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## RESEARCH ARTICLE

# IMPACT OF TBTCL ON LIPID CONTENT IN FRESHWATER PRAWN, *MACROBRACHIUM KISTNENSIS*

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### ABSTRACT

The effect of lethal concentrations as 0.33 ppm, 0.26 ppm, 0.17 ppm and 0.09 ppm of tributyltin chloride on lipid content in ovary, hepatopancreas, gill and muscle of a freshwater prawn, *Macrobrachium kistnensis* for 24, 48, 72 and 96 hours respectively has been investigated. The result showed that TBTCL induces significant alteration in lipid metabolic profiles in ovary, hepatopancreas, gill and muscle after exposure to lethal concentration. The depletion in lipid content during exposure of different concentration of TBTCL might be due to its utilization in energy production by oxidation of lipids under the influence of lipase enzyme to derive fatty acids precursor for TCA cycle.

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## INTRODUCTION

The steady development of industrialization over the past six decades in the countries and developing regions of the world depend upon the expansions of more and more chemical industries and technology. It is true that such development is really necessary for the growing needs of an increasing human population and for improving our standard of living. This rapid industrialization and green revolution introduced a large variety of chemicals into the environment. These chemicals create serious ecological problems particularly aquatic pollution.

Organotin compound were first developed as moth-proofing agents in 1920s, and later used as bactericides and fungicides. The wide spread use of organotin compounds as stabilizer in manufacturing Polyvinylchloride, as catalyst in the production of synthetic organic polymers and as biocidal agent in wood preservation, crop protection and mainly antifouling system has provided several points of entry for these compounds into aquatic environment. The release of organotin compounds into an aquatic environment has been decrease recently, but inputs still occur and previously contaminated sites continue to act as source (Stab *et al.*, 1995).

The biochemical changes occurring in the body gives important indication of stress. During stress an organism needs sufficient energy which is supplied from reserve materials i.e. protein, glycogen, lipid etc. If stress is mild then the stored glycogen is used as a source of energy, but when stress is strong, then the stored lipid and protein may also be mobilized.

The mode of action of toxicants and causes for death of poisoned aquatic animal is better understood from biochemical investigations beside mortality studies. Lipids are the predominant organic reserves of animals and serves as an alternate source of energy, particularly during stress conditions (Gilbert and O' Connor, 1970). The function and importance of lipid in the life is well known, they not only play a metabolic role and provides precursor for the endocrine processes to produce steroidal hormones but they are also important in maintaining the structural and physiological integrity of cellular and sub-cellular membranes (Shigmatsu and Takeshita 1959). Their role is to transfer the substrate via the circulatory system in both vertebrate and invertebrate is a vital one. The variation in the content and composition of depot-lipid is a function of both external and internal central system.

Some toxicologist focused certain attention on the impact of pollutants on the lipid reserves of aquatic animal. Villalan *et al.*, (1990) observed the reduction in lipid content in muscle due to chromium stress in *Macrobrachium idella*. Lomte and Muley (1993) reported the decrease in lipid level in the freshwater snail, *Thaيرا tuberculata* and *Parresia corrugata* exposed to copper toxicity. Sarvana *et al.*, (1997) reported that the reduction in lipid content in freshwater prawn, *Macrobrachium malcomsonii* when the prawn exposed to endosulfan. They suggested that the accelerated hydrolysis of lipid might be to cope up with the increased energy demand occurring due to pesticide toxicity.

Organotin compounds constitute a core group of aquatic pollutants and their excessive addition to aquatic ecosystem has evoked major environmental and health concern worldwide. However as the concentration of trace

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element in aquatic sources is dependent on several natural and anthropogenic factors, it has proven complex to measure in absolute terms. Organotin affects on biochemical and physiological system (Maguire, 2000). Industrial effluents contributing to aquatic pollution contains a vast array of toxic substances which include organotin compounds and heavy metals such as copper, which is used in antifouling paints in the form of copper sulphate and copper oxide.

Adequate literature is available on the toxicity of heavy metals, organopesticides, insecticides, hydrocarbon etc. on the toxicity in different aquatic animals related to effect on biochemical constituent levels in different tissues of animals. There was a paucity of information on the toxicity of organotin compounds on biochemical constituents in crustacean particularly prawn. Present study deals with the toxicity of tributyltin chloride which affects the levels of lipid in different tissues of prawn, *Macrobrachium kistnensis*.

