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A HISTOPATHOLOGICAL STUDY ON THE GILL AND MUSCLE OF INDIAN MAJOR CARP CATLA CATLA, EXPOSED TO AN ORGANOPHOSPHATE PLASTICIZER, TRI-ORTHO-CRESYL PHOSPHATE

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ABSTRACT: Tri-Ortho-Cresyl Phosphate (TOCP) is an organophosphate compound which is a toxic substance used as a plasticizer. The hazardous effect of TOCP on the histopathology of selected organs such as gill and muscle of the freshwater fish Indian major carp *Catla catla*, was investigated in the present study. Gill and muscle samples were collected after 15 days of exposure to sublethal concentrations of TOCP such as 0.25mg/l, 0.5mg/l and 1mg/l and were subjected to histopathological studies. The LC_{50} value for *Catla catla* with reference to TOCP was found to be 10 mg/litre. The present study revealed that degenerative alterations occurred in the gill and muscle tissues of *Catla catla* exposed to varying concentrations of TOCP.

KEYWORDS: TOCP, *Catla catla*, Gill, Muscle, LC_{50}.

1. INTRODUCTION:

Aquatic environments near industrial and urban centres are contaminated with a wide range of plastics that may be transformed into new potentially toxic compounds. The environmental conditions are not static and human influence has greatly stimulated the flow of environmentally deleterious changes by loading with chemicals to the aquatic system [5]. In the last few decades, a possible influence of environmental pollution on the aquatic environment has gained considerable interest. Fish have become a favourable subject for research in this area, because temperature changes, habitat and water quality deterioration as well as aquatic pollution adversely affect fish health, which may result in mortalities and population decline [12]. Fishes come into contact with multiple contaminants in the aquatic environment and biomagnifies the pollutants. These pollutants built up in the food chain are responsible for adverse effects and death in the aquatic organisms [6]. Fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bioindicators of environmental pollution [4,9,15].

Tri-Ortho-Cresyl Phosphate (TOCP) is an industrial chemical which is the most toxic isomer of TriCresyl Phosphate in acute and short-term exposure. TOCP is an organophosphate compound used commercially as a plasticizer in vinyl plastics, as a flame-retardant, as an additive to extreme pressure lubricants, and as a non-flammable fluid in hydraulic systems.

The measurement of concentrations of TOCP in water has shown only low levels of contamination. This reflects the low water solubility and ready degradability of the compound. TOCP has been found in surface waters and aquatic organisms near heavily industrialized areas, although concentrations are usually low. Accidental ingestion is the main cause of intoxication. Since the end of the nineteenth century, numerous cases of poisoning due to contamination of drink, food or drugs have been reported. Occupational exposure is principally via dermal absorption or inhalation, and some cases of poisoning have been reported. TOCP is toxic to aquatic and human life with long lasting effects as it is neurotoxic (WHO, 1990).

The investigation of histological changes in vital organs of fish is an accurate way to assess the effects of organophosphate compounds like TOCP in experimental studies. Hence, this study was undertaken to examine the effect of TOCP at different sublethal concentrations on histology of gills and muscle tissues of Indian major carp *Catla catla*. This investigation presents a reliable indicator of the aquatic ecosystem contamination and the possible negative impact of the surrounding environment.

2. MATERIALS AND METHODS:

2.1 Selection of Experimental Animal

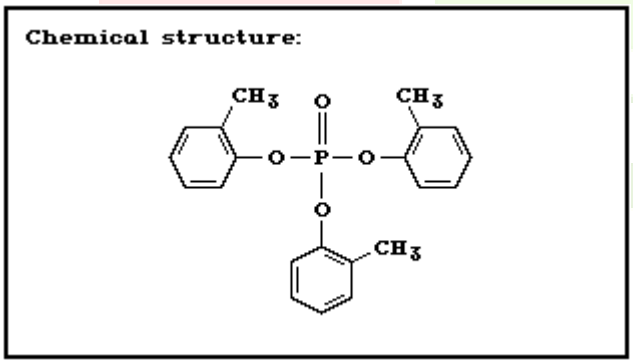
The freshwater fish *Catla Catla*, belonging to cyprinidae family was chosen as the experimental model for the present study. *Catla catla* is one of the commercially important and widely cultured Indian Major Carps. *Catla catla* is used for the various toxicology experiments in life sciences. *Catla catla* fingerlings of sizes ranging from 5 to 6 cms in length and 4 to 5 grams in weight were procured from Seed fish India- Poondi, Thiruvallur and brought to the laboratory in oxygen packs. The fishes were acclimatized for a week and maintained in aerated glass tanks filled with tap water. The stock fish were fed with commercial feed.

