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RESEARCH PAPER

Acute oxybenzone exposure induces behavioural, histological and haematological responses in *Labeo rohita* (Hamilton, 1822) fingerlings

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Abstract

The ecotoxicological impacts of oxybenzone gain much more attention due to its widespread use and release in aquatic environments. In the present study, *Labeo rohita* fingerlings were exposed to 3.8, 4, 4.2, and 4.4 mg L^{-1} concentrations of oxybenzone for 96 h. The 96-h median lethal concentration (LC_{50}) of oxybenzone was evaluated as 4.05 mg L^{-1} and also predicted survivability through GUTS-Model (General Unified Threshold Model). Acute exposure to oxybenzone has caused different behavioural deviations, including opercular movement, upside-down swimming and jumping tendency. Various tissue architectural alterations of the gill [inter lamellar space, partial fusion of Secondary Gill Lamellae (SGL), curling of SGL, blood congestion in the filament)] and liver (necrotic hepatocytes, blood congestion, pyknotic nucleus, large number of phagocyte cells, rupturing of the cell structure, fatty infiltration) were observed in treated groups. Haematological parameters, such as Hb content (Haemoglobin), TEC (Total Erythrocyte Count), PCV (Packed Cell Volume), MCH (Mean Corpuscular Haemoglobin), MCV (Mean Corpuscular Volume) and MCHC (Mean Corpuscular Haemoglobin Concentration), were significantly decreased in treated groups in a dose-dependent manner, whereas TLC (Total Leucocyte Count) level was increased significantly. Considering the toxicological effects of oxybenzone, the study emphasised the need to regulate its use and ensure safer disposal for environmental safety.

Keywords Ecotoxicology · Ultraviolet filter · Oxybenzone · Histology · Haematology · Survivability

Introduction

Oxybenzone [Benzophenone-3 (BP-3)] is an aromatic hydrocarbon commonly used in sunscreen agent that absorbs and reflects ultraviolet radiation (Calafat et al. 2008). Oxybenzone has also been used as an ultraviolet stabiliser in plastic surface coatings for food packaging to prevent food photodegradation (Suzuki et al. 2005) and in the formulation of a variety of cosmetic products like shampoo, fragrances, lipstick. (Calafat et al. 2008; Moreira et al. 2023). Oxybenzone is a hydrophobic and photo-stable compound which has more than 2.4 years of half-life on water surface (Zhang et al. 2021). The highest detection level of oxybenzone was about 5429 ng L^{-1}, observed in a surface water sample from Hong Kong (Du et al. 2017). In India, the highest detection level was observed in wastewater effluent with a concentration of 163 to 351 ng L^{-1} (Karthikraj and Kannan 2017). During the monsoon season in the Arkavathi river stream of India, the detection level of oxybenzone was found to vary between 30 and 112 ng L^{-1} (Gopal et al. 2021). Oxybenzone can be entered into the aquatic environment through direct and indirect inputs: direct input by bathing, swimming and industrial discharge and indirect input is mostly associated with wastewater discharge from household and wastewater-treatment plants (WWTPs) (Giokas et al. 2007; Balmer et al. 2005; Kim and Choi 2014; Scheele et al. 2023). Oxybenzone is known to persist in the aquatic environment and is

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bio-accumulative in the bodies of different aquatic organisms (Du et al. 2017). Oxybenzone has lethal and sub-lethal effects on a variety of aquatic organisms, including fish, algae and invertebrates (Downs et al. 2016; DiNardo and Downs 2018). It also has endocrine and neurological disruption ability and prevents reproduction of different aquatic organisms (Kinnberg et al. 2015; Simons et al. 2022).

Accordingly, the aim of the present study was to evaluate 96-h LC_{50} value and behavioural deviations by semi-quantitative oxybenzone exposure study. Furthermore, various histological changes in gill and liver and haematological parameters were also assessed. The Indian major carp, *Labeo rohita*, was selected as the model organism, considering its significance as a fast-growing, economically valuable aquaculture species (Karmakar et al. 2021; Nataraj et al. 2023). Assumingly, the outcomes of the present study can help evaluate and classify the ecotoxicological risk assessment of emerging pollutants such as oxybenzone.

Materials and methods

Procurement and handling of experimental species

Healthy fingerlings of *L. rohita* were procured from Bengal Fish Hatchery of Sonarpur, Kolkata and transported to the Wet Laboratory of the Faculty of Fishery Sciences under prescribed conditions. The average weight and length of experimental fish was 15.2 ± 1.7 g and 9.6 ± 1.4 cm respectively. The fish were kept in the rectangular fiber reinforced plastic (FRP) tank (800 L) with adequate oxygen and fed Growel FishMax feed (30% crude protein) at a rate of 3% body weight twice per day. The fish stock showed no mortality during 30 days of laboratory acclimatisation.

Test chemical

Oxybenzone (CAS No: 13-57-7) was purchased from HiMedia, India, with ≥ 98% purity. In the present experiment, absolute ethanol was used as a solvent. A desired stock solution of oxybenzone was prepared where the final concentration of solvent was maintained below 0.01%. The required volume of stock solution was added to the tanks to get the desired concentration of oxybenzone in the water test media.

Experimental setup

Acute toxicity bioassay and survival rate evaluation

The LC_{50} value of oxybenzone for *Labeo rohita* fingerlings was assessed in accordance with OECD guideline no. 203 (OECD 2019). Based on the range finding test, four concentrations viz., 3.8 (T_1), 4 (T_2), 4.2 (T_3) and 4.4 (T_4) mg L^{-1} of oxybenzone in duplicate along with control and positive control (with solvent) were arranged in a static system of 200 L FRP tank for 96-h definitive acute toxicity test. In each tank, 15 fish were stocked, and feed was not given throughout the experimental period. The physicochemical parameters of water such as temperature, pH, dissolved oxygen and alkalinity were analysed twice within 96-h using standard methodologies of APHA (2005). The determined concentration of oxybenzone stock solution was added into each tank, and observations were taken after 6 h, 12 h, 24 h, 48 h and 96 h, respectively. The cumulative mortality percentage and behavioural changes were noted down during the experimental period. The LC_{50} value of Oxybenzone on *L. rohita* fingerling was evaluated by Finney's probit analysis method (Finney 1971).

