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# HISTOPATHOLOGICAL EFFECTS OF BIOACCUMULATION OF PCB 126 ON LIVER TISSUE OF FRESHWATER FISH *PUNTIUS TICTO*

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**ABSTRACT:**

The present work attempts to determine the extent of bioaccumulation of PCB congener 126 during the chronic toxicity tests, at intervals of 10, 20 and 30 days and the histopathological changes accompanying bioaccumulation in the liver of the freshwater fish, *Puntius ticto*.

**Keywords:-** Histopathology, Puntius ticto, fresh water

## INTRODUCTION:

The properties of water are well known and it is a solvent for several inorganic and organic substances that are directly or indirectly beneficial to aquatic organisms including fish. Fish take in water through mouth for respiration. It passes over gills for intake of oxygen. There is also intake of water for nutrition; it passes to intestine, where there is absorption of digested food, passed to liver for biotransformation and to the kidney for excretion.

Human development has shown its own ill effects. There is release of a variety of substances foreign to normal metabolism of organisms, some of which are degraded by them while others persist in environment as pollutants. These pollutants may be particulate, in the form of pesticides sprayed on crops, which are given away as surface run offs from fields or by their leaching into groundwater. Another source of concern is the wastewater released by various industries into water bodies. Leakages and oil pills also contribute largely to pollution especially marine water.

Pollutants may not always dissolve in water but may settle down as sediments where they are taken up by the sediment dwelling organisms and others who feed upon them. Chemical contaminants taken up from the sediment at the bottom of the water body have been found in tissues of Russian sturgeon (*Acipenser gueldenstaedtii*) and European pond turtles (*Emys orbicularis*) (Swartz C.D. et. al, 2003), in benthic and pelagic fish and lobsters (Morgan E.J. and Lohmann, R., 2010).

PCBs (polychlorinated Biphenyls) are entirely man-made chemicals and began to be released into the environment by manufacturers and consumers in the 1900s. They are a group of widely versatile synthetic chlorinated compounds. Due to varied chemical structures due to substitution by Chlorine in different positions in the biphenyl structure, they have been assigned numerical congener numbers by IUPAC. PCB 126 is one such congener. In a



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concentrated form, they are either oily liquids or solids with no discernable taste or odor. These have a low degree of reactivity, high electrical resistance, good insulating properties, stability under heat and pressure and a non-inflammable nature, which make them perfect substances for use as dielectric fluids and as insulators for transformers and capacitors.

Further, it has been shown that a structure-activity relationship exists between the pollutant and organism, and bioaccumulation may vary with species as well as with the complexity of the compound (Boon J.P. et al, 1989). PCBs have been shown to cause oxidative stress and apoptosis in fish (Zhang, J., et al, 2009). DNA damage as a result of exposure to PCBs has been shown as a cause of environmental stress in fish (González-Mille, D.J., et al, 2010). It is reported that fish accumulate as many as 40,000 times of exposure concentrations of PCBs (Stalling D.L. and Lee Mayer F. Jr., 1972).

PCBs have been found to be toxic in developmental stages of birds, indicating that their transfer up the food chains from fish can be hazardous (Hoffman D.J. et al, 1996). PCBs have also been found in groundwater contaminated by equipment abandoned in mines (Bench D.W., 2003).

The chemical and biological stability of PCBs and their lipophilic nature is primarily responsible for their accumulation in the environment. They are seen to accumulate in sediment feeders such as *Daphnia magna* and then to be carried up into the food chain through predatory fish such as *Pimephalespromelas* (Dillon, T.M. and Burton, W.D.S., 1991). Detailed studies on subacute and chronic toxic effects of PCBs on mammals and birds, especially on their reproduction, have been documented (Kimbrough R. et al, 1978).

Uptake of such substances by fish leads to disturbances by interference in metabolic pathways as well as their deposition in different tissues, which increases over time due to their inability to dispose them. This is termed as bioaccumulation. Enzymes in metabolic pathways, tissues as well as whole systems of organisms have been seen to be affected by this phenomenon. Effects of PCBs on steroidgenesis and reproduction in fish have been shown to be deleterious (Freeman, H.C. and Idler, D.R., 1975).

