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Effects of organophosphate pesticide in fresh water fishes

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Abstract
Pesticides are one of the most potentially harmful toxic chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are quite significant. Aquatic ecosystems that run through agricultural or industrial areas have high probability of being contaminated by runoff and ground water leaching by a variety of toxic pesticides which pose a potential direct threat to freshwater organisms, particularly to sensitive animals, such as fish. Chlorpyrifos is an organophosphate insecticide and is highly toxic to freshwater fish. Fish have an important role in the food chain; therefore, investigation of the effects of toxic pesticides such as chlorpyrifos on fish has a diagnostic significance in evaluation of negative effects of pesticides to human health. Keeping in view of the above facts, in this review, an attempt has been made to elucidate the adverse impact of chlorpyrifos on the fish.

Keywords: Genotoxicity, blood variables, biochemical changes, histopathology, endocrine dysfunction, immune depression

Introduction
In India, pesticides constitute an important component in agriculture development and protection of public health since the tropical climate is very conducive to pest breeding [1]. Contamination by pesticides in aquatic ecosystem is a serious problem and fishes are more frequently exposed to these pollutants and may be taken in through gills, skin and contaminated foods [2]. Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems [3]. Chlorpyrifos is a widely used organophosphate pesticide, second largest selling in India and used for more than a decade to control pests on cotton, paddy fields, pasture and vegetable crops [4]. Its extensive use may increase the toxicity load to aquatic environment, causing adverse effects on non-target organism, fish. Acute and chronic toxic effects of chlorpyrifos in different fish species were extensively studied [5-15]. Sublethal toxicities of chlorpyrifos in aquatic environments can induce morphological, neurobehavioural, oxidative, biochemical, histopathological, haematological, developmental alterations etc. while the lethal levels cause mass mortalities in non-target organisms in general and fish in particular. This review is an attempt to document the toxic impacts of chlorpyrifos on fish, a non-target organism and provide a base line data for the further research investigations contemplated to elucidate the toxic impacts of various pesticide chemicals used in agri and aqua ventures.

Genotoxicity
Most of the pesticides are genotoxic [16] having the potential of causing DNA damage, increased incidences of neoplasia and adverse effect on vitality and progeny of aquatic animals, which may reduce the productivity of aquaculture. Anita *et al.*, [5] analyzed the incidence of nuclear anomalies in the blood cells of freshwater fish *Cirrhinus mrigala* using micronucleus (MN) assay, in which they found that MN induction was highest on day 14 at 0.08 mg/L concentration of chlorpyrifos. Alteration in cell morphology, presence of nuclear anomalies as broken egg and large size micronuclei, alteration in cell morphology besides micronuclei confirming the effect of chlorpyrifos on the nucleus were also clearly evident from their study.

Micronuclei assay results obtained by Palanikumar *et al.*, [17] reported increased abnormalities in *Chanos chanos* with increasing doses of chlorpyrifos. Ali *et al.*, [18] studied the MN induction in *Channa punctatus* on exposure to chlorpyrifos (203 µg/L) on days 1, 3, 5, 7, 14, 21, 28, and 35 and observed maximum MN induction (1.62%) on day 14. The highest DNA damage was observed on day 5, followed by a gradual nonlinear decline in the lymphocytes and gill cells. Toxicant produced a concentration dependent increase in DNA single-strand breaks in the form of comet induction and a time-dependent decrease in the damage, due to the DNA repair. Gollapudi *et al.*, [19] observed both concentration and time dependent increase in MN induction due to genetic toxicity of the chlorpyrifos in fish.

Haematological Alterations

The exposure of aquatic organisms to various stressors and pollutants including very low levels or sublethal concentration of pesticides in their environment generally cause rapid changes in various haematological characteristics of fish [20].

Haematological parameters are important for toxicological research and have been widely used in environmental monitoring, and as indicators of disease and environmental stress. Many studies have demonstrated changes in blood variables as a result of environmental conditions and presence of contaminants such as chlorpyrifos.