The survival rate of *L. rohita* to oxybenzone at acute level was evaluated by the GUTS (General Unified Threshold model for Survival) modelling which was executed by Open-GUTS standalone software. The parameters of the model are as follows: k_d (the dominat rate constant), m_w (median of the threshold distribution), h_b (the background hazard rate) and b_w (the killing rate which is only utilized for SD) (Jager et al. 2011; Jager and Ashauer 2018).

Behavioral variations

The behavioural variations in oxybenzone treated groups and control groups were observed at an interval of 12 h. Different behavioural changes were observed, including opercular movement, jumping tendency, mucus secretion, upside-down swimming, loss of equilibrium, rapid jerking movement, and frequent surfacing. The frequency of each alteration was categorised as none (−); mild (0–25%); moderate (25–50%); strong (50–75%) and very strong (> 75%) compared to the control fish.

Histological alterations

Gill and liver tissues were sampled from the experimental fish for histological analysis. The collected sample of tissue samples were fixed in Bouin's fluid for 48 to 72 h. After fixation, the samples were transferred to 70% ethyl-alcohol and kept overnight. The samples were then embedded in paraffin and sectioned at 6 µm after dehydration in graded series of ethyl alcohol concentrations (85%, 90%, and 100%). At each concentration, the tissues were immersed for 90 min with two changes of the solution. The sections were double stained in hematoxylin and eosin (H & E) for observation under an Olympus BX51 (Japan) microscope. The histopathological analysis was carried out as described by Roberts (2012).

Haematological indices

In the present study, fresh blood samples were collected and immediately transferred to EDTA tubes (2 ml) and shaken well to avoid coagulation. Haematological indices were assessed including Total Erythrocyte Count (TEC), erythrocyte index [Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)], haemoglobin content (Hb), Total Leucocyte Count (TLC) and Packed Cell Volume (PCV). Haemoglobin content and PCV were determined by haematocrit capillary tube and cyanomethamoglobin methods (Hesser 1960; Van Kampen and Zijlstra 1961). TEC, erythrocyte index, and TLC were estimated using Neubauer's counting chamber of a haemocytometer (Hendricks 1952; Shaw 1930).

Statistical analysis

After verifying the normality and homogeneity of the variance, the mean values were calculated using a one-way analysis of variance (ANOVA) in the SPSS software version 22.0 (SPSS Inc. Chicago, USA). In order to identify statistically significant differences, post-hoc comparisons of the experimental group mean using Duncan's multiple range test (DMRT) were performed. At the 5% probability level, a significant difference between the means was observed for all data ($p \leq 0.05$), and all data were displayed as mean ± standard deviation (SD).

Results

Behavioral changes and LC_{50} evaluation

In the present study, different types of behavioural changes were observed during exposure to oxybenzone in experimental tanks. The exposure to oxybenzone increased opercular movement, loss of equilibrium, upside-down swimming, mucus secretion, frequent surfacing, and jumping tendency, which is represented in Table 1. After 3 h of exposure to chemical, experimental fishes exhibited loss of equilibrium and jumping tendency from the tank with increased mucus secretion. The opercular movement was observed at 122 ± 4 beats/min in the control tank and an average of 142 ± 7 beats/min for the highest exposure concentration. The behavioural alterations were increased with increasing the exposure concentrations of oxybenzone. There were no observable behavioural changes in the control and positive control tanks. The 96 h LC_{50} value of oxybenzone on *L. rohita* fingerling was evaluated as 4.05 mg L^{-1}, which is represented in Fig. 1.

Survivability determination

The parameters of OpenGUTS model and forecasted LC_{50} value of oxybenzone in different investigation periods are represented in Tables 2 and 3, respectively. The k_d value determined by GUTS-SD (SD: Stochastic Death model) was higher that estimated by GUTS-IT (IT: Individual Tolerance model) and the estimated background rate of hazard (h_b) was approximately the same. Furthermore, based on AIC (Negative log likelihood of the model) value the fitted performance of GUTS-SD was better than GUTS-IT in the case of oxybenzone. The simulation results demonstrate that the GUTS-SD model is more efficient than GUTS-IT model in predicting the survival rate of *L. rohita* fingerlings under acute oxybenzone exposure condition.

Histological alterations

Histological changes in gills

Different types of histological changes in gill tissue were observed in different exposure concentrations of oxybenzone. Maximum changes were observed in the highest concentration of oxybenzone exposure (T_4), that included total fusion of SGL, aneurysms, epithelial lifting, and the tip of SGL swelling. Changes in T_1, T_2 and T_3 treatments

Table 1 Behavioural alterations in *L. rohita* fingerlings following acute oxybenzone exposure

Behaviour	Treatments					
	C	C^+	T_1	T_2	T_3	T_4
Frequent surfacing[a]	−	−	+	+++	++++	++++
Jumping tendency[a]	−	−	++	+++	+++	++++
Opercular movement	−	−	++	+++	+++	++++
Upside down swimming	−	−	++	+++	++++	++++
Mucus secretion	−	−	++	+++	+++	++++
Loss of equilibrium	−	−	++	++	++	++++
Rapid jerk movements[a]	−	−	+	++	+++	++++

None −, Mild +, Moderate ++, Strong +++, Very strong ++++

[a]At higher concentrations, the symptom was observed within the first 3 h of exposure to oxybenzone

