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THE TOXICITY ASPECTS OF CHLORPYRIFOS ON FRESH WATER FISHES WITH SPECIAL EMPHASIS ON *ANABAS TESTUDINEUS*

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Abstract- Chlorpyrifos (CPF) is an organophosphate pesticide (OP) used to control a variety of insects and pathogens in crops, fruits, and vegetables, as well as for various household purposes**.** The current study has been composed based on previous studies and findings on CPF toxicity to provide a brief overview of CPF-induced impairments in the aquatic ecosystem, with a focus on freshwater Anabas testudineus fishes. Comparison of other freshwater fishes has been drawn with *Anabas testudineus* by emphasizing deformity studies, reproductive toxicity, abnormalities leading to endocrine disruption, and immune dysfunction by exposure to CPF. Furthermore, the deformities and symptoms due to CPF exposure associated with freshwater fishes are discussed in this review.

Keywords- Organophosphate pesticides, Chlorpyrifos, Toxicity, Deformity. *Anabas testudines*, Fish.

1. Introduction –

Pesticides have a key role in daily lives such as; in agriculture, industries, and their exposure is also a global public health concern [1]. The vast exposure of pesticides costs more than 250,000 deaths every year and the cases of poisoning lead to more than 3 million according to the world health organization [2]. There might be many possible reasons for the increasing numbers of cases of pesticide poisoning including poor knowledge, less protection, Inadequate safety warning, and minimal understanding of farmers and agricultural workers. The persistent runoff of wide varieties of Eco toxicants such as pesticides is mixed into the agricultural fields which further leads to contaminate the oceanic biological system. It puts the aquatic organisms and living beings beneath a genuine danger. Due to the constant exposure to toxicants, fishes are more vulnerable to such pollution because of their bioaccumulation potential [3]. The most useful approach for assessing the impact of environmental contaminants on aquatic animals is through histo-cytological examinations [4][5][6]. *Anabas testudineus* is found natively and widely distributed in India and thus, in this review, this species is chosen as an ecotoxicological model for risk assessment and pesticide toxicity testing.

The toxic potentials of pesticides have been studied over a decade extensively and Organophosphates are considered to be highly toxic which possess great risk among the pesticides, as it includes most used insecticides in the world [7]. The Chlorpyrifos (CPF) [0, 0- diethyl 0-(3, 5, 6- tricloro-2- pyridinol) belongs to the group of organophosphorus pesticides (OPs) which are readily available and most commonly used globally for domestic and industrial purposes [2][8]. The toxicity mechanism of OPs works by binding the cholinesterase enzyme and limiting the activity through irreversible phosphorylation which leads to activating the Muscarinic and nicotinic receptors and increase in acetylcholine levels causing toxicity[9]. insecticides belong to OPs have a toxicity mechanism that allows them to be absorbed through the skin, breathed, or ingested by skin contamination [10]. The objective of this review is to explain the mechanism by which CPF works and its mode of action in the deformity studies on the growth and yield of fishes on the cellular and molecular level.

2. Chlorpyrifos –

CPF was first manufactured and introduced into the American market by Dow Elanco company in the USA in 1965 [11].). It belongs to the group of o organophosphate pesticides (OPs). The OPs have been used diversely in a wide variety of crops. Various health issues, outright bans on their use are reported in many parts of the world. Amongst OPs, CPF has been identified as the most diversely used insecticide across the world for agricultural and non-agricultural uses [12]. The known mode of action for OPs is to accumulate acetylcholine (AChE) causing stimulation of postsynaptic receptors which leads to toxicity [13][14][15]. However, CPF metabolizes a more potent inhibitor of AChE as it absorbs the corresponding oxygen analog (CPF-oxon)[16][17].

2.1 Hazardous effects of CPF –

OPs function by decreasing AChE enzymatic activity, peripheral nervous system (PNS), and altering serum paraoxonase-1 (PON1) activity in the brain. It can be utilized as a biomarker to assess OPs-induced toxicity (22). Generally, It accumulates in adipose tissue or frequently in conjunction with plasma albumin and mostly it is responsible for causing hepatic and immunological alteration, genotoxicity, embryotoxicity, teratogenicity, and neurobehavioral alterations (23)(24). CPF may cause toxicity through a variety of methods. Interfering with neurotransmitter receptors; Interacting with various enzymes; obstructing signal transduction pathways; and hampering Neuronal differentiation (23)(25).