## MATERIAL AND METHODS

The fresh water prawns *Macrobrachium kistnensis* were collected from Kham river near Aurangabad, Maharashtra. The prawns were maintained in plastic trough containing aerated tap water. They were acclimatized for a week in laboratory condition. The water was changed every 24 h. Prawns were fed with green algae at alternative days. 1ppm stock solution of TBTCI was prepared in acetone (Laughlin *et al.*, 1983). Matured healthy female prawns were selected for experiment. For each experiment 10 animals of approximately similar size ( $2.5 \pm 1$  cm in length) were exposed to 0.33, 0.26, 0.17 and 0.09 ppm LC<sub>50</sub> values for 24 h, 48 h, 72 h and 96 h respectively of tributyltin chloride. Along with experimental group a corresponding control group of 10 female prawns were also set up for the experimental period in non-contaminated freshwater medium to compare the results to investigate the effect of tributyltin chloride at different concentrations on the biochemical constituents of ovary, hepatopancreas, muscle and gills. After experimental period the ovary, hepatopancreas, muscle and gills were dissected out and wet tissues were weighed and further processes for the analysis of lipid in different tissues of prawn, *Macrobrachium kistnensis*.

### Estimation of Lipid

100 mg tissue was homogenized by adding 10 ml of chloroform Methanol (2:1) mixture. The homogenate was filtered and one ml of this (filtrate) was kept at room temperature in laboratory at 37°C for 2 days to dry. 1ml of concentrated sulphuric acid was added to the dry mixture and kept in boiling water bath for exactly 10 minutes, following by cooling rapidly under tap water. 0.2 ml of this solution was taken; 5 ml Vanillin reagent was added and kept aside 30 minutes at room temperature (37°C) before calorimetric estimator. The lipid was estimated by the method described by Barnes and Bardstock (1973). Lipid content expressed as mg lipid/100mg wet tissue. The data so obtained were statistically evaluated using students't test.

## RESULTS

Impact of different concentration of organotin tributyltin chloride compounds were studied to determine the biochemical constituent such as lipid were obtained in

various tissues such as ovary, hepatopancreas, gill and muscle of freshwater prawn, *Macrobrachium kistnensis*. The prawns tissues like ovary, hepatopancreas, gill and muscle were analyzed to observe the effect after 24, 48, 72 and 96 h for the lethal concentration (LC<sub>50</sub>) 0.33, 0.26, 0.17 and 0.09 ppm respectively. The data were supported to various statistical analysis and the variance, standard deviation and standard error of the mean were calculated. Students't test was used to find out significance. The level of significance was used in the present study ( $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ ).

In TBTCI exposed prawn *Macrobrachium kistnensis* for 24 h to 96 h the total lipid content decreased in all tested tissue as against in control. Among the body tissues the significant ( $p < 0.001$ ) decrease in the ovary were noticed for concentration of 0.26 ppm, 0.17 ppm and 0.09 ppm at 48 h, 72 h and 96 h respectively. The observed lipid content recorded was 14.14 % mg, 7.07 % mg and 1.76 % mg respectively (Table 1 and 2). In the control prawns ovary, lipid content was recorded as 19.45 % mg. The % content of lipid in hepatopancreas of control prawns was recorded as 37.13 % mg. Significantly decreased of lipid in the hepatopancreas was noticed and % lipid content decrease 37.13 % mg to 30.06 % mg, 19.45 % mg, 15.91 % mg and 8.84 % mg at 24 h, 48 h, 72 h and 96 h respectively (Table 1 and 2). In the hepatopancreas of tested prawns, a significant ( $p < 0.001$ ) decrease in the lipid as 19.45 % mg, 15.91 % mg and 8.84 % mg were observed when exposed to 0.26 ppm, 0.17 ppm and 0.09 ppm concentration of TBTCI. Total lipid content found in gills of control prawns was 15.91 % mg. The lipid content was significantly ( $p < 0.01$ ) decrease when exposed to 0.33 ppm of TBTCI. The significant ( $p < 0.001$ ) decreased were found as 5.30 % mg, 3.53 % mg and 1.76 % mg after exposure of 0.26 ppm, 0.17 ppm and 0.09 ppm concentrations of TBTCI respectively (Table 1 and 2). In TBTCI, exposed muscle produced a significant ( $p < 0.05$ ) decrease as 19.44 and % mg at 0.17 and 0.09 ppm concentration of TBTCI respectively (Table 2). Whereas the % lipid found in muscle of control prawns was 31.68 % mg. The maximum decrease of lipid content was found in hepatopancreas as compared to ovary, gill and muscle of TBTCI exposed prawn, *Macrobrachium kistnensis*.

## DISCUSSION

Industrialization has led to the production of large amount of complex waste, which is indiscriminately discharged in freshwater ecosystems inducing biochemical changes in aquatic animals. In the past two decades organotin compounds have developed into important industrial commodities. Tin is unsurpassed by any other metal in the number of its organic applications which includes uses as PVC stabilizers, antiyellowing agent, industrial catalyst, industrial and agricultural biocide and as antifouling agents in paint industries. The chemistry and toxicity of organotin compounds have been reviewed extensively, (WHO, 1980, Snoeij *et al.*, 1987, Indira, 1989, Blunden and Evans, 1990)

Lipid is a major constituent like protein and carbohydrate which contribute much towards oocyte development in the ovary. Lipids are important in the cellular and sub cellular membranes. The biochemical assessment of the functional impairment of such vital