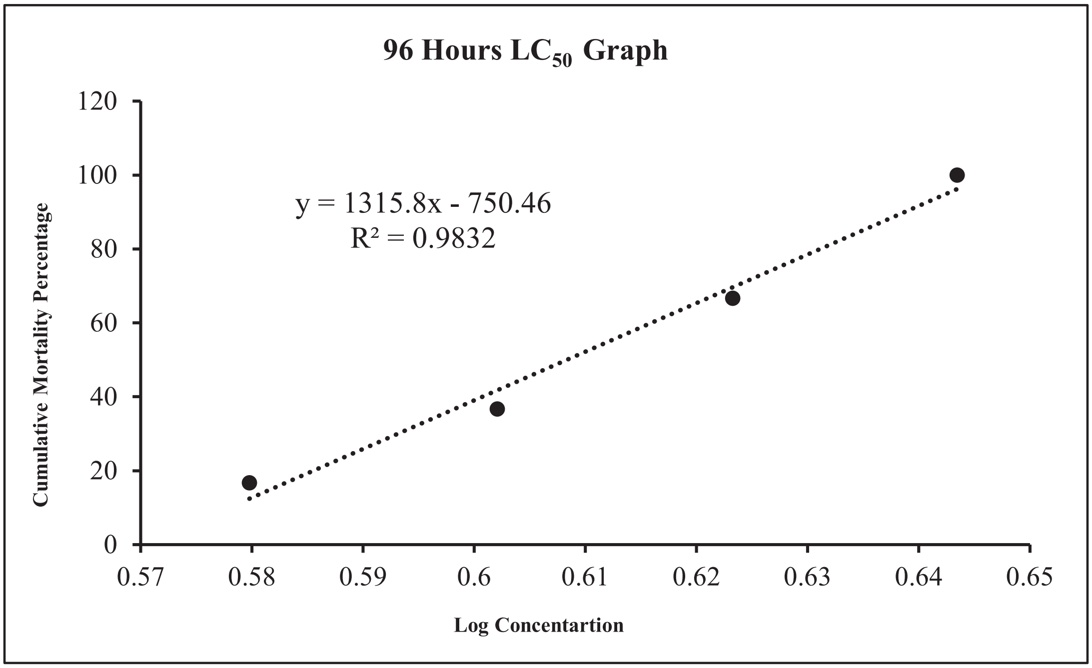

Fig. 1 96 h LC_{50} response of oxybenzone in *L. rohita* fingerlings

Table 2 Analysis of GUTS-model parameters during acute exposure of oxybenzone

Symbol	GUTS-REDUCE		Unit	AIC value	
	SD	IT		SD	IT
k_d	5.592 (3.895–13.66)	0.1493 (0.001641–0.7429)	day^{-1}	314.43	354.66
m_w	3.735 (3.642–3.776)	1.596 (0.01859–3.678)	ppm		
b_w	0.8461 (0.5801–1.157)	–	ppm^{-1} day^{-1}		
h_b	1E−6	1E−6	day^{-1}		
F_s	–	6.111 (2.181–16.07)	–		

k_d dominant rate constant, m_w: threshold for mortality, b_w: killing rate, h_b: background hazard rate, F_s: spread factor for the threshold distribution, AIC: negative log likelihood of the model

SD stochastic death model, *IT* individual tolerance model

Table 3 The estimated LC_{50} value of oxybenzone to *L. rohita* fingerlings

Time (day)	LC_{50} GUTS-SD (mg L^{-1})
1	5.123 (4.765–5.635)
4	3.982 (3.925–4.051)
7	3.867 (3.804–3.912)
14	3.798 (3.718–3.836)
28	3.765 (3.677–3.804)
50	3.752 (3.658–3.792)
100	3.743 (3.647–3.784)

were increased inter lamellae space, partial fusion of SGL, curling of SGL, blood congestion in the filament, epithelial upliftment, tip of the SGL is swelled and aneurysms which are represented in Fig. 2. In present study no changes were observed in the gill tissue sample of control and positive control group.

Histological changes in liver

Various types of histological changes in the liver were observed in different exposure concentrations of oxybenzone. In the present experiment, prominent changes were observed in the highest concentration (T_4), but in the control and positive control groups, no changes were observed. In the T_4 concentration, the noted changes were necrotic cells, degenerative cells, vacuolation and rupturing of the cell wall. In T_1, T_2, and T3 concentrations, major observable changes were necrotic hepatocytes, blood congestion,

