

**Effect of chlorpyrifos on the gonadal maturation of
freshwater catfish *Heteropneustes fossilis* (Bloch, 1794)**

SUMMARY

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SUMMARY

Chlorpyrifos (CPF, O, O'-diethyl-O-3, 5, 6-trichloro-2-pyridyl phosphorothionate) is the most extensively used broad-spectrum chlorinated organophosphate insecticide. The extensive use of pesticide has allowed it to reach the water bodies via surface runoff, air drift, overspray and improper disposal. It easily enters into different aquatic habitats and disturbs the normal physiology of aquatic animals including fish. CPF is an acetylcholinesterase inhibitor and causes acute and chronic effects, dragging towards death. Besides being acetylcholinesterase inhibitor, CPF is also an endocrine disruptor, genotoxic, mutagenic, teratogenic and developmental neurotoxic. Despite its toxicity, CPF is in demand because it degrades rapidly into other constituents via hydrolysis on exposure to light, soil and air. It is oxidized to more toxic metabolite chlorpyrifosoxon (CPO) via aerobic metabolism, photolysis and chlorination. CPO is a highly potent acetylcholine esterase (AChE) inhibitor having almost three times higher affinity toward the active site of AChE than the parent compound CPF and is considered to be more toxic to organisms including birds, mammals, freshwater fishes and invertebrates. Among the diverse range of wildlife species, fish serve as a useful bioindicator to assess the level of water contamination. *Heteropneustes fossilis* is a commercially important catfish found in India and its neighbouring countries. It is a seasonal breeder mainly found in freshwaters and brackish waters. It has high market demand because of its taste, nutritional and medicinal values. Its air breathing adaptation allows it to survive even in water bodies with poor oxygen content, making it an ideal fish species for aquaculture.

Although there are several reports to elaborate the extensive research performed on *H. fossilis*, there is still a dearth of knowledge in understanding the interaction of

environmental pollutant such as CPF with this fish. Therefore, the present study is an attempt to fill the gap along with some effective measures to reduce the toxic effects of such pollutant. The research work compiled in thesis form with a general introduction, two chapters and consolidated summary. To avoid repetition overall references are arranged at the last. The first chapter will elaborate CPF toxicity and their bioremediation with biochemical, enzymatic and histological methods. The second chapter will focus on CPF role on gonadal development in adult and fry stage.

In the first part of chapter I, the toxicity of chlorpyrifos (CPF) and its bioremediation by some powerful anti-oxidants curcumin, ascorbic acid and chitosan in freshwater fish, *Heteropneustes fossilis* was investigated. Different anti-oxidants [Curcumin (Cur), ascorbic acid (Asc) and chitosan (Chi)] alone and in different combinations were used for the first time to access their effectiveness against the CPF toxicity in *H. fossilis*. For the study, the live and healthy fish were exposed to sub-lethal concentration of CPF (0.174 μ M/l; 1/10th of 96 hrs LC₅₀) and effective concentrations of anti-oxidants alone and in different combinations. The finding of different experimental setup concluded that sub-lethal dose of CPF induces histopathological alterations in detoxifying organs (liver, kidney, gill) and increases anti-oxidative enzyme activities (catalase, peroxidase) and lipid peroxidation significantly. Fish in CPF medium when supplemented with anti-oxidative agents (Cur, Asc, Chi) found with suppressed toxic effects. In this study, the combination of Asc and Curcumin was found to be effective in attenuating the CPF toxicity as compared to other combinations. Chitosan was found to be ineffective in suppressing the CPF toxicity. The decrease in CPF toxicity becomes significant when tested in combinations of anti-oxidative agents as compared to their alone positive control groups. Therefore, co-incubation of curcumin and ascorbic acid was found to be

effective against CPF induced aberrations in anti-oxidative enzyme levels and anatomical changes.

In the second part of chapter I, the toxicity of CPF was studied on the biomolecular changes of important energy source compounds, glycogen, protein and lipid in tissues viz. muscles, liver, kidney, gill of fish and histotexture of nutrient absorbing organs (stomach, intestine). In this study, Cur and Asc was used as a bioremedial against CPF toxicity founding to be effective from the previous antioxidant enzyme activity study in freshwater fish, *H. fossilis*. The biomolecules such as glycogen, protein and lipid are essential for normal growth, metabolism and reproductive events. The live and healthy fish were exposed to a sub-lethal dose of CPF (0.174 μ M/l; 1/10th of 96 hrs LC₅₀) and effective concentration of antioxidants, Cur and Asc alone and in combination for 72 hrs to assess their effectiveness against the CPF toxicity. The understanding of different experimental setups concluded that a sub-lethal dose of CPF decreases the glycogen and protein content and increases the lipid content significantly. It also causes histopathological alterations in the stomach and intestine. However, co-incubation of curcumin and ascorbic acid was found to be effective in restoring CPF induced aberrations in biomolecular content and tissue anatomical changes.

In the first part of chapter II, interference of the CPF in gonad function at the steroidal level during prespawning phase of the freshwater catfish, *H.fossilis* was studied. The matured adult fish of early prespawning phase were exposed to different combinations of CPF(0.174 μ M/L, 1/10th of 96 hr LC₅₀), gonadotropin (hCG; 100IU/fish) and anti-oxidants (Cur; 10 mg/L and Asc; 5 mg/L) for 24 hrs to measure vitellogenin (Vtg) and ovarian steroid. Results showed that the CPF increased vitellogenin level in liver, serum

and ovary. The gonadotropin further induced this increase. Among steroidal cascade, CPF induced 17β -estradiol (E_2) many folds along with significant increase in progesterone (P_4) and $17\alpha,20\beta$ -dihydroxyprogesterone ($17\alpha, 20\beta$ -DHP) in comparison to the control group. The gonadotropin further increased CPF induced effects on vitellogenin and ovarian steroidal level. Though, anti-oxidants co-incubation with CPF did not produce any significant change on either vitellogenin or in ovarian steroids content. The results signal the major influence of CPF in gonadal physiology of freshwater catfish, *H. fossilis*.

In the second part of chapter II, the effect of CPF on the *NR5A1* (nuclear receptor subfamily 5, group A, member 1) expression during the fry stage of the freshwater catfish, *H. fossilis* was studied. The protein encoded by the *NR5A1* gene is a transcriptional activator that plays an important role in sex determination. It regulates various steroidogenic enzymes and several genes involved in steroidogenesis, reproduction and sexual differentiation. In this study, twenty days post hatch, fish fry were exposed to sub-lethal dose of CPF for 96 hrs to understand its influence on *NR5A1* gene expression pattern. CPF has been found to trigger *NR5A1* gene expression indicating its role in early gonad differentiation and development. The increase in the gene expression also indicates that exposure to endocrine disrupting chemical like CPF could alter estrogen dependent gonadal development in fish.

Thus, the present work is an effort to elaborate the hazardous effect of CPF on *H. fossilis*. In *H. fossilis*, CPF was found to induce alterations in anti-oxidant enzyme levels, biochemical content, histoarchitecture of tissues, vitellogenin content, steroidal levels and *NR5A1* expression pattern. These are the important parameters to assess the

toxic impact of the pesticide on fish. Fish serve as an ideal bioindicator to assess the health status of aquatic ecosystems. However, the toxic effects of CPF could be alleviated by the incorporation of antioxidants such as curcumin and ascorbic acid. In the present study, the combination of curcumin and ascorbic acid was found to be most effective in attenuating the toxic impact of the pesticide on fish. Thus, the usage of CPF should be optimized to protect the environment and the biota that could come in contact with such toxicant.