

## Review article

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**Toxicity and effects of chlorpyrifos in a non-target organism (Fish) – A review**

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**ABSTRACT:**

Agro chemicals are essentially meant for protecting agriculture crops against harmful insects and pests. They provide vital inputs required to augment food production and save crops from attack by a variety of pests in a safe and selective way. However, their chemical structure, improper preparation, application and storage may pose a serious threat to the environment. Aquatic ecosystems are the ultimate recipients of varying concentrations of different pesticide residues because of leaching, agricultural runoff, atmospheric transport, spray drift and improper disposal. When any pollutant is added to an aquatic ecosystem, it enters the tissues of various aquatic flora and fauna including fish leading to several biochemical and physiological alterations which may be adaptive or may lead to toxicity. Since major portion of the world's nutritional requirement is being supplied from fishery resources, it is therefore, important to secure the health of fishes. In this paper, we have reviewed the toxic effects of chlorpyrifos, an organophosphorus compound and the second largest selling insecticide in India. Considering its acute toxicity and detrimental effects such as behavioural, morphological and other impairments in fish, it is therefore poses a serious threat to aquatic organisms as well as to the health of human beings. This review provides a base line data for further studies on the effect of various toxic chemicals aiming at determination, interpretation and delineation mechanisms of their pollutant action, possible ways to mitigate adverse effects and future environmental management programmes.

**KEY WORDS:** Agro-chemicals, Aquatic ecosystems, Pesticide residues, Aquatic organisms, Food chain, Environmental management.

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**INTRODUCTION:**

In India, the use of various pesticides in agri-farming practices for profit maximization has been viewed as an integral part of the success of the agricultural sector. In India, 60% of all pesticides are used on cotton crops and it is alarming to note that about 17.53% of the

total pesticides are used only in Andhra Pradesh which stands first in the country (Mancini *et al.*, 2005). However, the indiscriminate use of pesticides has created lot of environmental problems affecting non-target organisms and contaminates soil and water. Organophosphorus (OP) pesticides are among the most widely used pesticides worldwide and are used in both agricultural and residential settings. They are popular because of their broad spectrum of applications and potent toxicity to insects, their relative inexpensive costs, and their decreased likelihood for pest resistance (Karalliedde *et al.*, 2001). Chlorpyrifos (O, O diethyl O -3, 5, 6 - trichloro - 2 - pyridyl phosphorothioate) is one of the earliest developed broad spectrum organophosphate pesticide, introduced in India in 1965 (Gayr, 2000). It is nonsystemic, general use pesticide used extensively in India in the management of domestic and agricultural pests with different trade names such as dursban, lorsban, radar, hilban, coroban etc. It was commercially used for more than a decade to control coleoptera, diptera, homoptera, and lepidoptera in soil or on foliage in over 100 crops (Reddy *et al.*, 2011; Tomlin, 2000). It is the second largest selling insecticide in India. The major route of chlorpyrifos to aquatic ecosystems is through rainfall runoff and air-drift (Xing *et al.*, 2012). Its release into the aquatic environment may have toxic effects to non-target aquatic species (Reddy *et al.*, 2011). It is a highly toxic pesticide for aquatic animals even at low concentrations (Humphrey *et al.*, 2004). A number of workers reported on the toxicity of chlorpyrifos on different aquatic animal models noticed that it is a potent neurotoxicant (Sturm *et al.*, 2007; Rao *et al.*, 2005). Fishes are useful bioindicators and integrators of contaminants because of their

wide distribution, being free swimmers, their ability to respond against environmental pollution, and their importance as an economic food source for human beings (Gupta *et al.*, 2009). In this review an attempt has been made to report the acute toxicity of chlorpyrifos and its effects on certain biomarkers in various fish species.