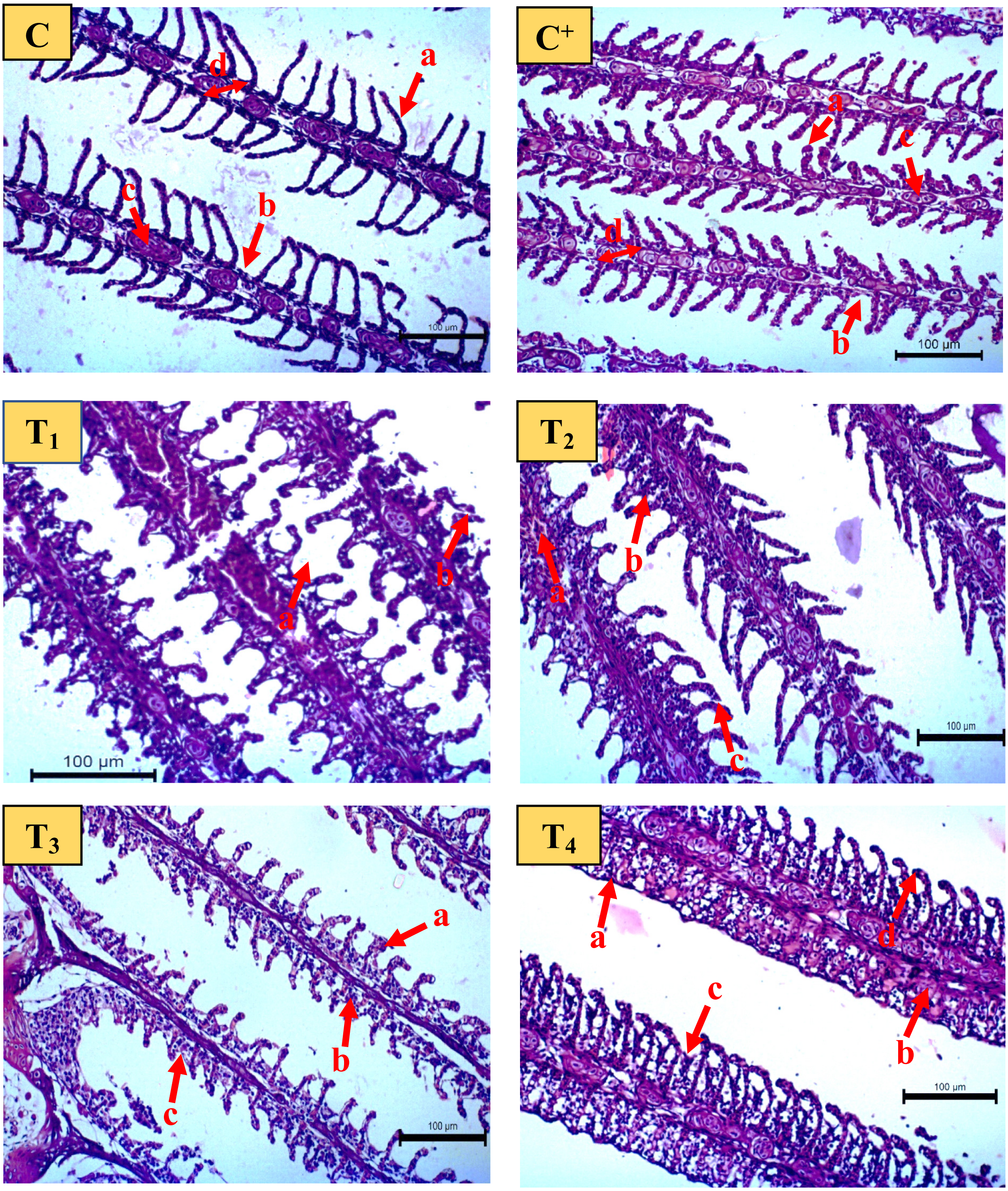

Fig. 2 Histological changes in the gills of *L. rohita* fingerlings exposed to oxybenzone. No prominent observable changes were noted in Control (**C**) and Positive Control (**C$^+$**) group[(a). SGL (b) intact epithelium layer (c) normal blood vessel (d). primary gill lamellae (PL)]. Changes observed in **T$_1$** concentration [(a) increased inter lamellae space, (b) curling of SGL]. In **T$_2$** concentration [(a) blood congestion in the filament, (b) partially fusion of SGL, (c) epithelial upliftment]. In **T$_3$** concentration [(a) tip of the SGL is swelled, (b) total fusion of SGL, (c) epithelial upliftment]. In **T$_4$** concentration [(a) total fusion of SGL, (b) aneurysms, (c) epithelial upliftment, (d) tip of SGL is swelled]

pyknotic nucleus, a large number of phagocyte cells, rupturing of the cell structure and fatty infiltration (Fig. 3).

Haematological indices

The changes in haematological indices of *L. rohita* fingerlings exposed to various concentrations of Oxybenzone are represented in Table 4. Treatment-wise, the value of Hb content, TEC, PCV, MCV, MCH and MCHC were significantly decreased ($p \leq 0.05$) compared with the control (C) and positive control (C^+) groups. The lowest value of all those parameters was observed in the T_4 treatment group. The value of TLC was significantly increased ($p \leq 0.05$) in oxybenzone-treated groups throughout the experimental period compared to the controls. The highest value of TLC was observed in T_4 treatment group.

Discussion

The present study largely illustrates different behavioural deviations, histological changes and haematological changes in *L. rohita* fingerlings, which were exposed to different concentrations of Oxybenzone.

According to Nagelkerken and Munday (2016), behavioural changes in an organism are the first indication of environmental changes. In the present experiment, different behavioural ailments were documented, including frequent surfacing, jumping tendency, opercular movement, upside-down swimming, mucus secretion, loss of equilibrium, and rapid jerk movements, which are similar to those observed in Bishphenol-A (BPA) exposed *Aristichthys nobilis* (Akram et al. 2021). Faheem et al. (2017) reported that jerky movement and erratic swimming were observed in *L. rohita* exposed to BPA, loss of balance, erratic swimming and altered swimming patterns were observed in *Ctenopharyngodon idella* (Krishnapriya et al. 2017) which support the findings of the present study. Omoregie et al. (2009) documented that a high opercular rate was observed due to the unfavourable conditions created by the toxicants. The abnormal behavioural changes were observed in the fish body because toxicants mainly affect the nervous system and other perceptive systems like secretion of mucus, equilibrium, swimming pattern and air gulping (Sharma and Chadha 2021). Sharma et al. (2019) reported that toxicants may damage the function of nerve cell bodies, axons and myelin sheaths and also can alter the synthesis and release of neurotransmitters, which may cause different behavioural abnormalities. Excessive mucus secretion was observed due to the direct contact between skin and the toxicant (Sharma and Chadha 2021). Excessive mucus creates a layer between the fish body and the toxicant to prevent skin irritation and inhibits the diffusion of oxygen during the gaseous exchange (Kumar et al. 2015). Similar findings were observed in *C. gariepinus* exposed to Diazinon (Adedeji et al. 2008).

In the present study, 96-h LC_{50} of Oxybenzone on *L. rohita* fingerlings was evaluated as 4.05 mg L^{-1}. Zhang et al. (2021) reported a 96-h LC_{50} value of 4.74 mg L^{-1} for Oxybenzone exposure in zebrafish embryos, while Du et al. (2017) found a 96-h LC_{50} value of 3.80 mg L^{-1} for *Oryzias latipes*. These findings are consistent with the results of the present study. The 96-h LC_{50} value of Oxybenzone in *Brachydanio rerio* was 3.90 mg L^{-1} (Zhang et al. 2021), which is also similar to the findings of the present observation. According to Pandey et al. (2011) the variability observed in LC_{50} value might be due to deviations in the preparation of stock solution, size and age of the experimental species, fluctuation in water quality parameters, etc.

Furthermore, the performance of the survival model offers compelling evidence supporting the ongoing selection of either the SD or IT model for consistent prediction of toxic effects across various patterns of toxicant exposure. These mechanistic effect models evaluate the impact of oxybenzone on test individuals. The higher predictive accuracy of the GUTS-SD model compared to the GUTS-IT suggests that fish exposed to oxybenzone share a critical threshold, beyond which the population is likely to experience random deaths. Toxicodynamic insights from the stochastic death model suggest that a shared survival threshold within the population promotes rapid activation of compensatory and damage repair mechanisms compared to individual tolerance model (Chukwuka et al. 2022). The faster recovery predicted by the stochastic model is attributed to the narrow tolerance range observed within the test population which justified the findings of the present study. This model may be incorporated to establish a standardized approach for future regulatory risk assessments of oxybenzone. (Medda et al. 2022).

