A preliminary studies to investigate the effect of Triphenyl phosphate in the alimentary tract of *Catla catla*

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ABSTRACT:

Triphenyl Phosphate (TPP) , an organophosphate flame retardant and plasticizer widely reported in the environment and biota samples. It has a structure resemblance with neurotoxic organophosphate pesticides. At present, studies relating to environment and health risk assessment of TPP are of prime importance due to the multiplex toxicity of this chemical compound. However, very few studies have paid attention to the impacts of TPP on aquatic biota. We investigated the histological alterations confronted by the alimentary tract (stomach and intestine) of *Catla catla*, when exposed to the sub lethal dosage (0.25mg/l , 0.5 mg/l and 1 mg/l) of TPP for a period of 15 days. As a conclusion, the findings of the present histological investigations demonstrates that the exposure of fresh water teleost fish, *Catla catla* to varying concentration of TPP caused vacuolation, edema, necrosis, congestion of cells in inner lining of stomach and rupture in the intestinal lining cells, haemorrhage, necrosis of intestinal villi cells, widening of intestinal villi. Hence, the study provides systematic analysis of TPP induced toxic histopathological alterations in alimentary tract (stomach and intestine) of *Catla catla*.

Keywords: TPP, *Catla catla*, Plasticizer, Toxic effect.

INTRODUCTION:

Fishery resources are an important source of both macro- and micro-nutrients for humans. Internationally fish accounts for about 17 percent of animal protein ingestion show that fish is the most important animal-source food in the diets of more than one billion people (Tacon and Metian, 2009). Due to feeding and living in the aquatic environments fish are particularly uncovered and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants (Yarsan and Yipel, 2013; Mahboob *et. al.*, 2014). Fish, in contrast with invertebrates, are more sensitive to many toxicants and are a difficult test subject for suggestion of ecosystem health (Adams and Ryon, 1994; Zaki *et. al.*, 2014). The many sources of water pollution cause difficult consequences to aquatic life. Fish and aquatic life those are at the top of the aquatic food chain are exposed to higher levels of toxins directly from the polluted water and by feeding on other fishes who are already exposed to high levels of toxins in water (Kivi, 2010; Jezierska and Witeska, 2001). TPP is one of the most important flame retardant and plasticizer found in various compartments in the environment due to the fact that they are prone to leaching, abrasion and volatilization from the manufactured products as they are not chemically

bonded with the products (Wei *et. al.*, 2015). Water pollution with TPP affects various physiological process in fishes and the effects of TPP are related to the uptake and accumulation by the fishes, resulting in TPP induced disturbances in the histoarchitecture and function of various tissues and organs.

Hence, in the present study attempt has been made to observe possible histopathological changes in alimentary tract, stomach and intestine of freshwater Major Carp, *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:

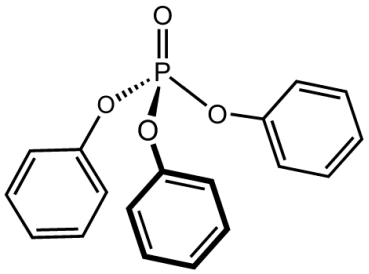
Catla catla, is the experimental animal model used in this study which belongs to the family of Cyprinidae of order cypriniformes. They are economically important South Asian fresh water fish, native to rivers and lakes. It is one of the most important aqua - cultured species. It is also grown in polyculture ponds with rohu and mrigal carp. It is a surface and mid water feeder. The experimental fish fingerlings of size (2 – 4 gm), 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 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:



Appearance: Colourless crystalline Solid

Chemical Name: Triphenoxyphosphine oxide

CAS Number: 115-86-6.

Molecular Formula: $C_{18}H_{15}O_4P$

Molecular Weight: 326.29 g/mol

Water Solubility: 1.9 mg/l at room temperature.

Solubility in Other Solvents: TPP is soluble in other organic solvents such as benzene, chloroform, dimethyl ether, acetone and is moderately soluble in ethanol.

