.8, T4: 1.0 mg L⁻¹ of TCS]

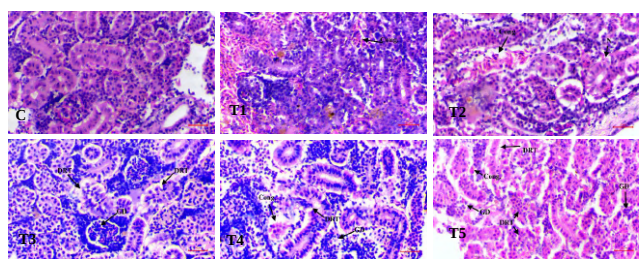


Figure 2: Photomicrographs showing histological sections of liver at various acute concentrations of TCS. H&E stain, 40X magnification. TD: Tissue degeneration, N: Necrosis, V: Vacuolization, E: Edema. [C: control, T1: 0.2, T2: 0.4, T3: 0.6, T4: 0.8, T4: 1.0 mg L⁻¹ of TCS]

The kidney tissue exhibited various histological lesions including congestion, necrosis, degeneration of renal tubule, glomerular degeneration (Figure 3). Severe lesions were seen in T₅ and T₄ groups; whereas, T₃ and T₂ had moderate level on lesion, as compared to control group. Congestion in kidney tissue was observed in T₁ group; whereas, the histological sections of the control group displayed normal

kidney tissue, without any significant micro-anatomical variations. The fish kidney consists of many renal corpuscles featuring well-developed glomeruli and a network of tubules. It receives the largest share of post-branchial blood. The teleostean kidney is among the initial organs to be impacted by pollutants in the water (Hadi and Alwan, 2012). The kidneys function as the principal hematopoietic organ in most teleost and these metabolic processes formed as a result of detoxification-metabolism of toxic compounds, can lead to pathophysiological injuries. As a chief organ responsible for decontamination and removal of xenobiotics, the kidney is extremely vulnerable to structural damage when exposed with acute concentrations (Badroo et al., 2020; Smorodinskaya et al., 2023). Similar histological finding were reported by Akram et al. (2022) and Naz et al. (2023), in *Aristichthys nobilis* exposed to TCS and *Labeo rohita* exposed to copper sulfate toxicity, respectively.

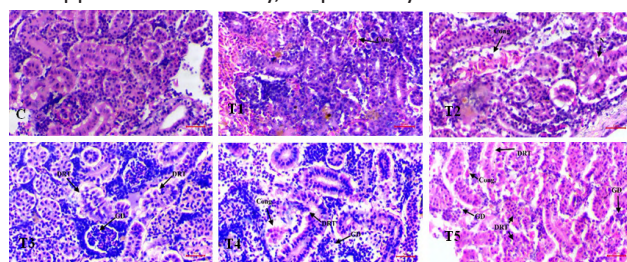


Figure 3: Photomicrographs showing histological sections of kidney at various acute concentrations of TCS. H&E stain, 40X magnification. Cong.: Congestion, N: Necrosis, DRT: Degeneration of renal tubule, GD: Glomerular degeneration. [C: control, T1: 0.2, T2: 0.4, T3: 0.6, T4: 0.8, T4: 1.0 mg L⁻¹ of TCS]

Conclusion

The findings of the present study showed that acute exposure to TCS makes a profound toxicological impact on the commercially important freshwater teleost, *L. rohita*. It caused a progressive increase in behavioural abnormalities in the fish with respect to concentration of TCS and time of exposure. Severe respiratory distress and erratic swimming behavior reveals the underlying tissue degradation caused by acute concentrations of TCS. Histological alterations in major target organs (gill, liver and kidney) reported from this study can also be used as an indication of adverse effect posed by TCS in aquatic organism. Epithelial uplifting in gills, vacuolization in liver tissue and degeneration of renal tubule could be used as key markers of tissue damage caused by TCS. This study highlights the acute susceptibility of *L. rohita* to TCS pollution, emphasizing on the importance of integration of behavioral and organ specific histological evaluation of risk posed by emerging pollutants. Further investigations can be done to study the recovery dynamics to assess whether the behavioral and histological impairments, as obtained in the present study, could be reversed following TCS removal from the aquatic environment.

