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Mugdha M Desai
PG Scholar, Department of Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Vikas V Karande
Professor & Head, Department of Veterinary Pharmacology and Toxicology, Kranti Sinha College of Veterinary Science, Maharashtra Animal and Fishery Science University, Shirwal, Satara, Maharashtra, India

BC Ghumare
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Kranti Sinha College of Veterinary Science, Maharashtra Animal and Fishery Science University, Shirwal, Satara, Maharashtra, India

CS Mote
Professor, Department of Veterinary Pharmacology and Toxicology, Kranti Sinha College of Veterinary Science, Maharashtra Animal and Fishery Science University, Shirwal, Satara, Maharashtra, India

SN Jadhav
Associate Professor, Department of Veterinary Biochemistry, Kranti Sinha College of Veterinary Science, Maharashtra Animal and Fishery Science University, Shirwal, Satara, Maharashtra, India

MD Meshram
Professor, Department of Veterinary Medicine, Kranti Sinha College of Veterinary Science, Maharashtra Animal and Fishery Science University, Shirwal, Satara, Maharashtra, India

TC Shende
Associate Professor, Department of AGB, Kranti Sinha College of Veterinary Science, Maharashtra Animal and Fishery Science University, Shirwal, Satara, Maharashtra, India

Corresponding Author:
Vikas V Karande
Professor & Head, Department of Veterinary Pharmacology and Toxicology, Kranti Sinha College of Veterinary Science, Maharashtra Animal and Fishery Science University, Shirwal, Satara, Maharashtra, India

Medial lethal concentration 50 (LC_{50}) of a Chlorantraniliprole-based insecticide in *Danio rerio*

Mugdha M Desai, Vikas V Karande, BC Ghumare, CS Mote, SN Jadhav, MD Meshram and TC Shende

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Abstract
Chlorantraniliprole (CAP) is a widely used insecticide whose potential toxicity to non-target aquatic organisms remains a concern. This study evaluates the acute toxicity, developmental toxicity of a chlorantraniliprole-based insecticide in zebrafish (*Danio rerio*), a standard model for toxicological research. One-hour post-fertilization embryos were exposed to varying concentrations (7.4–74 µg/ml) to determine the median lethal concentration (LC_{50}). The LC_{50} was 17.56 µg/ml, with dose-dependent mortality observed. Initial mortality was low during the first 72 hours but increased sharply post-hatching, indicating time-dependent toxicity mediated by the protective chorion. These findings reveal CAP's emphasizing the need for stringent environmental monitoring of this insecticide.

Keywords: Chlorantraniliprole, Zebrafish, LC_{50}, *Danio rerio*

Introduction
Chlorantraniliprole (CAP), an insecticide developed by DuPont, is widely used in agriculture globally. This anthranilic diamide effectively controls a broad spectrum of insect pests by rapidly stopping their feeding upon exposure, thereby protecting crops. CAP's insecticidal action is similar to that of flubendiamide. Both target ryanodine receptors in insect muscle cells, leading to muscle paralysis, lethargy, and eventual death in insects. A significant advantage of CAP is its high selectivity for insect ryanodine receptors, which means it has reduced toxicity to mammals and enhanced safety for humans. Toxicological assessments, including studies on manufacturing personnel and agricultural workers, have demonstrated that CAP presents no significant acute toxicity or adverse effects on skin, eyes, or reproductive health (Bentley *et al.*, 2009) [3].

Despite being designed with safety in mind for humans and the environment, research indicates that pesticides do not always meet these safety expectations. Although safety standards are established, a residual risk of adverse effects always remains. It is inherently challenging to definitively ascertain a pesticide's safety across all conditions and to predict its performance in varied scenarios. The market for diamide insecticides has experienced significant growth, with sales reaching $2.429 billion in 2019, marking the fastest growth rate (+9.7%) in the global pesticide market. Consequently, diamide insecticides have become the fourth most commonly used class of insecticides worldwide, representing 8% of total global insecticide sales. However, specific studies raise concerns even for pesticides considered less toxic. For instance, demonstrated that chlorantraniliprole, despite its perceived lower toxicity, can negatively impact the liver and kidneys in rats. These adverse effects manifested as alterations in body weight, liver enzyme levels, kidney function, and protein levels.