Melting point: 49°C

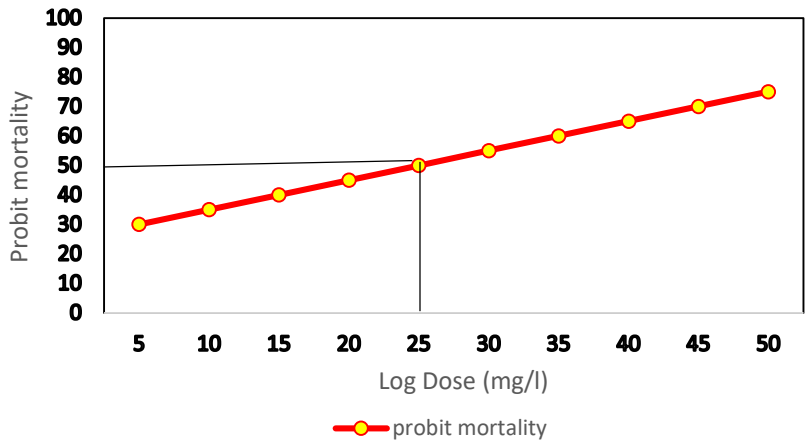
Boiling point: 370 - 500°C

Vapour pressure: 1.2×10-3 Pa at 20°C and 2.4×10-3 Pa at 25°C.

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 24hrs, 48hrs, 72hrs. 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 (graph 1).

Graph 1: Showing the LC_{50} 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.

Histopathological studies:

Histopathological studies were carried out on tissues taken from stomach and intestine by dissecting the control and experimental fishes exposed to sub lethal concentrations of TPP. Tissues were fixed in 10% neutral buffered formalin. Thin sections of 5µ thickness were prepared, stained with haematoxylin and eosin, observed under the light microscope. Following procedure was adopted for histopathology: (Bancroft and stevens, 1977; Bancroft and Cook, 1984)

1. Fixation: Organ tissues were kept in Bouin solution for 24 hrs. Solution consisted of aqueous picric acid, formalin and glacial acetic acid in a ratio (75: 20: 5) respectively.

2. Dehydration: After fixation, tissues slices were kept in tissue baskets with tagging. Dehydration was done in Alcoholic ascending grade series as 30% (for 30 minutes), 50 %(for 30 minutes), 70%, 90% (for 2 hours each) and 100% for 2 hours two times.

3. Clearing: After dehydration, tissue baskets were transferred to clearing agent, Xylene for 1 hour and 2 hours.

4. Infiltration: After clearing, tissues were placed in molten paraffin wax for 45 minutes and 60 minutes at 58-69 °C in oven.

5. Embedding: Tissues were removed and embedded in moulds with paraffin wax. Wax blocks with embedding tissues were freezed for solidification.

6. Sectioning: Tissues were cut at 5 micron by using Microtome. Ribbons were spread on glass slide containing adhesive material as glycerine and albumin. Sections were placed in oven at 37°C.

7. Staining: Wax was removed from sections with xylene for 2 minutes for 3 times. Hydration was done by immersing the tissues in descending series of alcohol for 1-2 minutes each. (100%, 90%, 70%, 50% and 30%). Tissues were stained with haematoxylin stain for 2-5 minutes and then washed the slides under running tap water to remove excess stain. Counter stained with Eosin (1%) for 15 seconds to 2 minutes. Washed with water, dried in oven for 2 minutes. Mounted with Canada balsam.

8. Examination: Slides were examined under light microscope and photographed at 40 x10 X objective lens. The histopathological changes in experimental groups were observed and compared with that of control group.

RESULTS:

Assessment of LC_{50}:

The LC_{50} of TPP in *Catla catla* was found to be 25 mg/l . The LC_{50} value was calculated by constructing the regression line, taking test doses and their corresponding mortalities in logarithmic values using Behreus and Karbeur (1953) probit analysis. The $1/25^{th}$ value of LC_{50} that is 1mg/l, $1/50^{th}$ value of LC_{50} that 0.5 mg/l and $1/100^{th}$ value of LC_{50} that 0.25 mg/l was chosen for sub lethal toxicity study.

