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Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fresh water Major carp *Catla catla* exposed to Triphenyl phosphate

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ABSTRACT

Plasticizers are the xenobiotics which have attracted the attention in recent days due to their extensive use in commercial applications and growing concern of potential threat they pose to human health and ecosystem. Triphenyl phosphate (TPP), is an organophosphate flame retardant widely used as a plasticizer in hydraulic fluids, lacquers and varnishes, which is highly toxic to non – target organisms. Fish has been used as indicator species for aquatic pollution. *Catla catla* an Indian major carp is an edible fish and is highly sensitive to slight stress. Recently, fish biomarkers are widely used to determine the impact caused to aquatic ecosystem by release of these chemicals. *Catla catla* were exposed to sublethal concentrations (0.25mg/l , 0.5 mg/l and 1 mg/l) of TPP to determine the alterations in the biomolecules (protein, carbohydrate and lipid profiles) in tissues (gill, muscle and intestine). Significant alterations in the protein, carbohydrate and lipid profiles in tissues were observed which are indications of severe cellular damage in tissues of respective organs of TPP treated fish. The results of the present investigation suggest that gill, muscle and intestine exposed to Sub lethal concentrations (0.25mg/l , 0.5 mg/l and 1 mg/l) of TPP were adversely affected with increasing concentration of toxicant which are highlighted by significant alterations in protein, carbohydrate and lipid profiles. Thus, the study can be effectively used as a potential biomarker for monitoring of organophosphorus plasticizer in aquatic environment. Further, TPP should be used with caution to protect natural waters and aquatic organisms.

Key words : *TPP, Catla catla, Plasticizer, Toxic effect.*

Introduction

Anthropogenic activities concerning with xenobiotics such as plasticizers, pharmaceuticals, personal care products, pesticide residues and gasoline additives can result in the release of toxic organic compounds with carcinogenic, teratogenic and endocrine disrupting properties into the environment. Plasticizers are xenobiotics that are of particular concern because of the extent of their use in commercial applications and growing recognition of the potential threats they pose to human health and ecosystems (Gomez – Hens and Aguilar - Caballos, 2003; Rahman and Brazel, 2004). Plasticizers are used to impart properties such as flexibility and workability to plastics for effective processing and tailoring of plastic formulations (Sears *et al.,* 1982). Their importance as industrial chemicals is demonstrated by their rate of use, which is estimated to be millions of tonnes annually. Unfortunately, plasti-

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cizers are not chemically bound to the polymers and can migrate from plastic products during normal use and following their disposal (Fromme *et al.*, 2002). Thus, many studies have shown their wide distribution in the environment and their occurrence in surface waters that receive treated effluents (Fromme *et al.*, 2002; Fauser *et al.*, 2003; Gavala *et al.*, 2003; Martineen *et al.*, 2003). One of the most common organophosphate compound is, TPP which is a phosphate ester, having much utility both as a flame retardant in polyurethane foam and as a plasticizer in hydraulic fluids, lacquers and varnishes (CPSC, 2005; Van der Veen and de Boer, 2012). Due to the fact that TPP is not bonded to manufactured products, it is relatively prone to be released into the environment through leaching, abrasion and volatilization (Wei *et al.*, 2015).

Hence, in the present investigation, an attempt has been made to observe changes in biochemical parameters like protein, carbohydrate and lipid profiles of vital organs like gill, muscle and intestine of Indian major carp *Catla catla*, exposed to sub – lethal concentrations (0.25 mg/l, 0.5 mg/l, 1 mg/l) of TPP technical grade, for an exposure period of 15 days.

Materials and Methods

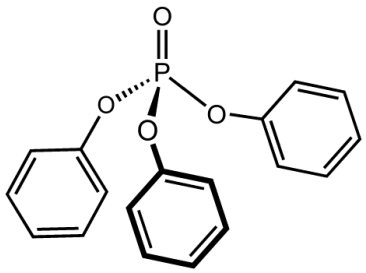
Animal selection and acclimatization

Catla catla, is the experimental animal model used in this study which belongs to the family of Cyprinidae of order cypriniformes. They are economically important South Asian 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. It is a surface and mid water feeder. The experimental fish fingerlings of size (2 – 4 g), were procured from Tamil Nadu Fish Seed Farm, Poondy, Thiruvallur and 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 60 cm 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.

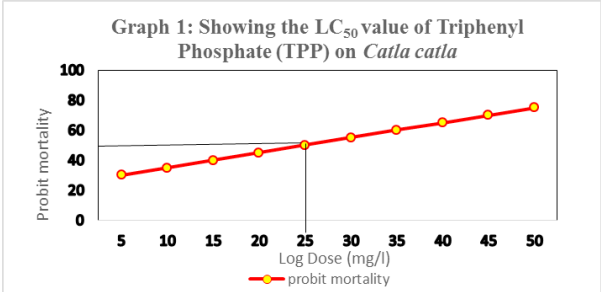


Structure of TPP

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. It 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.

