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Haematological profile of *Channa striatus* exposed to sublethal concentrations of endocrine disruptor chemical Triclosan

Jacob Ann Mary*and Kaippallil Jose D

Department of Zoology, St Berchmans College Changanacherry, Kottayam, Kerala 686101

ABSTRACT

The endocrine disruptor chemical triclosan (5-chloro-2-(2, 4-dichlorophenoxy phenol) is an antibacterial agent used in a variety of personal care and in household products. Triclosan has recently attracted special attention of the scientific community, regulatory agencies, and the public as a result of their widespread use and endocrine related effects. Triclosan has been detected in public wastewater treatment plants, rivers etc.TCS act as a potential endocrine disruptor, the impact of this estrogenic endocrine disrupting chemical (EDCs) on the immune system is ambiguous Hence the present study was conducted to analyze the sublethal toxicity level of triclosan on freshwater fish *Channa striatus*. Fishes were grouped into two, Control and experimental groups, Control was maintained along with the experimental period. LC50 was found to be 0.602ppm. Experimental fishes were exposed to 1/10th (0.060ppm) and 1/5th (0.120ppm) of LC50 concentrations of triclosan for an interval of 7,14,21,28 days along with the control group. Hematological parameters such as red blood cell count (RBC), white blood corpuscles (WBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean cell volume (MCV), were observed in the treated fish and compared with the control group. Exposure to triclosan at both sublethal concentrations showed reduction in RBC, Hb this may be due to the hemolytic ability of triclosan. WBC content was significantly increased to get over the stress induced by triclosan. The results revealed that sublethal concentrations of triclosan produced changes in all the measured hematological parameters. The results are statistically significant at $P < 0.05$ level.

KEYWORDS: Triclosan,Endocrine Disruptor Chemical,*Channa Striatus*.LC50 ,Hemolysis.

***Corresponding author**

Ann Mary Jacob

Department of Zoology, St Berchmans College Changanacherry, Kerala 686101

Email:annjacob2015@gmail.com, Mobile no: 09567649885

INTRODUCTION

Endocrine disruptors come from a wide range of sources, such as pesticides, plastics, pharmaceuticals, household chemicals, and industrial chemicals. They alter the hormonal functions, which prevent normal hormonal binding and block synthesis[8] .Due to keen scientific interest and various actions councils led by citizens have created awareness among people about the threats of endocrine disruptor chemicals[29].As the name indicates EDC'S cause disruption to the endocrine system interfere with natural hormonal functions[22]. Triclosan is one of the most extensively used antimicrobial agent in use. Owing concern about the effect of this chemical in the environment has evolved due to their endocrine disrupting properties. It was first registered as a pesticide with the Environmental Protection Agency (EPA) in 1969. Triclosan has been detected in aquatic organisms ranging from algae, fishes as well as in human urine, blood and breast milk [20,1] .Triclosan has been detected not only in surface water and estuarine sediment but also in freshwater at concentrations of up to 800 ng/kg, researchers have detected triclosan contamination in both aquatic and terrestrial environments and also detected its bioaccumulation in aquatic biota [31]. Aquatic organisms are highly sensitive to changes in the water bodies, environment pollution or habitat destruction will be reflected in their biological makeup. Fishes are susceptible to any alteration in the physico-chemical characteristics of their habitat [25,2] stated that haematological tests are the valuable indicator of stress or disease in fish as circulatory system has a close association with the environment..In fish, exposure to chemical pollutants can alter haematological levels . Haematological studies could be used to figure out the health condition of fish and water quality [11]. Fish haematological parameters are often determined as an index of their health status [4].Thus fish could be used as a warning system to indicate the presence of pollutants in natural water bodies [18]. Haematological studies are used to determine the effect of toxicants in environment as a result of the close association between the circulatory system and the external environment [6,32]. In recent years haematological parameters have been used more to determine the sublethal concentrations of chemicals [34].The present study was undertaken to evaluate the toxic effect of sublethal concentrations of triclosan on haematological parameters on freshwater fish *Channa sriatus.*