Histological indices are a vital tool to evaluate the early toxicological effect of any contaminants (Liu et al. 2015). Dos Santos et al. (2021) elicited various histological alterations in the gill tissue of *Poecilia reticulate* exposed to different concentrations of oxybenzone for 96-h, which are comparable with the findings of the present study. Changes like epithelial lifting, fusion of secondary lamellae, hyperplasia were induced as defence mechanisms to prevent contact between blood and the external environment (Elshaer et al. 2013). As a result, the ventilation rate is increased and the oxygen uptake is impaired in the fish body (Fernandes 2003; Cengiz and Unlu 2006), which is also associated with the findings of the present study and supported by the increased opercular movement of the exposed fish in the present experiment. An aneurysm is a localized, balloon-like swelling in a blood vessel filled with blood which indicates the presence of toxicants in the environment as well as body of the affected fish. It poses a serious risk of epithelial rupturing and haemorrhage (Rajkumar et al. 2016).

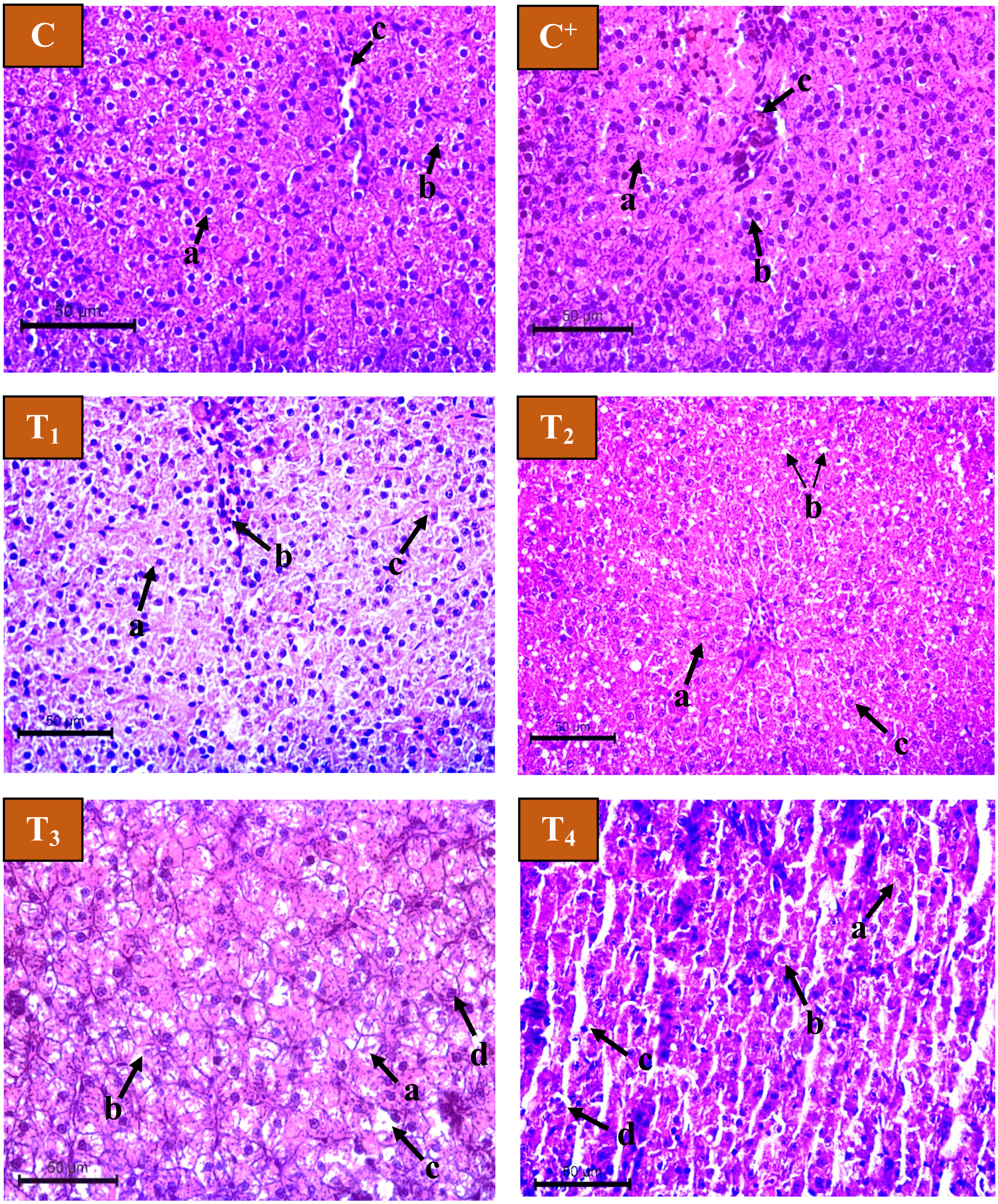

Fig. 3 Histological changes in the liver of *L. rohita* fingerlings exposed to oxybenzone. In the liver tissue of the Control (C) and Positive Control (C^+) groups, no changes were observed [(a) compact cell, (b) proper nuclei structure, (c) phagocyte cells]. Changes observed in $\mathbf{T_1}$ concentration [(a) rupturing of cell wall, (b) a large number of phagocyte cells, (c) swelled cell wall]. In $\mathbf{T_2}$ concentration [(a) degenerative cells, (b) fatty infiltration, (c) pycnotic nuclei]. In $\mathbf{T_3}$ concentration [(a) necrotic cells, (b) degenerative cells, (c) vaculation, (d) swelling of structure of the cell]. In $\mathbf{T_4}$ concentration [(a) necrotic cells, (b) degenerative cells, (c) vaculation, (d) rupturing of the cell wall]

Table 4 Results of haematological indices (mean ± SD) of *L. rohita* fingerlings exposed to different concentrations of oxybenzone

