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RESEARCH ARTICLE

EFFECT OF TRICLOSAN ON TOTAL PROTEIN CONTENT IN FRESH WATER FISH, *CHANNA PUNCTATUS*

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ABSTRACT

Triclosan (2,4,4' –trichloro-2'-hydroxydiphenyl ether) is a chlorinated aromatic compound used as a broad-spectrum antimicrobial agent against many types of Gram-positive, Gram-negative bacteria and some fungi. Triclosan is used in many personal care products such as soaps, skin cleansers, deodorants, lotions, creams and toothpastes. The indiscriminate use of Triclosan leads to the contamination of surface waters, and poses threats to aquatic organisms. Fish species are ideal aquatic animal models for assessing the toxicity like perturbations in behavior, oxygen uptake and biochemical profiles etc. The objective of the present study is to investigate the aquatic toxicity of Triclosan by studying the total protein content modifications in fish, *Channa punctatus.* The experimental fishes are divided into four groups- control, low (0.1 ppm), medium (0.5 ppm) and high (1.0 ppm) concentrations and are exposed to technical grade Triclosan for 96 hrs. The live fishes are sacrificed after 96hrs and total protein content is analyzed from five different tissues - Brain, Liver, Kidney, Gill and Muscle. The total protein content was maximum in liver with depletion of 31.64% in 0.1 ppm, 46.39% in 0.5 ppm and 64.36% in 1.0 ppm, compared to control. The total protein content was minimum in brain with depletion of 18.20% in 0.1 ppm, 39.92% in 0.5 ppm and 59.11% in 1.0 ppm, compared to control. The decrease of total protein content may be due to enhanced proteolytic activity and decreased anabolic activity of protein under stress condition towards Triclosan. The results suggest that Triclosan causes hazardous effects to aquatic organisms especially fishes even at lower concentrations.

INTRODUCTION

The chemicals present in personal care products belong to a relatively new group of contaminants referred to emerging contaminants and are dominating in urban waste waters (Ellis, 2006). Triclosan [5-chloro-2-(2, 4-dichlorophenoxy) phenol; TCS] is an active ingredient used in many personal care products as a broad spectrum antibacterial agent (Bester, 2003). Products that contain trichlosan, wash down through drains and enter into waterways, making it a common contaminant (Heidler and Halden. 2007)**.** Triclosan is commonly detected in aquatic ecosystems leading to the continuous exposure of aquatic organisms along with its bioaccumulation potential, which has led to detectable levels of this antimicrobial agent in a number of aquatic species (Dann, Hontela, 2011). Triclosan has an estimated half-life of 60 days in water (Halden and Paull, 2005). Triclosan is toxic to algae, phytoplankton, and other aquatic life (Yang *et al*. 2008, DeLorenzo and Fleming, 2008). Triclosan has also been shown to have genotoxic and cytotoxic effects in algae(Ciniglia *et al.*, 2005) Biodegradation and photolytic degradation mitigate the availability of Triclosan to aquatic organisms; however the by-products such as methyltriclosan and other chlorinated phenols may be more resistant to degradation and have higher toxicity than the parent compound. There is more concern on Triclosan as it will accumulate and spread through aquatic and terrestrial food webs (Coogan *et al*.,2007)

MATERIALS AND METHODS

The experiment was initiated by collecting healthy set of *Channa punctatus* (Bloch), weighing average of 100 - 110 g and 25.5±1.21 cm in length, which were procured from Wardhannapet water body, Warangal and acclimatized for one week in large plastic tubs of 25 litres capacity already filled with dechlorinated tap water and proper aeration under laboratory conditions. The fish were fed *ad labium* with standard commercial food. Continuous aeration was supplied and water was changed for every 24 hrs. They were starved for one day before experiment (Butlerworth, 1972). If mortality occurred during the experimental period, dead fish were removed immediately to avoid depletion of dissolved oxygen (DO) level which adversely affects other fish (Schreck and Brouna, 1975). For estimation of proteins, the live fishes were sacrificed after 96 hours and the vital tissues like muscle, brain, liver, gill and kidney were taken from the control and experimental groups.

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Triclosan

For present study, commercial grade Triclosan was procured from ViVi Med Labs, Hyderabad. (Batch No. TCS0212028). In toxicity studies for water insoluble aromatic hydrocarbons, they are dissolved in organic solvents, diluted to achieve a target concentration, and then test organisms are exposed to solution of these compounds to assess potential effects (Okumura *et al*., 2001). As Triclosan is a chlorinated aromatic compound and it is insoluble in water, a stock solution was prepared in acetone and later it was used to get required dilutions (0.1 ppm, 0.5 ppm & 1.0 ppm) in water.