Determination of LC_{50}

Determination of LC_{50} was done according to Behreus and 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 72 hrs was taken as the LC_{50} value. The percentage mortality was converted



into probit values and plotted against the log dose values (graph 1).

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 biochemical parameters such as quantitative analysis of proteins, carbohydrates and lipids were estimated by standard procedures in three tissues – gill, muscle and intestine of the healthy fish (Control) and of those from the fish exposed to sub-lethal concentrations (0.25mg/l, 0.5 mg/l and 1 mg/l) of TPP for a period of 15 days before sacrifice for the biochemical analysis. The proteins, carbohydrates and lipids were estimated by methods of Lowry *et al.,* (1951), Roe (1955) and Folch *et al.,* (1957) respectively.

Results

Protein

The protein content in the tissue extracts of gill, muscle and intestine of the group I control were found to be 0.106 mg/g, 0.151 mg/g and 0.120 mg/g, respectively. In group II treated with acetone the protein concentration of tissue extracts of gill, muscle and intestine was found to be 0.119 mg/g, 0.109 mg/g and 0.115 mg/g, respectively. In group III treated with TPP (0.25 mg/l) the protein content in the tissue extracts of gill, muscle and intestine was noted as 0.096 mg/g, 0.098 mg/g and 0.095 mg/g respectively. In group IV treated with TPP (0.5mg/l) the protein content in the tissue extracts of gill, muscle and intestine was observed as 0.093 mg/g, 0.090 mg/g and 0.098 mg/g, respectively. In group V treated with TPP (1 mg/l) the protein content in the tissue extracts of gill, muscle and intestine was found to be 0.084 mg/g, 0.089 mg/g and 0.086 mg/g, respectively. (Graph 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).

Carbohydrate

The carbohydrate content in the tissue extracts of gill, muscle and intestine of the group I control were found to be 0.07 mg/g, 0.05 mg/g and 0.06 mg/g respectively. In group II treated with acetone the carbohydrate concentration of tissue extracts of gill, muscle and intestine was found to be 0.06 mg/g, 0.04 mg/g and 0.05 mg/g respectively. In group III treated with TPP (0.25 mg/l) the carbohydrate content in the tissue extracts of gill, muscle and intestine was noted as 0.05 mg/g, 0.03 mg/g and 0.04 mg/g respectively. In group IV treated with TPP (0.5mg/l) the carbohydrate content in the tissue extracts of gill, muscle and intestine was observed as 0.04 mg/gm, 0.02 mg/g and 0.03 mg/g respectively. In group V treated with TPP (1 mg/l) the carbohydrate con-

Table 1. Effect of sub lethal dosages of Triphenyl Phosphate (TPP) (0.25, 0.5, 1 mg/g B.wt.) on protein (Gill, Muscle and Intestine) of *Cattla cattla* (15 days)

Treatment	Protein (mg/g of wet tissue)		
	Gill	Muscle	Intestine
Group I - Control	0.119 ± 0.012	0.151 ± 0.010	0.12 ± 0.008
Group II - Treated Acetone	0.106 ± 0.011	0.109 ± 0.005	0.115 ± 0.007
Group III - Treated Triphenyl phosphate (0.25 mg/gm b.wt)	0.096 ± 0.003	0.098 ± 0.003	0.095 ± 0..006
Group IV - Treated Triphenyl phosphate (0.5 mg/gm b.wt)	0.093 ± 0.005	0.09 ± 0.008	0.094 ± 0.01
Group V - Treated Triphenyl phosphate (1 mg/gm b.wt)	0.084 ± 0.007	0.089 ± 0.009	0.086 ± 0.01

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

tent in the tissue extracts of gill, muscle and intestine was found to be 0.03 mg/g, 0.01 mg/g and 0.02 mg/g, 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).

Lipid

The lipid content in the tissue extracts of gill, muscle and intestine of the group I control were found to be 0.37 mg/g, 0.323 mg/g and 0.365 mg/g respectively. In group II treated with acetone the lipid concentration of tissue extracts of gill, muscle and intestine was found to be 0.212 mg/g, 0.176 mg/g and 0.237 mg/g, respectively. In group III treated with TPP (0.25 mg/l) the lipid content in the tissue extracts of gill, muscle and intestine was noted as 0.183 mg/g, 0.143 mg/g and 0.172 mg/g respectively. In