2.2 Selection of Plasticizer

Tri-Ortho-Cresyl Phosphate (TOCP) an organophosphate compound of commercial importance, was used as the test material for the present study.

2.3 PHYSICO-CHEMICAL PROPERTIES

2.3.1. *Chemical Structure*



Molecular Formula: $C_{21}H_{21}O_4P$

Synonyms: Tri Cresyl Phosphate (TCP), Tri-o-tolyl phosphate (TOTP).

***CAS Chemical Name*:** Phosphoric acid, Tri-o-tolyl ester.

CAS Registry Number: 78-30-8

2.3.2. *Physical Properties*

It is a non-flammable, non-explosive, colourless, odourless, viscous liquid, although commercial samples are typically yellow.

Molecular Weight: 368.37g/mol.

Water Solubility: 0.34 mg/l, sparingly soluble in water, slightly soluble in acetone.

Solubility In Other Solvents: Acetone and all other organic solvents

Melting Point: 11˚C, 52˚F.

Boiling Point: 410 ˚C, 770˚F.

Vapour Pressure: 1.96 X 10-6 mm Hg at 25˚C, 1.7x10-6 mmHg, (77°F): 0.00002 mmHg.

Density: 1.1955 g/cu cm at 20 ˚C.

2.4 ASSESSMENT OF LC_{50}

The assessment of LC_{50} was done following the procedure of Finney's probit analysis[8]. The assessment of toxicity was done based upon the percentage mortality of fishes against the test doses. The LC_{50} bio-assay method in the present study involves placing groups of organisms (10 fishes per group) and the mortality rate was observed and recorded at time intervals of 24 hours, 48 hours, 72 hours and 96 hours. The concentrations which produced 50% mortality at 96 hours was taken as the LC_{50} value. The LC_{50} value was calculated by constructing the regression line and the percentage mortality was converted into probit values and plotted against the log dose values.

2.5 EXPERIMENTAL DESIGN FOR SUB LETHAL STUDY

Four experimental groups were maintained with ten fingerlings in each glass tank filled with 20 litres of aerated tap water along with suitable control without the toxicant for the sublethal study. The plasticizer Tri-Ortho-Cresyl Phosphate was weighed and dissolved in equal volumes of acetone at varying concentrations of 0.25mg/l, 0.5mg/l and 1mg/l and mixed directly in water, in which the fishes were introduced and maintained. Experimentation was carried out for a duration of 15 days.

2.5 HISTOPATHOLOGICAL STUDIES

Histopathological studies were carried out on control fishes and on experimental fishes, which were sacrificed after a period of 15 days. The gills, muscle, stomach, liver, kidney and intestine were removed and preserved separately in neutral buffered formalin. They were processed by the routine histological techniques as follows.

1. Fixation in neutral buffered formalin for 48 hours.
2. Tissue dehydrated by placing in graded series of alcohol (30%, 50%, 70%, 90%, and 100%) for about 2 hours each.

3. Two changes in absolute alcohol, 12 hours each.
4. Clearing in xylene-two changes of 3hours each.
5. Wash in water.
6. Blocks were prepared by embedding the tissues in paraffin wax.
7. Sections of 5µ thickness were obtained using microtome.
8. Strips of sections each containing four were mounted onto a glass slide.
9. Glass slide with tissue mounted were placed in xylene to remove the wax.
10. Hydration was done by passing through graded series of alcohol (100%, 90%, 80%, 70%, 50% and 30%).
11. Washed in water.
12. Staining was done using haematoxylin and eosin. Cover slip was placed using DPX and slides were made permanent and examined for histopathological changes.

3. RESULTS:

3.1 Determination Of LC_{50}

The LC_{50} value for *Catla catla* with reference to Tri-Ortho-Cresyl Phosphate was found to be 10 mg/litre. (Graph 1). From this lethal concentration, the $1/10^{th}$ value of LC_{50} that is 1mg/l, the $1/20^{th}$ value of LC_{50} that is 0.5mg/l and the $1/40^{th}$ value of LC_{50} that is 0.25mg/l was chosen for the sub lethal toxicity study.