#### ACUTE TOXICITY TO FISH:

The most common way of direct assessment of potential impact of various toxic pesticides is through the use of standardized laboratory toxicity experiments that expose a single species to a single pesticide over a range of concentrations for a specified period of time. Comparison of such toxicological results among different pesticides or for a pesticide among test species indicates the relative toxicity of those pesticides or the relative toxicity of that pesticide to those test animals under standardized test conditions (Nowell *et al.*, 2014). Period of exposure for which the LC<sub>50</sub> value is determined is of considerable importance in toxicity evaluation of various toxic chemicals in aquatic organisms, and in general depending upon the nature of the toxic material LC<sub>50</sub> values are assessed at 24 or 48 or 72 or 96 hours or even more after due acclimatization to the laboratory conditions (David and Philip, 2005). Various researchers reported the toxicity of chlorpyrifos in various fish fauna (Table 1). Observed variations in LC<sub>50</sub> values of chlorpyrifos are due to a number of biological and physico-chemical factors induced by various environmental and regional influences (Magesh and Kumaraguru, 2006).

Table 1: Acute toxicity of chlorpyrifos to different fish species

S. No	Name of the fish	Value of LC <sub>50</sub>	Exposure period	Reported by
1	<i>Cirrhinus mrigala</i>	0.44 mg/L	96h	Anita et al., 2016
2	<i>Oreochromis mossambicus</i>	0.0022 mg/L	96h	Padmanabha et al., 2015
3	<i>Oreochromis niloticus</i>	1.023 mg/L	96h	Diaz and Giron, 2014
3	<i>Hetropneustes fossilis</i>	2.84 ppm	96h	Khatun and Mahanta, 2014
4	<i>Chanos chanos</i>	3.56 µg/L	96h	Palanikumar et al., 2014
5	<i>Gambusia affinis</i>	0.284 ppm	96h	Sharma, 2014
6	<i>Danio rerio</i>	0.16 µg/L	96h	Tiwari and Ansari, 2014
4	<i>Oncorhynchus mykiss</i>	9 µg/L	96h	Topal et al., 2014
7	<i>Cyprinus carpio</i>	203 µl/L	96h	Banaee et al., 2013
8	<i>Channa punctatus</i>	0.253 µl/L	96h	Devi and Mishra, 2013
9	<i>Clarias gariepinus</i>	0.92 mg/L	96h	Okechukwu et al., 2013
10	<i>Carassius auratus</i>	0.2 ml/L	96h	Vaidehi et al., 2013
11	<i>Puntius chola</i>	0.219 mg/L	96h	Verma and Saxena, 2013
12	<i>Channa punctatus</i>	811.98 µg/L	96h	Ali and Kumar, 2012
13	<i>Cyprinus carpio</i>	2.08 mg/L	96h	Gündüz et al., 2012
14	<i>Hetropneustes fossilis</i>	1.76 mg/ L	96h	Srivastav et al., 2012
15	<i>Cyprinus carpio</i>	580 µg/L	96h	Xing et al., 2012
16	<i>Clarias batrachus</i>	16.5 mg/L	96h	Reddy et al., 2011
17	<i>Poecila reticulata</i>	0.176 mg/L	96h	Sharbidre et al., 2011
18	<i>Oreochromis niloticus</i> (Juvenile)	98.67 µg/L	96h	Oruç, 2010
19	<i>Oreochromis niloticus</i> (Adult)	154.01 µg/L	96h	Oruç, 2010
20	<i>Lepidocephalichthys irrorata</i>	0.00510 mg/L	72h	Vidyarani et al., 2010
21	<i>Cyprinus carpio</i>	0.160 mg/L	96h	Halappa and David, 2009
22	<i>Cyprinus carpio</i>	5.28 mg/L	24h	Ramesh and Saravanan, 2008
23	<i>Gambusia affinis</i>	297 mg/ L	96h	Kavithaa and Rao, 2008
24	Hybrid catfish ( <i>Clarias macrocephalus</i> x <i>Clarias gariepinus</i> )	33 mg/L	96h	Chawanrat et al., 2007
25	<i>Oreochromis niloticus</i>	1.023 mg/L	96h	Girón-Pérez et al., 2006
26	Nile tilapia	1.57 mg/L	96h	Gül, 2005
31	<i>Gambusia affinis</i>	297 µg/L	96h	Rao et al., 2005
32	<i>Oreochromis mossambicus</i>	25.7 µg/L	96h	Rao et al., 2003
33	<i>Oreochromis niloticus</i>	25.9 µg/L	96h	
34	<i>Oryzias latipes</i> larvae	0.30 mg/L	48h	Carlson et al., 1998
35	<i>Pimephales promelas</i>	162.7 µg/L	48h	Moore et al., 1997
36	<i>Oryzias latipes</i>	0.25 mg/L	48h	Rice et al., 1997
37	<i>Notemigonus crysoleucas</i>	35 ppb	96h	Barron and Woodburn, 1995
38	<i>Pimephale promelas</i> larvae	0.12 mg/L	96h	Jarvinen et al., 1988
39	<i>Oncorhynchus mykiss</i>	0.015 mg/L	96h	Mayer and Ellersieck, 1986