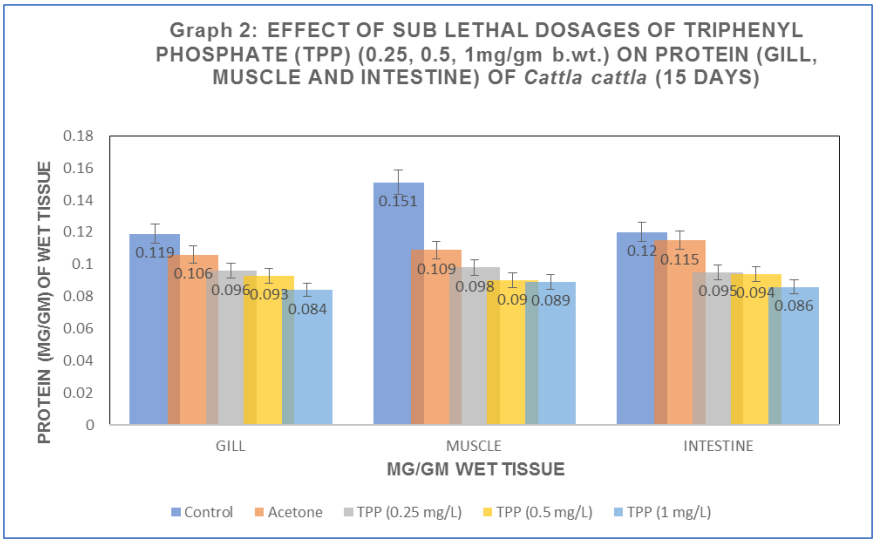


Table 2. Effect of Sub Lethal Dosages of Triphenyl Phosphate (Tpp) (0.25, 0.5, 1mg/g B.wt.) on Carbohydrate (Gill, Muscle and Intestine) of *Cattla cattla* (15 days)

Treatment	Carbohydrate (mg/g of wet tissue)		
	Gill	Muscle	Intestine
Group 1- Control	0.07 ± 0.008	0.05 ± 0.01	0.06 ± 0.01
Group 2- Treated Acetone	0.06 ± 0.003	0.04 ± 0.009	0.05 ± 0.01
Group 3- Treated Triphenyl phosphate (0.25 mg/g b.wt)	0.05 ± 0.006	0.03 ± 0.005	0.04 ± 0..005
Group 4- Treated Triphenyl phosphate (0.5 mg/g b.wt)	0.04 ± 0.009	0.02 ± 0.004	0.03 ± 0.005
Group 5- Treated Triphenyl phosphate (1 mg/g b.wt)	0.03 ± 0.003	0.01 ± 0.005	0.02 ± 0.005

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

Table 3. Effect of sub lethal dosages of triphenyl phosphate (TPP) (0.25, 0.5, 1mg/g b.wt.) on lipid (gill, muscle and intestine) of *cattla cattla* (15 days)

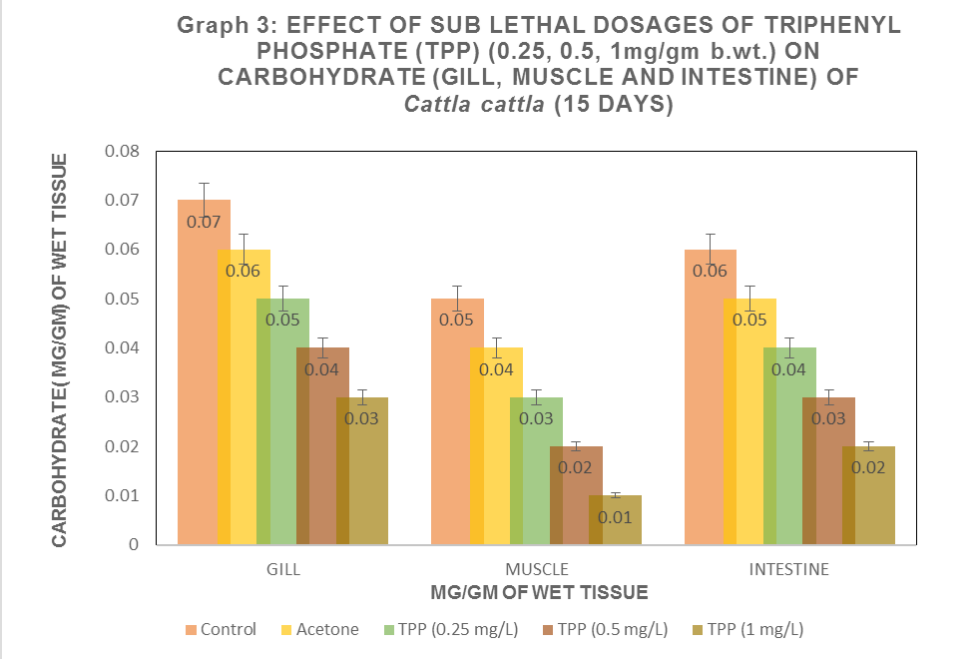
Treatment	Lipid (mg/gm of wet tissue)		
	Gill	Muscle	Intestine
Group 1- Control	0.37 ± 0.007	0.323 ± 0.05	0.365 ± 0.03
Group 2- Treated Acetone	0.212 ± 0.02	0.176 ± 0.06	0.237 ± 0.008
Group 3- Treated Triphenyl phosphate (0.25 mg/g b.wt)	0.183 ± 0.009	0.143 ± 0.007	0.172 ± 0.008
Group 4- Treated Triphenyl phosphate (0.5 mg/g b.wt)	0.143 ± 0.007	0.101 ± 0.004	0.155 ± 0.006
Group 5- Treated Triphenyl phosphate (1 mg/g b.wt)	0.091 ± 0.01	0.076 ± 0.005	0.078 ± 0.04