Zebrafish were chosen as the model organism for this study due to their numerous experimental advantages. These include transparent embryos and larvae, allowing for easy observation, as well as higher throughput, shorter test periods, lower cost, and reduced compound requirements. Their use is also approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) for toxicity and safety assessments in Investigational New Drug (IND) approvals.

Furthermore, zebrafish share over 85% genetic homology with humans and exhibit significant similarities in organogenesis and functional mechanisms. Given the limited existing research on chlorantraniliprole toxicity in aquatic organisms, this study aimed to evaluate the median lethal concentration, developmental toxicity caused by chlorantraniliprole-based insecticides. The research was conducted in accordance with OECD (2006) [8] guidelines, specifically utilizing TG 236 for the Fish Embryo Toxicity (FET) test.

Zebrafish Maintenance

For this study, 75 wild-type zebrafish (*Danio rerio*), specifically 25 females and 50 males aged 8 months, were obtained from Tarun Fish Farm in Chennai, India. These fish were kept at Krantisinh Nana Patil College of Veterinary Science in Shirwal and given seven days to acclimatize before any experiments began.

The zebrafish were housed in aerated glass tanks maintained at a temperature of 26-28°C. A controlled light cycle of 14 hours light and 10 hours dark was established using blinds to prevent any outside light interference. Feeding schedules followed the details in Table 1. Tanks were routinely cleaned, with a deep cleaning performed every 15 days to ensure the beneficial microbial population remained intact. Fish were separated into different tanks based on the specific needs of each experiment.

Experimental Design

The study evaluate the LC_{50} of a chlorantraniliprole (CAP)-based insecticide in zebrafish.

LC_{50}: A preliminary range-finding study using 14 different concentrations of CAP-based insecticide was conducted to determine the highest non-lethal and lowest lethal concentrations, which were found to be 0.04 µl/ml (7.4 µg/ml) and 0.4 µl/ml (74 µg/ml), respectively. Based on this, the following concentrations were selected for LC_{50} and developmental toxicity testing: 7.4, 9.25, 11.1, 12.95, 14.8, 16.65, 18.5, 37, 55, and 74 µg/ml.

The test formulation used was Coragen® 18.5% w/w SC (suspension concentrate), containing 200 g/L of chlorantraniliprole as the active ingredient. As the formulation was applied in µl/ml, the active ingredient concentration was calculated in µg/ml. The unequal intervals between test concentrations resulted from conversion adjustments based on the formulation strength. Stock solutions were prepared using E3 medium, a standard buffer that supports zebrafish embryo development by maintaining ionic and osmotic stability. Twenty embryos at one-hour post-fertilization (HPF) were exposed to each concentration in 24-well plates. A control group was maintained in E3 medium.

Mortality observations were made at 24, 48, and 72 HPF, assessing parameters outlined in OECD Test Guideline 236 (2006): egg coagulation, absence of heartbeat, failure of tail detachment, and developmental delays. The occurrence of any one of these endpoints was considered lethal. Cumulative mortality and survival were calculated, and LC_{50} was determined using the geometric mean method. A graph of mortality percentage versus concentration was plotted.

Results

Median Lethal Concentration (LC_{50}) Estimation

Table 1: Per cent mortality of embryos exposed to Chlorantraniliprole across different concentrations till 72 hrs. post exposure

Sr. No.	Concentrations µg/ml	Total no embryo	Cumulative		Mortality %
			Live	Dead	
1	7.40	20	118	0	0.00
2	9.25	20	98	4	3.92
3	11.10	20	82	8	8.89
4	12.95	20	66	13	16.46
5	14.8	20	51	19	27.14
6	16.65	20	37	29	43.94
7	18.5	20	27	39	59.09
8	37.0	20	17	50	74.63
9	55.0	20	8	62	88.57
10	74.0	20	0	82	100.00
					LC_{50} = 17.56