Ali and Kumar [9] reported decreased erythrocyte, leukocyte, haemoglobin and hematocrit mean levels in *Channa punctatus* exposed to two sublethal concentrations (203 and 68.0 µg/L) of chlorpyrifos for 1, 3, 7, 14 and 21 days. Chlorpyrifos had a negative effect on the hematological parameters and the antioxidant enzyme systems of *Cyprinus carpio*. DNA damage by chlorpyrifos in *Channapunctatus* using micronucleus (MN) and comet assays has also been investigated by Ali *et al.* [18]. They found that MN induction in the blood was highest on day 14 at 203 µg/L of chlorpyrifos. The highest DNA damage was observed on day 5, followed by a gradual nonlinear decline in the lymphocytes and gill cells. Malla *et al.*, observed significant changes in *Channa punctatus* induced by chlorpyrifos in haematological parameters like erythrocyte sedimentation rate (ESR). The ESR (mm/h) ranges from 2.41 to 2.75 mm/h with an average of 2.56 mm/h. An increase in ESR (mm/h) from 2.57 mm/h to 2.83 mm/h for 24 h to 96 h respectively was recorded in 10 ppm of chlorpyrifos, when compared to control group. Similar increase in ESR (mm/h) from 2.61 mm/h to 2.86 mm/h for 24 h to 96 h respectively was recorded in 15 ppm of chlorpyrifos. Chlorpyrifos after 24 h showed decrement in RBC (-72.43%) and haemoglobin (-18.35%) and an increment in WBC (+57.94%) compared to controls in *Cyprinus carpio* [13]. Chlorpyrifos effect reported by Chindah *et al.* in *Tilapia guineensis* showed decreased indices of blood after exposure to chlorpyrifos at different concentrations. Sub-lethal levels of chlorpyrifos at different concentrations (0.0006, 0.00125, 0.0025, and 0.005 ppm) for 8 weeks showed significant variations in erythrocyte, leucocyte, and haematocrit were observed between treatments.

Biochemical Alterations

Pesticides can cause serious impairment to physiological and health status of fish. Therefore, biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides and can be a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish. Chlorpyrifos induced biochemical alterations in fish reported by various researchers suggested they are good parameters which help to see the effects of toxicants on metabolism of fish. Padmini and Rajaram studied the effect of different concentrations of chlorpyrifos viz. 0.04, 0.045, 0.05, 0.055, 0.06 and 0.065 ml/L on protein, glycogen and lipid in liver and kidney of *Channa gachua* for 96 h and reported decreased levels in both the tissues in comparison with control. Sub-lethal dose of chlorpyrifos (0.284 ppm) administered in *Heteropneustes fossilis* for 5, 10, 15, 20, 25 and 30 days induced alternations in serum concentration of tri-iodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH). In particular, possible impairment of thyroid function was demonstrated by the significant decrease ($p<0.01$) in serum T_3, T_4 and TSH levels. Chlorpyrifos significantly decreased total protein, catalase, glutathione Stransferase and induced lipid peroxidation in *Chanos chanos*. Maximum effects of protein, catalase, lipid peroxidation and glutathione s-transferase were obtained in response to 23.µg/L of chlorpyrifos.[17] Sharma observed an increase in ACP, ALP, LDH activities and decrease in ATPase at different concentrations (0.284, 0.094 and 0.284) of chlorpyrifos for 15, 30 and 45 days. Topal30 observed decreased CA enzyme activity in the gills and a time-dependent decrease in CA activity in liver tissues of rainbow trout exposed to 2.25, 4.5 and 6.75 µg/L of chlorpyrifos for 24, 48, 72, and 96 h.

Banaee *et al.*, [8] reported hyperglycemia, increased blood triglyceride, and increased levels of AST, LDH and CK activities *Cyprinus carpio* exposed to chlorpyrifos for 10, 20 and 30 days. The most important alterations in the blood biochemical parameters were measured in the specimens exposed to 40 µg/L chlorpyrifos on the 20th and 30th day of the trial. Varied response of protein profiles in liver tissue of *Carassius auratus* exposed to chlorpyrifos (0.025 ml/L) for 15, 60 and 180 m were reported by Vaidehi *et al*. Khan and Sharma reported that sublethal concentrations of chlorpyrifos (10%, 20% and 30% of 96 h LC_{50} i.e. 0.028 ppm) for 15, 30 and 45 days caused increased activity of acid phosphatase and alkaline phosphatase in liver and kidney tissues of *Gambusia affinis.*

Reddy *et al.*, [10] studied the effect of sublethal concentrations of chlorpyrifos on protein metabolism in gills, kidney, liver, and muscle of *Clarias batrachus* exposed to 0.825 mg/L and 1.65 mg/L for 7, 14, 21, and 28 days. Total protein, amino acid, and ammonia contents were decreased in all tissues for 28 days. Urea and glutamine levels were elevated, except in kidneys. The activities of protease, alanine, and aspartate aminotransferases, and acid and alkaline phosphatases were elevated in the tissues for 28 days exposure at both concentrations. Saradhamani and Binukumari noticed significant decrease in glycogen, protein and lipid in some tissues of *Oreochromis mossambicus* after long term exposure of chlorpyrifos. An increase in MDA levels and a decrease in non-enzymatic antioxidant levels were reported in different tissues of *Poecilia reticulata* exposed to chlorpyrifos. Tripathi and Shasmal reported inhibitory effect of chlorpyriphos on LDH activities in different tissues of the fish. Decreased levels of antioxidant enzymes SOD, CAT and GR activities were also

observed in the exposed fish. Increased levels of GST activity was observed in *Oreochromis niloticus* with chlorpyrifos exposure [11].

