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Biochemical toxicity of organophosphate derivatives in fishes

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Abstract
Organophosphorus compounds have replaced organo-chlorines to a greater extent and are frequently sprayed on agricultural crops. These xenobiotics primarily find their way into aquatic ecosystems thereby induce toxicity to non-target species. Among the various harms caused to fishes by organophosphates, alteration in their biochemical indices is important as it induces severe damage to various vital metabolic processes in fish. The primary effect of organophosphate toxicity, which is their mechanism of action as well, is the inhibition of acetylcholinesterase enzyme in synapses which leads to severe morbidity and loss of balance and motion or even paralysis. Various other biochemical metabolisms like protein metabolism, lipid metabolism, carbohydrate metabolism and liver enzymes get severely impaired due to the toxicity induced by organophosphates. Therefore, it is necessary to protect the natural water bodies from getting contaminated with pesticides to allow fishes to grow healthier and harmless for human consumption. The present paper reviews the work done on biochemical toxicity of organophosphorus compounds in fish.

Keywords: Aquatic ecosystems, biochemical alterations, fish, organophosphates, toxicity

Introduction
Life is possible only through the metabolic processes of cells, mainly by requiring constant supply of nutrients, and oxygen and by constant removal of waste products. In vertebrates, metabolic processes were done in the blood vascular system [1], which is regulating the life activities are seen in this between homeotherms and poikilotherms because it is the communicating tissue and medium for all the cells of a body. Fishes belonging to different taxonomic groups are adopted variously depending on different prevailing ecological conditions. Haematological characteristics are an important tool that can be used to understand as an effective and sensitive index to monitor physiological and pathological changes in fishes. Changes in haematological parameters depend upon the aquatic biotope, fish species, age, and sexual maturity and health status [1-9]. Various blood parameters in fish have been established by different investigators in fish physiology and pathology [7, 11, 15, 17]. Organophosphates constitute a large group of chemicals used over past 60 years to protect the crops, livestock and human as well as is used as warfare agents. Based on the structural characteristics, they have broadly been divided into 13 types, including phosphates, phosphonates, phosphinates, phosphorothioates (S=), phosphorothioates (S=), phosphoro-thioates (S substituted), phosphonothioates (S substituted), phosphorodithioates, phosphoro-trithioates and phosphoramidothioates [19-22]. These are probably the most pervasive compounds, many of which form important biochemical substances like DNA, RNA and various co-factors, essential for the sustenance of life. In addition, organophosphates represent a diverse class of insecticides or nerve agents acutely toxic to bees, wild life and human populations. Organophosphates were developed in Germany during 1930's as a potential chemical warfare and are presently used to control insects on fruits, vegetables, grain crops and stored seeds. In household, these compounds are efficient in controlling cockroaches, houseflies, termites, etc. and effective in protecting the horticultural and field crops from insect pest attacks [23-30]. Use of organophosphate insecticides is on rise as they, unlike organochlorine compounds, are less persistent in the environment. They quickly are transformed into nontoxic compounds, despite the fact that of having high non-specific acute toxicity that may lead to frequent intoxication of non-target organisms [31, 32]. However, under specific conditions some organophosphates remain active in soils upto six months after their application, thus increasing the duration which may be harmful to non-target species [33-39].

In natural waters, they have several mechanisms of degradation such as oxidation, photodegradation and hydrolysis [40]. This is what actually determines the pattern of insecticide usage in India which is 76% against 44% total insecticide use globally [41-49]. Organophosphorus compounds like other classes find their way into aquatic habitats such as rivers, lakes and ponds [50]. They have been found toxic to the organisms contributing substantially to the food chain and water bodies [51-59]. Fishes which encounter with organophosphorous compounds develop various metabolic abnormalities [60, 62, 63]. (John, 2007). Pesticides move into environmental components either by spray drift, aerial spray or washing from the atmosphere, by precipitation, leaching, percolation and/or runoff from agricultural land [60]. Most insecticides ultimately find their way into rivers, lakes and ponds [61-69]. The problems associated with organophosphates include their lack of specificity and high water solubility. They are able to enter aquatic environment where they prove toxic to both vertebrates and invertebrates [70]. This illegitimate entrance of organophosphate xenobiotics leads to bio-magnification when contaminant load increases significantly with each successive trophic level [16-19, 21, 39, 49, 53]. Bio-accumulation and bio-magnification of these substances in aquatic organisms including fish leads to chronic toxicity and behavioural changes in extreme cases. In fish, the uptake of pesticides take place through absorption by gills and residue uptake through gills and is related to the metabolic rate and body size [11-19, 25, 32, 41, 51]. These xenobiotics not only lead to higher instances of toxicity but alter various biochemical indices including some important physiological processes and enzyme kinetics in fishes. There are various biochemical alterations caused by the toxicity of organophosphorus compounds in fishes.