CPF was firstly introduced as a substitute for the organochlorine pesticide DDT (Dichlorodiphenyltrichloroethane) in 1965. Later it became part of a pattern known as "regrettable substitution," where banned chemical compounds are replaced with one that is potentially as dangerous or even worse[18]. Few researchers have compared CPF to other pesticides to determine toxicity levels, and one investigation on freshwater shrimp (*Paratya australiensis* Kemp) discovered that alpha-cypermethrin generated the highest levels of chronic toxicity followed by CPF, Carbaryl, dimethoate, fenarimol, and diuron [19]. Another study indicated that OPs are more hazardous than pyrethroid pesticides. Two OPs (CPF and acephate) and a triazole fungicide (tetraconazole) were tested in honey bees versus nine pyrethroid pesticides (*Apis mellifera* L.) Studies and experiments indicate, CPF can cause a variety of deformities as a result of both acute and chronic exposures [20] .

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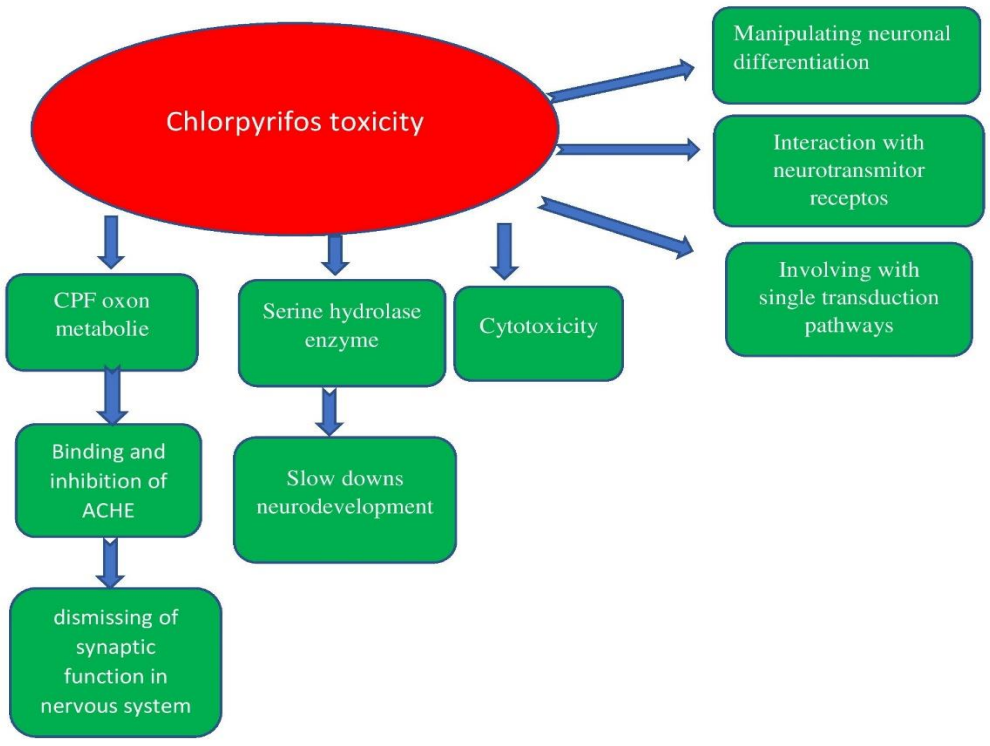
Figure-1– mode of action