Treatments	Parameters						
	Hb contents (g %)	TEC (million/Cu mm.)	TLC (Per Cu mm.)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (gm/dl)
C	4.91 ± 0.03^{d}	1.67 ± 0.03^{e}	4600 ± 100^{a}	12.56 ± 0.04^{d}	30.51 ± 0.24^{c}	91.45 ± 0.12^{d}	32.14 ± 0.48^{e}
C^{+}	4.88 ± 0.03^{d}	1.65 ± 0.04^{e}	4700 ± 173.21^{a}	12.54 ± 0.60^{d}	30.02 ± 0.55^{c}	90.37 ± 0.43^{d}	32.30 ± 0.39^{e}
T_1	4.05 ± 0.05^{c}	1.44 ± 0.07^{d}	5800 ± 200^{b}	12.57 ± 0.87^{d}	28.34 ± 0.74^{b}	86.32 ± 1.10^{c}	30.08 ± 0.66^{d}
T_2	3.84 ± 0.86^{c}	1.31 ± 0.05^{c}	6566.7 ± 208.17^{c}	10.43 ± 0.78^{c}	27.71 ± 1.14^{b}	83.35 ± 1.25^{b}	27.04 ± 0.63^{c}
T_3	3.32 ± 0.17^{b}	1.16 ± 0.04^{b}	7200 ± 200^{d}	9.31 ± 0.20^{b}	23.22 ± 0.41^{a}	81.32 ± 0.41^{a}	24.61 ± 0.70^{b}
T_4	2.27 ± 0.20^{a}	1.03 ± 0.08^{a}	7666.7 ± 208.17^{e}	8.32 ± 0.16^{a}	22.36 ± 0.42^{a}	80.06 ± 0.66^{a}	22.20 ± 0.86^{a}

Significant differences between the control and treatment groups are expressed through different alphabetical superscripts

In the present study, histological changes in the liver tissue were analogous to the findings of Dos Santos et al. (2021) in *P. reticulate* exposed to acute oxybenzone. The findings of the present research are also comparable with the findings of Liu et al. (2015) in *Carassius auratus* exposed to different concentrations of Benzophenone UV filters. When fishes come in contact with the toxicant, blood flow increases rapidly to the liver. As a consequence, blood congestion and sinusoid dilation were observed, which ensured the hepatocyte detoxification process and also justified the findings of the present study (Hued et al. 2012). Due to this hepatocytic detoxification, cell rupturing and haemorrhage were reported (Dos Santos et al. 2021), which might be similar to the findings of the present experiment. According to Faheem et al. (2016), degeneration of cells and necrosis of cells may be observed due to the congestion of the central vein. Liu et al. (2015) noted that the pyknotic nucleus might be due to the deposition of fats and glycogen, which supports the results of the present study.

According to Sudová et al. (2009), the evaluation of haematological indices mainly represents the typical physiological function of fish body and the impact of toxicants. The results of the present study are similar to the study of Krishnapriya et al. (2017) in Indian major carp *L. rohita* exposed to BPA. Senthil et al. (2011) elaborated that Hb content decreased in *C. gariepinus* exposed to Nonylphenol due to blood cell lyses and decreased the hemopoietic activity of the kidney, which sustenance the outcomes of the present study. The PCV value was elevated might be due to anaemia (Senthil et al. 2011). Karmakar et al. (2021) justified that the level of TEC is elevated, which increases in free radicals, which inhibits the activity of antioxidant enzymes. Krishnapriya et al. (2017) documented that the values of MCV and MCH were significantly decreased due to the shrinkage of erythrocytes in *L. rohita* exposed to BPA, which supports the observation of the present study. According to Krishnapriya et al. (2017), BPA exposure reduced the Hb content of *L. rohita*, which resulted in elevated value of MCHC. The TLC level in the present study was significantly increased to stimulate the immunity function against the toxicological effect of oxybenzone, which was also documented by Krishnapriya et al. (2017) in yellow perch exposed to BPA for a period of 7 days. Uribe et al. (2011) explained that WBC cells produce a defence mechanism through secretion of antibodies against any foreign particle entering the body. As a result, the TLC level might be increased, as documented in the present study.

Conclusion

The present study reveals various acute toxicological impacts of oxybenzone in *L. rohita* fingerlings exposed for a period of 96-h. Exposure to this toxic pollutant resulted in various abnormalities, including behavioural changes, histological alterations in gill and liver tissues, and disruptions in haematological parameters. The outcome of the present study may help policymakers to understand the eco-toxicological impacts of oxybenzone and regulate its use and disposal.

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Author contributions Suman Karmakar: Investigation, validation, software and original draft preparation; Kuntal Krishna Bera: Formal analysis, data curation; Sayantan Bhattacharjee: Investigation, validation; Sutanu Karmakar: Methodology, visualization, validation, review and editing of manuscript; Sangram Keshari Rout: Visualization, validation, editing of manuscript; Gour Hari Pailan: Review and editing of manuscript; Bipul Kumar Das: Conceptualization, supervision, reviewing and editing of manuscript.

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Data availability All the relevant data are included in the manuscript.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests, legal, commercial or professional relationships that could have appeared to influence the work reported in this paper.

Ethical approval All experimental techniques and fish care protocols used in the present experiment were as per the standing guidelines of the Institute Animal Ethics Committee (763/GO/Re-S/ReRc-L/2003/CCSEA) of the West Bengal University of Animal and Fishery Sciences, Kolkata.

References

Adedeji, O.B., Adedeji, A.O., Adeyemo, O.K., Agbede, S.A.: Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*). Afr. J. Biotechnol. **7**, 5 (2008)

Akram, R., Iqbal, R., Hussain, R., Jabeen, R.F., Ali, M.: Evaluation of oxidative stress, antioxidant enzymes and genotoxic potential of bisphenol A in fresh water bighead carp (*Aristichthys nobils*) fish at low concentrations. Environ. Pollut. **268**, 115896 (2021). https://doi.org/10.1016/j.envpol.2020.115896

APHA (American Public Health Association, American Water Works Association and Water Environmental Federation): Standard Methods of Examination of Water and Wastewater, 21st edn. APHA, Washington (2005)

Balmer, M.E., Buser, H.R., Müller, M.D., Poiger, T.: Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. Environ. Sci. Technol. **39**, 953–962 (2005). https://doi.org/10.1021/es040055r

Calafat, A.M., Wong, L.Y., Ye, X., Reidy, J.A., Needham, L.L.: Concentrations of the sunscreen agent benzophenone-3 in residents of the United States: National Health and Nutrition Examination Survey 2003–2004. Environ. Health Perspect. **116**(7), 893–897 (2008). https://doi.org/10.1289/ehp.11269

Cengiz, E.I., Unlu, E.: Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: a microscopic study. Environ. Toxicol. Pharmacol. **21**(3), 246–253 (2006). https://doi.org/10.1016/j.etap.2005.08.005