- **Biochemical alterations in fishes due to organophosphorus toxicity**

Teleosts exhibit severe biochemical alterations due to the toxicity of organophosphorus pesticides. These xenobiotics are potent to cause physiological dysfunction thereby affecting the organisms at organ, cellular and molecular levels which, in turn, is responsible for biochemical changes in fish. Various biochemical alterations in fish due to organophosphorus toxicity include inhibition of acetylcholinesterase enzyme, impaired metabolism of carbohydrate, protein and lipid metabolism and alterations in enzymes. Behavioural changes in fish due to acetylcholinesterase enzyme are the prime indicator of organophosphorus toxicity in fish. Plasma enzymes levels in fish have also been proposed to be good indicators of extreme stress and provide information of organ dysfunction [10-18]. Enzymes are increasingly used not only as indicators of pathological processes but also as indicators of stress (Jiminez and Stegeman, 1990). Biochemical and physiological indicators such as enzymes could be used as biomarkers to identify possible environment contamination before the health of aquatic organisms is seriously affected [27, 38, 44, 55]. Changes in plasma enzyme activity are used as indicators of tissue injury, environmental stress or a diseased condition. The rate of increase in plasma enzyme activity depends on the concentration of enzyme in cells, the rate of leakage caused by injury and the rate of clearance of enzyme from plasma [17, 56, 58].

- **Inhibition of acetylcholinestrase**

Due to their effect on wide class of organisms, organophosphorus insecticides are widely sprayed in orchards and agricultural fields. They depict cholinergic property (carbamates group of pesticide also act on the same enzyme but through a different mechanism), hence act as neurotoxins to a broad spectrum of pests. The inhibition of enzyme has been observed in different tissues of fishes including their brain [27, 29, 31]. Inhibition of this enzyme leads to the accumulation of acetylcholine in the synapse, resulting in increased firing of the postsynaptic neuron or increased neuro-effector activity. The consequences of increased cholinergic activity in parasympathetic autonomic nervous system (muscarinic receptors) may include increased mucous secretion, loss of balance, frenzied movements with alternate quiescent stages, hypoxia and erosion of gills. The effects of increased neuroeffector activity on skeletal muscles may include muscle fasciculation, cramps, muscle weakness and depolarization type paralysis [9, 10, 16, 19]. In addition to the acute effects, some organophosphorus compounds have been found associated with delayed neurotoxicity, known as organophosphorus-induced delayed neuropathy (OPIDN) [29, 32]. Inhibition of acetylcholinesterase due to organophosphorus compounds and a positive correlation between them in fishes has been studied by several workers [8, 19, 29, 38, 44, 57].

Protein metabolism

Proteins are primarily involved in cell architecture, which is a chief source of nitrogenous metabolism and source of energy during chronic periods of stress. Protein metabolism in fishes reflects decreased alterations in total protein content levels after an acute exposure of organophosphorus pesticides [35, 39, 44, 57]. During stress conditions, fish needs more energy to detoxify toxicants; hence more protein is required to meet the increased energy demands to overcome stress conditions. Thus depletion of protein fraction in liver may have been due to their degradation and possible utilization for metabolic purposes. [69, 72, 79, 80] who studied the effects of sub-lethal doses of dichlorvas 76% on *Labeo rohita* and *Cirrhina mrigala* fingerlings drew similar conclusions. [80] Reported decrease in total proteins, glycogen and even in DNA and RNA contents due to organophosphorus toxicity. Decrease in protein levels may also be due to their degradation or destruction of hepatocytes or due to necrotic changes occurring in organophosphate toxicity and consequent impairment in protein synthesis machinery [75, 79, 80]. Oxidative stress due to organophosphate toxicity has also been found responsible for protein degradation in fishes [9, 11, 13, 17, 29, 30, 42, 56, 70] while working on biochemical changes induced by dimethoate in the liver of fresh water fish *Puntius ticto* (Hamilton) reported a significant decrease in protein levels in testis, ovary and brain; slight decrease in intestines, muscles, liver and gills, and increase in protein levels in kidney. Chronic toxicity reflected a decrease in the levels of protein content in ovary, brain, intestine, muscles, gills and liver to 2.506 and 1.253 ppm exposure; whereas in testis protein level was increased to 1.253 ppm. [79, 80] found a significant decrease in total protein levels, both in albumin and globulin content after acute exposure of *Heteropneustes fossilis* to an organophosphorus pesticide Nuvan. Thus, it may be concluded that exposure to organophosphates leads to

severe hindrances in vital metabolic processes so induce severe alterations in lipid metabolism in fishes.

