

**A toxicological study of organophosphorous
pesticide, chlorpyrifos exposure on reproductive
performance of freshwater catfish
“Heteropneustes fossilis”**

ABSTRACT

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The organophosphorous pesticides (OPs) are ubiquitous in the environment, commonly used as agricultural and vector control. They released into the environment in large amount. It may contaminate the environment and accumulate in food chains there by posing hazardous effect to human and aquatic animal health. The application of pesticides to flooded areas, along with accidental spillage, spray drift, leaching, runoff or drainage from treated agricultural lands has the potential to expose the non-target organisms especially the fish. Contamination of surface water has been well documented worldwide and constitutes a major issue at local, regional, national, and global levels. They alter the physico-chemical properties of water or riverine system. The significant increase of chemical emissions of the water resources had led to deleterious effect for aquatic organism.

The primary mechanism of action of OPs is as neurotoxic agents. They showed their toxic activity by inhibiting acetylcholinesterase enzyme (AChE). Among OPs, chlorpyrifos (CPF) (O,O-diethyl-O-3,5,6-trichlor-2-pyridyl) phosphorothioate) is a emulsifiable broad spectrum organophosphorous insecticide (OP), that has been widely used all around the world. It is second largest selling OP and third ranked among all pesticides in use. CPF inhibits the acetylcholinesterase (AChE) enzyme which caused accumulation of acetylcholine resulting in acute toxicity, delayed neuropathy and ultimately death of an organism.

Fish are the non-target organism of pesticidal exposure as they play an important in food chain. Fish are used as an indicator of environmental suitability. So that any change in fish behavior or morphology indicated the water quality deterioration. Aquatic contamination of pesticides cause acute and chronic poisoning in fish and other organisms directly or indirectly via food chain. Recent evidences indicate that fish, an extremely valuable resource, are quickly becoming scares. A vast scale mortality of fish in various water bodies has been recorded and the reason behind was the huge application of pesticides and insecticides in agricultural practices which further contaminate the aquatic environment. Pesticidal exposure cause fish mortality, reduced fish reproduction and increased amount of pesticide in edible fish can also affect the human health.

Therefore, the present study was undertaken to evaluate the effect of organophosphorous pesticide, chlorpyrifos on survival, behavior, morphological, histological, embryogenesis, oocyte maturation and follicular steroids of freshwater catfish, *Heteropneustes fossilis*.

Chapter I: Effect of organophosphorous pesticide, chlorpyrifos on mortality, behavior, morphology and ovarian histology of freshwater catfish, *Heteropneustes fossilis* (Bloch, 1794)

The present investigation was undertaken to explore the toxic effect of organophosphorous pesticide, chlorpyrifos (CPF) to assess the mortality, behavior, morphology and ovarian histology of freshwater catfish, *Heteropneustes fossilis* (Bloch, 1794). This study was conducted by exposing the fish to different concentrations of CPF (0.002-2.28 mM/l) for 96 hr experimental duration along with control group of fish in different reproductive phases (resting, preparatory and pre-spawning phase). The Finney's probit analysis (1971) was used to determine median lethal concentration (LC₅₀). The safe concentration of the studied pesticide was calculated as per Hart et al. (1945) and Henderson et al. (1959) in all respective seasons. The obtained LC₅₀ were found to be 1.547, 0.678, 0.299 and 0.174 mM/l in resting phase, 0.332, 0.193, 0.152 and 0.123 mM/l in preparatory phase and 0.296, 0.107, 0.044 and 0.026 mM/l in pre-spawning phase for 24, 48, 72 and 96 hr respectively. The safe concentrations were 0.039 mM/l in resting phase, 0.19 mM/l in preparatory phase and 0.004 mM/l in pre-spawning phase. Susceptibility of catfish, *H. fossilis* to CPF was found to be duration and concentration dependent. Their tolerance to CPF was increasing with inactiveness of gonadal activity (resting season). At higher concentration of CPF, fish showed alterations in morphological and behavioral responses, especially erratic and jerky swimming, frequent surfacing and ingulping, mucus secretion, an increase in opercular movement and copious secretion of mucus all over the body. The histological assessment resulted that CPF caused reduction in size of oocytes, vacuolation in cytoplasm, damaged ovarian follicles, increase in inter-follicular space, breaking of ovarian wall, occurrence of necrosis, damaged structure of ovarian follicles, formation of fragmented ova, atretic oocytes and nucleolar extrusion. It is

concluded that CPF is highly toxic to catfish and severely affect their physiology and behavior.