Chukwuka, A.V., Saha, S., Mukherjee, D., Banerjee, P., Dhara, K., Saha, N.C.: Deltamethrin-induced respiratory and behavioral effects and adverse outcome pathways (AOP) in short-term exposed *Mozambique tilapia*, *Oreochromis Mossambicus*. Toxics **10**(11), 701 (2022). https://doi.org/10.3390/toxics10110701

DiNardo, J.C., Downs, C.A.: Dermatological and environmental toxicological impact of the sunscreen ingredient oxybenzone/benzophenone-3. J. Cosmet. Dermatol. **17**(1), 15–19 (2018). https://doi.org/10.1111/jocd.12449

dos Santos, A.S., Silva, O.V., Ribeiro, D.M., Luiz, B.L., Teixeira, S.M., Lopes, R.T., Luiz, E.F.: Environmentally relevant concentrations of benzophenone-3 induce differential histopathological responses in gills and liver of freshwater fish. Environ. Sci. Pollut. Res. **28**(33), 44890–44901 (2021). https://doi.org/10.1007/s11356-021-13839-0

Downs, C.A., Kramarsky-Winter, E., Segal, R., Fauth, J., Knutson, S., Bronstein, O., Loya, Y.: Toxicopathological effects of the sunscreen UV filter, oxybenzone (benzophenone-3), on coral planulae and cultured primary cells and its environmental contamination in Hawaii and the US Virgin Islands. Arch. Environ. Contam. Toxicol. **70**(2), 265–288 (2016). https://doi.org/10.1007/s00244-015-0227-7

Du, Y., Wang, W.Q., Pei, Z.T., Ahmad, F., Xu, R.R., Zhang, Y.M., Sun, L.W.: Acute toxicity and ecological risk assessment of benzophenone-3 (BP-3) and benzophenone-4 (BP-4) in ultraviolet (UV)-filters. Int. J. Environ. Res. Public Health **14**(11), 1414 (2017). https://doi.org/10.3390/ijerph14111414

Elshaer, F., Abu-Shaeir, W., Bakry, S.: Histopathological changes in the Kidney of mosquito fish, *Gambusia affinis* and guppy fish, *Poecilia reticulata* exposed to Bisphenol A. Egyp. J. Aquat. Biol. Fish. **17**(4), 83–93 (2013). https://doi.org/10.21608/ejabf.2013.2188

Faheem, M., Jahan, N., Lone, K.P.: Histopathological effects of bisphenol-A on liver, kidneys and gills of Indian major carp, *Catla catla* (Hamilton 1822). J. Anim. Plant Sci. **26**(2), 514–522 (2016)

Faheem, M., Khaliq, S., Ahmad, H.U., Lone, K.P.: Bisphenol-A (BPA) alters plasma thyroid hormones and sex steroids in female Pakistani major carp (*Catla catla*; Cyprinidae). Pak. Vet. J. **37**(3), 326–330 (2017)

Fernandes, M.N., Mazon, A.F.: Environmental pollution and fish gill morphology. In: Val, A.L., Kapoor, B.G. (eds.) Fish Adaptation, pp. 203–231. Science Publishers, Enfield (2003)

Finney, D.J.: Probit Analysis, 3rd edn., p. 333. Cambridge University Press, Cambridge (1971)

Giokas, D.L., Salvador, A., Chisvert, A.: UV filters: from sunscreens to human body and the environment. Trends Anal. Chem. **26**(5), 360–374 (2007). https://doi.org/10.1016/j.trac.2007.02.012

Gopal, C.M., Bhat, K., Ramaswamy, B.R., Kumar, V., Singhal, R.K., Basu, H., Balakrishna, K.: Seasonal occurrence and risk assessment of pharmaceutical and personal care products in Bengaluru rivers and lakes India. J. Environ. Chem. Eng. **9**(4), 105610 (2021)

Hendricks, L.J.: Erythrocyte counts and hemoglobin determinations for two species of suckers, genus *Catostomus*, from Colorado. Copeia **4**, 265–266 (1952)

Hesser, E.F.: Methods of routine fish hematology. Progr. Fish Culturist **22**, 164–171 (1960). https://doi.org/10.1577/1548-8659

Hued, A.C., Oberhofer, S., de los Ángeles, B.M.: Exposure to a commercial glyphosate formulation (Roundup®) alters normal gill and liver histology and affects male sexual activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). Arch. Environ. Contam. Toxicol. **62**(1), 107–117 (2012). https://doi.org/10.1007/s00244-011-9686-7

Jager, T., Ashauer, R.: Modelling Survival Under Chemical Stress: A Comprehensive Guide to the GUTS Framework. Toxicodynamics Ltd, York (2018)

Jager, T., Albert, C., Preuss, T.G., Ashauer, R.: General unified threshold model of survival—a toxicokinetic-toxicodynamic framework for ecotoxicology. Environ. Sci. Technol. **45**(7), 2529–2540 (2011). https://doi.org/10.1021/es103092a

Karmakar, S., Karmakar, S., Jana, P., Chhaba, B., Das, S.A., Rout, S.K.: Nonylphenol exposure in *Labeo rohita* (Ham.): evaluation of behavioural response, histological, haematological and enzymatic alterations. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. **247**, 109058 (2021). https://doi.org/10.1016/j.cbpc.2021.109058

Karthikraj, R., Kannan, K.: Mass loading and removal of benzotriazoles, benzothiazoles, benzophenones, and bisphenols in Indian sewage treatment plants. Chemosphere **181**, 216–223 (2017). https://doi.org/10.1016/j.chemosphere.2017.04.075

Kim, S., Choi, K.: Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review. Environ. Int. **70**, 143–157 (2014). https://doi.org/10.1016/j.envint.2014.05.015

Kinnberg, K.L., Petersen, G.I., Albrektsen, M., Minghlani, M., Awad, S.M., Holbech, B.F., Holbech, H.: Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, *Danio rerio*. Environ. Toxicol. Chem. **34**(12), 2833–2840 (2015). https://doi.org/10.1002/etc.3129

Krishnapriya, K., Shobana, G., Narmadha, S., Ramesh, M., Maruthappan, V.: Sublethal concentration of bisphenol A induces hematological and biochemical responses in an Indian major carp *Labeo* *rohita*. Ecohydrol. Hydrobiol. **17**(4), 306–313 (2017). https://doi.org/10.1016/j.ecohyd.2017.06.003

