Environment and Ecology 43 (4) : 965—970, October—December 2025
Article DOI: https://doi.org/10.60151/envec/WWYS4909
ISSN: 0970-0420 (Print), ISSN: 3049-3625 (Online)

Toxic Effects of Chlorpyrifos on Early Developmental Stages of Fish *Anabas testudineus* (Bloch 1972)

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Received 16 July 2025, Accepted 11 September 2025, Published on 16 October 2025

ABSTRACT

Chlorpyrifos is one of the most widely used insecticide in and around Northeast, Assam, India. In this study an experiment was conducted to examine the effects of chlorpyrifos on embryonic development of *Anabas testudineus*. From the fertilized eggs and hatched larvae LC_{50} value was calculated at 24 h, 48 h and 72 h. Four sub-lethal concentration was selected for the toxicity treatment on embryological and larval stages i.e., 0.1 μgL^{-1}, 0.2 μgL^{-1}, 0.4 μgL^{-1} and 0.6 μgL^{-1}. About 100 numbers of fertilized eggs were introduced to each mention sub-lethal concentration of chlorpyrifos. The LC_{50} values of chlorpyrifos pesticide for embryo and larvae of *Anabas testudineus* at 24 h was 0.261 μgL^{-1} respectively. Results showed increasing concentrations decreased hatching success and increased embryonic mortality rate. In embryos, various abnormalities were observed including egg shell broken, yolk sac damage, yolk sac elongation. The LC_{50} values of chlorpyrifos pesticide for larvae of *Anabas testudineus* 24, 48, 72 h were 0.6 μgL^{-1}, 0.39 μgL^{-1} and 0.311 μgL^{-1} respectively. Various physical deformities, including inflammation of swim bladder, abnormalities in notochord, yolk sac edema, lordosis, tail ulceration and irregular caudal region were evident in larvae in response to different concentrations of chlorpyrifos. The finding of the current study indicates that chlorpyrifos pesticide exerts various developmental toxicity to *Anabas testudineus* embryos and larvae.

Keywords Chlorpyrifos, Acute toxicity, *Anabus testudineus*, Embryo, Larvae.

INTRODUCTION

In everyday life, pesticides are used extensively in industry and agriculture, and their exposure poses a threat to public health worldwide (Yaqub *et al.* 2014). The World Health Organization estimates that the widespread use of pesticides results in over one million unintentional pesticides poisonings with severe manifestations occur annually, leading to approximately 20,000 deaths (WHO 1990). Pesticides and other persistent runoff of a wide range of ecotoxicants are combined with soil in agricultural fields. It eventually contaminates the biological system of the ocean. Organophosphate pesticides chlorpyrifos is widely used in global farming practices (Hasanu-

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zzaman *et al.* 2018, De Silva and Samayawardhena 2002). Different formulation of chlorpyrifos was used in Northeast specially in Assam one of the most aquatic biodiversity rich parts of India. Pesticides are exposed to human and other animals in various ways, including chronic, domestic exposure and exposed to polluted water (Dawood *et al.* 2020). Fish are especially susceptible to pollutants because of their propensity for bioaccumulation as a result of their ongoing exposure to toxicants (Benjamin and Kutty 2019). In fishes, along with adults early developmental stages were highly susceptible to any changes in environmental parameters (Sumon *et al.* 2016). So, the findings of the study will be useful for evaluating negative impact of pesticides in aquaculture system and advantageous for finding different strategies for larval rearing of fishes for commercial purpose.

MATERIALS AND METHODS

Broodstock collection and maintenance

The captive breeding experiments were done in the laboratory of Pandu College (affiliated College under Guwahati University) located in Guwahati, Assam, India. The ripe and mature broods were collected from the local fish market of Pandu Bazar, Guwahati, Assam, India and kept in a medium-sized aquarium fish tank for 2 to 5 days to adapt the nature of the new environment. Brood fish were also fed twice daily at 3%–7% body weight with supplementary feed. Water was changed daily for better survival of the brooders. No mortality was recorded during the stocking period. The length and weight of the male fishes are 11±2.5 cm & 16.87±4.1 g approximately and female fishes are 12±3.2 cm & 22.47±3.6 g approximately.

Collection of pesticide

The chlorpyrifos 20 EC was collected locally in liquid form as a commercial insecticide. Chemical was handled carefully and required concentration was prepared by de-chlorinated tap water.

Induced breeding and collection of fertilized eggs

Free oozing males and ripe females were selected in the ratio of 1:2 respectively. Synthetic hormone S-GnRHa (ovasis) were injected below the pectoral fin. Releasing and spawning of eggs was monitored after 11–12 hrs of injection. After spawning the females and males were removed from the tank immediately. Eggs that have missed fertilization appeared with a coating of fungus non-productive and hence removed from the tank to avoid any contamination. Just after spawning, fertilized eggs were collected in the petri dishes using droppers for further study. Hatching time is observed between 18 to 19 hr at 26–28°C.