MATERIALS AND METHODS

Channa striatus (Order: Perciformes, Family: Channidae) collected for the study was from culture ponds in Tiruvalla, Kerala (9°16′N 76°47′E). Healthy fishes were selected for the study. Fishes were acclimatized in lab conditions for 14 days prior to the experiment. Fishes ranging from (42±4.8gm) and length (17±4.5cm) were selected for the study. The fishes were disinfected with KMNO4 solution prior to acclimatization. They were then transferred to aquarium tanks for

acclimatization.Water quality characteristics were noted. The mean values for test water qualities were as follows: temperature 28.5 ± 1.5°C, pH 7.1 ± 0.03, dissolved oxygen 6.5 ± 0.2 mg/l. A minimum of 10 fishes was introduced into each tank, the tanks were covered with black paper in order to avoid exposure to sunlight. The water was changed in alternative days, Feeding was carried out daily during the acclimatization period. Then the feeding was stopped during the experimental period. Triclosan was procured from Sigma Aldrich Mumbai. A Stock solution was prepared by dissolving 400mg of triclosan in 40ml acetone. Test concentration was made by diluting appropriate aliquots of the stock triclosan solution. LC50 of triclosan was determined by using probit analysis (Finney 1950). The LC50 value of triclosan was found to be 0.602 ppm. For the analysis, two sublethal concentrations (ie,1/5th and 1/10th of LC50 concentrations) were selected along with control. Observations were done on 7th, 14th, 21st and 28th day respectively.

Haematological Estimations

Different haematological parameters like total erythrocyte count, haemoglobin, haematocrit and erythrocyte constants like mean cell volume and mean cell hemoglobin were estimated. Fishes exposed to sublethal concentration were selected for haematological analysis.

Collection of Blood

Properly washed fishes were taken out on corresponding days. Blood was collected using the heparinized needle from the caudal peduncle. The collected blood was analyzed for studies immediately.

Total erythrocyte count (TEC)

The haemocytometer method was employed for determining TEC. Blood was diluted to 200 times in RBC pipette by using Haem's fluid. The diluted blood was poured into Neubaur Counting chamber and erythrocytes were counted on prescribed columns under a compound microscope and expressed as the total number of erythrocyte per cubic millimeters of blood. Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were calculated in 10^6 mm-^{3} [35].

Total WBC count

Total white blood cells (WBC) were counted using an improved Neubaur haemocytometer. Blood was diluted 1:20 with Turk's diluting fluid and placed in haemocytometer. 4 large (1 sq mm) corner squares of the haemocytometer were counted under the microscope. The total number of WBC was calculated in mm^3 x10^3 [35].

Haemoglobin (Hb)

Cyanmeth haemoglobin method was adopted for the study. 0.02 ml of blood was added to 5 ml Drabkins reagent in a test tube, mixed well and kept for 5-10 minutes. Commercially available methaemoglobin standard was used and readings of standard and test solution were taken in colorimeter using a green filter (540nm).

$$\text{Hb (gm\%)} = \frac{\text{OD of test solution} \times \text{Concentration of standard}}{\text{OD of standard solution}}$$

Haematocrit (Ht)

The capillary tube method was employed for haematocrit determination. A column of blood about 7.5 cm length was drawn into a glass capillary tube of about 10 cm length and 1mm bore diameter. One end of the capillary tube was sealed with bee wax.

Ht (%) $= \frac{LI}{L2} X10$; where L1 and L2 are length of settled and serum content respectively.

Mean Cell Calculations

From the estimated values mean cell volume (MCV) and mean cell haemoglobin (MCH) can be calculated using a standard formula as below:

Mean Cell Volume(MCV) $= \frac{Ht}{TEC} X10$

Mean Cell haemoglobin(MCH) $= \frac{Hb}{TEC} X10$

Statistical analysis

Values of experimental groups were compared statistically with control by student's t-test. Significance was established at $P < 0.05$ by using SPSS package.

RESULTS AND DISCUSSIONS

No mortality was observed during the sublethal study period. However as the time progressed, fishes were showed lethargy, altered swimming movements,fast gulping etc .The 96 hour LC50 value of triclosan according to probit analysis was 0.602ppm with 95% lower and upper confidence limit was 0.537ppm and 0.675ppm respectively.