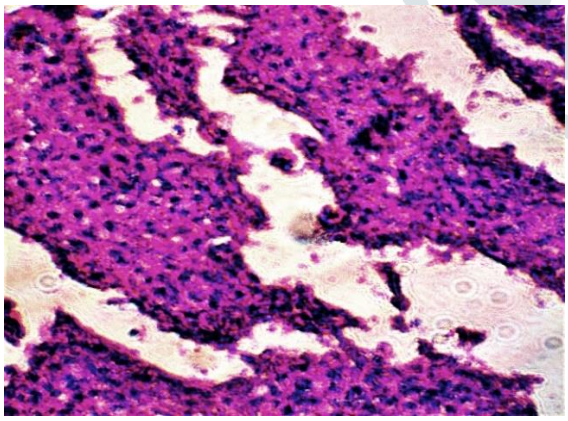
HISTOPATHOLOGY:

Stomach:

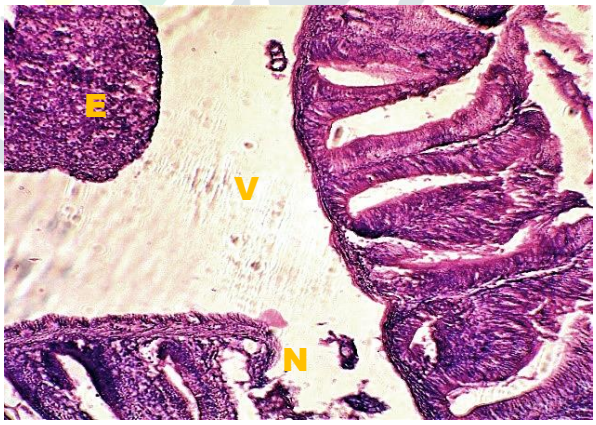
The section of stomach showed normal architecture in the case of control group (Fig.1). The section of stomach tissue of *Catla catla* exposed to sub lethal dosages of TPP (0.25mg/l, 0.5 mg/l, 1 mg/l) for 15 days, showed vacuolation, edema, necrosis and congestion of cells in inner lining of stomach is seen in the focal area (Fig.2, 3, 4 & 5).

Intestine:

The section of intestine showed normal architecture in the case of control group (Fig.6). The section of intestine tissue of *Catla catla* exposed to sub lethal dosages of TPP (0.25mg/l, 0.5 mg/l, 1 mg/l) for 15 days, showed rupture in the lining cells and haemorrhage, necrosis of intestinal villi cells. Widening of intestinal villi cells is seen in the focal area (Fig. 7, 8, 9 & 10).

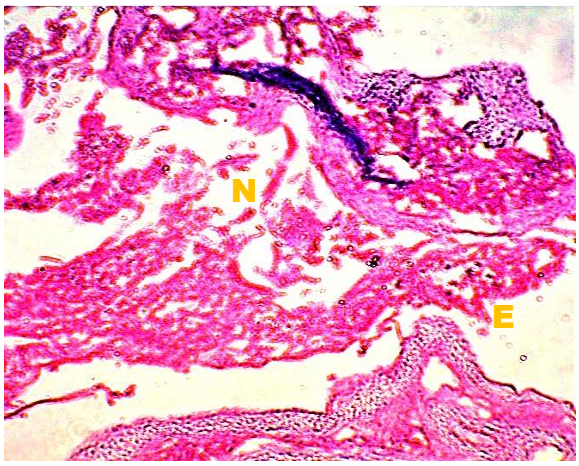


Haematoxylin and Eosin stained photomicrograph showing the histology of a Control stomach tissue of *Catla catla* – (X 100) (Fig 1)



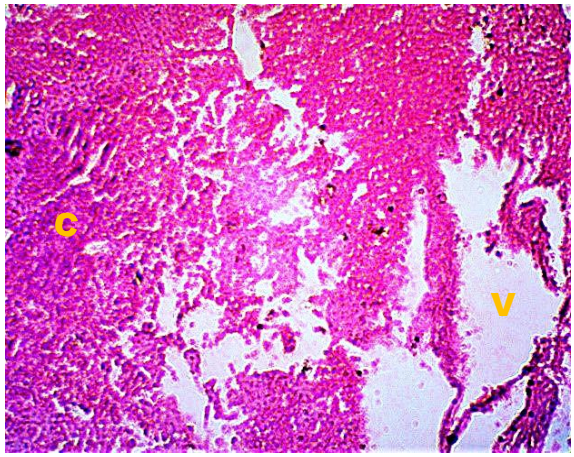
Haematoxylin and Eosin stained photomicrograph showing the histology of Acetone treated stomach tissue of *Catla catla* - (X 100) (Fig 2).

V – Vacuolation, E – Edema, N - Necrosis



Haematoxylin and Eosin stained photomicrograph showing the histology of stomach tissue of *Catla catla* treated with (0.25 mg/l) b.wt. of Triphenyl Phosphate - (X 100) (Fig 3).

N – Necrosis, E – Edema.