Srinivasa Rao *et al.*, observed insignificant alteration in proteins at the end of 48 h initially in *Labeo rohita* exposed to pesticide (0.1891 ppm) for 2 days. Significant and maximum depletion was observed in the head and minimum in muscle.

Kavitha and Rao observed an elevated lipid peroxidation level in *Gambusia affinis* exposed to lethal concentration of chlorpyrifos for 96 h. Ramesh and Saravanan[13] observed decrement in plasma protein (-16.46%) and an increment in plasma glucose (+26.35%) in *Cyprinus carpio* exposed to chlorpyrifos. A fall in glycogen levels and an increase in enzyme levels in the fish *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* on exposure to sublethal concentrations of chlorpyrifos were also evident. Tilak *et al.*, observed the total protein depletion in tissues of muscle, liver, kidney, brain and gill of *Labeo rohita* exposed to both technical as well as 20% EC of chlorpyrifos. Elevation in blood glucose levels was observed in *Sarotherodon mossambicus* due to the effect of acute exposure of chlorpyrifos. Hypocalcemic response has also been noted in *Heteropneustes fossilis* exposed to high sublethal concentrations of chlorpyrifos.

AChE activity is considered to be a specific biomarker of exposure to organophosphorus insecticides like chlorpyrifos. In aquatic animal models, most of the observations estimates lie in the range of 70 to 90% AChE reduction for tissues when exposed to chlorpyrifos. However, Sharbidre *et al.*, reported that brain AChE showed dose-dependent inhibition up to 66% in *Poecila reticulata*, exposed to chlorpyrifos. *Ctenopharyngodon idellus* exposed to 1.44 µg/L and 2.41 µg/L of chlorpyrifos altered the activity of acetylcholinesterase in liver, kidney and gills at regular intervals of 15, 30 and 60 days of chronic exposure, and at the same intervals showed recovery response with a direct relationship between the inhibition of acetylcholinesterase activity and increase in the pesticide concentration and exposure period. A decreases in the AChE activity levels in different tissues of fish were observed after exposure to chlorpyrifos.

Reduction in AChE activity in *Channa punctatus* exposed to two sublethal concentrations (203 and 68 µg/L) of chlorpyrifos for 1, 3, 7, 14 and 21 days was evident [9] in which the highest reduction in AChE activity was in brain followed by gill and blood plasma in both sublethal concentrations. Kavitha and Rao reported an inhibition of AChE activity in *Gambusia affinis* exposed to lethal concentration of chlorpyrifos for 96 h. Chawanrat *et al.*, [14] observed variability in acetylcholinesterase upon exposure to sublethal concentrations chlorpyrifos in brain, liver, muscle and gill tissues of hybrid catfish (*Clarias macrocephalus* X *Clarias gariepinus*) for 4 days. AChE inhibition increased rapidly with insecticide concentration. Relative inhibition of AChE was higher in larger fish but did not differ significantly with sex. Relative inhibition of AChE accompanying insecticide exposure was highest in the brain tissues and progressively less in the liver, muscle and gill tissues. Insecticide concentrations and AChE inhibition in the brain increased over the 4-day sublethal exposure. Sub-lethal concentration (60 µg/L) of chlorpyrifos on *Gambusia affinis* carried out *in vivo*, for 20 days altered the locomotor behavior in relation to bioaccumulation and interaction with AChE resulted in the inhibition of the enzyme activity (40 to 55%) in brain and also bioaccumulation of the toxicant in different parts of fish. The accumulation of chlorpyrifos was maximum in viscera followed by head and body. Sandahl *et al.*, studied the relationship between AChE activity and behavior of *Oncorhynchus kisutch* exposed to chlorpyrifos (0-2.5 mg/L) for 96 h. Chlorpyrifos inhibited tissue AChE activity in a dose-dependent manner.

