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Research Article

A Study of Total Carbohydrate Modifications induced by Triclosan on *Channa punctatus* (Bloch)

Ravi Kumar Kola[1] and Prameela Devi Yalavarthy[2]*

[1,2]*Department of Zoology, Kakatiya University, Warangal, Telangana, India.*

*Corresponding author:
E-mail: prameeladeviy@yahoo.co.in

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ABSTRACT

Triclosan (2,4,4′ –trichloro-2′-hydroxydiphenyl ether) is a chlorinated aromatic compound, used as a synthetic broad-spectrum antimicrobial agent in many personal care products like soaps, hand-washes, toothpastes, mouth washes, deodorants, antiperspirants and shaving creams. The products that contain Triclosan wash down through drains and into water systems and it had become a common contaminant. Triclosan can persist in the environment and exhibit toxicity towards a number of aquatic organisms. Fishes are the most widely distributed organisms and play a central role in aquatic ecosystems. Hence, understanding toxic responses in fish is of high ecological relevance. To assess the aquatic toxicity caused by Triclosan in fresh water fish, *Channa punctatus* the total carbohydrate modifications were studied. The fishes were exposed to sub lethal and lethal concentrations for 96 hours. Estimation of total carbohydrates in the five tissues-muscles, gill, liver, kidney and brain was done by Carroll *et al.*, method. The total carbohydrate content decreased with the increase of Triclosan concentration. The total carbohydrate levels depletion was observed maximum in Kidney as 36.31% and minimum percentage in brain as 18.93% in sub lethal concentration and maximum in Liver as 42.37% and minimum in Gill as 30.17% in lethal concentrations when compared with the control. The levels of total carbohydrates were found to decrease because of utilization of carbohydrates to meet the energy demands during stress conditions. It was observed that low concentration of Triclosan (0.37 ppm) is also very toxic and causes alterations in vital organs of fish, *Channa punctatus*
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1. Introduction

With the advent of science and technology man has started producing various new chemical substances for use in Personal care Products. These chemicals substances pose a significant challenge in understanding about their adverse environmental and health impacts. Triclosan (2,4,4′ –trichloro-2′-hydroxydiphenyl ether), Chemical Abstract Service Registry Number 3380-34-5) is a chlorinated aromatic compound and its functional groups include both phenols and ethers. It is used in variety of common household and personal care products, including soaps, mouthwashes, deodorants, toothpastes, acne creams and hand sanitizers (Daughton *et al.*, 1999). It was introduced in 1968 and is being increasingly used in various other products (Oliveira, *et al.*, 2009). Over the last decade, there has been a rapid increase in the use of Triclosan-containing products (AMA, 2000). In 2008 the Environmental Working Group reported Triclosan in more than 140 types of personal care and home products (EWG, 2008). The indiscriminate use of Triclosan in various personal care products lead to the discharge of this chemical into domestic waste streams (Okumara and Nishikawa, 1996). Triclosan is now one of the most commonly detected organic products found in wastewater (Gaume *et al.*, 2012). Fishes are generally considered as good model organisms for monitoring of the aquatic environment (van der Oost *et al.*, 2003). The fishes are the most susceptible and more vulnerable to containments than any other aquatic animals (Shivani Sharma *et al.*, 2014). The biochemical alterations are usually the first detectable and quantifiable responses to environmental changes of animal health and internal environment of the organism (Agrahari *et al.*, 2007). There is limited literature regarding the effect of Triclosan toxicity on total carbohydrate modifications in fresh water fishes. Hence, the present study was undertaken to assess the aquatic toxicity by studying the total carbohydrates modifications in five different tissues of fresh water fish, *Channa punctatus.* The fishes were exposed to different concentrations of sub lethal and lethal for 96 hours.

2. Material and Methods

2.1. *Experimental animal and acclimation*

The teleost fish, *Channa punctatus* was selected in this study because of its wide availability and ability to tolerate harsh aquatic environmental conditions. The teleosts are good models to evaluate the toxicity of pollutants because they possess similar biochemical responses like those of mammals and other vertebrates (Li *et al.,* 2011).

Fresh water fishes, *Channa punctatus* were collected from local water bodies of Warangal District, Telangana State (South India). They were transported to the laboratory and used for the experimental purpose. The fishes were washed three times in dechlorinated tap water and treated with 1% $KMnO_4$ for 2-3 minutes to remove external parasitic infections (Santhanam *et al.,* 2005). Fishes were acclimatized to laboratory conditions for 10 days. The fishes were maintained in dechlorinated tap water and water was renewed every day to provide freshwater rich in oxygen. Fish food pellets were provided *ad libitum* (Affonso *et al.,* 2002). Continuous artificial aeration was maintained throughout the acclimation and exposure periods. After ten days, fishes of similar sizes (15 ±5 cm) and weight (± 170.25 gm) were sorted out and separated into 9 groups of 10 fish each and kept in plastic tubs (30 L) under light-dark (12:12 hrs) cycle. Only normal and healthy fishes were selected for the present biochemical experiment. The fishes were fastened for 24 hrs prior to the experiment. After 96 hours live fishes were removed from three groups (control, sub lethal and lethal) and immediately stunned with a blow on the head, and were dissected out to collect the five different tissues - Liver, kidney, brain, muscle and gills. These are the most vulnerable tissues of a fish exposed to the medium containing any type of toxicant.

2.2. *Test chemical*

The commercial-grade Triclosan used in this study was obtained from ViVi Med Labs, Hyderabad.