40	<i>Cyprinodon variegatus</i>	0.136 mg/L	96h	Clark et al., 1985
41	<i>Fundulus similis</i>	0.0041 mg/L	96h	Schimmel et al., 1983
42	<i>Menidia menidia</i>	0.0017 mg/L		
43	<i>Mugil cephalus</i>	0.0054 mg/L		
44	<i>Pimephale promelas</i>	0.248 mg/L	48h	Holcombe et al., 1982
45	<i>Ictalurus punctatus</i>	0.280 mg/L	96h	Johnson and Finley, 1980

### MORPHOLOGICAL ALTERATIONS:

Pesticides such as organophosphates are known to cause morphological alterations in the fish. Morphological abnormalities, as a result of sublethal toxicity due to pesticides, can reduce the health, fitness and well-being of aquatic organisms (Rice et al., 1997). Chlorpyrifos induced morphological alterations in different fish species were studied by various researchers. Jindal and Kaur (2015) studied the effect of pesticide on scales of *Ctenopharyngodon idellus* using SEM at various exposure periods (15, 30 and 60 days) at different sublethal concentrations (1.44 µg/L and 2.41 µg/L). Significant alterations such as damaged radii, focus, circuli and severely damaged lepidonts were observed. The intensity of scale damage was found to be toxicant concentration and exposure period dependent and also prolonged exposure to the pesticide damaged the fish scales severely. Devi and Mishra, 2013 assessed the effect of chlorpyrifos toxicity on morphological manifestation of fry fish of *Channa punctatus*. The prevalence of morphological deformities such as shedding of scales, discoloration, lesion of skin, split and necrosis of fins, eye deformities, scoliosis, damaged skull, lower lip extension and copious amount of mucus secretion all over the body were observed and the percentage of these deformities was dose and duration dependent. Srinivasa Rao et al. (2010) observed morphological deformities in *Labeo rohita* exposed to chlorpyrifos (0.1891

ppm) for 2 days. Halappa and David, 2009 observed caudal bending in *Cyprinus carpio* exposed to 0.0224 mg/L and 0.0112 mg/L for 1, 7 and 14 days. Caudal bending persisted even under recovery periods. The extent of caudal bending was pronounced in the highest toxicant concentration. Hyper extension of fins, dullness in body colour and fish body became lean towards abdomen were also observed with time and concentration of the pesticide. *Cyprinus carpio* exposed to chlorpyrifos (5.28ppm 24h LC<sub>50</sub> for 1 day) showed darkening of skin on dorsal side (Ramesh and Saravanan, 2008).

### ALTERATIONS IN OXYGEN CONSUMPTION:

Studies on oxygen consumption form a suitable tool in the assessment of stress due to toxic pollutants on the aquatic organisms and give an index of energy expenditure mechanisms for environmental variations (Franklin et al., 2010). Determination of oxygen consumption by the fish is useful for assessment of lethal effects and is one of the important indicators which reflect physiological state of animal. In an aquatic ecosystem, toxicants present above the normal level i.e. at lethal concentrations bring about mortality of fish and also increase the rate of oxygen consumption in survived fish (Tilak et al., 2007). Rao et al., 2003 reported that the disturbance in oxidative metabolism leads alteration in whole animal oxygen consumption in *Oreochromis mossambicus* exposed to chlorpyrifos in which fish showed

reduced oxygen consumption when exposed to the toxicant. The changes in the respiratory activity of fish have been used by several researchers. Exposure to sublethal concentrations is reported to increase respiratory activity, resulting in increased ventilation and hence increased uptake of the toxicant in various fish species. Hence, changes in oxygen consumption can be measured as a response to toxicants (Jen *et al.*, 2010).