**Table 1. Changes in Lipid level in Different tissues of Freshwater prawn, *Macrobrachium kistnensis* exposed to different concentration of TBTCL**

Exposure period	Tissue	% of Lipid Control (%S.D.)	Experimental (% S.D.)	Significance
24 h	Ovary	19.45 ± 0.02	17.684±0.005	
	Hepatopancreas	37.13 ± 0.01	30.06± 0.01	**
	Gill	15.91 ± 0.005	10.61±0.005	**
	Muscle	31.68 ± 0.02	19.4 ±0.005	
48 h	Ovary	19.45 ± 0.02	14.14±0.005	***
	Hepatopancreas	37.13 ± 0.01	19.45±0.005	***
	Gi	15.91 ± 0.005	5.30 ± 0.02	***
	Muscle	31.68 ± 0.02	14.14.± 0.02	***

Values are expressed as Mean ± S. D.

Symbol indicates significance \* p < 0. 05 \*\* p < 0. 01 \*\*\* p < 0.001

**Table 2. Changes in Lipid level in Different tissues of Freshwater prawn, *Macrobrachium kistnensis* exposed to different concentration of TBTCL**

Exposure period	Tissue	% of Lipid Control (%S.D.)	Experimental (% S.D.)	Significance
72 h	Ovary	19.45 ± 0.02	07.07 ± 0.01	**
	Hepatopancreas	37.13 ± 0.01	15.91±0.005	***
	Gill	15.91±0.005	03.53 ± 0.01	***
	Muscle	31.68 ± 0.02	5.30 ± 0.01	***
96 h	Ovary	19.45 ± 0.02	1.76 ± 0.005	**
	Hepatopancreas	37.13 ± 0.01	8.84 ± 0.005	***
	Gill	15.91±0.005	1.76 ± 0.005	***
	Muscle	31.68 ± 0.02	3.53 ± 0.01	***

Values are expressed as Mean ± S. D.

Symbol indicates significance \* p < 0. 05 \*\* p < 0. 01 \*\*\* p < 0.001

organs is assumed as an important role as a biochemical tool for understanding environmental toxicity, as the toxicity induced such vital organic constituents like lipid alterations precede the onset of morphological and physiological manifestation of the toxicity (Livingstone *et al.*, 1988).

In the present study the freshwater prawn, *Macrobrachium kistnensis* was exposed to 0.33 ppm, 0.26 ppm, 0.17 ppm and 0.09 ppm LC<sub>50</sub> of 24 h, 48 h, 72 h and 96 h respectively of TBTCL as a model. In the present investigation, reduction in lipid content was observed in selected tissue of *Macrobrachium kistnensis* when exposed to all concentrations at 24 h, 48 h, 72 h and 96 h. The values obtained for ovary hepatopancreas and gill were significantly decreased for 0.33 ppm, 0.26 ppm, 0.17

ppm and 0.09 (Table 1 and 2). Among the vital organs hepatopancreas is chief metabolic organ in invertebrates like bivalves, crustaceans, etc. and is involved in the regulation of general metabolism by storage and release of reserves such as lipids under normal or stress conditions. The results of the present study indicated that hepatopancreas suffered greater damage following gill, ovary and muscle in *Macrobrachium kistnensis*. Similar results were reported that maximum total lipid alteration found in the hepatopancreas than the other tested tissue exposed to antifouling agents in acute and chronic exposures (Muley and Lomte 1983 and Patel and Eapen 1989). When animal is under stress, stored reserved material such as lipid in different tissues, get oxidized rapidly by lipase. The toxicant like copper oxide and

copper sulphate, TBTO, TBTCI and other antifouling agents affects multiple physiological processes as enzyme-and / or membrane specific reactions by oxidation of lipolytic enzyme was reported by several workers (Winston and Di- giulio 1991 and Elia *et al.*, 2003). In present investigation the elevated hydrolysis of lipid under such condition might be due to cope up with increased energy demand occurred due to toxicity stress conditions caused by TBTCI. These findings are substantiate with earlier findings. Bhilve *et al.*, (2000) stated that considerable decrease in total lipid in tested tissues might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress conditions after glycogen, lipid content may be used for energy production overcome the toxic stress. Similar results were observed in different aquatic animals by some researchers (Sarojini *et al.*, 1990, Lomte and Muley 1993, Geraldine *et al.*, 1999 Amanulla *et al.*, 2004 and Vijayavel *et al.*, 2006).

In conclusion, present study substantiate earlier findings that prawn, *Macrobrachium kistnensis* when exposed to lethal concentrations of TBTCI, lipid level decreased under stress condition. A perusal of data available for aquatic animal exposed to different toxic pollutants, biochemical constituents level decrease indicated that the toxic and adaptive mechanism under stress conditions. The depletion in lipid content during exposure of TBTCI might be due to its utilization in energy production by oxidation of lipids under the influence of lipase enzyme to derive fatty acids precursor for TCA cycle.

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