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Studies on the changes in cholesterol and glucose levels in *Catla catla*, exposed to Triphenyl phosphate (TPP)

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ABSTRACT

Fresh water Major carp, *Catla catla*, were exposed to the plasticizer Triphenyl phosphate (TPP) for 72hrs and the LC_{50} value was determined to be 25 mg/L. The fishes were exposed to three different sub – lethal concentrations of TPP (0.25 mg/L, 0.5 mg/L and 1 mg/L) for a period of 15 days. Cholesterol and glucose content was observed from different tissues (gill, muscle and intestine) after exposure period. The results showed that there was a significant decline in the cholesterol and glucose level of gill, muscle and intestine with respect to increase in the concentration of TPP. The decrease in cholesterol and glucose levels in gill, muscle and intestine might be an indication of TPP toxicity stress which suppresses the activity of number of enzymes responsible for lipid and glucose transformation, thus affecting the metabolic cycle of *Catla catla.*

Key words: *TPP, Toxicity, Cholesterol, Glucose, Catla catla.*

Introduction

TPP is an unsubstituted aryl phosphate ester historically used as a high-production volume flame retardant within polyvinyl chloride, polymers, printed circuit boards, photographic films, and hydraulic fluids (Brooke *et al.,* 2009). Since 2005, the use of TPP as a flame retardant within polyurethane foam likely increased within the United States following the phase-out of penta brominated diphenyl ether and subsequent replacement with alternative TPP containing flame retardant formulations (USEPA, 2005a). TPP is used as a plasticizer and a fire retardant in a wide variety of settings and products. It has been used as a flame retardant for a variety of materials, including electronic equipment, PVC, hydraulic fluids, glues, in nail polishes, and casting resins. TPP is also used as a plasticizer in lacquers, varnishes, and hydraulic fluids. TPP is an additive flame retardant that can migrate from end-use products into indoor and outdoor environmental media (Van der Veen and de Boer, 2012). As such, environmental exposure to TPP may pose a health risk to humans and ecological species, particularly aquatic organisms like fishes, planktons, etc.

Hence, the present investigation has been made to observe possible changes in the cholesterol and glucose content in gill, muscle and intestine of *Catla catla*, exposed to Sub lethal concentrations (0.25 mg/L, 0.5 mg/L and 1 mg/L) of TPP for a period of 15 days.

Materials and Methods

Animal selection and acclimatization

Catla catla, is the experimental animal model used in

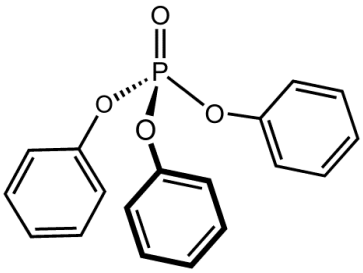
this study which belongs to the family of Cyprinidae of order cypriniformes. It is a fresh water fish, native to rivers and lakes. It is one of the most important aqua - cultured species. It is also grown in polyculture ponds with rohu and mrigal carp. The experimental fish fingerlings of size 2 – 4 g, were procured from Tamil Nadu Fish Seed Farm, Poondy, Thiruvallur. Shifted in plastic bags containing fresh water filled with oxygen to the research laboratory. The fingerlings of fish were immersed in 0.1% $KMnO_4$, for 2 – 3 minutes in order to sterilize before acclimatization.

The process of acclimatization was carried out for a period of one week in glass aquaria of size 30cm x 60cm x 45 cm filled with water before the start of the experiment in the laboratory. Ten fish fingerlings were kept in each glass aquaria. The fish fingerlings were fed with commercial artificial feed at 2 - 3 % of wet body weight during the acclimatization period. Proper aeration was supplied continuously to all the glass aquaria with electric air pump. The water in the glass aquaria was replaced with fresh water for every two days.

Physio – Chemical Properties of TPP

The present study involves TPP, technical grade as chemical, whose toxic effect in tissues of vital organs of Cattla carp has been evaluated.

Structure of TPP



TPP is the chemical compound with the formula $OP(OC_6H_5)_3$. This colourless solid is the ester (tri ester) of phosphoric acid and phenol.

Determination of LC_{50}

Determination of LC_{50} was done according to Behreusand Karbeur (1953). In the present study 72hrs LC_{50} bio – assay method was followed in which the ten fishes per group was placed at various concentrations of TPP in each group. The mortality rate was observed and recorded at time intervals of 24 hrs, 48 hrs, 72 hrs. The concentration of TPP which gave 50% mortality at 72hrs was taken as the LC_{50} value. The percentage mortality was converted into probit values and plotted against the log dose values (Fig. 1).