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Author's Contributions

Mayuri Nag: Writing - original draft, Investigation; Saurav Kumar: Conceptualization, Supervision, Methodology, Investigation; Sinchana R. Mahale: Data curation; Mayur Bhadarka: Writing - review & editing; Tapas Paul: Writing - review & editing, Data curation; Bhautik D. Savaliya: Data curation; Kundan Kumar: Funding acquisition; S.P. Shukla: Investigation, Methodology.

Ethical Statement

The authors declare that no generative artificial intelligence tools were used in drafting or preparing this manuscript. The manuscript was written, interpreted and revised solely by the authors, who take full responsibility for its content.

References

- APHA, 2017. *Standard Methods for Examination of Water and Wastewater*, 23rd Edition. (Eds.) Rice, E.W., Baird, R.B. and Eaton, A.D. American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). American Public Health Association, Washington DC.
- Akram, R., Ghaffar, A., Hussain, R., Khan, I., Santana, V.L.A., Mehmood, K., Naz, S., Iqbal, R., Imran, H.M., Qamar, M.R., Zhu, H., 2022. Hematological, serum biochemistry, histopathological and mutagenic impacts of triclosan on fish (bighead carp). *Agrobiological Records* 7, 18-28. DOI: <https://doi.org/10.47278/journal.abr/2021.009>.
- Arias-Cavieles, A., More, J., Vicente, J.M., Adasme, T., Hidalgo, J., Valdés, J.L., Humeres, A., Valdés-Undurraga, I., Sánchez, G., Hidalgo, C., Barrientos, G., 2018. Triclosan impairs hippocampal synaptic plasticity and spatial memory in male rats. *Frontiers in Molecular Neuroscience* 11, 429. DOI: <https://doi.org/10.3389/fnmol.2018.00429>.
- Badroo, I.A., Nandurkar, H.P., Khanday, A.H., 2020. Toxicological impacts of herbicide paraquat dichloride on histological profile (gills, liver and kidney) of freshwater fish *Channa punctatus* (Bloch). *Environmental Science and Pollution Research* 27(31), 39054-39067. DOI: <https://doi.org/10.1007/s11356-020-09931-6>.
- Benli, A.Ç.K., Köksal, G., Özkul, A., 2008. Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus* L.): Effects on gill, liver and kidney histology. *Chemosphere* 72(9), 1355-1358. DOI: <https://doi.org/10.1016/j.chemosphere.2008.04.037>.
- Bera, K.K., Kumar, S., Paul, T., Prasad, K.P., Shukla, S.P., Kumar, K., 2020. Triclosan induces immunosuppression and reduces survivability of striped catfish *Pangasianodon hypophthalmus* during the challenge to a fish pathogenic bacterium *Edwardsiella tarda*. *Environmental Research* 186, 109575. DOI: <https://doi.org/10.1016/j.envres.2020.109575>.
- Bhattacharjee, S., Rout, S.K., Prasad, G.S., Karmakar, S., Chowdhury, A., 2022. Acute toxicity (96 hours) study of clofibric acid on *Labeo rohita* (Hamilton 1822): Determination of LC₅₀ value and behavioral changes. *Environment and Ecology* 40(4B), 2397-2401.
- Boyd, C.E., 2017. General relationship between water quality and aquaculture performance in ponds. In: *Fish Diseases: Prevention and Control Strategies*. (Ed.) Jeney, G. Academic Press, Elsevier. pp. 147-166. DOI: <https://doi.org/10.1016/B978-0-12-804564-0.00006-5>.
- Capkin, E., Ozcelep, T., Kayis, S., 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. DOI: <https://doi.org/10.1016/j.chemosphere.2017.05.093>.
- Çelebi, H., Gök, O., 2018. Effect of triclosan exposure on mortality and behavioral changes of *Poecilia reticulata* and *Danio rerio*. *Human and Ecological Risk Assessment: An International Journal* 24(5), 1327-1341. DOI: <https://doi.org/10.1080/10807039.2017.1411783>.
- Chakraborty, P., Mukhopadhyay, M., Sampath, S., Ramaswamy, B.R., Katsoyiannis, A., Cincinelli, A., Snow, D., 2019. Organic micropollutants in the surface riverine sediment along the lower stretch of the transboundary river Ganga: Occurrences, sources and ecological risk assessment. *Environmental Pollution* 249, 1071-1080. DOI: <https://doi.org/10.1016/j.envpol.2018.10.115>.
- Chalew, T.E.A., Halden, R.U., 2009. Environmental exposure of aquatic and terrestrial biota to triclosan and triclocarban. *JAWRA Journal of the American Water Resources Association* 45(1), 4-13. DOI: <https://doi.org/10.1111/j.1752-1688.2008.00284.x>.
- Chen, X., Mou, L., Qu, J., Wu, L., Liu, C., 2023. Adverse effects of triclosan exposure on health and potential molecular mechanisms. *Science of the Total Environment* 879, 163068. DOI: <https://doi.org/10.1016/j.scitotenv.2023.163068>.
- Dann, A.B., Hontela, A., 2011. Triclosan: Environmental exposure, toxicity and mechanisms of action. *Journal of Applied Toxicology* 31(4), 285-311. DOI: <https://doi.org/10.1002/jat.1660>.
- Dar, O.I., Sharma, S., Singh, K., Sharma, A., Bhardwaj, R., Kaur, A., 2020. Biochemical markers for prolongation of the acute stress of triclosan in the early life stages of four food fishes. *Chemosphere* 247, 125914. DOI: <https://doi.org/10.1016/j.chemosphere.2020.125914>.
- Dhillon, G.S., Kaur, S., Pulicharla, R., Brar, S.K., Cledón, M., Verma, M., Surampalli, R.Y., 2015. Triclosan: Current status, occurrence, environmental risks and bioaccumulation potential. *International Journal of Environmental Research and Public Health* 12(5), 5657-