3.2 Histopathological Studies

The histopathological changes in the various organs such as gill, muscle, liver, kidney, intestine and stomach are as follows:

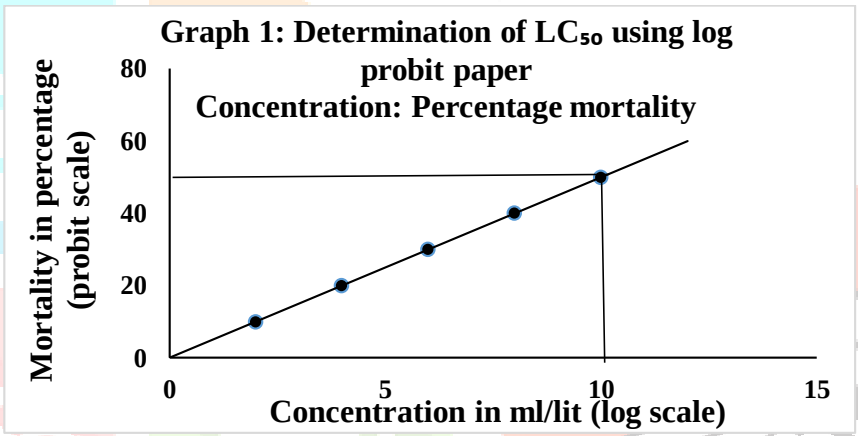
3.2.1 Gill

The section of gill showed normal histological appearance in the control group of *Catla catla*, (Fig. 1) whereas mild changes like epithelial necrosis, edema, primary lamellae uprooted from their bases, hyperplasia and degeneration of secondary lamellae were noticed in the gill section treated with Acetone. (Fig. 2). The gill section treated with 1mg/l of TOCP showed fusion of secondary lamellae, disruption of secondary lamellae, hyperplasia, epithelial necrosis and primary lamellae uprooted from their bases. (Fig. 3). The gill section treated with 0.5mg/l of TOCP showed vacuolization, edema, necrosis of primary lamellae, fusion of secondary lamellae and

hyperplasia. (Fig. 4). The gill section treated with 0.25mg/l of TOCP displayed primary lamellae uprooted from their bases, necrosis of secondary lamellae, edema and hyperplasia. (Fig. 5).

3.2.2 Muscle

The section of muscle in the control group of *Catla catla* showed a normal architecture, (Fig. 6) on the other hand the muscle section treated with Acetone showed mild changes such as disruption of muscle bundles, muscular necrosis, shortening of muscle fibres, vacuolation and intercellular space. (Fig. 7). The muscle section treated with 1mg/l of TOCP displayed vacuolation, intercellular space, fragments of muscle bundle, disruption of muscle bundle and separation of muscle bundle. (Fig. 8). The muscle section treated with 0.5mg/l of TOCP revealed swelling of muscle bundles, vacuolation, haemolysis, dystrophy and severe intramuscular edema. (Fig. 9). There was muscular necrosis, splitting muscle fibres, vacuolation, intercellular space and shortening of muscle fibres in the muscle section treated with 0.25mg/l of TOCP. (Fig. 10).



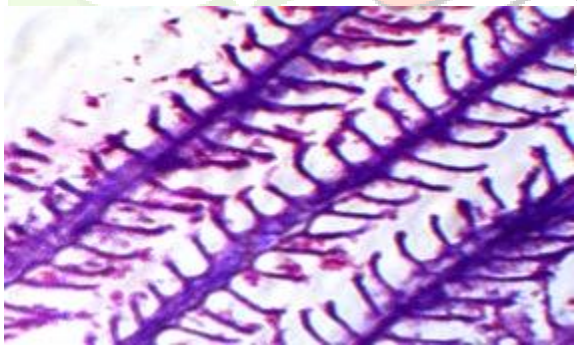


Fig. 1 Photomicrograph showing C.S of gill - Control - stained in haematoxylin and eosin × 100.