Table 1: Showing haematological parameters of fish *Channa striatus* exposed to sublethal concentrations of Triclosan

Parameter	*Days*	*Control*	*1/5th Concentration*	*1/10th Concentration*
RBC count(million/mm^3)	*7 days*	*3.81 ± 0.11*	** 2.66 ± 0.20*	** 3.20 ± 0.11*
	14days	*3.79 ± 0.10*	**2.17 ± 0.01*	**3.11 ± 0.6*
	21days	*3.77 ± 0.20*	** 2.11 ± 0.9*	**2.97 ± 0.01*
	28days	*3.74 ± 0.11*	** 1.83 ± 0.10*	**2.67 ± 0.13*
WBC count(cells/mm^3)	*7 days*	*16.740 ± 0.49*	*24.120 ± 2.00*	**19.82 ± 0.25*
	14days	*16.865± 1.42*	**26.64 ± 0.95*	*21.14 ± 2.37*
	21days	*17.15± 0.45*	**27.12 ± 0.77*	*21.92± 0.77*
	28days	*17.67± 0.83*	**27.90 ±0.40*	*22.12 ± 0.62*
Hb(%)	*7 days*	*11.7 ± 0.15*	*10.6± 0.15*	**11.4 ± 0.15*
	14days	*11.6 ± 0.57*	**10.0± 0.57*	**11.1 ± 0.57*
	21days	*11.5 ± 0.30*	**9.6± 0.30*	*10.8. ± 0.2*
	28days	*11.5 ± 0.10*	**9.0± 0.10*	**10. 1± 0.15*
PCV(%)	*7 days*	*43.82 ± 3.26*	*37.02 ± 0.83*	*39.64 ± 2.43*
	14days	*43.51 ± 2.72*	**36.8 ± 0.30*	*39.11 ± 0.5*
	21days	*43.22 ± 1.9*	*35.76 ± 1.1*	**38.93 ± .19*
	28days	*42.10 ± 1.42*	**33.16 ± 0.81*	*37.01 ± 1.90*
MCH(pg)	*7 days*	*30.7± .50*	**39.8± 1.2*	**35.62± 0.52*
	14days	*30.6± .60*	**46.08± 1.04*	**35.69± 1.05*
	21days	*30.7± .13*	**45.49± 1.4*	**36.36± 2.2*
	28days	*30.7± .35*	**44.77± 1.53*	**37.82± 0.80*
MCV(fL)	*7 days*	*115± 1.52*	**139.2±3.65*	*123.9± 0.90*
	14days	*114.8± 1.9*	**169.6± 1.70*	**125.8± 0.40*
	21days	*114.6± 1..10*	** 169.5± 1.40*	**131.1± 2.90*
	28days	*112.6± 2.70*	**165± 0.50*	**138.6± 3.2*

Each value is mean ±SD of 6 observations

* indicates significant ($P < 0.05$).

Fish blood is being studied as a major bio marker for monitoring environmental parameters [17].The results of the present investigation show that the triclosan exposure imposed a drastic reduction in the total count of RBC's. Triclosan treated fish showed a stastically significant decrease in RBC. The reduction was dosage dependent [21].In this study sub lethal concentrations of triclosan caused a significant reduction in RBC count , packed cell volume ,Hb content of *Channa striatus* (Table1). The reduction observed in red blood cell count of triclosan treated fish, which is considered as the most ideal bio marker with respect to endocrine disruptors. Red blood cell plays a pivotal role in the transport of oxygen and carbon dioxide in the body. Hence , a reduced red blood cell count implied a reduction in the level of oxygen that would be transported to the tissues as well as the level of carbon dioxide returned to the lungs [30,26,13].