2.2 Chlorpyrifos as an endocrine disruptor-

Endocrine disruptors (EDs) are particles found both within the environment and within the diet that meddled with typical hormone regulation [25]. These substances can influence enzymatic pathways involved in hormone biosynthesis, bioavailability, or digestion system & might act as agonists or antagonists of hormone receptors [26] .CPF are known to deliver antagonistic impacts on the endocrine homeostasis, It is alluded to as endocrine-disrupting chemicals (EDCs) due to its interfering property of normal hormonal regulation [25]. OPs such as CPF can trigger AChE concentrations to increase in the synaptic cleft, causing the failure of translation of hormonal regulatory impulses and deterioration of endocrine homeostasis in the body[27].CPF has been reported of causing harmful effects on the sex steroid metabolism system causing gonadal decay in aquatic fauna [28]. Many studies have been conducted on CPF where it has been evident that it alters with the normal hormonal regulation [29]. The number of substances that are causing endocrine disruption, has been identified recently. Hence it is more crucial to study their environmental complications thoroughly. The study of pesticide impacts on fish has diagnostic value because of their commercial importance[30]. The histopathological changes in brain cells of catfish and its association with behavioral abnormalities have been analyzed for four days of CPF exposure (1.92 mg/L), It is found that CPF exposure to brain cells caused downregulation of brain cell performance, altered brain activity, poor swimming and decreased motility[31]. Amongst already identified endocrine disruptors at coral reefs, CPF is one of them which is known to be a thyroid disruptor with negative effects on fish behavior [32] [33]. It potentially interfered with the zebrafish embryos by altering the expression pattern of the estrogen-responsive genes VTG and Erα, while exposed to the various concentrations [34]. CPF is also used in a variety of pests across the globe with endocrine-disrupting properties to alter developmental patterns, gonads, and neurodevelopment, Its long-term sublethal exposure was shown to have a deleterious impact on the nervous system and hormone metabolism[35]. It is an environmental pollutant, and its toxicity has been detected in many countries. According to a study on carp, immunotoxicity caused by CPF exposure can promote deterioration of biological signaling pathways of the spleen [36]. Under the toxicity properties, CPF also severely disrupted the gonadal quality and sex hormones of goldfish. It also produced a decrease in the amounts of Testosterone and 17-β-estradiol in the serum of red blood cells [37]. A study discovered endocrine disruption in lake Van fish, revealing that a higher than 1µM dose of CPF can cause oxidative stress and DNA damage in primary gill cell culture [38].

3. Immune dysfunction –

Numerous recent studies have proved that immunological alteration is found in fish existing in polluted waterways. Retrospective studies have been linked with the CPF causing the elevation in CD26-positive peripheral lymphocytes (T and B lymphocytes and macrophages) and higher autoantibody titers in individuals [39]. Toxicity of CPF has been studied on *Oreochromis niloticus* to study immune dysfunction which leads to having disoriented macrophages, depressed phagocytic function, and lower total pronephros cell count, as compared to controls at the concentration of 1 ug/L. Their data suggest that the immune cells in the hematopoietic compartment have been altered due to the exposure of environmentally relevant concentrations of CPF [40].

4. Reproductive toxicity –

According to various research, CPF operates as a gene expression suppressor and a mechanism related to hypothalamic gonadotropin production or steroidogenesis, as well as an androgen receptor (AR) antagonist. This suppression may lead, oxidative damage to the Leydig cells which may lead to reduce amount of testosterone. It also reduces other gonad abnormalities and reduces the activity of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [41]. Various environmental toxicants including CPF have been associated with the deterioration of the male reproductive system, infertility, and the induction of various reproductive system abnormalities [42]. Reduction in testicular weight which is associated with degenerative and necrotic changes in the seminiferous tubules, widening of the interstitial spaces, reduction of germ cells has been identified as a major reproductive defect in males as a result of CPF exposure [43][44][41].

In the case of females, the OP insecticides predominantly affect the reproductive system and germ cells, causing abnormalities in the ovarian cycle, estrous cycle arrest, alterations in steroidogenic enzymes, decreased ovarian weight and follicle formation, and in some cases infertility [45]. CPF's mechanism of action involves causing lower levels of serum sex hormones such as LH, progesterone, estrogen and it acts to modify processes involving embryonic, cell proliferation, and apoptosis by imitating estrogen [46][45].

5. Deformity studies –

Fishes are considered to be as best bioindicators hence they are used as an ecotoxicological model for risk assessment of pesticides. In a study, *Anabas testudineus* was used to study the mechanism of toxicity against CPF. CPF was studied on *Anabas testudineus* to see if it could be used as a pollutant indicator when exposed to various concentrations, as well as to analyze histo-cytological alterations that could later be identified by Scanning electron microscope (SEM) and Transmission electron microscope (TEM); a sensitive and low-cost approach for detecting the early effects of toxicants in organisms.

6. **Histo-Cytological examinations-**

Histo-cytological tests have been discovered to be an effective method for assessing pollution's environmental impact and effects on aquatic animals [4][5][6]. This study is relevantly easy and useful to determine an individual's health and aptness. There have been developed biomarkers for detecting the impacts of pollution for a wide spectrum of histo-cytological abnormalities in fish [47].