- **Carbohydrate metabolism**

The blood glucose level serves as an indicator of biological stress caused by pollutants such as pesticides and metals [51] reported that a lethal concentration of 6 mg/L sumithion, an organophosphate pesticide, increased blood glucose level and phosphorylase activity in freshwater fish *Sarotherodon mossambicus*. A reduction in hepatic glycogen indicating impairment in the carbohydrate metabolism has been reported in *Anabas testudineus* treated with acute lethal and sublethal concentrations of Furadan [41, 51]. A reduction in the glycogen content was noted in whole body homogenate of fingerlings of *Labeo rohita* and *Cirrhina mrigala* exposed to different sublethal concentrations of nuvan [59, 79, 80] studied the acute effect of dimethoate on some aspects of carbohydrate metabolism of hepatic tissue in cat fish, *Clarias batrachus*, after 1, 2, 4 and 8 days of exposure to sublethal concentration. They reported depletion in glycogen content and increase in lactate level. The activity levels of glycogen phosphorylase in the hepatic tissue also increased. [80] observed that the fish exposed to dimethoate seemed to induce glycogenolysis, possibly by increasing the activity of glycogen phosphorylase to meet the energy demand under stress condition or the toxicant might have affected glycogenesis by inhibiting the activity of carbohydrate metabolism. These results suggest that carbohydrate metabolism was adversely affected in hepatic tissue by organophosphate pesticide dimethoate. Fall in the glycogen level in gills of *Punctius ticto* indicates its rapid utilization to meet the enhanced energy demands under dimethoate toxicity through glycolysis or hexosmonophosphate pathway [39, 48, 56]. It could also be due to the prevalence of hypoxic or anoxic conditions, which normally enhances glycogen utilization [71]. Enhanced utilization of glycogen and its consequent depletion in tissues may be attributed to hypoxia as it increases carbohydrate consumption. Under hypoxic conditions, the animals derive their energy from anaerobic breakdown of glucose, available to the cell by the increased glycogenolysis [29, 34, 49, 55]. Further, the decline in glycogen might partly be due to its utilization in the formation of glycoprotein and glycol-lipids, which are the essential constituents of various cells and other membranes.

The effect of fenitrothion on the energy metabolism of European eel, *Anguilla anguilla* was studied by [59] who reported no significant change in liver glycogen level but increase in blood glucose levels. Similarly freshwater cat fish, *Clarias batrachus*, when exposed to sublethal concentrations of an organophosphate and a carbamate pesticide reflected hyperglycaemic response to both the pesticides [80]. General hypoglycemia and reduction in hepatic glycogen content in fishes exposed to experimental concentrations of different pesticides has previously been reported [9, 19, 25, 32, 41, 51] observed a decrease in carbohydrate content of fish *Labeo rohita* exposed to sublethal concentration of monocrotophos. Increased blood glucose level was found in the same fish in response to the toxicity of cypermethrin [29, 30]. Exposure of fishes to organophosphates elicits consistent hyperglycemia apart from liver and muscle glycogenesis [39, 59]. Apart from decrease in total protein due to organophosphorus toxicity, various other alterations such as increase in plasma glucose [25, 32, 41, 51], amino acids [41, 51] and ammonia [24-39] has also been reported. [29, 34, 45, 64] reported that glycogen, cholesterol and ascorbic acid contents in the gills decreased after organophosphorus exposure. [38, 46, 80] studied the acute effect of dimethoate on carbohydrate metabolism of hepatic tissue in cat fish, *Clarias batrachus*, after 1, 2, 4 and 8 days of exposure to sublethal concentration and found that the glycogen content was depleted and lactate level increased. The activity levels of glycogen phosphorylase in hepatic tissues also showed increase. These results conclude that carbohydrate metabolism is adversely affected in hepatic tissue by organophosphate pesticide toxicity.

