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Triclosan toxicity alters behavioral and hematological parameters and vital antioxidant and neurological enzymes in *Pangasianodon hypophthalmus* (Sauvage, 1878)

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ABSTRACT

Triclosan and its metabolites are detected in a diverse aquatic environment and are major concerns for various aquatic organisms. The present study investigated the impact of acute and sub-lethal exposure of triclosan on behaviour, activities of acetylcholinesterase and selected antioxidant enzymes, haematological and serum gas-electrolyte parameters of *Pangasianodon hypophthalmus*. The 96 h LC_{50} of triclosan for *P. hypophthalmus* was estimated as 1458 μg L^{-1}. Further, sub-lethal triclosan exposure to 1/15th (97 μg L^{-1}), 1/10th (145 μg L^{-1}) and 1/5th (291 μg L^{-1}) of 96 h LC_{50} concentration for a period of 45 days lead to decrease in total erythrocyte count, haemoglobin content and packed cell volume of blood while total leukocyte count increased significantly ($p < 0.05$) as compared to control. A concentration-dependent increase in the serum chloride and decrease in partial pressure of oxygen in blood serum was noted on 45th day. An increased activity of catalase and superoxide dismutase in gill and liver tissues and inhibition of acetylcholinesterase activity in brain was observed on 15th, 30th and 45th day of exposure which was dependent on both - concentration of triclosan and duration of exposure. A significant high activity of glutathione-S-transferase in gill and liver tissue was observed in triclosan exposed groups in comparison to control during the experimental period. The study shows that long-term sub-lethal exposure of triclosan to fish can lead to several physiological alterations such as enzymatic scavenging of oxygen radicals and the normal neurological functions mediated by the enzyme acetylcholinesterase. With increasing anthropogenic activity, the study provides a convincing evidence for the necessity of a regulated use and safer disposal of triclosan to the environment.

1. Introduction

Triclosan (TCS, 5-chloro-2-(2, 4-dichlorophenoxy)-phenol) is a synthetic broad-spectrum antibacterial or antimicrobial agent in many soaps, detergents, toothpaste, mouthwash, deodorants and in disinfectants, in addition to innumerable other personal care, veterinary, industrial and household products which inhibits the activity of bacteria, viruses and fungi (Allmyr et al., 2008; USEPA, 2010). Concentrations of TCS in personal care products are typically in the range of 0.1%-0.3% of product weight (Sabaliunas et al., 2003). The multi-purpose application of TCS in various industrial products provides a number of pathways for the compound to enter the environment, particularly through wastewaters. About 96% of TCS that originates from consumer products is mixed with residential drains which in turn enter the wastewater treatment plants (WWTP) (Adolfsson-Erici et al., 2002). The biological sewage treatment is known to remove up to 98% of TCS from the water, but it can accumulate in the sludge and sediment where the remediation is a cumbersome process (Heidler and Halden, 2007). The concentration range of triclosan has been reported worldwide from 1.4 to 40,000 ng L^{-1} in surface waters, 20–86,161 ng L^{-1} in wastewater influent and 23–5370 ng L^{-1} in wastewater effluent. Further, a relatively high concentration of triclosan has been found in freshwater and marine sediment (< 100 – 53,000 μg kg^{-1} (dry weight) and 0.02–35 μg kg^{-1} (dry weight)), biosolids of WWTP (580–15,600 μg kg^{-1} (dry weight)), sludge (580–15,600 μg kg^{-1} (dry weight)) and pore water (0.201–328.8 μg L^{-1} (dry weight)) (SCCS, 2010).

Recently, the potential threats of TCS to aquatic organisms have received much attention due to associated potential ecotoxicological risks (Liang et al., 2013). It has been reported that TCS is highly toxic to algae, invertebrates and mainly effects developmental and reproductive stages of fish (Orvos et al., 2002). The sensitivity of each organism on

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TCS is dependent on concentration and duration of exposure. TCS with environmental concentration from 0.05 to 500 μg L^{-1} with a No Observed Effect Concentration (NOEC) of 0.21 μg L^{-1} causes an increase of bacterial mortality than bio-film algae (Ricart et al., 2010). Some microalga like *Selenastrum capricornutum* was reported approximately 30–80 folds more sensitive towards TCS than bacteria and fish (Tatarazako et al., 2003). In addition, it has been reported that TCS adversely affect the behaviour of fish, which includes loss of equilibrium, locking of the jaw, quiescence, and erratic swimming movements and it is also lethal to fish at higher concentration depending on species (Drummond and Russom, 1990). The median lethal concentration (LC_{50}) values of TCS for *Danio rerio* (Zebrafish), *Lepomis macrochirus*, *Oncorhynchus mykiss*, *Oryzias latipes* (medaka), and *Pimephales promelas* ranged from 270 to 602 μg L^{-1} (Orvos et al., 2002; Tatarazako et al., 2003; Oliveira et al., 2009).

Fish thrive in intimate contact with aquatic environment and are therefore highly susceptible to changes in the aquatic environment. The adverse changes in water quality are likely to be reflected in their blood and serum parameters. Hematological parameters are successfully used as a parameter to assess the toxicity of triclosan in the fish, *Oreochromis niloticus* (Vijitha et al., 2017). Similarly, behavioral alternations in fishes are considered as the indication of the degree and form of toxicity in toxicological experiments (Drummond and Russom, 1990; Robinson, 2009). Several studies demonstrated the antioxidant enzyme activities like catalase, superoxide dismutase and glutathione-S-transferase as effective biomarkers to assess stress and toxicity in fishes (Uner et al., 2005; Zhang et al., 2008). Acetylcholinesterase is also a widely used biomarker for neurotoxicity assessment and successfully demonstrated the neurological effects of glyphosate and endosulfan in fish (Glusczak et al., 2006; Dutta and Arends, 2003). Therefore, the above parameters were chosen for the current study to evaluate the acute and chronic toxicity of TCS in *P. hypophthalmus*, which is a common aquaculture species in Southeast Asia from food security view-point, and a candidate species for wastewater aquaculture (Ghosh, 2018).