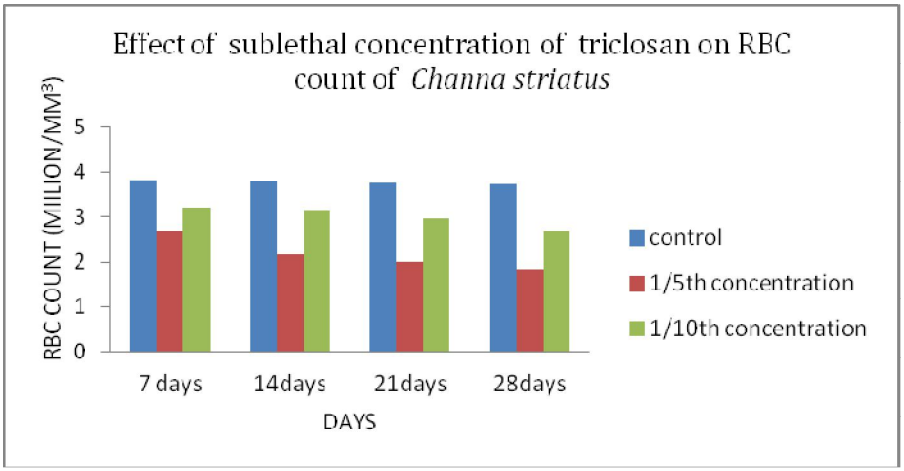

Figure 1: Showing the effect of sublethal concentration of triclosan on RBC count of *Channa striatus*

Triclosan treated fishes showed a significant increase in WBC in time-dependent and dose -dependent manner when compared to the control group and this may be due to the stress induced by triclosan. The increase in WBC observed in the present study could be due to a stimulation of the immune system in response to tissue damage caused by Triclosan. [10] suggested that excitement of immune system is in response with the increase in WBC. The physiological stress resulting from triclosan is clearly reflected by the blood patterns of the experimental fish. An increase in the number of leucocytes is mainly due to the squeezing of leucocytes in peripheral blood and also due to the increase in the number of thrombocytes.WBC are involved in the regulation of immunological activities in the body [23].An increase in the number of WBC shows an increased rate of stress [9].Similar results were observed by [24,3,16,23] .Sublethal assay performed on the toxic effects of triclosan on *Channa striatus* showed that triclosan has significant effects on the haematological parameters observed.

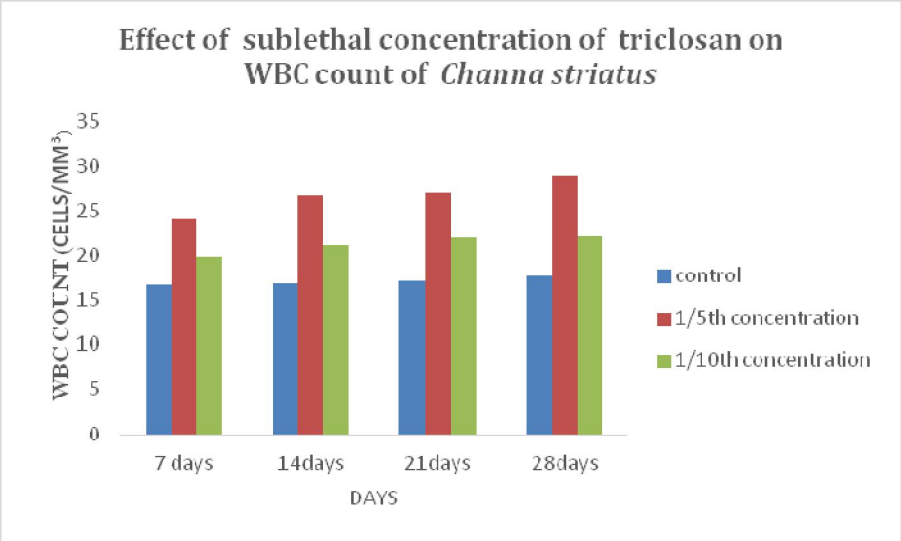

Figure 2: Showing the effect of sublethal concentration of triclosan on WBC count of *Channa striatus*

The decreased haemoglobin concentration observed in this study shows less oxygen release to the tissues. erythrocytes act as a carrier of haemoglobin. during respiration haemoglobin reacts with oxygen in the blood to form oxyhaemoglobin [14,7] .The decreased haemoglobin concentration showed the reduced supply of adequate oxygen to the tissues and resulted in a decline of physiological activities [18].