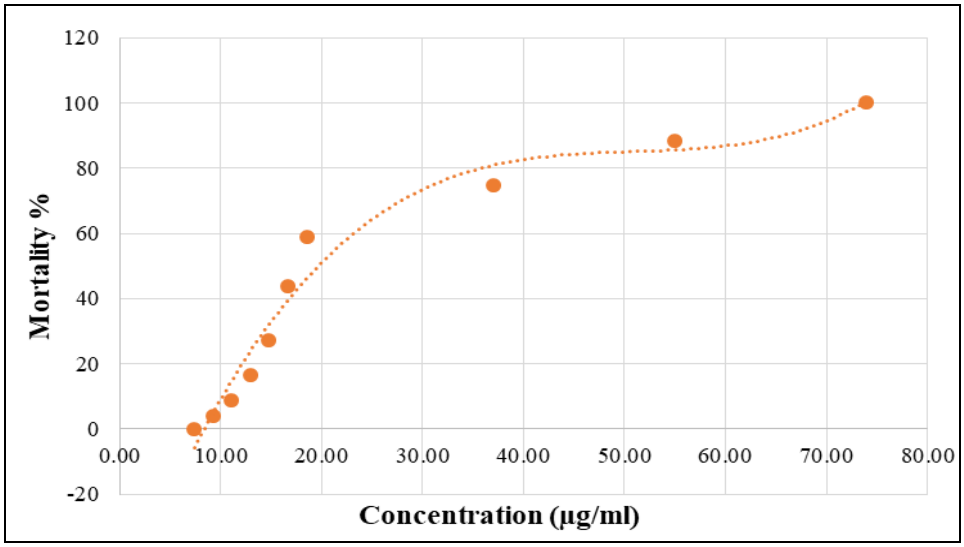


Fig 1: The graph showing concentration of test drug versus per cent mortality in zebrafish embryos exposed to Chlorantraniliprole at 72hrs post exposure

At the end of the 72-hour exposure period, the calculated median lethal concentration (LC_{50}) of the chlorantraniliprole-based insecticide was 17.56 µg/ml. This value was derived using the geometric mean method using result displayed in Table 1 and Figure 1.

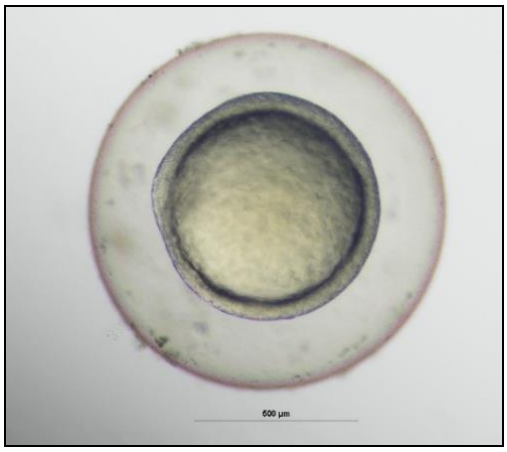
$$LC_{50} = \sqrt{c_{low} \times c_{high}}$$

Were, C_{low} is the highest concentration below 50% mortality C high is the lowest concentration above 50% mortality Graphically a sigmoidal dose-response curve was observed (Figure 3). At lower concentrations (7.4 to 9.25 µg/ml), mortality remained close to 0%, suggesting negligible toxicity at these levels. The no observed adverse effect concentration (NOAEC) was determined to be 7.4 µg/ml, as no mortality occurred at this dose. This finding aligns with Lahm *et al.* (2009) [7], who reported low toxicity at sublethal exposures.