5684. DOI: <https://doi.org/10.3390/ijerph120505657>.
- Gopal, C.M., Bhat, K., Ramaswamy, B.R., Kumar, V., Singhal, R.K., Basu, H., Udayashankar, H.N., Vasantharaju, S.G., Praveenkumarreddy, Y., Shailesh., Lino, Y., Balakrishna, K., 2021. Seasonal occurrence and risk assessment of pharmaceutical and personal care products in Bengaluru rivers and lakes, India. *Journal of Environmental Chemical Engineering* 9(4), 105610. DOI: <https://doi.org/10.1016/j.jece.2021.105610>.
- Gyimah, E., Dong, X., Qiu, W., Zhang, Z., Xu, H., 2020. Sublethal concentrations of triclosan elicited oxidative stress, DNA damage and histological alterations in the liver and brain of adult zebrafish. *Environmental Science and Pollution Research* 27, 17329-17338. DOI: <https://doi.org/10.1007/s11356-020-08232-2>.
- Hadi, A.A., Alwan, S.F., 2012. Histopathological changes in gills, liver and kidney of fresh water fish, *Tilapia zillii*, exposed to aluminum. *International Journal of Pharmacy and Life Sciences* 3(11), 2071-2081.
- Hemalatha, D., Rangasamy, B., Nataraj, B., Ramesh, M., 2019a. Assessment of triclosan impact on enzymatic biomarkers in an Indian major carp, *Catla catla*. *The Journal of Basic and Applied Zoology* 80, 23. DOI: <https://doi.org/10.1186/s41936-019-0094-2>.
- Hemalatha, D., Nataraj, B., Rangasamy, B., Shobana, C., Ramesh, M., 2019b. DNA damage and physiological responses in an Indian major carp *Labeo rohita* exposed to an antimicrobial agent triclosan. *Fish Physiology and Biochemistry* 45, 1463-1484. DOI: <https://doi.org/10.1007/s10695-019-00661-2>.
- Karmakar, S., Karmakar, S., Jana, P., Chhaba, B., Das, S.A., Rout, S.K., 2021. Nonylphenol exposure in *Labeo rohita* (Ham.): Evaluation of behavioural response, histological, haematological and enzymatic alterations. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 247, 109058. DOI: <https://doi.org/10.1016/j.cbpc.2021.109058>.
- Kumar, G., Kumar, S., Paul, T., Pal, P., Shukla, S.P., Kumar, K., Jha, A.K., Pradeep, S., 2024. Ecotoxicological risk assessment of triclosan, an emerging pollutant in a riverine and estuarine ecosystems: A comparative study. *Marine Pollution Bulletin* 205, 116667. DOI: <https://doi.org/10.1016/j.marpolbul.2024.116667>.
- Lu, H., Ma, H., Tao, G., 2009. Spectrophotometric determination of triclosan in personal care products. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 73(5), 854-857. DOI: <https://doi.org/10.1016/j.saa.2009.04.007>.
- Majumder, R., 2024. Comparative acute toxicity studies of chlorpyrifos technical grade with its emulsifiable concentrate (20% EC) on *Labeo rohita*, a freshwater major carp and *Mystus vittatus*, a freshwater catfish. *Bulletin of Environmental Contamination and Toxicology* 113, 27. DOI: <https://doi.org/10.1007/s00128-024-03936-4>.
- Maurya, P.K., Jyoti, S., Maurya, P.K., 2024. Acute toxicity of dithane M-45 in freshwater fish *Labeo rohita*: A laboratory study. *Environment Conservation Journal* 25(4), 1113-1119. DOI: <https://doi.org/10.36953/ECJ.28752902>.