Estimation of total proteins

Total protein content was estimated by the method Lowry *et al*., (1951). 100 mg of tissue was homogenized in 5 ml of cold distilled water. 5 ml of 30% TCA was immediately added to precipitate the protein. Precipitate was collected by centrifugation at 3000 rpm for 15 minutes. The supernatant was discarded. The pellet was repeatedly washed with distilled water to remove the traces of TCA precipitated. Protein was redissolved in 0.1 N NaOH and estimated by this method. 0.5 mL of the solution was transferred in to a test tube and 4 mL of alkaline copper sulphate (50 ml of 2% Na_2CO_3 and 1ml of 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartrate) reagent was added followed by 0.4 mL of diluted commercial Folin's reagent (diluted with distilled water in 1:1 ratio). The optical density of blue color developed was read at 750 nm after 30 minutes of addition of the reagent using a spectrophotometer. Bovine serum albumin was used as standard. The protein content in the tissue was expressed micrograms/100mg wet weight of the tissue.

RESULTS

The calculated values for total proteins and percent changes in different concentrations of Triclosan over control along with Standard deviation are given in Table-1 and are graphically represented in Fig-I. The protein levels in muscle, liver, gill, brain and kidney of *Channa punctatus* exposed to three different concentrations of Triclosan showed significant decrease when compared to control fish. In the control fish, *channa punctatus* the total protein content is in the order of: Muscle > Liver >Gill> Brain > Kidney. During the exposure of three different concentrations of Triclosan (0.1 ppm ,0.5 ppm and 1.0 ppm) for 96 hours the percent change of total protein depletion was in the order of Liver > Muscle > Gill > kidney > Brain.

Table1 Levels of Protein in different tissues of *channa punctatus* after treatment with three concentrations of Triclosan for 96 hours

Tissue	Control	Concentration of Triclosan		
		0.1 ppm	0.5 ppm	1.0 ppm
Muscle	8.28± 0.33	6.03±0.51	4.53±0.47	3.27±0.68
Liver	6.51±0.21	4.45±0.32	3.49±0.56	2.32±0.36
Gill	5.57±0.28	4.21±0.36	3.26±0.22	2.23±0.31
Brain	4.01±0.13	3.28±0.18	2.41±0.44	1.64±0.35
Kidney	3.64±0.26	2.86±0.28	2.08±0.21	1.42± 0.33

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

The maximum percentage of protein depletion was observed in liver as 31.64% in 0.1 ppm, 46.39% in 0.5 ppm & 64.36% in 1.0 ppm and minimum percentage was observed in brain as 18.20% in 0.1 ppm, 39.92% in 0.5 ppm and 59.11% in 1.0 ppm when compared with the control.

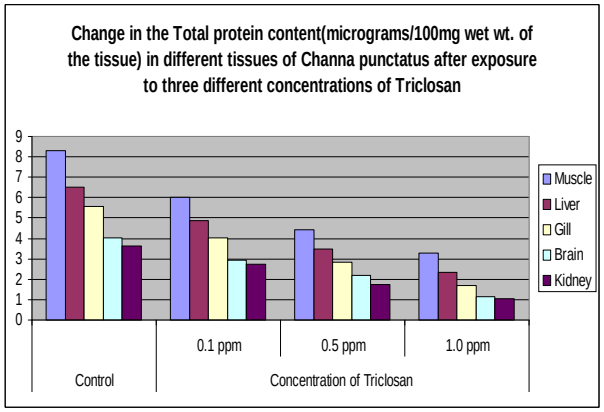

Figure I Graphical representation of the changes in Total protein content of *channa punctatus* exposed to Triclosan

DISCUSSION

The changes in biochemical parameters are important to indicate the susceptibility of organ systems to toxicants by altering their function (Verma and Tonk, 1983). Proteins are of primary importance in the living organisms not only of their unique specificity but also because of the fact that they appear to confer their biological specificity among various type of cells (Bhushan *et al*., 2002). The present study revealed the reduction in protein levels in the tissues of the fresh water fish, *Channa punctatus* by following acute exposure of toxicant Triclosan. The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress (Remia *et al*., 2008). It was reported that reduction in protein content could be due to its utilization to mitigate the energy demand when the fishes are under stress (Venkatramana *et al*., 2006). The depletion of protein fraction in the tissues may be due to their degradation and possible utilization for metabolic purpose (Chezhian *et al*., 2010). Similar change was also observed in *Channa punctatus* exposed to technical grade malathion by Agrhari *et al*., (2006). Tilak *et al*., (2003) explained the reduction of protein content of liver, brain and ovary of *Channa punctatus* exposed to toxicant, fenvalerate. Based on these investigations, the changes and decrease in protein levels was also due to inhibition of metabolizing enzymes after exposure of *Channa punctatus* to Triclosan.

CONCLUSION

The present study revealed that there was significant change in the protein content of vital organs in *Channa punctatus* which are brought about by exposure to Triclosan. Triclosan is affecting fresh water organisms even at very low concentrations of 0.1 ppm. The effects of these deleterious chemical goes unnoticed because most of the changes occurring in the body of the fish are minute and if the indiscriminate usage of Triclosan continues it will become irreparable when it gets accumulated and spread through aquatic and terrestrial food webs.

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