Anabas testudineus was subjected to toxicity research after sublethal exposure to CPF, in which the morphological alterations on the gills, scales, and erythrocytes were examined using a scanning electron microscope (SEM). The fish were divided into three groups and given sublethal amounts of 125, 250, and 375 µg L-1, as well as a control treatment that did not include any pesticides for 21 days. In this study, epithelial hyperplasia and fusion of secondary lamellae were observed in fish subjected to 125 µg L-1 treatment, whereas highly mucous cells were observed, along with hyperplasia and fusion in secondary lamellae, in fish exposed to 250 & 375 µg L-1 treatment. Lobopodial projection and oozed out cytoplasmic contents were exhibited in erythrocytes to the fishes exposed to the nominal concentrations where as normal scale structure had circuli present all over in the control treatment [48].

Another CPF toxicity examination was carried out on antagonistic histological and cytological effects in *Anabas testudineus* gills. Gills provide important activities such as gas exchange, ionic and acid-base control, nitrogen excretion, and they have a higher surface area to deal with the external environment [49]. Therefore, fish gills are considered as the best indicator of fish to analyze the toxicity impact [50]. The confluence of secondary lamellae and epithelium in the gill tissue was the most obvious histological consequence in the finding, Side effects included epithelial hypertrophy, aneurysm, necrosis, the elevation of the lamellar epithelium, and desquamation of epithelial cells. Among the cytological effects were epithelial detachment, a wide subepithelial space, and necrotic, apoptotic, presence of macrophages, swelling of mitochondria, distension of the tubular system, the presence of some big vacuoles, excessive mucous deposition, and nucleus abnormalities [51].

7. **AChE activity –**

An experiment was carried out on native climbing perch *Anabas testudineus* fingerlings to see how CPF affected the activity of acetylcholinesterase (AChE) in the brain. This study was performed in a static non-renewable system to conduct acute and subacute toxicity tests in which the fingerlings were treated with five lethal & sublethal CPF concentrations. These tests were carried out to see how sensitive AChE activity in the brain was and how quickly it recovered. In this test, CPF was found to be mildly toxic to climbing perch, with a 96-hour LC50 of 1.7 mg/L. For the four highest test dosages, AChE inhibition was 70% after 96 hours, indicating that the CPF is moderately dangerous. After 7 days of recovery in CPF-free water, it was revealed that brain AChE activity was still much lower in control than the rest of the four highest nominal concentrations[52]. Because of its propensity to block the acetylcholinesterase (AChE) enzyme, CPF is well known to produce nervous system malformations, and its toxic effects have been thoroughly investigated in freshwater fish and aquatic invertebrates [53][54].

Table 1. Master table on fish species studies with chlorpyrifos and its impact–

Fish species studied on	Exposed hours	LC50 value	impact	References
Cyprinus caprio	24 hr	5.28 ppm	Haematological parameters (RBC, Haemoglobin and plasma protein were decreased)	[55]
Cyprinus carpio (Fingerlings)	96 hr	0.160 mg/L	Impaired behavioural responses, loss of equilibrium and erratic swimming.	[56]
Gambusia affinis.	96 hr	297 µg/L	loss of equilibrium, loss of feeding, and erratic swimming	[33]
Oncorhynchus mykiss	96 hr	30 µg/L	cellular injury in the brain	[57]
Labeo rohita	96 hr	442.8 µg/L	different neurotoxic behavioral responses	[58]
(Oreochromis niloticus L.) Larvae	96 hr	1.57 mg/l.	Behavioural changes (upside down swimming, Colour fading)	[59]

Labeo Bata	24 ,48,72,96 hr	257.03,208.92,177.82,109.64 µg /L^{-1}	Anaemia with decreased in RBC count, packed cell volume, Haemoglobin (Hb) and Mean corpuscular volume (Mcv)	[60]
Pimephales promelas	96 hr	122.2 µg/L	increased deformities and a reduction in growth	[61]
Clarias gariepinus	96 hr	0.861 mg/ l^{-1}	Protein concentration decline, Lowered Red and white blood cell count	[62]
Heteropneustes fossilis (Average length 18 ± 2 cm and weight 48 ± 2 g)	96 hr	1.90 mg/L	Significant decrease in acetylcholinesterase (Ache) activity in brain	[63]
Heteropneustes fossilis (Adult)	96 hr	23.10 ppm	erratic swimming, gulping, mucus secretion, increased opercular movement and profuse emission of mucus all above the body	[64]
Oryzias latipes	24 & 48 hr	0.30 & 0.25 mg/L	continuous forward posturing of the pectoral fins, hemorrhage (vertebral area), and scoliosis in the caudal region	[65]
Oryzias latipes	96 hr	266.79 µg /L^{-1}	Increased mortality	[66]
Puntius chola	96 hr	0.219 ppm	Abnormal behaviour and increased mortality	[67]
Channa punctatus	96 hr	20.32 ppm	erratic swimming, gulping, mucus secretion, increased opercular movement and profuse emission of mucus all above the body	[64]
Anabas testudineus	96 hr	16.61 ppm	erratic swimming, gulping, mucus secretion, increased opercular movement and profuse emission of mucus all above the body	[64]
Batasio tengana	96 hr	13.94 ppm	erratic swimming, gulping, mucus secretion, increased opercular movement and profuse emission of mucus all above the body	[64]