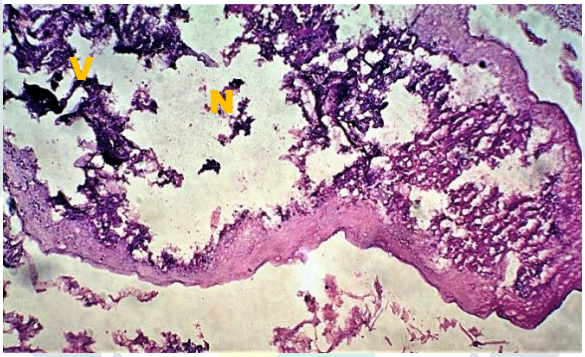


E

Haematoxylin and Eosin stained photomicrograph showing the histology of stomach tissue of *Catla catla* treated with (0.5 mg/l) b.wt. of Triphenyl Phosphate - (X100) (Fig 4).

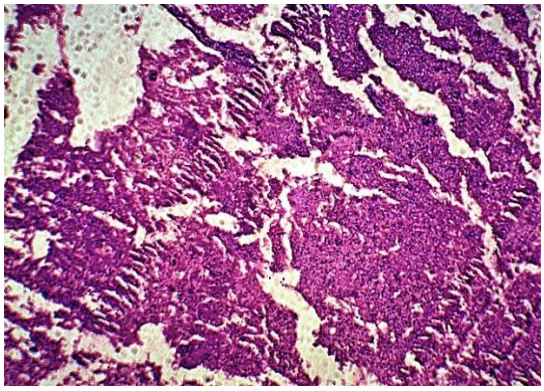
V – Vacuolation, C – Congestion, E – Edema.

E

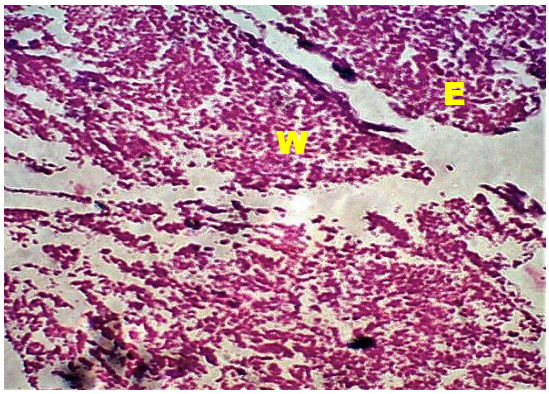


Haematoxylin and Eosin stained photomicrograph showing the histology of stomach tissue of *Catla catla* treated with (1 mg/l) b.wt. of Triphenyl Phosphate - (X 100) (Fig 5).

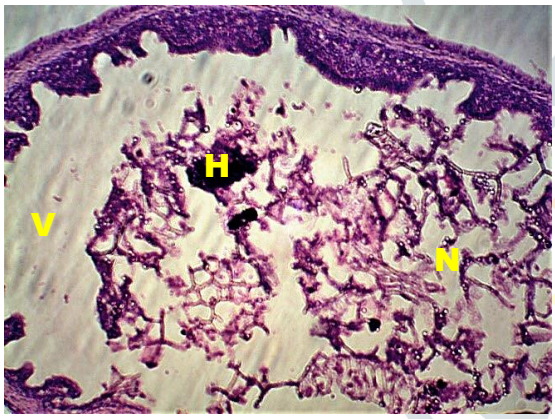
V – Vacuolation, N – Necrosis, E – Edema.



Haematoxylin and Eosin stained photomicrograph showing the histology of a Control intestine tissue of *Catla catla* – (X 100) (Fig 6)

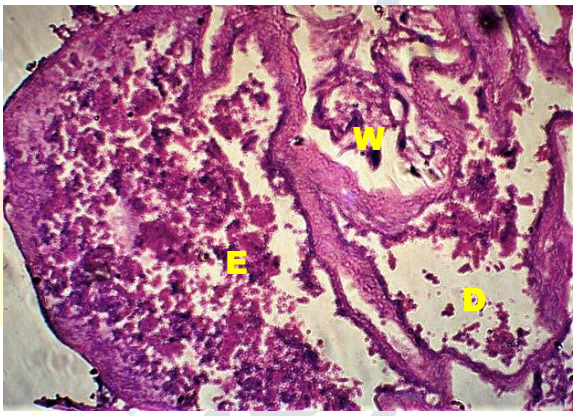


Haematoxylin and Eosin stained photomicrograph showing the histology of Acetone treated intestine tissue of *Catla catla* - (X 100) (Fig 7).
W – Widening of Villi, E – Edema.