Experimental design and acute toxicity test

The experiment was conducted in small glass aquaria of (20×30×40) cm. For the experiment, LC_{50} value of fertilized eggs and larvae was calculated by Probit analysis method (Finney 1971). 100 number of fertilized eggs were distributed into 4 prior arranged sets of aquaria containing 4 different concentrations (0.1 μgL^{-1}, 0.2 μgL^{-1}, 0.4 μgL^{-1}, 0.6 μgL^{-1}) of chlorpyrifos, each of which carried out in triplicates and control group without pesticides were maintained. Dead embryos were counted after 24 h and recorded along with hatching success were also noted in each treated and control group. Similarly, 100 number of larvae were exposed to above four concentrations for 24 h, 48 h and 72 h period of time. From the records of mortality larvae LC_{50} value were calculated. Deformities of embryos and larvae snapped under a microscope (Labomed, CSM2).

Acquisition of water quality parameters

During the experimental trial water quality was maintained within the normal range (APHA 2023). Partial water exchange was done for better breeding response among brooders during the induced breeding. Temperature, pH and dissolved oxygen were monitored by automated YSI professional plus multiparameter instruments (Brannum Lane, USA).

Data analysis

Data were analyzed by one way ANOVA to assess the statistical significance of differences. Statistical significance set at $p < 0.05$ level using SPSS 16.0 version.

RESULTS

Toxicity of chlorpyrifos pesticides on embryos of *Anabas testudineus*

The LC_{50} values of chlorpyrifos pesticide for embryo of *Anabas testudineus* at 24 h was 0.261 μgL^{-1}. There was a significant increase ($p<0.05$) in mortality of embryos and increase in hatching in respect to increase in concentration of chlorpyrifos (Table 1). Time period of different embryonic stages after fertilization in normal and chlorpyrifos treated fish *A. testudineus* was represented in Table 2. Various abnormalities were seen such as egg shell broken after 14 h of exposed to 0.1 μgL^{-1} of chlorpyrifos yolk sac damaged after 15.5 h of exposer to 0.2 μgL^{-1} of chlorpyrifos, yolk sac damaged after 15 h of exposure to 0.4 μgL^{-1} of chlorpyrifos, yolk sac elongation after 16.5 h exposure to 0.6 μgL^{-1} of chlorpyrifos (Fig. 1).

Table 1. Toxicity of chlorpyrifos on the embryos of *Anabas testudineus* (n=100). *Significance level ($p<0.05$).

Concentrations (μgL^{-1})	No. of dead embryos at 24 h	Hatching success
0.0	2±1.7	98.1
0.1	27±3.1*	73.3
0.2	41±2.5*	59.5*
0.4	76±3.8*	23.4*
0.6	96±4.2*	3.26*

Toxicity of chlorpyrifos pesticides on the larvae of *Anabas testudineus*

The LC_{50} values of chlorpyrifos pesticide for larvae of *Anabas testudineus* 24, 48, 72 h were 0.6 μgL^{-1}, 0.39 μgL^{-1}, 0.311 μgL^{-1} respectively. Significant ($p<0.05$) mortality of larvae was observed in response to increase in concentrations of chlorpyrifos pesticide, (Table 2) while experimenting. Various deformities were observed due to exposure at different concentration of chlorpyrifos pesticides, in case of larval development such as inflammation of swim bladder, abnormalities in notochord, yolk sac edema, lordosis, tail ulceration and irregular caudal region. Deformities were prominent when larvae were exposed to 0.6 μgL^{-1} concentration of chlorpyrifos (Fig. 2). Abnormalities like swollen yolk sac after 22 h of exposed to 0.1 μgL^{-1} of chlorpyrifos, inflammation of swim bladder after 22.5 h of exposer to 0.2 μgL^{-1} of chlorpyrifos, abnormalities in notochord and yolk sac edema after 24 h of exposure to 0.4 μgL^{-1} of chlorpyrifos, lordosis after 48 h exposure to 0.6 μgL^{-1} of chlorpyrifos, tail ulceration and irregular caudal region after 72 h exposure to 0.6 μgL^{-1} of chlorpyrifos were prominent (Table 3).

Table 2. Time of different embryonic stages after fertilization in normal and treated fish *A. testudineus* woth chlorpyrifos.