Bioaccumulation not a phenomenon restricted in isolation to a single affected organism due to presence of food chains and interdependence of organisms for food. This results in passage of the accumulated substance in the higher trophic levels of the food chain, resulting in biomagnification.

Man, one of the top consumers in most food chains, also does not escape deleterious effects of these pollutants. All U.S. residents have been reported to have measurable PCBs in their adipose tissue. PCBs have been found in human milk as well (Norén K. et al, 1990). The toxic coplanar PCB congeners 126 and 169 have been found in human as well as wildlife tissues (Tanabe S. et al, 1989), (Safe S.H., 1994).

Thus the phenomena of bioaccumulation and biomagnification have widespread deleterious effects.

**MATERIAL & METHODS**

PCB 126 (3,3',4,4',5-pentachlorobiphenyl) of Dr. Ehrenstorfer, Germany, Make was used for the present study. It has been reported that congeners with IUPAC numbers 77, 126, and 169 are the three most potent congeners from toxicity point of view, due to their coplanar configuration and absence of ortho substitutions (Safe S. et al, 1985), (McFarland V.A. and Clarke J.U., 1989)

Freshwater fish *Puntiusticto* were acquired as test organisms from a local freshwater body. They were acclimatized in dechlorinated tap water.

Static bioassays for determination of acute toxicity were carried out according to methods recommended in APHA Standard Methods (1976), ISI (1971) and US EPA (1975, 1978). This method of bioassay has been widely used (Davis J.C. and Mason B.J., 1973), (Lee J.S. et al, 2005). Appropriate controls were invariably maintained. A safe dose of 12.5 µg $l^{-1}$of PCB 126 dissolved in dichloromethane was calculated in the static bioassays, based on the $LC_{50}$ values obtained.

Further, chronic toxicity tests were carried out using a flow-through system where 20 litres of solution containing the safe dose of PCB 126 was introduced into a 20 liter aquarium every 24 hours. Fish were thus exposed to the PCB 126 in this solution. Appropriate controls were maintained.

Fish were taken dissected and liver tissue was taken out for estimation of bioaccumulation of PCB 126 every $5^{th}$ day, whereas tissue for histopathological study was collected every $10^{th}$ day. All tissue was fixed in Buoin's fixative (aqueous) and later stained using the HE (Haematoxylin –Eosin) staining technique for observing histopathological changes.

Tissue for analysis of bioaccumulation was fixed in 4% formaldehyde (Deubert K.H. et al, 1973). In the present experimental work, Dichloromethane has been used for extraction of PCB 126 from liver tissue, as indicated in literature (Satyanarayan S. and Ramakant, 2004). Elution of PCB 126 was carried out as per clean up procedure available in literature by extraction in dichloromethane and passing repeatedly through columns of Celite 545 filter aid. Excess dichloromethane was allowed to evaporate and the eluate was analysed for bioaccumulation using GC-MS of Thermo Fisher Make.

The GC-MS was calibrated for measurement of PCB 126. Mass spectra, calibration curve and standard chromatogram (Fig.1) were obtained.

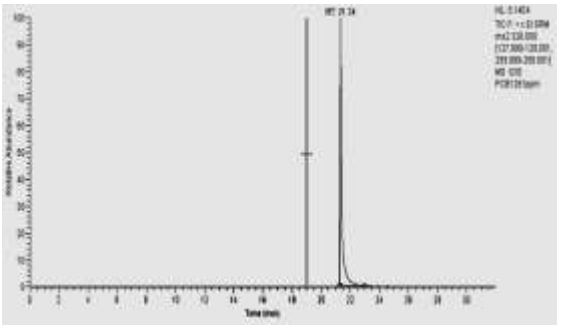


**Fig.1: Standard Chromatogram of PCB 126 (3,3',4,4',5- Pentachlorobiphenyl)**

**RESULT & DISCUSSION:**

**a) BIOACCUMULATION**:

Results were obtained based on chromatograms for PCB 126 obtained from eluates of liver samples of fish exposed to 5,10 (Fig.2) ,15,20, 25 and 30 days.