Haematoxylin and Eosin stained photomicrograph showing the histology of intestine tissue of *Catla catla* treated with (0.25 mg/l) b.wt. of Triphenyl Phosphate - (X100) (Fig 8).

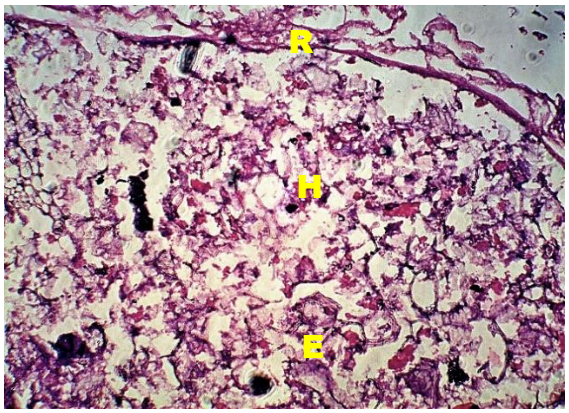
H-Haemorrhage, N-Necrosis,
V - Vacuolation



Haematoxylin and Eosin stained photomicrograph showing the histology of intestine tissue of *Catla catla* treated with (0.5 mg/l) b.wt. of Triphenyl Phosphate - (X100) (Fig 9).

W – Widening of villi, D – Disruption of Villi, E - Edema

N



Haematoxylin and Eosin stained photomicrograph showing the histology of intestine tissue of *Catla catla* treated with (1 mg/l) b.wt. of Triphenyl Phosphate - (X 100) (Fig 10).

H – Haemorrhage, R – Rupture of villi,
N – Necrosis, E – Edema.

DISCUSSION:

Any organ can function normally only when its structure is normal but any structural damage to it is likely to affect the function of that organ. Virchow, (1958); Bell, (1968) & Brown *et. al.,* (1968) suggested that there is a clear correlation between pathological condition of cell or tissue and it's affected functions. Thus, a study on histology provides a very important and useful data concerning changes in cellular or sub cellular structure of an organ much earlier than external notification. Histological criteria serve as a working approach for assessing toxicity in number of animals. Unfortunately, however, the effect of toxic substances on fish has to some extent been hampered because of the lack of proper histological literature on various fish organs. Such an experimental study helps in determining the extent of pollutant stress, well in advance to avoid any future disasters. The present study was done to investigate the histopathological alterations in alimentary tract, stomach and intestine of freshwater Major Carp, *Catla catla* after exposure to sub lethal dosage of TPP (0.25mg/l, 0.5 mg/l, 1 mg/l). The histopathological modifications in the stomach of *Catla catla*, after exposure to sub lethal dosage (0.25mg/l, 0.5 mg/l, 1 mg/l) of TPP in the present study includes, vacuolation, edema, necrosis, congestion of cells in inner lining of stomach. These findings are also in agreement with (Senapati *et. al.,* 2013; Crespo *et. al.,* 1986; Establier *et. al.,* 1978; Sastry and Gupta 1978). The histopathological modifications in the intestine of *Catla catla*, after exposure to sub lethal dosage (0.25mg/l, 0.5 mg/l, 1 mg/l) of TPP in the present study includes, rupture in the intestinal lining cells, haemorrhage, necrosis of intestinal villi cells, widening of intestinal villi. These finding are also agreement with (Muniyan, 1999; Das and Mukherjee, 2000; Cengiz *et. al.,* 2001; Yildirim *et. al.,* 2006).

Thus, the findings from the present study envisage that TPP has a potential to induce detrimental response in histoarchitecture of aquatic organisms when they come in contact with them either through direct or indirect mode.

CONCLUSION:

In summary, the present study revealed that TPP exposure caused severe pathological alterations in stomach and intestine of *Catla catla*, under laboratory condition. Pathological lesions displayed stronger responses under laboratory condition compared to field study. Finally, these pathological alterations to this plasticizer, TPP exposure could be considered as indicators to evaluate fish health status under stressed conditions in freshwater ecosystem, and careful handling and monitoring should be taken to minimize the utilization of this hazardous plasticizer, TPP to save our environment.

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