Stage of development	Time after fertilization	Time after fertilization (treated with 0.1 $\mu g\ L^{-1}$ chlorpyrifos)	Time after fertilization (treated with 0.2 $\mu g\ L^{-1}$ chlorpyrifos)	Time after fertilization (treated with 0.4 μgL^{-1} chlorpyrifos)	Time after fertilization (treated with 0.6 μgL^{-1} chlorpyrifos)
Late blastula	12 h	13 h	13.5 h	14 h	14.5 h
Early neurula	13 h	14 h	14.5 h	15 h	15.5 h
Somite showing head and tail formation	14 h	15 h	15.5 h	16 h	16.5 h
Newly hatched larvae	18 h	19 h	19.5 h	20 h	20.5 h

Table 3. Toxicity of chlorpyrifos on the larva of *Anabas testudineus*. *Significance level ($p<0.05$).

Concentrations (μgL^{-1})	No. of dead larvae at 24 h	No. of dead larvae at 48 h	No. of dead larvae at 72 h
0.0	2±1.2	5±2.8	8±3.6
0.1	8±4.1	12±3.8	22±6.2*
0.2	11±5.6*	18±7.2*	25±7.3*
0.4	28±6.2*	44±8.3*	59±8.0*
0.6	46±6.7*	60±8.8*	71±9.5*

DISCUSSION

The large applications of pesticides and insecticides

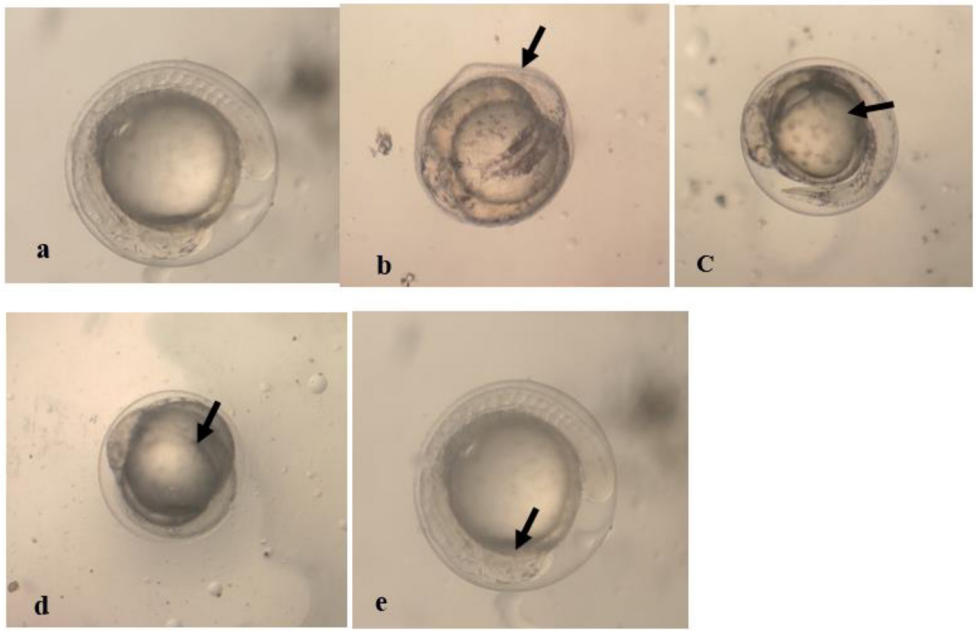

Fig. 1. Abnormalities observed in embryos of *A. testudineus* exposed to chlorpyrifos. a. Normal embryo after 12 h, b. Egg shell broken after 14 h of exposed to 0.1 µgL^{-1} of chlorpyrifos, c. Yolk sac damaged after 15.5 h of exposer to 0.2 µgL^{-1} of chlorpyrifos, d. Yolk sac damaged after 15 h of exposure to 0.4 µgL^{-1} of chlorpyrifos, e. Yolk sac elongation after 16.5 h exposure to 0.6 µgL^{-1} of chlorpyrifos.

are undesirable, both are very unhealthy especially when used frequently, because they are potent chemicals, effects when over applied are disastrous and always have a negative impact. The extensive use of these chemicals not only harm the target organisms but also have harmful effects on non-target organisms such as fish. So, in this present investigation, it is seen that the hatching period remarkably decreases with