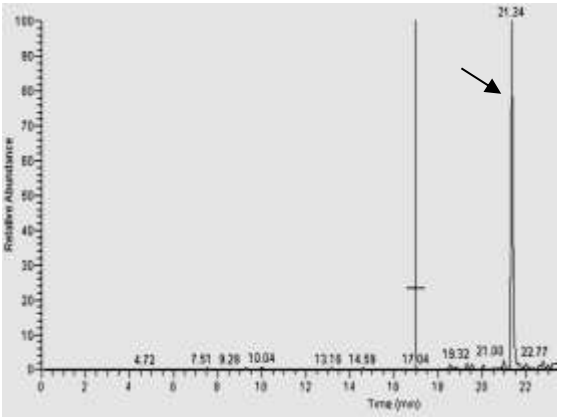



**Fig.2: Chromatogram showing PCB 126 in *Puntius* Liver (10 Days)**

The results have been tabulated in Table 1.

**Table 1: Bioaccumulation of PCB 126 in Liver of *Puntius ticto*.**

| Exposure time, Days | Values in µg $g^{-1}$ wet weight of the Liver tissue |
|---|---|

| | |
|---|---|
| 5 | 2.54 |
| 10 | 8.56 |
| 15 | 9.98 |
| 20 | 12.12 |
| 25 | 12.96 |
| 30 | 14.15 |

Bioaccumulation showed an increasing trend over the period of exposure (Graph 1).

**b) HISTOPATHOLOGY**:

As an indicator of exposure to contaminants, histology represents a useful tool to assess the degree of pollution, particularly for sub-lethal and chronic effects (Bernet D. et al, 1999). Experimental animal studies have shown toxic effects of PCBs to the liver (US EPA, 1999).

The fish liver is typically composed of a large number of polyhedral hepatic cells containing a granular cytoplasm. The nuclei of liver cells are vesicular with large nucleoli. Numerous bile ductules, bile capillaries and sinusoids are noticed. Sinusoids, which are irregularly distributed between the hepatocytes are few in number, and lined by endothelial cells with prominent nuclei.

Pollutants are known to have deleterious effects on liver. Literature cites instances of neoplasms, hypertrophy, lesions, tumors, necrosis, hydrophic vacuolation and bile duct hyperplasia in liver.

Post-embryonic larval and metamorphic stages of summer flounder, and potentially other fish species with complex life histories, are vulnerable to the effects of dioxin-like compounds, including lethality, developmental delay, and malformations (Soffientino B. et. al; 2010).

Experimental animal studies have shown toxic effects of PCBs to the liver (US EPA, 1999).

**OBSERVATIONS:**

Puntius exposed to PCB 126 showed a complete degeneration followed by diffused vacuolation in liver tissue. This trend became more significant as the period of exposure increased.

Exposure of *Puntius* to PCB 126 for 10 days showed no significant change other than a degeneration of sinusoids and formation of large vacuoles around them (Fig.4).

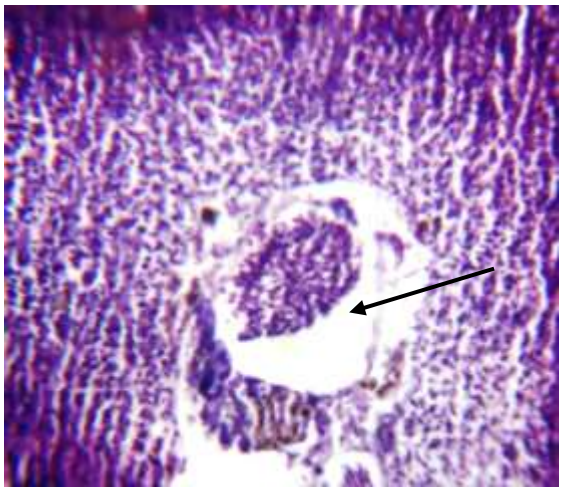


**Fig.4: T.S. Liver of *Puntiusticto* exposed to PCB 126 for 10 days (Magnification: 40X)**

A 20 day exposure showed further degeneration of hepatic tissue with collection of blood in the hepatic interstitium (Fig.5).