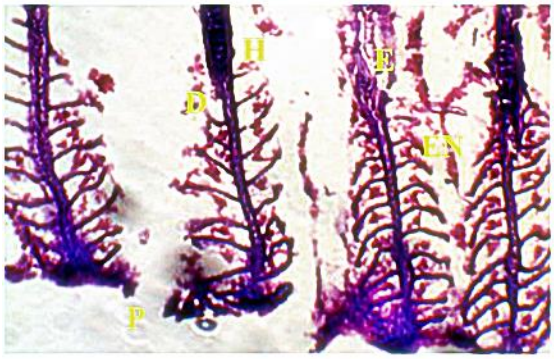


Fig. 2 Photomicrograph showing C.S of gill- Treated with Acetone- stained in haematoxylin and eosin ×100. EN-Epithelial Necrosis, E-Edema, P- Primary lamellae uprooted from their bases, H- Hyperplasia, D- Degeneration of secondary lamellae.

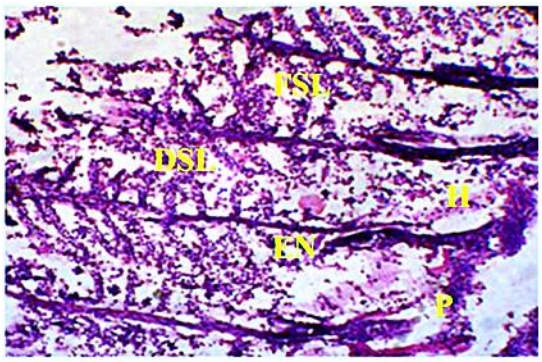


Fig.3 Photomicrograph showing C.S of gill- Treated with Tri -Ortho -Cresyl Phosphate (1mg/l) - stained in haematoxylin and eosin ×100. FSL- Fusion of Secondary Lamellae, DSL-Disruption of Secondary Lamellae, H-Hyperplasia, EN- Epithelial Necrosis, P-Primary lamellae uprooted from their bases.

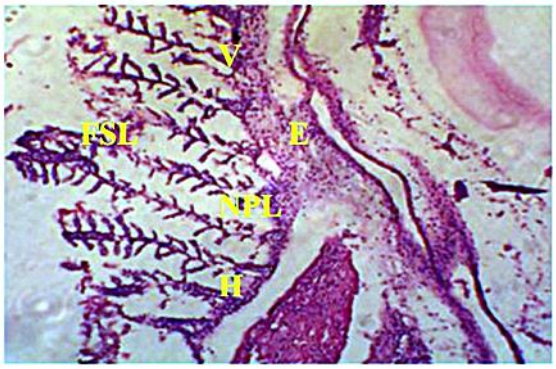


Fig. 4 Photomicrograph showing C.S of gill- Treated with Tri -Ortho -Cresyl Phosphate (0.5mg/l) - stained in haematoxylin and eosin × 100. V-Vacuolization, E-Edema, NPL-Necrosis of Primary Lamellae, FSL- Fusion of Secondary Lamellae, H- hyperplasia.

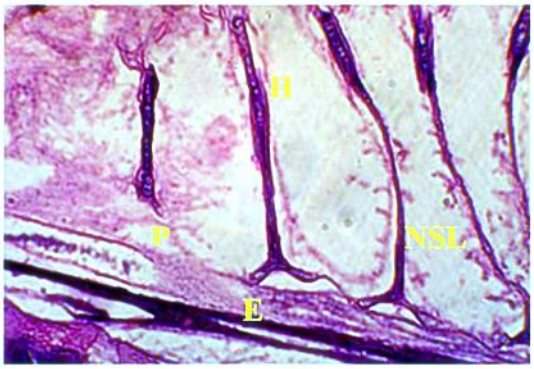


Fig. 5 Photomicrograph showing C.S of gill- Treated with Tri-Ortho-Cresyl Phosphate (0.25mg/l) - stained in haematoxylin and eosin × 100. P- Primary lamellae uprooted from their bases, NSL-Necrosis of Secondary Lamellae, E-Edema, H-Hyperplasia.

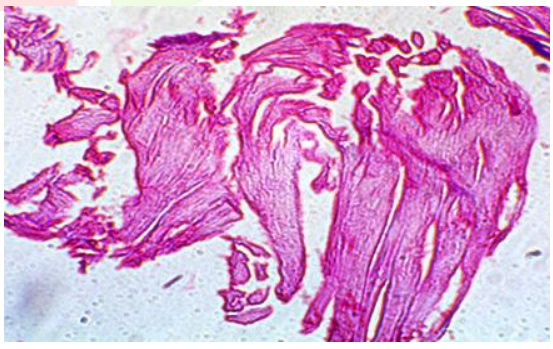


Fig. 6 Photomicrograph showing C.S of muscle- Control- stained in haematoxylin and eosin × 100.