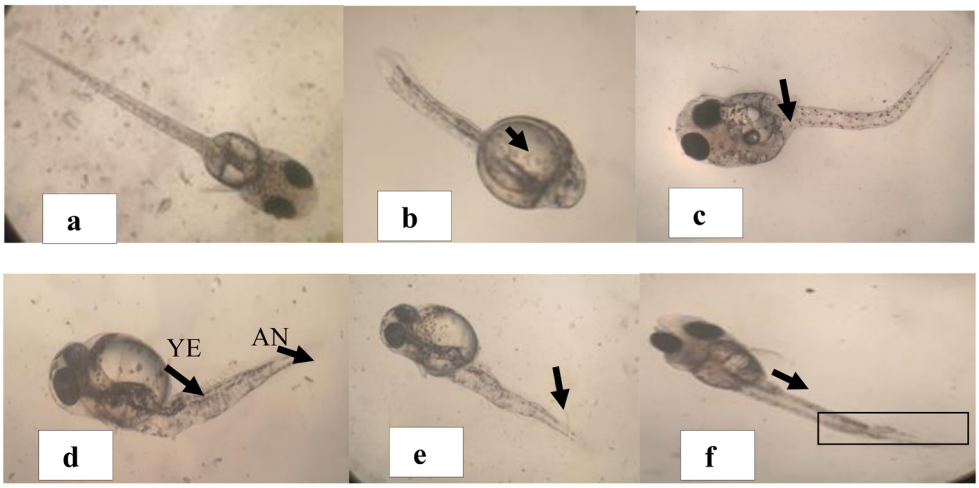

Fig. 2. Abnormalities observed in larva of *A. testudineus* exposed to chlorpyrifos. a. Normal larva after 72 h, b. Swollen yolk sac after 22 h of exposed to 0.1 µgL^{-1} of chlorpyrifos, c. Inflammation of swim bladder after 22.5 h of exposer to 0.2 µgL^{-1} of chlorpyrifos, d. Abnormalities in notochord (AN) and yolk sac edema (YE) after 24 h of exposure to 0.4 µgL^{-1} of chlorpyrifos, e. Lordosis after 48 h exposure to 0.6 µgL^{-1} of chlorpyrifos, f. Tail ulceration and irregular caudal region after 72 h exposure to 0.6 µgL^{-1} of chlorpyrifos.

increasing concentrations of chlorpyrifos pesticide. The incubation time and survivability of embryos and larvae were also affected after exposure to different concentrations. Fishes while exposed to other organophosphate insecticides are reported to have behavioral as well as metabolic changes (Dembele *et al.* 2000). *Tilapia guineensis* was experience higher mortality rate with increased concentration and treatment duration of chlorpyrifos (Chindah *et al.* 2005). Chlorpyrifos has been revealed to have significant impacts on growth of *O. niloticus* (Majumder and Kaviraj 2019). Previous work, on 96 h LC_{50}, it has been detected that in *Cirrhinus mrigala*, it was 0.44 mg L^{-1} (Bhatnagar *et al.* 2016), *Oncorhynchus mykiss* was 24 µg L^{-1} (Mayer and Ellersieck 1986) *Nile tilapia* was 1.57 mg L^{-1} (Gul 2005) *Oreochromis mossambicus* was 25.7 µg L^{-1} (Venkateswara *et al.* 2003) and *Cyprinus carpio* was 580 µg L^{-1} (Xing *et al.* 2012). Present study value of LC_{50} was highly lowered as it was for fish embryos and larvae.

For instance, when zebrafish were exposed to varying amounts of dimethoate, the rate of hatching significantly decreased (Ansari and Ansari 2011). Similar results were reported with increasing concentrations of organophosphate sumithion with increase in mortality of embryos and larvae of zebrafish. Interestingly, the percentage of death in embryos was higher than in larvae, suggesting that zebrafish larvae are less susceptible to organophosphate toxicity than embryos (Rahman *et al.* 2020). One experiment by Kabir *et al.* (2013) on effect of the organophosphate insecticide, sumithion on larval development in *Heteropneustes fossilis* revealed that sumithion exerted developmental toxicity on *Heteropneustes fossilis* larvae, resulting in deformities and mortality. Similar findings were noted in this present study with organophosphate chlorpyrifos. Study highlights the urgent need for less toxic pesticides to protect aquatic ecosystems. Several deformities observed in this study on the embryos and larvae of *Anabas testudineus* fish were evident due to exposure of chlorpyrifos pesticides to a varying concentration. A number of abnormalities in case of both embryo and larvae, were seen particularly at higher concentrations. Similar deformities were observed in the embryos and larvae of zebrafish after exposure to different concentrations of sumithion (Rahman *et al.* 2020, Marimuthu *et al.* 2013). Similarly banded gourami when exposed to chlorpyrifos, deformities were seen at different concentrations and it increases with increase time period (Sumon *et al*. 2016). As concentrations of insecticides raised, so did the formation of edema in embryos and post-hatch larvae, this could be because of osmoregulation failure linked to pesticide accumulation (Cook *et al*. 2005). Other abnormalities such as scoliosis and lordosis in the spine, embryo and larvae exposed to toxins, may be caused by insufficient neuromuscular coordination and unequal buildup of toxicants (Ekrem *et al*. 2012).

ACKNOWLEDGMENT

The authors would like to acknowledge the Department of Zoology, Pandu College, Assam 781012, India, for providing experimental facilities to conduct this study.

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