2. Materials and methods

2.1. Experimental fish

Experimental fish, *P. hypophthalmus* fingerlings with an average weight of 12.0 ± 1.2 g (mean ± SD) were procured from local fish hatchery and transported to Central Wet Laboratory, ICAR-Central Institute of Fisheries Education, Mumbai under prescribed conditions. The fishes (n = 400) were held (1 g L^{-1} of water) at 25.6 ± 0.7 °C in adequately aerated big circular fibreglass reinforced plastic (FRP) tank (1000 L capacity), filled with de-chlorinated tap water (pH, 7.6–8.1) under natural photoperiod (12:12 h light-dark). During the three week acclimatization, the fish were fed with a commercial fish diet composed of 30% crude protein at the rate of 3% of body weight. Partial water exchange (20–25%) was done every alternative days to maintain the water quality.

2.2. Chemicals

Triclosan or Irgasan (CAS ID 3380-34-5) was obtained from Sigma Aldrich, USA (purity: > 97%) and used for the present study. A stock solution of 1000 mg L^{-1} of triclosan was prepared using 0.01 N NaOH (sodium hydroxide) solutions as a solvent.

2.3. Experimental design

2.3.1. Acute toxicity study

Before acute experimental exposure, a toxicity range-finding test was conducted following the method of static acute toxicity test (EPA, 2002). Randomly selected fishes were exposed to widely spaced sample dilutions in a logarithmic series 10^5, 10^4, 10^3, 10^2 μg L^{-1} triclosan with 0.0 μg L^{-1} as control (5 fishes per concentration). The cumulative mortality percentage was recorded for 96 h. Based on the result of range-finding test, five experimental concentration (1200, 1400, 1600, 1800 and 2000 μg L^{-1} triclosan) and 0.01 N NaOH solution as control, were selected for 96 h acute toxicity tests expected to cause 0–100% mortality. The acute toxicity test of TCS was carried out according to EPA (2002) in a static system by using 150 L capacity glass tanks filled with 80 L de-chlorinated water. The research was conducted in an indoor experimental outfit under the natural 12 h: 12 h light-dark photoperiod. The ethical guidelines for the Animal Care of ICAR- Central Institute of Fisheries Education, Mumbai, India were strictly adhered to conduct the present study. Each concentration was prepared in triplicate and used for stocking of ten fishes in each replication. Feed was not offered to the fish 48 h before and 96 h of test period. The physico-chemical parameters of the test water analyzed daily using standard methods APHA (2005). Observation for fish mortality and behavioural changes were noted every 24 h and dead fishes were removed immediately using scoop net. Finney's probit analysis method (Finney, 1971) was used to determine the median lethal concentration (LC_{50}) of the triclosan (96 h) for *P. hypophthalmus* and was calculated as 1458 μg L^{-1}.

2.3.2. Chronic toxicity study

The chronic toxicity study was performed with 1/15th (97 μg L^{-1}), 1/10th (145 μg L^{-1}) and 1/5th (291 μg L^{-1}) of 96 h LC_{50} of TCS concentration, including a control (0.1 N NaOH solution without triclosan) for 45 days. Ten fish each, from acclimatized group was distributed randomly to the experimental tanks in triplicate (30 fishes per exposure concentration). The treatment solutions were 100% renewed every four-day interval with fresh working test solution of desired concentration of triclosan to maintain the water quality (Table 1) of the test media and the concentration of TCS. Feeding and other conditions were the same as acclimatization conditions. Blood and serum of test fishes along with control were collected on 45th day while tissues (liver, gill and brain) were collected on 15th, 30th and 45th days of exposure for analysis.

2.4. Collections of blood, serum and preparation of tissue homogenate for enzyme analysis

On 45^{th} day two fishes from each tank (replicate) were anaesthetized using clove oil at a dose of 50 μL L^{-1} water to minimize the stress. Blood was withdrawn from caudal vein of the fish using a medical syringe (23 G), which was previously rinsed with 2.7% EDTA solution. The collected blood was then transferred immediately to EDTA coated vials (as anticoagulant) and was shaken well to prevent blood clotting. Another portion of blood was collected without anticoagulant and was centrifuged for 10 min at 3000 × *g* in a refrigerating centrifuge to obtain the serum used for electrolyte analysis.

The whole brain and a piece of gill and liver were excised from six fishes per treatment including control groups at every 15 day interval of experimental period of 45 days. The brain, liver and gill samples (organs) from each experimental group were pooled and weighed further, homogenized in a chilled 0.25 M sucrose (1:19 w/v). The homogenates were centrifuged at 12,000 × *g* for 10 min at 4 °C then the supernatants

Table 1
Physico-chemical parameters of water of different treatment groups during the experimental period of 45 days.

Treatments (TCS concentration)	Temperature (°C)	pH	DO (mgL^{-1})	Free CO_2	Alkalinity (mgL^{-1})
0 μg L^{-1}	25.8–27.4	7.8–8.3	6.9–7.3	ND	281–289
97 μg L^{-1}	25.3–26.2	7.6–8.0	6.8–7.6	ND	279–286
145 μg L^{-1}	25.0–26.1	7.5–8.2	6.7–7.5	ND	280–288
291 μg L^{-1}	25.6–27.4.	7.7–8.4	6.6–7.2	ND	282–290

DO = Dissolved oxygen, ND = Not detected.