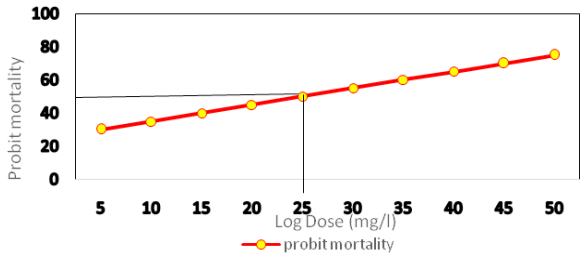


Fig. 1. Showing the LC50 value of Triphenyl Phosphate (TPP) on *Catla catla*

Experimental Design For Sub – Lethal Study

The sub – lethal study was carried out by placing five groups which contain ten fishes per group and the group comprising of group – I – Control, group – II – Acetone treated, group – III – Sublethal treated with 0.25 mg/L of TPP, group – IV – sublethal treated with 0.5 mg/L of TPP and group – V – treated with 1 mg/L of TPP in 20 litres of water. The water in the glass aquaria was changed for every two days and freshly prepared toxicant was added to maintain the concentration of TPP at constant level. The experiment was carried out for a duration of 15 days.

Biochemical analysis

The fishes were sacrificed immediately after exposure period and the gill, muscle and intestine were processed for the biochemical estimations. Cholesterol content was estimated by (Zak B, 1977) and glucose content was estimated by glucose oxidase method (Bergmeyer HU, 1974) respectively.

Results

Cholesterol

The cholesterol content in the tissue extracts of gill, muscle and intestine of the group I control were found to be 424.34 mg/dL, 485.65 mg/dL and 487.65 mg/dL respectively. In group II treated with acetone the cholesterol concentration of tissue extracts of gill, muscle and intestine was found to be 373.93 mg/dL, 453.90 mg/dL and 457.39 mg/dL respectively. In group III treated with TPP (0.25 mg/L) the cholesterol content in the tissue extracts of

gill, muscle and intestine was noted as 286.33 mg/dL, 253.52 mg/dL and 330.40 mg/dL respectively. In group IV treated with TPP (0.5 mg/L) the cholesterol content in the tissue extracts of gill, muscle and intestine was observed as 153.58 mg/dL, 206.93 mg/dL and 284.11 mg/dL respectively. In group V treated with TPP (1 mg/L) the cholesterol content in the tissue extracts of gill, muscle and intestine was found to be 128.6 mg/dL, 143.73 mg/dL and 265.83 mg/dL respectively. (Fig. 2). The mean and SD values of group I, II, III, IV and V were found to be significantly different from control at 5% probability level (Table 1).

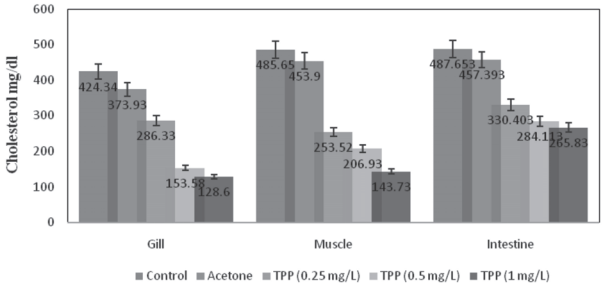


Fig. 2. Effect of sub Lethal Dosages of Triphenyl phosphate (TPP) (0.25, 0.5, 1 mg/L b.wt.) on Cholesterol level of *Catla catla*

Glucose

The glucose content in the tissue extracts of gill, muscle and intestine of the group I control were found to be 161 mg/dL, 143.55 mg/dL and 166.33 mg/dL respectively. In group II treated with acetone the glucose concentration of tissue extracts of gill, muscle and intestine was found to be 128.33 mg/dL, 129 mg/dL and 151.66 mg/dL respectively. In group III treated with TPP (0.25 mg/L) the glucose content in the tissue extracts of gill, muscle and intestine was noted as 92 mg/dL, 115.33 mg/dL and 98.66 mg/dL respectively. In group IV treated with TPP (0.5 mg/L) the glucose content in the tis-

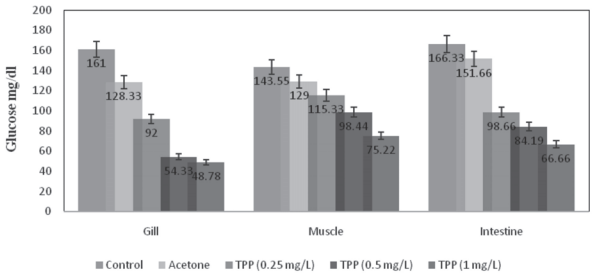


Fig. 3. Effect of sub Lethal Dosages of Triphenyl phosphate (TPP) (0.25, 0.5, 1 mg/L b.wt.) on glucose level of *Catla catla*