Kumar, M., Kumar, P., Devi, S.: Toxicity of copper sulphate on behavioural parameter and respiratory surveillance in freshwater catfish *Clarias batrachus* (Lin). Res. J. Chem. Environ. Sci. **3**(1), 22–28 (2015)

Liu, H., Sun, P., Liu, H., Yang, S., Wang, L., Wang, Z.: Hepatic oxidative stress biomarker responses in freshwater fish *Carassius auratus* exposed to four benzophenone UV filters. Ecotoxicol. Environ. Saf. **119**, 116–122 (2015). https://doi.org/10.1016/j.ecoenv.2015.05.017

Medda, S., Saha, N.C., Bhattacharya, R., Chatterjee, A., Pal, S.: Quinalphos Induced toxicity and potential attenuation of growth, hematological, biochemical, enzymological and oxidative stress biomarkers in *Tubifex tubifex* and *Oreochromis mossambicus*: a multiple biomarker approach. Scholars Acad. J. Biosci. **12**, 332–352 (2022). https://doi.org/10.36347/sajb.2022.v10i12.002

Moreira, A.L.P., Paiva, W.S., de Souza, A.M., Pereira, M.C.G., Rocha, H.A.O., de Medeiros, S.R.B., Luchiari, A.C.: Benzophenone-3 causes oxidative stress in the brain and impairs aversive memory in adult zebrafish. Environ. Toxicol. Pharmacol. (2023). https://doi.org/10.1016/j.etap.2023.104164

Nagelkerken, I., Munday, P.L.: Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. Glob. Change Biol. **22**(3), 974–989 (2016). https://doi.org/10.1111/gcb.13167

Nataraj, B., Hemalatha, D., Malafaia, G., Maharajan, K., Ramesh, M.: "Fishcide" effect of the fungicide difenoconazole in freshwater fish (*Labeo rohita*): a multi-endpoint approach. Sci. Total Environ. **857**, 159425 (2023). https://doi.org/10.1016/j.scitotenv.2022.159425

OECD: Test Guideline No. 203; Fish, Acute Toxicity Testing. OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris (2019)

Omoregie, E., Ajima, M.N., Keke, R.I., Wieski, K.: Effect of single superphosphate fertilizer on survival and respiratory dynamics of Nile tilapia, *Oreochromis niloticus* (Actinopterygii: Perciformes: Cichlidae). Acta Ichthyol. Piscatorial **39**(2), 103 (2009)

Pandey, A.K., Nagpure, N.S., Trivedi, S.P., Kumar, R., Kushwaha, B., Lakra, W.S.: Investigation on acute toxicity and behavioral changes in *Channa punctatus* (Bloch) due to organophosphate pesticide profenofos. Drug Chem. Toxicol. **34**(4), 424–428 (2011). https://doi.org/10.3109/01480545.2011.585650

Rajkumar, K.S., Kanipandian, N., Thirumurugan, R.: Toxicity assessment on haemotology, biochemical and histopathological alterations of silver nanoparticles-exposed freshwater fish *Labeo rohita*. Appl. Nanosci. **6**(1), 19–29 (2016). https://doi.org/10.1007/s13204-015-0417-7

Roberts, R.J.: Fish Pathology Wiley, New Delhi (2012)

Scheele, A., Sutter, K., Karatum, O., Danley-Thomson, A.A., Redfern, L.K.: Environmental impacts of the ultraviolet filter oxybenzone. Sci. Total Environ. **863**, 160966 (2023). https://doi.org/10.1016/j.scitotenv.2022.160966

Senthil, K.S., Kavitha, C., Ramesh, M., Grummt, T.: Toxicity studies of nonylphenol and octylphenol: hormonal, hematological and biochemical effects in *Clarias gariepinus*. J. Appl. Toxicol. **31**(8), 752–761 (2011). https://doi.org/10.1002/jat.1629

Sharma, M., Thakur, J., Verma, S.: Behavioural responses in effect to chemical stress in fish: a review. Int. J. Fish. Aquat. Stud. **7**(1), 01–05 (2019)

Sharma, P., Chadha, P.: Bisphenol A induced toxicity in blood cells of freshwater fish *Channa punctatus* after acute exposure. Saudi J. Biol. Sci. **28**(8), 4738–4750 (2021). https://doi.org/10.1016/j.sjbs.2021.04.088

Shaw, A.B.: A direct method for counting the leukocytes, thrombocytes and erythrocytes of birds's blood. J. Pathol. Bacteriol. **33**(3), 833–835 (1930). https://doi.org/10.1002/path.1700330326

Simons, K., Thibault, O., Nolen, R., Umeki, Y., Faulkner, P., Petersen, L., Hala, D.: A systems toxicological analysis of oxybenzone effects on the metabolic physiology of embryo-larval zebrafish (*Danio rerio*). Environ. Adv. **9**, 100308 (2022). https://doi.org/10.1016/j.envadv.2022.100308

Sudová, E., Piačková, V., Kroupová, H., Pijáček, M., Svobodová, Z.: The effect of praziquantel applied per os on selected haematological and biochemical indices in common carp (*Cyprinus carpio* L.). Fish Physiol. Biochem. **35**(4), 599–605 (2009). https://doi.org/10.1007/s10695-008-9269-3

Suzuki, T., Kitamura, S., Khota, R., Sugihara, K., Fujimoto, N., Ohta, S.: Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilizers and sunscreens. Toxicol. Appl. Pharmacol. **203**(1), 9–17 (2005). https://doi.org/10.1016/j.taap.2004.07.005

Uribe, C., Folch, H., Enríquez, R., Moran, G.J.V.M.: Innate and adaptive immunity in teleost fish: a review. Vet. Med. **56**(10), 486 (2011)

Van Kampen, E.J., Zijlstra, W.G.: Standardization of hemoglobinometry II. The hemiglobincyanide method. Int. J. Clin. Chem. **6**(4), 538–544 (1961). https://doi.org/10.1016/0009-8981(61)90145-0

Zhang, Y., Shah, P., Wu, F., Liu, P., You, J., Goss, G.: Potentiation of lethal and sub-lethal effects of benzophenone and oxybenzone by UV light in zebrafish embryos. Aquat. Toxicol. **235**, 105835 (2021). https://doi.org/10.1016/j.aquatox.2021.105835