The LC50 value of Triclosan was determined in the laboratory. Ninety fishes were randomly distributed into nine aquarium tanks (30L) filled with different concentration of triclosan (0.60, 0.70, 0.80, 0.90, 1.00, 1.10, 1.20, 1.30 and 1.40mg/L). The mortality was recorded for 96h. The LC50 of triclosan calculated with the help of probit analysis (Finney, 1971). The 96 hr concentration (1.11 mg/L) of calculated LC50 was selected. One-third (1/3) of LC_{50} was taken for **Sublethal study** (0.37mg/L). The fishes were exposed to sub lethal and lethal concentrations of Triclosan and one group was maintained as control, the results were observed after 96 hrs time interval. Since Triclosan is a chlorinated aromatic compound and it is insoluble in water, stock solution of 1mg/mL Triclosan was prepared in acetone. In many of the pesticide toxicity studies, acetone has been used as the solvent. Acetone at low concentrations was found to be non-toxic to fish (Shivakumar and David, 2004) and the concentrations were prepared by using the stock solution.

2.3. *Estimation of Carbohydrates*

Total carbohydrates were estimated by using the Anthrone method of Carroll *et al.,* (1956). The control and experimental fishes were homogenized in 5ml of 10% Trichloroacetic acid (TCA) and centrifuged at 1000 rpm for 15 minutes. 0.5 ml of supernatant was taken for the estimation of total carbohydrates. To this supernatant 4ml of Anthrone reagent was added and vortexed for proper mixing. Then the samples were incubated in the boiling water bath for about 15 min and cooled to room temperature. The colour developed was read in a Digital-visible spectrophotometer at 620 nm against the blank. D-glucose was used as standard and the amount of carbohydrates present was calculated. The values were expressed as µg for 100mg wet weight of the tissue.

3. Results and Discussion

The total carbohydrate level in the selected five tissues of control and Triclosan treated fishes were given in the (**Table-1**). Among the five tissues, the total carbohydrate levels were found to be high in the order of Liver >Kidney> Muscle > Gill>Brain (Table-1) in the control fish.

But, the carbohydrate levels were found to be decreased in the exposed groups i.e., sub lethal and lethal concentrations. The decrease in total carbohydrate levels of the fishes treated with Triclosan was found to be in the order of :
Sub-lethal: Kidney>Liver>Muscle>Gill>Brain and
Lethal: Kidney>Liver>Muscle>Brain>Gill

Among all the tissues maximum percentage of total carbohydrates depletion was observed in Kidney as 36.31% in sub-lethal and 55.21% in lethal and minimum percentage was observed in brain as 18.93% in sub-lethal and in gill as 30.17 % in lethal when compared with the control.

Table-1: Levels of Carbohydrates in different tissues of *Channa punctatus* after treatment with sub lethal and lethal concentrations of Triclosan for 96 hours

Tissue	Control	Concentration of Triclosan			
		Sub-lethal	% change	Lethal	% change
Liver	26.62±0.41	19.21±0.37	27.83	15.34±0.28	42.37
Brain	4.12±0.12	3.34±0.38	18.93	2.85±0.46	30.82
Gill	6.43±0.32	5.14±0.41	20.06	4.49±0.28	30.17
Muscle	11.43± 0.47	8.56±0.44	25.10	7.45±0.35	34.82
Kidney	15.45±0.34	9.84±0.19	36.31	6.92± 0.32	55.21

*Each value is mean of ± SD of six (6) individual observations
*Values are expressed in µg/100 mg wet wt. of the tissue

The contamination of aquatic systems attracted the attention of researchers all over the world (Dutta and Dalal, 2008). Aquatic ecosystems were contaminated with a wide range of pollutants and become a matter of concern over the last few decades (Vutukuru, 2005). Biochemical parameters are used of as bio indicator for determination the pollution in aquatic ecosystems (Mason, 1991). Alteration in the biochemical values in fish gives an indication to understand the mode of action and type of toxic chemicals. Most of the pollutants acts as metabolic depressor in the environment and generally causes pressure on biologically active molecules such as proteins, carbohydrates and lipids (Agrahari, and Gopal, 2009). Carbohydrates are the primary and immediate source of energy (Lehninger, 1978). In the present study, total carbohydrates levels in muscle, liver, gill, brain and kidney of *Channa punctatus* exposed to two different concentrations of Triclosan showed significant decrease when compared to control fish. The total carbohydrates levels decreased with increased concentration of Triclosan. Similar change was observed with Gautam and Gautam, (2001) in *Channa punctatus*, the decrease in carbohydrate metabolism after the pesticide exposure. The decrease in total carbohydrate levels in the liver, muscle, gill, kidney and brain of fishes treated with Triclosan indicates utilization of carbohydrates to meet energy demands. In fishes carbohydrates play a major role as energy precursors under stress condition (Yildiz and Benli, 2004). When animals were subjected to toxic pollutants, metabolism of carbohydrates is disturbed (Olaganathan and Patterson, 2013). The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition (Thenmozhi *et al.*, 2010). The excess demand of energy is perhaps achieved by rapid glycogenolysis through activation of glycogen transferase (Revathi et al., 2005)

4. **Conclusion**:

The results indicate that Triclosan has deleterious effect on total carbohydrate levels of the fresh water fish, *Channa punctatus* even at lower concentration. Pollution of fresh waters by Triclosan is known to disturb the sensitive equilibrium of the aquatic ecosystem. There is a possibility of Triclosan accumulation in fish organs, even when the exposure is low. This could lead to biomagnification in the food chain, ultimately being toxic to human beings.

Competing Interests

The authors have declared that no competing interests exist.

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