### NEUROBEHAVIOURAL ALTERATIONS:

The behaviour of an animal is controlled by its neuro-muscular coordination and metabolic balance, and hence it reflects the physiological status of the animal. As a result of this, the alterations in the physiology of an animal can be visualized through its abnormal behaviour. Behavioral changes are the most sensitive indicators of potential toxic effects. The behavioral and the swimming patterns of the fish exposed to different insecticides include changes in swimming behavior, feeding activities, predation, competition, reproduction and social interactions such as aggression (Cong *et al.*, 2009). Several reports are now available on the ethology of fishes exposed to chlorpyrifos and most of them revealed abnormal behaviors of fishes under toxicant burden. The extent of abnormality in the behaviour of fish serves as an index of the damage caused by the pesticide to it. Hence, along with the toxicity evaluation of a particular pesticide, the behavioural responses are also generally investigated.

Padmanabha *et al.*, 2015 reported respiratory distress (such as gasping in air), loss of balance and erratic swimming prior to death in *Oreochromis mossambicus* exposed to two sublethal concentrations viz., 0.0044 ppm and 0.0022 ppm along with lethal concentration

(0.022 ppm) as reference for 48h. Results indicated that lethal concentrations of chlorpyrifos had profound effect than sublethal concentrations and toxicant had a significant effect on functional activity of test animal by altering respiration rate and impairing feeding behavior. Jindal and Kaur (2014) observed erratic, speedy and jerky movements in *Ctenopharyngodon idellus* at lower concentration (1.44µg/l) and hyperactivity, violent behaviour and jumping out of the tanks violently (escape behaviour) at the higher concentration (2.41µg/L) of chlorpyrifos. The intensity of altered behaviour was found to be dependent on concentration of the toxicant and period of exposure. Prolonged exposure (i.e. 30 days and 60 days) to the toxicant, the fish became hypoactive, struggled for breathing, restricted swimming movements finally led to lethargic condition and loss of equilibrium. Tiwari and Ansari (2014) observed respiratory distress such as gasping in air, loss of balance and erratic swimming prior to death in *Danio rerio* exposed to chlorpyrifos (0.16 µg/L). Banaee *et al.*, 2013 reported unbalanced swimming, swimming in the surface in *Cyprinus carpio* exposed to chlorpyrifos (40 µg/L) for 10, 20 and 30 days of exposure. Devi and Mishra, 2013 assessed the effect of chlorpyrifos toxicity on the behavior of *Channa punctatus*. Behavioural changes such as convulsions, swimming erratically, vertical hanging, coughing, loss of balance, abnormal opercular movement, and lateral flexure, with tail beat were shown and finally fish became lethargic and settled at the bottom and their belly turned up before death. Opercular beat frequency (OBF/min.), tail beat frequency (TBF/min.), in gulping air or escaping attempt/min. and surfacing etc. increased with increasing concentration of CPF up to 24h after that decreased following 96 hrs of

exposure tenure. These quantal behaviour responses were significant at  $p < 0.001$ .

Investigation of acute toxicity of chlorpyrifos-ethyl on *Clarias gariepinus* by Okechukwu *et al.*, 2013 revealed that fish exposed to 0.64 mg/L, 0.80 mg/L, 0.96 mg/L, 0.12 mg/L, 0.28 mg/L for 96h showed highly significant ( $p < 0.001$ ) effects of concentration and duration on rates of tail fin movement of fish. There was significant decrease ( $p < 0.05$ ) in the opercular ventilation of treated fish when compared with control. Also both the main effects of concentration and duration of exposure of chlorpyrifos-ethyl and the effect of their interactions on AGI were highly significant ( $p < 0.001$ ). Highest mortality was recorded from 1-12h period of exposure and the mortality at 12h was significantly ( $P < 0.05$ ) higher than the mortality recorded from 24h-96h periods. The acute toxicity of chlorpyrifos-ethyl elicited dose and duration dependent behavioural changes that led to mortality of fish. Verma and Saxena, 2013 observed behavioural changes in *Puntius chola* exposed to different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 ppm) of chlorpyrifos at 4, 8, 24, 48, 72 and 96h of exposure period. In 0.1 ppm treatment till 8h post-exposure, movement was normal; after which movement became slow. At 72 and 96h, settling of the fishes at the bottom of the tanks and increase in mucus production were observed. In 0.2 ppm treatment, slightly fast movement was observed in comparison to control, which became slow as time elapsed. In 0.3 ppm treatment, fast and jerking movement with increased mucus production. As time elapsed, fish exhibited vertical hanging and settling at the bottom. In 0.4 ppm and 0.5 ppm treatments, fishes exhibited stress symptoms, vertical hanging in the water and mortality. Fishes of all treated groups showed hyper activity and frequent surfacing