Table 1. Effect of sub lethal dosages of Triphenyl phosphate (TPP) (0.25, 0.5, 1 mg/Ltb.wt.) on Cholesterol (Gill, Muscle and Intestine) of *Cattlacattla*

Treatment	Cholesterol (mg/dl)		
	Gill	Muscle	Intestine
Group 1- Control	424.34 ± 9.97	485.65 ± 9.64	487.65 ± 6.55
Group 2- Treated Acetone	373.93 ± 5.51	453.90 ± 8.50	457.39 ± 6.94
Group 3- Treated Triphenyl phosphate(0.25 mg/ltb.wt)	286.33 ± 7.048	253.52 ± 8.85	330.40 ± 7.92
Group 4- Treated Triphenyl phosphate (0.5 mg/ltb.wt)	153.58 ± 5.73	206.93 ± 8.68	284.11 ± 8.155
Group 5- Treated Triphenyl phosphate (1 mg/ltb.wt)	128.6 ± 9.57	143.73 ± 9.37	265.83 ± 9.32

Values are expressed as mean ± Standard Deviation.
Significantly different from Control at 5% probability level.

Table 2. Effect of sub lethal dosages of Triphenyl phosphate (TPP) (0.25, 0.5, 1 mg/Ltb.wt.) on Glucose (Gill, Muscle and Intestine) of *Cattlacattla*

Treatment	Glucose (mg/dL)		
	Gill	Muscle	Intestine
Group 1- Control	161 ± 9.54	143.55 ± 9.58	166.33 ± 8.18
Group 2- Treated Acetone	128.33 ± 5.67	129 ± 5.86	151.66 ± 9.68
Group 3- Treated Triphenyl phosphate (0.25 mg/ltb.wt)	92 ± 8.17	115.33 ± 7.18	98.66 ± 9.66
Group 4- Treated Triphenyl phosphate (0.5 mg/ltb.wt)	54.33 ± 7.34	98.44 ± 6.36	84.19 ± 7.04
Group 5- Treated Triphenyl phosphate (1 mg/ltb.wt)	48.78 ± 6.03	75.22 ± 8.002	66.66 ± 8.17

Values are expressed as mean ± Standard Deviation.
Significantly different from Control at 5% probability level.

sue extracts of gill, muscle and intestine was observed as 54.33 mg/dL, 98.44 mg/dL and 84.19 mg/dL respectively. In group V treated with TPP (1 mg/L) the glucose content in the tissue extracts of gill, muscle and intestine was found to be 48.78 mg/dl, 75.22 mg/dL and 66.66 mg/dL respectively. (Graph 3). The mean and S.D values of group I, II, III, IV and V were found to be significantly different from control at 5% probability level (Table 2).

Discussion

The sub – lethal exposure results showed that the cholesterol and glucose content was decreased in gill, muscle and intestine with increase in concentration (0.25 mg/L, 0.5 mg/L and 1 mg/L) of TPP. The decrease in concentration of cholesterol may be due to TPP toxicity stress which supresses the activity of number of enzymes responsible for lipid transformation ultimately causing disturbance in lipid metabolism, which eventually leads to decreased value of cholesterol. Similar results were observed by Choudhary and Gaur (2001); Shindhe *et al.,* (2002); Vaseem *et al.*, 2013; Latif *et al.*, 2014. Tissue glucose has been shown to be a sensitive biochemical indicator of environmental stress for any chemical pollutant including pesticides (Silbergeld, 1974; Wedemeyer and Yasutake, 1977; David *et al.*, 2005; Zutshi *et al.,* 2010). The glucose level represents a dynamic balance between the rate at which the sugar is entering the blood from the liver and the rate at which it is being removed by the body tissue from the blood (Saskin, 1941). The decrease in concentration of glucose may be due to TPP toxicity stress, which leads to hypoglycemic conditions in experimental fishes.

Conclusion

The use of biochemical responses in TPP, an organophosphorus plasticizer biomonitoring in fishes is very effective as a biomarker of pollution incidents, both in the field and in the laboratory. The results from the present study show that the decreased level of both cholesterol and glucose in gill, muscle and intestine tissues can serve as an indicator saying that in future this TPP can cause an alarming damage to the environment. It will also spoil the ecosystem and fauna which live in. The physiological disturbances can latter lead to fatal death in environmental monitoring. However, there is a need for further research on joint action toxicity of TPP and continuous bio - monitoring of organophosphate plasticizer pollution in aquatic environment.

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