- **Lipid metabolism**

Lipid metabolism in fishes is seriously impaired due to organophosphate exposure. Several studies support the opinion that lipid metabolism is altered due to the acute organophosphorus toxicity. However, [29, 36, 42, 55] reported that cythion and hexadrin caused no significant variations in liver and ovarian lipid content in freshwater fish, *Heteropneustes fossilis*. On the other hand, [15, 22, 74, 80] studied the combined and individual effects of carbaryl and fenthoate on the levels of total lipids, free fatty acids, cholesterol and lipase activity in fish *Channa punctatus* and reported that free fatty acids, cholesterol and lipase activity were increased while total lipid was decreased. [1-19] studied the effect of two pesticides, an organophosphate and an organochlorine, on total lipid and its various fractions. Except for the elevated liver lipid in males in response to malathion, no significant change in total lipid was observed following pesticide exposure. The organophosphate methyl-parathion was found to reduce the efficiency of hormone sumaach to increase the ovarian activity in freshwater fish *Channa punctatus* and lipid content of the ovary under treatment was reduced with sumaach [80]. There was a decrease in liver cholesterol content (25%) in fish *Ctenopharyngodon idella* exposed to sublethal doses of fenvalerate at the end of 1st week of exposure and it became 48% at the end of 4th week [72, 79, 80]. Plasma cholesterol and triglyceride contents decreased during 96 hour treatment with sublethal concentration of diazinon in fish *Anguilla anguilla* [62, 65]. They reported that similar to lipid content there was a reduction in cholesterol content as well, even under the effect of sumaach, whereas in control fishes the sumaach was able to increase the amount of ovarian cholesterol concentration. A decrease in total lipid content of liver, muscle and gill of two different size groups of *Tilapia mossambica* exposed to phosalone suggested that lipids might have been channeled for energy production under stress condition [24, 29, 30, 80] reported an increase in cholesterol content of testes in fish *Glossogobius giuris* treated with sublethal concentration of fenthion. [29, 33, 49, 79] reported malathion induced depletion of cholesterol in heart muscles of fish *Glossogobius giuris*. Therefore, it is evident that organophosphates induce toxicity in fish, thereby impair lipid metabolism in them.

- **Impact on enzymes**

Insecticides may cause serious impairment to physiological and health status of fish. Biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides [16-19, 21, 39, 49, 53] and serves as a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish. Enzyme

activities in fishes are severely disrupted due to pesticide exposure. Liver enzymes show alterations directly proportional to the time of exposure. Blood biochemistry test indicates the happening in fish body exposed to insecticides. When different tissues are injured, the damaged cells release specific enzymes into plasma and one can recognize their abnormality levels in blood. Then it helps to locate the lesions. Also, if certain organs are not eliminating certain waste products or are not synthesizing certain important materials, this suggests that they are not functioning properly. In some cases due to the severity of damage to the tissues, particularly liver, the synthesis of many biochemical parameters may reduce significantly in cells, which may decrease some biochemical factors in blood of fish exposed to insecticides. These changes were reported in *Heteropneustes fossilis* [29], *Cirrhinus mrigala* ([53] and *Oncorhynchus mykiss* [17] exposed to dimethoate, cypermethrin, and diazinon, respectively. The activities of aspartate and alanine amino transferases (AAT and AAT) which serve as strategic links between protein and carbohydrate metabolisms are known to alter under several physiological and pathological conditions [9]. Glutamate dehydrogenase (GDH), a mitochondrial enzyme, catalyses the oxidative deamination of glutamate and provides α-ketoglutarate to the Kerb's cycle [6]. This enzyme has metabolic functions with great physiological significance and is closely associated with the detoxification mechanisms of tissues. GDH in extra-hepatic tissues could be utilized for channeling of ammonia released during proteolysis for its detoxification into urea in liver. Hence, the activities of AAT, ALT and GDH are considered as sensitive indicators of stress [17]. Levels of these enzymes get elevated on account of acute organophosphate exposure indicating stress conditions in fishes due to toxicity [2, 9, 11]. Similar results were reported by [7] who worked on biochemical alterations induced in *Channa punctatus* by an organophosphate triazophos. [36-49, 61, 79, 80] suggested that the steady rise in the activities of GDH, AAT and ALT in the organs of fish exposed to sublethal concentration of cypermethrin may be due to the synthesis of these enzymes under sub- acute cypermethrin stress. The increase in these enzyme activities could be helpful to the fish for structural reorganization of proteins and incorporation of keto acids into TCA cycle to favour gluconeogenesis or energy production. The activity of LDH reportedly decreased on account of organophosphorus exposure to fishes [71-80] reported increase in the activity of enzymes AAT, ALT and LDH due to the toxic stress of alachlor in *Channa punctatus*. Increase in the activity of LDH has also been reported by Amanullah *et al*. (2010) in gills, hepato-pancreas and muscle. [40-45] reported increased alkaline phosphatase, acid phosphatase and cholesterol while many workers reported decrease [16-19, 21, 39, 49, 53] studied the effects of organophosphate, diazinon, on some biochemical changes in *Rutilus frisii* kutum male brood stocks and reported that diazinon caused an increase in AST, ALP and adrenaline. Various enzymes like serum creatinine, bilurubin and serum urea and different ions like blood potassium, calcium, magnesium and liver chloride were elevated by altered levels while as blood sodium, chloride and liver sodium, potassium and calcium levels were significantly decreased [24-29, 31, 59, 77, 79].

Conclusion

Organophosphorus insecticides induce serious biochemical alterations in fishes and, in extreme cases, paralyze them completely by inhibiting acetylcholinesterase enzyme in synapse. Hence, it is essential to prevent natural water bodies from pesticide contamination by having checks and balances at the point and non-point sources of pollution.

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