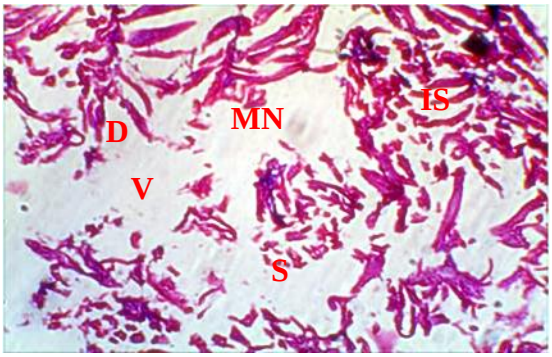


Fig. 7 Photomicrograph showing C.S of muscle-Treated with Acetone- stained in haematoxylin and eosin × 100. D- Disruption of muscle bundle, MN-Muscular Necrosis, S-Shortening of muscle fibres, V-Vacuolation, IS- Intercellular Space.

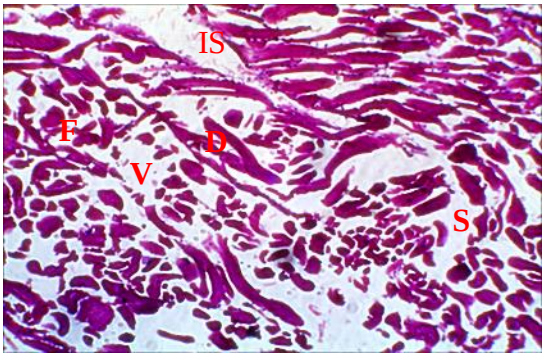


Fig. 8 Photomicrograph showing C.S of muscle-Treated with Tri-Ortho-Cresyl Phosphate (1mg/l-stained in haematoxylin and eosin × 100. V-Vacuolation, IS-Intercellular Space, F- Fragments of muscle bundle, D-Disruption of muscle bundle, S-Separation of muscle bundle.

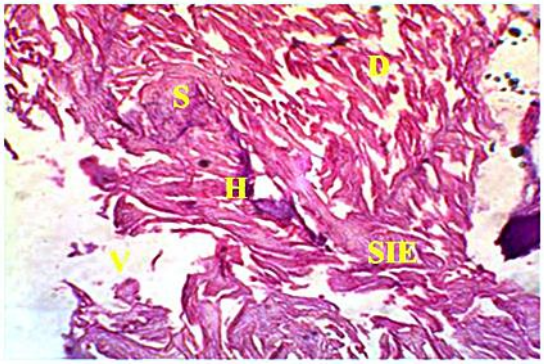


Fig.9 Photomicrograph showing C.S of muscle-Treated with Tri-Ortho-Cresyl Phosphate (0.5 mg/l) - stained in haematoxylin and eosin × 100. S-Swelling of muscle bundle, V-Vacuolation, H-Haemolysis, D- Dystrophy, SIE- Severe Intramuscular Edema.

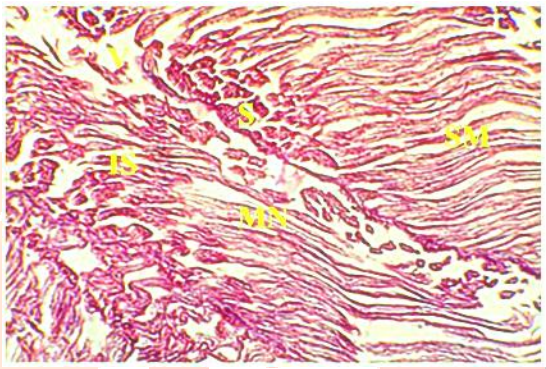


Fig.10 Photomicrograph showing C.S of muscle-Treated with Tri-Ortho-Cresyl Phosphate (0.25 mg/l) - stained in haematoxylin and eosin × 100. MN- Muscular Necrosis, SM-Splitting Muscle fibres, V-Vacuolation, IS-Intercellular Space, S-Shortening of muscle fibres.