Rao *et al.*, [4] observed 90% inhibition of acetylcholinesterase activity in *Oreochromis mossambicus* after exposure to chlorpyrifos. Benchmark concentration (BMC) approach by Sandahl and Jenkins reported that, chlorpyrifos significantly inhibits brain AChE activity at concentrations below a part per billion. *In vitro* studies conducted by Carr *et al.* on *Gambusia affinis*demonstrated that muscle AChE is more sensitive to organophosphates than that in brain. Altered locomotor behaviour of fish could be attributed to the accumulation of acetylcholine which interrupted coordination between the nervous and muscular junctions. Considering the role of AChE in neurotransmission in both central nervous system and at neuromuscular junctions, the inhibition of AChE activity could be correlated to behavioural changes observed in various fish exposed to chlorpyrifos.

Histopathological Alterations

Histological information, along with physiological and biochemical data may provide a more complete and accurate description of the activity leading to death of the organisms due to a chemical agent. Histopathology may therefore prove to be a cost effective tool to determine the health status of fish populations and hence reflect the health of the entire aquatic ecosystem in the biomonitoring process. Chlorpyrifos induced alterations in histopathology of various fish species are extensively studied. Manjunatha and Philip observed vacuolization and presence of sinusoid spaces in liver tissue of *Danio rerio* exposed to 200µg/L of chlorpyrifos for 24, 48, 72 and 96 h. Topal *et al.*, observed hyperaemia and degenerative changes in liver and lamellar hyperaemia, lamellar oedemas, clumping, cellular degeneration, hyperplasia, and lamellar atrophy in gill of rainbow trout exposed to 2.25, 4.5 and 6.75 µg/L of chlorpyrifos for 24, 48, 72, and 96 h. Devi and Mishra reported that sublethal concentrations of chlorpyrifos (1.46 µl/L and 0.538 µl/L) for 3 and 7 days caused histopathological changes in liver and gill tissues of *Channa punctatus.* The main histopathological changes in gills exposed to the highest concentration were edema, lifting of lamellar epithelia and an intense vasodilatation of the lamellar vascular axis. Lamellar fusion caused by the filamentary epithelium proliferation and some lamellar aneurisms were also found. Hypertrophy; hyperplasia and lifting of epithelial cells and fusion of secondary lamellae in gill were pronounced in all treatment.

The liver showed vacuolation and necrosis. These hepatic alterations were more evident at higher concentrations and long term exposure tenure. Liver showed hypertrophy of hepatocytes, infiltration of leukocytes, necrosis and fibrosis. Even low dose of chlorpyrifos produced severe pathological lesion for long term exposure in gills as well as liver.

Melanomacrophage aggregations, cellular atrophy, pyknotic nucleus, cytoplasmic vacuolation, cytoplasmic and nuclear degeneration, cellular rupture, necrosis, and nuclear and cellular hypertrophy in the liver tissues of *Cyprinus carpio*

after 14 days of exposure of chlorpyrifos in doses of 1 and 100 µg/L.55 *Cyprinus carpio* exposed to chlorpyrifos showed alterations in structure of the gills and liver. The liver tissue revealed different degree of hydropic degeneration, vacuolisation, pyknotic nuclei, and fatty infiltration while the gills displayed varied degrees of epithelial hypertrophy, telangiectasis, oedema with epithelial separation from basement membranes, general necrosis, and epithelial desquamation. Shrinkage of glomeruli with wide urinary spaces was reported in fish after 3 weeks of treatment with 2.64 µm Lorsban have been noted. Aniladevi *et al.*, observed histopathological alterations in gill and liver tissues of *Oreochromis mossambicus* exposed to sublethal concentration (82 µg/L) of chlorpyrifos for a period of 21 days. In liver, for a period of 7 days, hepatocytes appeared swollen with granular cytoplasm. Pancreatic acini appeared to loss the normal structure and were found in necrotic state. Cytoplasm of hepatocytes became more basophilic indicating the protein precipitation, which led to necrosis of cell. The cells became more rounded-off showing acute necrosis and also glycogen depletion. 21 days of long-term exposure resulted in necrosis of the hepatic tissue. Completely vacuolated areas were observed with fat deposition. Biliary hyperplasia was observed at certain regions of the hepatic tissue. On short-term exposure, the changes observed in gills were hyperemia, clubbing and edema. After 21 days of pesticide exposure, gills became edematous with prominent clubbing. Separation of primary gill lamellae, and hemorrhage in the blood vessels outside the secondary gill lamellae were observed. Hyperemia of the gill filaments that engorged with blood vessels appeared. Hyperplasia was observed in secondary gill lamellae, which led to fusion of adjacent primary and secondary gill filaments.