Cirrhinus mrigala	96 hr	0.44 mg /L^{-1}	Alterations in behaviour such as Air Ingulping (AI), Operculum Beat Frequency (OBF), Surfacing Movement (SM), Vertical Hanging (VH) and Tail Beat Frequency (TBF)	[68]
Etroplus Maculatus	96 hr	6.61µg/L	Jerky vertical movement, loss of equilibrium, surface swimming, scollosis, darkening of skin and bulging of eyes.	[69]
Trichogaster fasciata	96 hr	880 µg/L	hemorrhage, hypertrophy, necrosis, pyknosis, vacuums, splitted gill lamellae and missing of gill lamellae were observed in the gills	[70]
Ceriodaphnia dubia	96 hr	0.13 µg/L	Mortality	[71]
Oreochromis niloticus (Juvenile and adult)	96 hr	98.7 and 154.0 µg/L	Impair steroid hormone levels.	[72]
Poecilia reticulata	96 hr	175.8 µg/L	Antioxidant defense system (ADS) is fluctuated with inhibition of ACHE.	[73]

8. Discussion-

In this review, *Anabas testudineus* is chosen as a test animal to review the toxicity aspects of Chlorpyrifos, because these are the most preferable animals and well suited for long-term scientific testing as well as because of its importance in the food chain. These animals have also a high commercial value due to their nutritional content and taste [74]. According to the finding of a study, CPF was found out to be mildly toxic compared to other insecticides such as diazinon and fenobucarb where the climbing perch were exposed in similar conditions. CPF was slightly more sensitive with a higher LC50 value [75][76]. But compared to other freshwater fishes *Anabas testudineus* is found to be less sensitive to CPF. CPF 96-hour LC50 values for juvenile and adult Nile tilapia (*Tilapia niloticus*), Common carp (*Cyprinus carpio*), Mosquito fish (*Gambusia affinis*), and Guppy (*Poecilia reticulata*) were found out to be much higher and highly sensitive respectively[72][36][33][73].

One of the key traits of Climbing perch fishes are recognized for their obligatory air-breathing, which means they must breathe air regularly even if the dissolved oxygen (DO) levels in the water are high; otherwise, they will suffocate[77]. This species is also capable of adapting to difficult environmental circumstances [78][79].The most prevalent CPF deformity finding is irregular swimming, loss of stability, and other neurotoxic behavioral responses in fish. Although in few experiments, it has been shown to increase fish mortality.

CPF has been studied with *Oryzias latipes, Ceriodaphnia dubia, and Puntius chola* fishes in multiple investigations, and it has been shown to increase fish mortality. Many other factors can trigger after exposure to OPs insecticides such as lower AChE activity in the brain, higher oxygen consumption, and changes in the behavior of the examined fish such as - decreased activity, irregular swimming, and rapid gill movements. All of this occurred, showing that the fish nervous system was subjected to sublethal consequences. These findings are congruent with these of several other researchers who observed similar changes as a result of OP insecticide-induced brain AChE inhibition [80][81][82][83][84][85]. CPF can generate long-term sublethal effects on aquatic organisms. For instance, in a study, it was discovered that the activity of brain AChE in *Orechromis mossambicus* was entirely restored 22 days after exposure to monocrotophos (containing oxon group, P = 0), however, exposure to OPs including a thiol group

took 36 days to recover [86]. However, detoxification ability is dependent on the physiochemical characteristics of the pesticide and varies between fish species [87][88].

9. Conclusion-

The present review provides a brief explanation of CPF-induced toxic effects and possible mechanisms of action on fishes e.g., acetylcholinesterase inhibition, histo-cytological alteration, reproductive toxicity, immune dysfunction, and endocrine disruption in fishes with special emphasis on *Anabas testudineus*. It is evident that CPF is more likely to trigger sublethal effects on the fish nervous system, which in turn could affect fish growth and yield. This should be examined further by using CPF in conjunction with other pesticides on *Anabas testudineus* as well as in other organisms.

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