- Mushigeri, S.B., David, M., 2005. Fenvalerate induced changes in the Ach and associated AchE activity in different tissues of fish *Cirrhinus mrigala* (Hamilton) under lethal and sub-lethal exposure period. *Environmental Toxicology and Pharmacology* 20(1), 65-72. DOI: <https://doi.org/10.1016/j.etap.2004.10.011>.
- Nag, S.K., Sarkar, S.D., Manna, S.K., 2018. Triclosan - an antibacterial compound in water, sediment and fish of River Gomti, India. *International Journal of Environmental Health Research* 28(5), 461-470. DOI: <https://doi.org/10.1080/09603123.2018.1487044>.
- Naz, S., Hussain, R., Guangbin, Z., Chatha, A.M.M., Rehman, Z.U., Jahan, S., Liaquat, M., Khan, A., 2023. Copper sulfate induces clinico-hematological, oxidative stress, serum biochemical and histopathological changes in freshwater fish rohu (*Labeo rohita*). *Frontiers in Veterinary Science* 10, 1142042. DOI: <https://doi.org/10.3389/fvets.2023.1142042>.
- Oliveira, R., Domingues, I., Grisolia, C.K., Soares, A.M.V.M., 2009. Effects of triclosan on zebrafish early-life stages and adults. *Environmental Science and Pollution Research* 16, 679-688. DOI: <https://doi.org/10.1007/s11356-009-0119-3>.
- Orvos, D.R., Versteeg, D.J., Inauen, J., Capdevielle, M., Rothenstein, A., Cunningham, V., 2002. Aquatic toxicity of triclosan. *Environmental Toxicology and Chemistry* 21(7), 1338-1349. DOI: <https://doi.org/10.1002/etc.5620210703>.
- Parvathy, A.J., Das, B.C., Jifirya, M.J., Varghese, T., Pillai, D., Rejish Kumar, V.J., 2023. Ammonia induced toxicophysiological responses in fish and management interventions. *Reviews in Aquaculture* 15(2), 452-479. DOI: <https://doi.org/10.1111/raq.12730>.
- Paul, T., Shukla, S.P., Kumar, K., Poojary, N., Kumar, S., 2019. Effect of temperature on triclosan toxicity in *Pangasianodon hypophthalmus* (Sauvage, 1878): Hematology, biochemistry and genotoxicity evaluation. *Science of the Total Environment* 668, 104-114. DOI: <https://doi.org/10.1016/j.scitotenv.2019.02.443>.
- Paul, T., Kumar, S., Shukla, S.P., Pal, P., Kumar, K., Poojary, N., Biswal, A., Mishra, A., 2020. A multi-biomarker approach using integrated biomarker response to assess the effect of pH on triclosan toxicity in *Pangasianodon hypophthalmus* (Sauvage, 1878). *Environmental Pollution* 260, 114001. DOI: <https://doi.org/10.1016/j.envpol.2020.114001>.
- Peng, Y., Cui, H., Shu, C., Wei, Z., Ni, X., Liu, J., 2024. Triclosan induces liver injury in long-life exposed mice via activation of TLR4/NF- κ B/NLRP3 pathway. *Ecotoxicology and Environmental Safety* 273, 116115. DOI: <https://doi.org/10.1016/j.ecoenv.2024.116115>.
- Priyatha, C.V., Chitra, K.C., 2018. Acute toxicity of triclosan on the native freshwater fish, *Anabas testudineus* (Bloch, 1792): Behavioral alterations and histopathological lesions. *International Journal of Life Sciences* 6(1), 166-172.