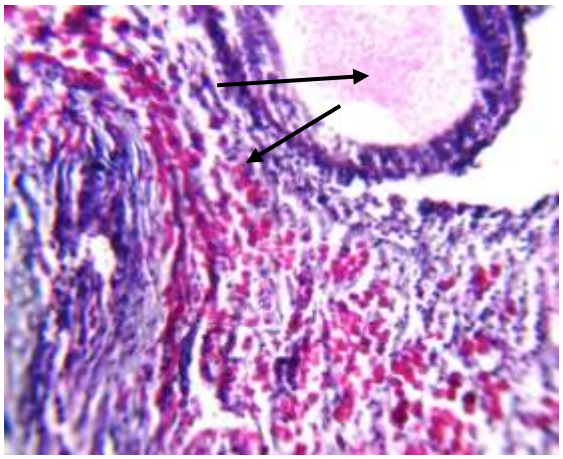


**Fig.5: T.S. Liver of *Puntiusticto* exposed to PCB 126 for 20 days (Magnification: 40X)**

A 30 days' exposure of *Puntius* to PCB 126 showed degeneration of liver tissue along with necrotic patches in some places (Fig.6).

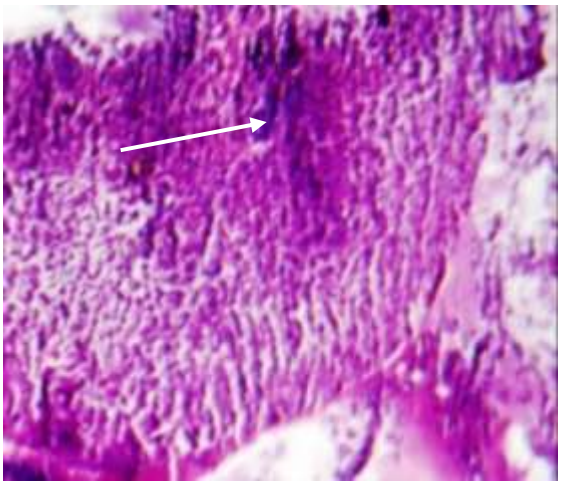


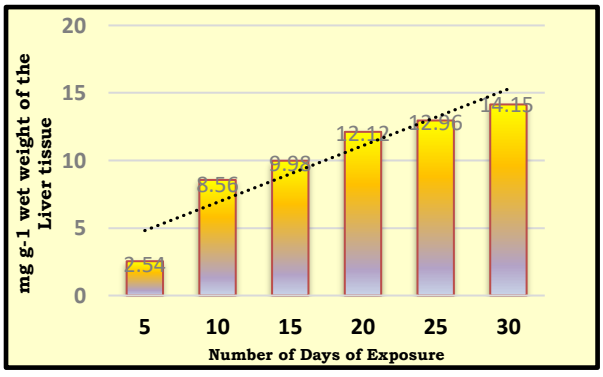
**Fig.6: T.S. Liver of *Puntius ticto* exposed to PCB 126 for 30 days (Magnification: 40X)**

**CONCLUSION :**

PCB 126 accumulated in the liver tissue 2.54µg $g^{-1}$to 14.15µg $g^{-1}$wet weight, initially showing a sharp rise over the first 10 days and then increasing gradually over the remaining 20 days. The increase from the initial 5th day to the 30th day was more than about 6-fold.

The bioaccumulation manifested itself in tissue degeneration, haemorrhages in the tissue interstitium and necrosis in the liver. It can be seen that as exposure period increases, tissue degeneration and necrosis set in.

Such degenerative changes over longer periods may affect enzymatic activity in liver, leading to mortality.

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