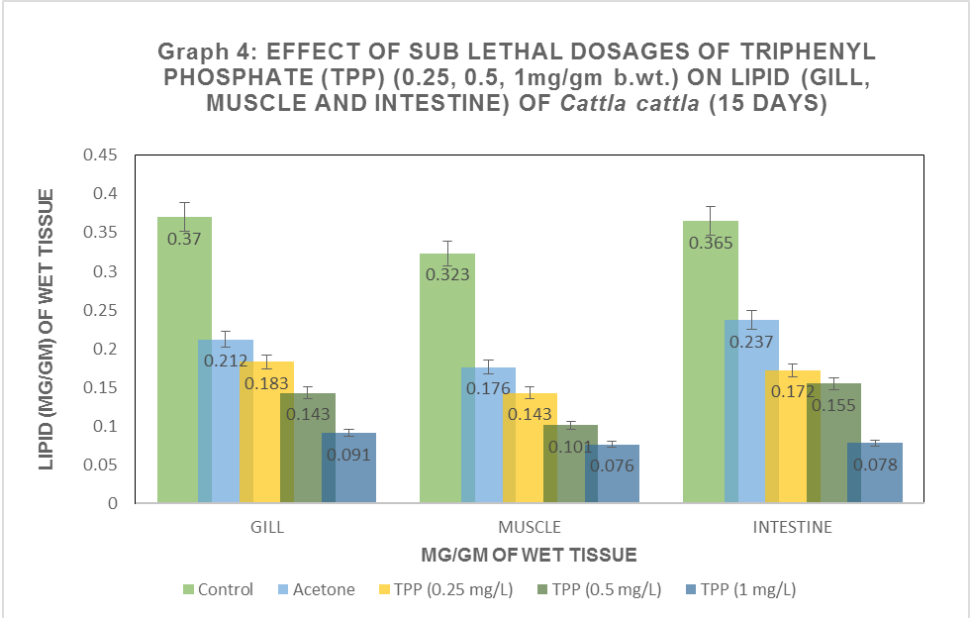
Values are expressed as mean ± standard deviation.
Significantly different from Control at 5% probability level.

group IV treated with TPP (0.5mg/l) the lipid content in the tissue extracts of gill, muscle and intestine was observed as 0.143 mg/g, 0.101 mg/g and 0.155 mg/g respectively. In group V treated with TPP (1 mg/l) the lipid content in the tissue extracts of gill, muscle and intestine was found to be 0.091 mg/g, 0.076 mg/g and 0.078 mg/g respectively (Graph 4). The mean and SD values of group I, II, III, IV and V were found to be significantly different at 5% level (Table 3).

Discussion

During the present investigation TPP caused significant decline in the protein, carbohydrate and lipid levels in tissues of gill, muscle and intestine of *Catla catla* at different sub lethal dosages (0.25 mg/l, 0.5 mg/l, 1 mg/l). The toxicant TPP act as a kind of strees and the organism respond by developing necessary potential occurring in body to give first indi-





cation of stress. During stress an organism needs sufficient energy which is supplied from reserve food materials such as proteins, carbohydrates , lipids, etc., Decrease in protein, carbohydrate and lipid content was observed throughout the exposure period. The sub lethal exposure results shows that there is a progressive decline in the protein, carbohydrate and lipid profiles of *Catla catla*, with respect to increase in concentration of TPP. The toxicity of TPP showed a direct correlation with concentration and time of exposure. Similar observation has been observed by Singh and Bhati (1996), Agarwal, (1992); Vutukuru, (2005), Sastry and Sunita, (1983) and Gill and Pant, (1981).

Conclusion

The present investigation provides a tool for assessing the toxic nature of TPP, which produced pronounced changes in biochemical parameters (proteins, carbohydrates and lipids) in *Catla catla*, with respect to increase in concentration and exposure time period. This change may probably affect the enzyme mediated bio-defense mechanism of these fishes. Hence, it may be concluded that the use of TPP must be checked especially near the water bodies. Further research should focus on the effect of TPP toxicity on *Catla catla,* at cellular and sub cellular levels.

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