4. DISCUSSION:

The aquatic environment is continuously being contaminated with toxic chemicals from industrial activities. The run off from treated areas enters the river and aquaculture ponds that are supplied by rivers [1]. The contamination of fresh water with a wide range of pollutants has become a matter of concern over the last few decades [14]. In the present study, the toxicity tests were conducted to evaluate the acute toxicity of Tri-Ortho-Cresyl Phosphate on the freshwater fish, *Catla catla*. The LC_{50} values are useful measure of acute toxicity of tested chemical used under certain environmental conditions. The LC_{50} value for *Catla catla* with reference to Tri -Ortho -Cresyl Phosphate was found to be 10 mg/l.

Histopathological studies on fish are a noteworthy and promising field to understand the structural organization that occurs in the organs due to pollutants in the environment. Histopathological studies are conducted to establish fundamental relationships of contaminant exposure and its biological responses [11].The structural

changes in the organs at microscopic cellular and organ level leads to alterations of the function systems [2]. Changes, occurring specifically of histological alterations, in fish populations due to chemical stress, are manifestations resulting and can give a relatively rapid indication of how environmental conditions affect fish populations. These structural changes vary with the body parts, nature of the pollutant, medium and duration of exposure. Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies. Tissue changes in test organisms exposed to a sub-lethal and lethal concentrations of toxicant are a functional response of organisms which provides information on the nature of the toxicant [7]. Exposure to sub-lethal concentrations of environmental chemicals may lead to the histological structure alterations which can significantly alter the function of tissues and organs. Histological and ultrastructural changes in cells, tissues or organs can afford good biomarkers of pollutant stress [10].

In the present study the section of gill tissue of *Catla catla* in the control group showed normal organization whereas the gill tissue exposed to Acetone showed mild changes like epithelial necrosis, edema, primary lamellae uprooted from their bases, hyperplasia and degeneration of secondary lamellae. The gill section of *Catla catla* exposed to 1mg/l of TOCP revealed fusion and disruption of secondary lamellae, hyperplasia, epithelial necrosis and primary lamellae uprooted from their bases. The section of gill tissue exposed to 0.5mg/l of TOCP displayed vacuolization, edema, necrosis of primary lamellae, fusion of secondary lamellae and hyperplasia. The gill section exposed to 0.25mg/l of TOCP showed primary lamellae uprooted from their bases, necrosis of secondary lamellae, edema and hyperplasia. Major alterations in gills may be defense mechanisms serving as barriers to the entry of contaminants [3]. Since gills are the respiratory organs that frequently encounter hazardous pollutants which are present in water in different forms, these pollutants may lead to the alteration in the normal area which causes the reduction in oxygen consumption and physiological imbalance in the organism [13].

In the present investigation the muscle section in the case of control fish exhibited a normal histological appearance whereas the muscle tissue of *Catla catla* exposed to Acetone showed mild alterations like muscular necrosis, vacuolation, intercellular space, disruption and shortening of muscle bundles,. The section of muscle exposed to 1mg/l of TOCP displayed vacuolation, intercellular space, fragments, disruption and separation of muscle bundle. The muscle section exposed to 0.5mg/l of TOCP displayed swelling of muscle bundles, vacuolation, haemolysis, dystrophy and severe intramuscular edema. The section of muscle of the fish exposed

to 0.25mg/l of TOCP revealed muscular necrosis, splitting and shortening muscle fibres, vacuolation and intercellular space.

Hence the histopathological studies performed in gill and muscle tissues of *Catla catla* exposed to sub-lethal concentrations of Tri-Ortho-Cresyl Phosphate reported significant damage in the vital tissues indicating that it was a useful methodology for monitoring the long-term effects of Tri-Ortho-Cresyl Phosphate on the fish. Tissue injuries and damages in organs can result in the reduced survival, growth and fitness, the low reproductive success or increase of susceptibility to pathological agents.

5. CONCLUSION:

Present work showed that Tri-Ortho-Cresyl Phosphate is strongly toxic and severely affects histology of gill and muscle tissue of *Catla catla*, making the fish less fit for better survival. These histopathological changes can alter various physiological activities of the fish. From the viewpoint of public health, fish that are permanently exposed to such plasticizers can be dangerous and cause diseases if the amounts of these substances in their bodies exceed the standard ranges for human consumption. Obviously, such changes in the fish body as a bioindicator warn human health because the food chain is affected by the environment. Thus this study will enhance our understanding for reduced and more effective use of plasticizers and other contaminants.

6. ACKNOWLEGEMENTS:

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7. CONFLICT OF INTEREST:

The authors declared no potential conflicts of interests with respect to the authorship and publication of this paper.

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