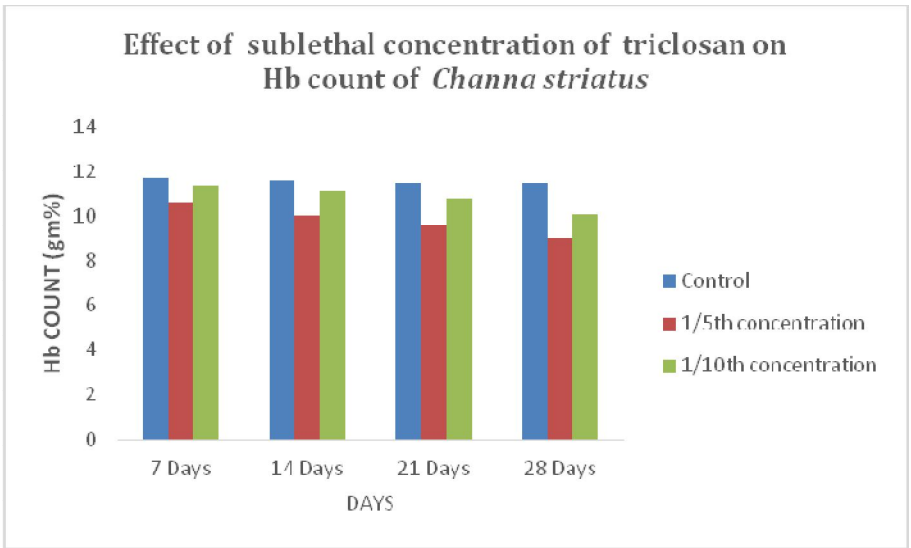

Figure 3: Showing the effect of sublethal concentration of triclosan on Hb count of *Channa striatus*

Decrease in RBC count, Hb content and PCV were showing fish suffer anemia [15].Decreased Hct and Hb values coupled with decreased erythrocytes count were observed in *Clarias batrachus* treated to mercuric chloride [17].Decrease rate of PCV is due to the destruction of RBC [28].

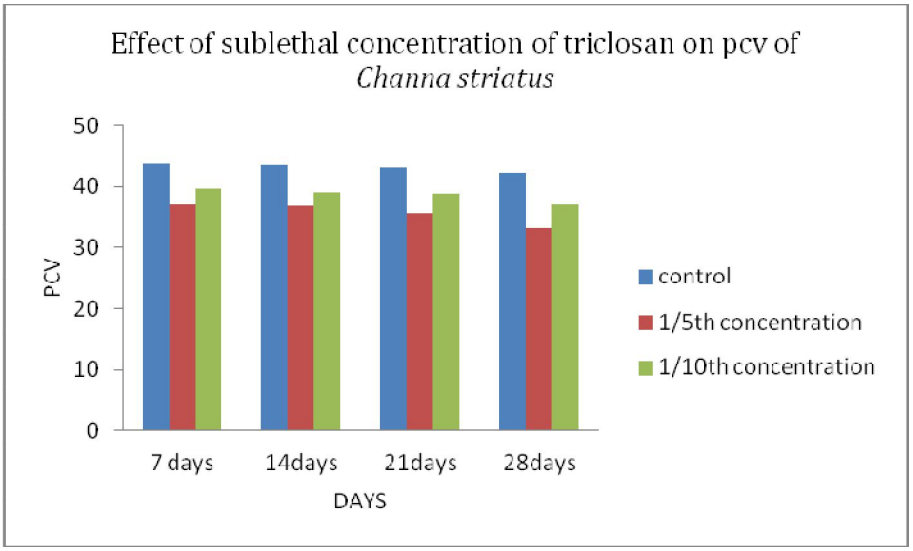

Figure 4: Showing the effect of sublethal concentration of triclosan on PCV count of *Channa striatus*

The increase in the number of MCV may also due to an increase of immature RBC [5]. The increase in MCV values indicates that the anemia was of a macrocytic type [28].A Similar result was observed on acute effect of diazinon on carp [27]. The increase in number of MCV may also due to an increase of immature RBC [5].