to gulp air. It was also noticed that as time passed, they continued to swim near the water surface and tried to jump out from the holding tanks. Once the fishes were exhausted, they sank to the bottom of the tanks with no opercular movement and finally succumbed with their mouth opened.

Sharbidre *et al.*, 2011 observed abnormal swimming behavior in *Poecilia reticulata* as a response to chlorpyrifos exposure which resulted in aggressive behaviour, rapid gulping of water, increased opercular movement and abnormal and erratic swimming movements. Fish was stressed progressively with time before death. They were lethargic and at the time of death exhibited transient hyperactivity before collapsing. Sledge *et al.*, 2011 demonstrated that clorpyrifos caused selective long term neurobehavioral alterations in zebrafish which showed persistent neurobehavioral impairment, where tests of sensorimotor response (tap startle response and habituation), stress response (novel tank diving test) and learning (3-chamber tank spatial discrimination) were conducted after early developmental clorpyrifos exposure. Persisting behavioral effects such as decreased habituation, decreased escape dividing response, increased swimming activity and lower learning rate were observed.

Eddins *et al.* (2010) observed a significant decrease in whole brain activity of zebrafish after exposure to clorpyrifos. Srinivasa Rao *et al.*, (2010) observed almost similar behavioural alterations in *Labeo rohita* when the fish was exposed to the pesticide (0.1891 ppm) for 2 days. Reduction in feeding behavior under toxic environmental condition might be profitable to lower the energetic costs of digestion which was evidenced by the findings of Halappa and David (2009) where

*Cyprinus carpio* was exposed to chlorpyrifos. Fish in toxic media exhibited irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and sinking to the bottom. The carps were found under stress, but mortality was insignificant in both the sublethal (0.0224 and 0.0112 mg/L) concentrations. Ramesh and Munniswamy (2009) reported an excess secretion of mucus in *Cyprinus carpio* exposed to chlorpyrifos. *Cyprinus carpio* exposed to chlorpyrifos (5.28ppm 24h LC<sub>50</sub> for 1 day) showed abnormal behavioral changes like fast swimming activity, profuse secretion of mucus, hypersensitivity, jerky movement, loss of equilibrium, etc. (Ramesh and Saravanan, 2008).

Rao et al., 2005 reported locomotor behavior in mosquito fish *Gambusia affinis* in response to the sublethal exposure [60µg/L (1/5<sup>th</sup> of LC<sub>50</sub>)] to chlorpyrifos *in vivo*, for 20 days. They observed that fish were under stress, and reduced their locomotor behavior like distance travelled per unit time (m/min) and swimming speed (cm/s) with respect to the length of exposure. Sandahl et al., 2005 observed the behavior of coho salmon (*Oncorhynchus kisutch*) exposed to chlorpyrifos (0–2.5 mg/L) for 96h and recorded spontaneous swimming and feeding behaviors in the fish. Stressful behavior such as erratic swimming, increased obf and tbf, regular visit to the surface to gulp in air, loss of balance, restlessness and finally death of fish was observed by Chindah et al., 2004 in *Tilapia guineensis* treated with acute concentrations of chloropyrifos.

#### CONCLUSION:

From the present review it is evident that, chlorpyrifos seems to exert significant effects on the behavior and morphology of the fish at different exposure periods and at various concentrations. Behavioural changes and

morphological alterations in fish exposed to chlorpyrifos were proven to be more sensitive diagnostic endpoints than was the mortality caused by toxicant. Chlorpyrifos is highly toxic and had a detrimental impact on the behavioural responses and morphology of the fish. Studies on toxic potential of chlorpyrifos to different aquatic organisms especially to fish directly and indirectly to humans are needed to explore the negative consequences and to formulate the future management strategies for safeguarding aquatic environment and its associated fauna.

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