- Ramaswamy, B.R., Shanmugam, G., Velu, G., Rengarajan, B., Larsson, D.G.J., 2011. GC-MS analysis and ecotoxicological risk assessment of triclosan, carbamazepine and parabens in Indian rivers. *Journal of Hazardous Materials* 186(2-3), 1586-1593. DOI: <https://doi.org/10.1016/j.jhazmat.2010.12.037>.
- Raut, S.A., Angus, R.A., 2010. Triclosan has endocrine-disrupting effects in male western mosquitofish, *Gambusia affinis*. *Environmental Toxicology and Chemistry* 29(6), 1287-1291. DOI: <https://doi.org/10.1002/etc.150>.
- Roberts, R.J., 2012. *Fish Pathology*. John Wiley & Sons, Blackwell Publishing Ltd. p. 592. DOI: <https://doi.org/10.1002/9781118222942>.
- Roseboom, D.P., Richey, D.L., 1977. Acute toxicity of residual chlorine and ammonia to some native Illinois fishes. In: *Report of Investigation (Illinois State Water Survey) RI-085*. Illinois State Water Survey. p. 42. URL: <https://hdl.handle.net/2142/77795>.
- Sahu, V.K., Karmakar, S., Kumar, S., Shukla, S.P., Kumar, K., 2018. Triclosan toxicity alters behavioral and hematological parameters and vital antioxidant and neurological enzymes in *Pangasianodon hypophthalmus* (Sauvage, 1878). *Aquatic Toxicology* 202, 145-152. DOI: <https://doi.org/10.1016/j.aquatox.2018.07.009>.
- Sharma, M., 2019. Behavioural responses in effect to chemical stress in fish: A review. *International Journal of Fisheries and Aquatic Studies* 7(1), 01-05.
- Sharma, S., Dar, O.I., Singh, K., Thakur, S., Kesavan, A.K., Kaur, A., 2021. Genomic markers for the biological responses of Triclosan stressed hatchlings of *Labeo rohita*. *Environmental Science and Pollution Research* 28, 67370-67384. DOI: <https://doi.org/10.1007/s11356-021-15109-5>.
- Singh, M., Barman, A.S., Devi, A.L., Devi, A.G., Pandey, P.K., 2019. Iron mediated hematological, oxidative and histological alterations in freshwater fish *Labeo rohita*. *Ecotoxicology and Environmental Safety* 170, 87-97. DOI: <https://doi.org/10.1016/j.ecoenv.2018.11.129>.
- Smorodinskaya, S., Kochetkov, N., Gavrilin, K., Nikiforov-Nikishin, D., Reznikova, D., Vatlin, A., Klimuk, A., Odorskaya, M., Nikiforov-Nikishin, A., Ponomarev, A., Marsova, M., Danilenko, V., 2023. The Effects of acute bisphenol a toxicity on the hematological parameters, hematopoiesis and kidney histology of zebrafish (*Danio rerio*). *Animals* 13(23), 3685. DOI: <https://doi.org/10.3390/ani13233685>.
- Thilakan, A.P., Kumar, K., Shukla, S.P., Kumar, S., Saharan, N., Karmakar, S., 2019. Occurrences of triclosan in Versova creek of Mumbai, India and its toxicity on selected aquatic organisms. *Journal of Experimental Zoology India* 22(2), 737-742.
- Wang, F., Guo, X., Chen, W., Sun, Y., Fan, C., 2017. Effects of triclosan on hormones and reproductive axis in female Yellow River carp (*Cyprinus carpio*): Potential mechanisms underlying estrogen effect. *Toxicology and Applied Pharmacology* 336, 49-54. DOI: <https://doi.org/10.1016/j.taap.2017.10.005>.
- Wang, F., Xu, R., Zheng, F., Liu, H., 2018. Effects of triclosan on acute toxicity, genetic toxicity and oxidative stress in goldfish (*Carassius auratus*). *Experimental Animals* 67(2), 219-227. DOI: <https://doi.org/10.1538/expanim.17-0101>.
- Wu, C., Zhou, L., Zhou, Y., Zhou, C., Xia, S., Rittmann, B.E., 2021. Dechlorination of 2,4-dichlorophenol in a hydrogen-based membrane palladium-film reactor: Performance, mechanisms and model development. *Water Research* 188, 116465. DOI: <https://doi.org/10.1016/j.watres.2020.116465>.
- Yancheva, V., Velcheva, I., Stoyanova, S., Georgieva, E., 2016. Histological biomarkers in fish as a tool in ecological risk assessment and monitoring programs: A review. *Applied Ecology and Environmental Research* 14(1), 47-75. DOI: https://doi.org/10.15666/aeer/1401_047075.