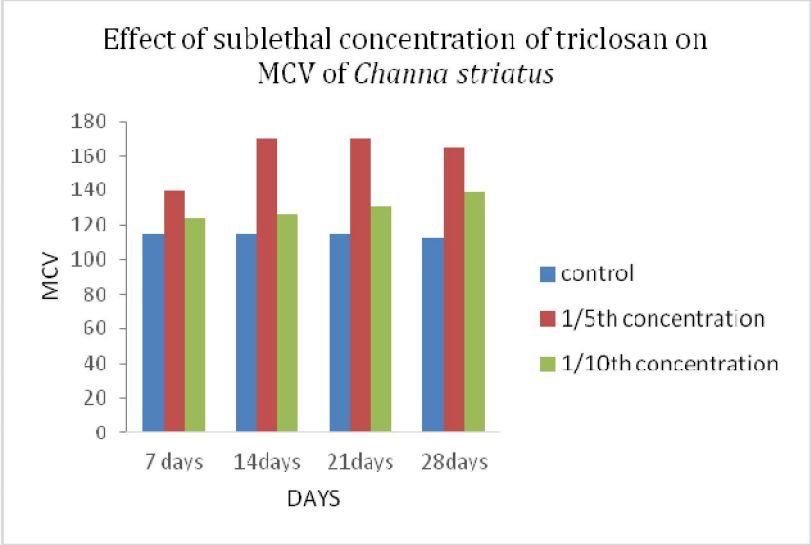

Figure 5: Showing the effect of sublethal concentration of triclosan on MCV count of *Channa striatus*

It was observed that the MCH and MCV have increased significantly. A similar observation was made for *Cyprinus carpio* exposed to cadmium exposure [15.] The significant change in the MCH may be due to the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood [12].

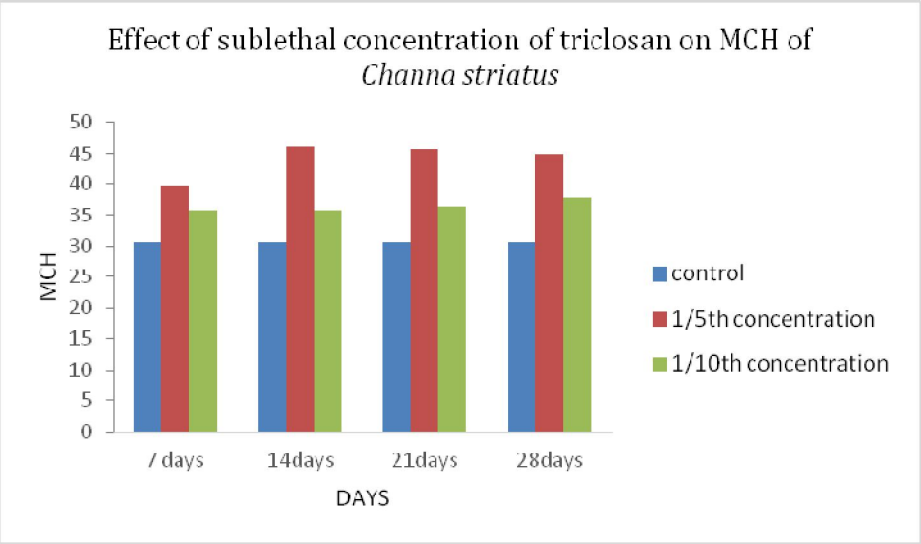

Figure 6: Showing the effect of sublethal concentration of triclosan on MCH count of *Channa striatus*

CONCLUSION

The present investigation showed that, the RBC, haemoglobin and haemotocrit values are significantly decreased in triclosan treated fish when compared to control. The decreased rate of RBC might be due to hemolysis. The decreased value of Hb content shows anaemia.The anaemia could be due to the destruction of RBC. The variation in the MCH in the present investigation, clearly indicates that the concentration of haemoglobin in the red blood cells were lower in the treated fish than in the control fish, thereby, showing an anaemic condition.. The haematological parameters levels were decreased such as RBC, PCV, and MCH where as increased level of WBC, MCV, for sublethal concentration of triclosan exposure to *Channa striatus* were observed for the period of 7,14,21 and 28 days respectively . Fluctuations in haematological parameters were noted due to exposure of endocrine disruptor chemical triclosan on *Channa striatus*. This imbalance in haematological parameters may further lead to abnormality and malfunction in different systems of fish. In conclusion results of the present investigation show that triclosan caused immunological and haematological impairments in *Channa striatus*, which suggests that the endocrine disruptor chemical triclosan may weaken the immune system and may result in severe physiological problems, ultimately leading to the lethality of fish. It could be concluded that triclosan induced deleterious

effectson the haematological parameters in fish.

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