Chapter II: Effect of organophosphorous pesticide, chlorpyrifos on oocyte maturation and follicular steroids in freshwater catfish, *Heteropneustes fossilis* (Bloch, 1794)

The objective of the present study was to investigate *in vitro* effect of organophosphorous pesticide, chlorpyrifos (CPF) on hCG-induced oocyte maturation (OM) in the freshwater catfish, *Heteropneustes fossilis*. In this observation, interplay of different steroids involved in oocyte maturation was also measured. For this, the post-vitellogenic follicles were incubated with human chorionic gonadotropin (hCG) alone to induce oocyte maturation in a concentration and duration-dependent manner and with CPF on hCG induced OM post-vitellogenic follicles. The result showed that CPF caused an inhibitory effect on hCG induced GVBD in co-incubation experiments at the dose of 0.26 pM/ml. Pre- and post-incubation of follicles with CPF resulted a significant inhibition in hCG induced oocyte maturation in all durations and maximum inhibition 87.67% and 76% was recorded at 12 hr incubation duration ($p < 0.05$, Newman-Keuls test). Effective dose of hCG (1.7 mg/ml, 8 hr) induced OM coincided with a significant increase in follicular pregnenolone, progesterone, deoxycorticosterone (DOC) and dihydroxyprogesterone (DHP) as well as significant decrease in estradiol-17 β (E₂) as compared to control group of post-vitellogenic follicles. Under CPF exposure follicles elicited a sharp significant decrease in pregnenolone, progesterone and DHP. However, DOC and E₂ were increased significantly. Co-incubation study revealed that CPF suppressed hCG induced concentrations of DHP, pregnenolone and progesterone significantly but side by side support DOC increase and maintain high E₂. Thus, the present results suggested that the CPF inhibited hCG induced oocyte meiotic maturation due to suppression of maturation inducing steroids within follicle.

Chapter III: Effect of organophosphorous pesticide, chlorpyrifos embryogenesis of freshwater catfish, *Heteropneustes fossilis* (Bloch, 1794)

The present investigation was aimed to study the effect of chlorpyrifos on hatchlings of freshwater catfish, *Heteropneustes fossilis*. For this, *in vitro* and *in vivo* experiments were performed. In *in vitro* experiment, the fertilized eggs stripped from normal control catfish were exposed to different concentrations of chlorpyrifos viz., 0.52, 0.26, 0.052, 0.026, 0.013, 0.006, 0.004 $\mu\text{M/l}$. For *in vivo* study, the induced female catfish with synthetic hormone (hCG) was exposed to same concentrations of chlorpyrifos. The experiment was done in triplicates along with the control group. The result showed that both, fertilization and hatching rate was decreased significantly in exposed group in comparison to control. *In vivo* exposure showed significantly low embryo mortality ($27.2 \pm 0.76\%$) ($F=893.67$; $p < 0.05$) and high hatching rate ($90.5 \pm 0.5\%$) ($F=271.87$; $p < 0.05$) as compared to *in vitro* study ($35.2 \pm 0.81\%$ and $85.4 \pm 1.25\%$) ($F=1070.28$, $F=267.23$; $p < 0.05$) in lower concentration of CPF (0.004 $\mu\text{M/l}$). The percentage of malformed hatchlings was higher in *in vitro* experiment as compared to *in vivo*. The occurrence of all studied parameters was observed in a concentration dependent manner, as the concentrations of CPF were increased, the percentage of embryo mortality and malformed hatchlings was decreased. However, the hatching rate was increased. The result showed that exposed group of hatchlings were affected by many morphological and notochordal deformities viz., ventral or lateral tail flexure, abnormal spinal bending, irregular head shape and size, loss of eye, reduced barbel, pericardial edema, yolk sac edema, notochordal defect, tail fin flexure, reduction of brain development and reduction of pigmentation. The result suggested that OPs play an important role in inducing the muscular dystrophy and morphological deformities during embryonic development and organogenesis.