Tilak *et al.*, reported degenerative changes such as degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels, necrosis and disappearance of hepatocyte wall, deposition of hepatic cords, decrease in size of nucleus, pycnotic and vacuolar degeneration within the nucleus in *Catla catla* under chlorpyrifos toxicity. These degenerative changes are further intensified in lethal exposures. Rao *et al.*, [4] observed the bulging of secondary lamellae at the terminal ends, lesions and erosions at the base of lamellae on 12th day of exposure of *Oreochromis mossambicus* to chlorpyrifos. A thick coat of mucus on the gill filaments was persisting on 18th day of exposure. De Silva and Samayawardhena observed shorter gill lamellae, fusion, complete destruction of lamella, increased vacuolation, irregular appearance of gill lamellae in *Poecilia reticulata* exposed to chlorpyrifos. Shrinkage of glomeruli with wide urinary spaces was reported in catfish exposed to 2 mg/L and 113 µg/L of chlorpyrifos.

Endocrine Dysfunction

Srivastav *et al.*, reported that short-term exposure of coroban (0.8 of 96 h LC_{50} value i.e. 1.76 mg/L) for 96 h caused decrease in the serum calcium levels. No change was noticed in the prolactin cells of chlorpyrifos treated fish. In long-term exposure (i.e. 0.44 mg/L) for 7, 14, 21, and 28 days provoked hypocalcemia. The prolactin cells of treated fish exhibited slight degranulation after 21 days whereas the nuclear volume remained unchanged. After 28 days, the prolactin cells exhibited further degranulation and the nuclear volume recorded an increase. Cytolysis and vacuolization were also visible in test animal *Heteropneustes fossilis*. In *Oreochromis niloticus*, chlorpyrifos exposure caused decrement in serum estrogen and testosterone levels. Estradiol level after 15 days of exposure decreased by 60.45%, 48.65%, 56.93% after 5, 10, 15 ppb chlorpyrifos treatments. Cortisol level was also found to be lower than that of control level after 10 ppb (59.97%) and 15 ppb (39.41) chlorpyrifos treatments.[11]

Immunological Alterations

Fish immune system, important for defense against a variety of harmful pathogens is very sensitive to homeostatic adjustments via endocrine regulation and is influenced by biochemical profile of the nervous system. Insecticides can alter the immune functions of the body and result in immune-depression, uncontrolled cell proliferation, and alterations of the host defense mechanism against pathogens. Effects of chlorpyrifos on the immune factors of fish such as mRNA levels of IL-1β and IFN- γ2b in immune organs of common carp have been reported by Wang *et al*. Díaz and Girón7 studied the immune response parameters of *Oreochromis niloticus*. Results indicated that chlorpyrifos at 0.051 mg/L induced a diminishment in concentration of lgM in plasma. On the other hand, organisms exposed to high concentration of the pesticide showed an increase in the lysozyme activity. Girón *et al.*, [15] reported that *O. niloticus* exposed to 0.422 and 0.211 mg/L of chlorpyrifos during 96 h caused significant decrease in the phagocytic capacity and in the percentage of phagocytic cells present in blood.

Developmental Alterations

Sreedevi *et al.*, observed potential developmental effects of non-lethal concentrations (400 µg/L, 600 µg/L, 800 µg/L and 1000 µg/L) of chlorpyrifos in *Danio rerio*. Fertilized eggs of the same developmental stage, 4 h post fertilization showed delay in hatching at higher concentrations. Abnormalities like edema, difference in yolk sac size and decrease in pigmentation were also observed in embryos before hatching, where as in larvae edema, shrinking of yolk sac and dorsal curvature of the body was noticed. Richendrfer *et al.*, observed that the administration of sub-chronic dose of 1 µM chlorpyrifos to zebrafish larvae from 0 to 7 day post fertilization significantly impacted body morphology.

Exposure to different concentrations of chlorpyrifos was shown to cause significant spatial discrimination impairments, response latency, reduction in swimming activity and impaired learning. Levin *et al.*, found that embryonic exposure of zebrafish to clorpyrifos caused significant impairment in discrimination learning and swimming speed.

Conclusion

It is evident that chlorpyrifos presented in aquatic ecosystems can affect aquatic fauna in different ways. Alterations in physico-chemical properties of water, destruction of delicate balance in the environment, entry into the food chains and physiological damage to the vital tissues of aquatic fauna are the threatening issues of the modern day pesticides. Long term exposure to these products causes countless abnormalities and reduces the life span of organisms. Finally, we conclude that chlorpyrifos is highly toxic to fish, and impose life threatening effect on

fish at both lethal and sublethal concentrations. Altered biochemical, histopathological and haematological responses can be used as tools in bioassessment to monitor ecotoxicological risks associated with pesticides such as chlorpyrifos to various fish.

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