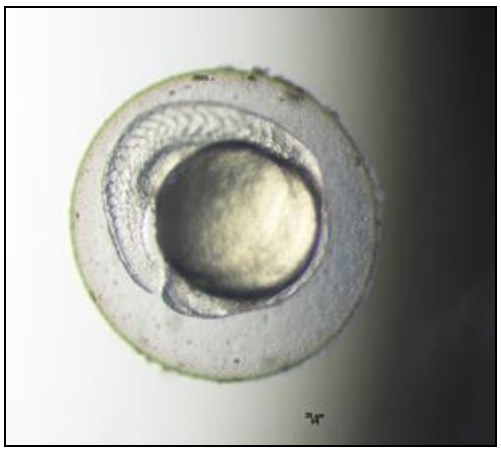
A steep increase in mortality was recorded between 11.1 and 18.5 µg/ml, indicating a clear threshold effect. The lowest observed effect concentration (LOEC) was likely around 9.25 µg/ml, supporting the classification of chlorantraniliprole as a high-potency insecticide due to its specific action on ryanodine receptors (Cordova *et al.*, 2006) [4].

Discussion: The LC_{50} value of 17.56 µg/ml for chlorantraniliprole in zebra fish embryos at 72 hours post-fertilization aligns with previous reports of its toxicity to aquatic organisms, although sensitivity varies by species and developmental stage. For example, *Daphnia magna*, a freshwater invertebrate, exhibited an LC_{50} of 8.5 µg/L at 48 hours, indicating greater vulnerability at earlier exposure durations. This supports the general observation that aquatic invertebrates are more sensitive than vertebrates to chlorantraniliprole exposure (Shi *et al.* 2025). Among fish species, *Labeo rohita* showed an LC_{50} of 12.748 mg/L after 96 hours (Bantu *et al.* 2013a) [1], and *Channa punctatus* reported an LC_{50} of 14.42 mg/L (Bantu *et al.* 2013b) [2]. The relatively higher LC_{50} values in these species compared to zebra fish embryos suggest that early developmental stages possess heightened sensitivity, likely due to immature detoxification pathways.

Chlorantraniliprole's toxic effect arises from modulation of ryanodine receptors, causing sustained calcium release in muscle tissue, leading to paralysis and death (Ebbinghaus-Kintscher *et al.* 2006) [5]. The mortality plateau observed beyond 37 µg/ml reflects receptor saturation, beyond which lethality stabilizes. Although initially promoted for selective toxicity toward lepidopteran pests (Lahm *et al.* 2009) [7], increasing evidence demonstrates adverse effects on non-target species such as pollinators and aquatic organisms (Bantu *et al.* 2013; He *et al.* 2024) [1, 6].



0–6 hour post fertilization embryo



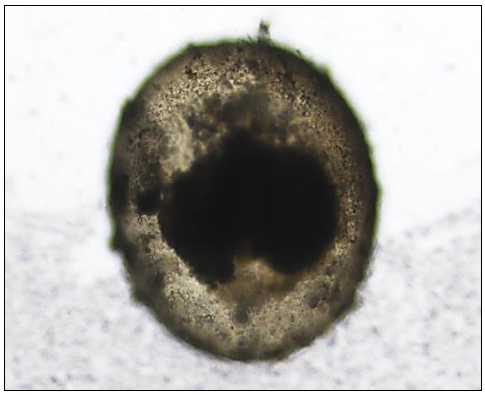
24-hour post fertilization embryo

48-hour post fertilization embryo

72-hour post fertilization larva

144-hour post fertilization larva

Plate 3.1: Control group of LC_{50} embryos showing normal developmental stages



A

Plate 3.2: Zebrafish embryos (24 hpf) showing coagulation at 74 µg/ml concentration

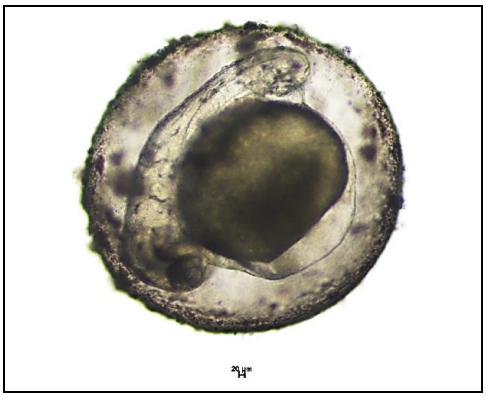
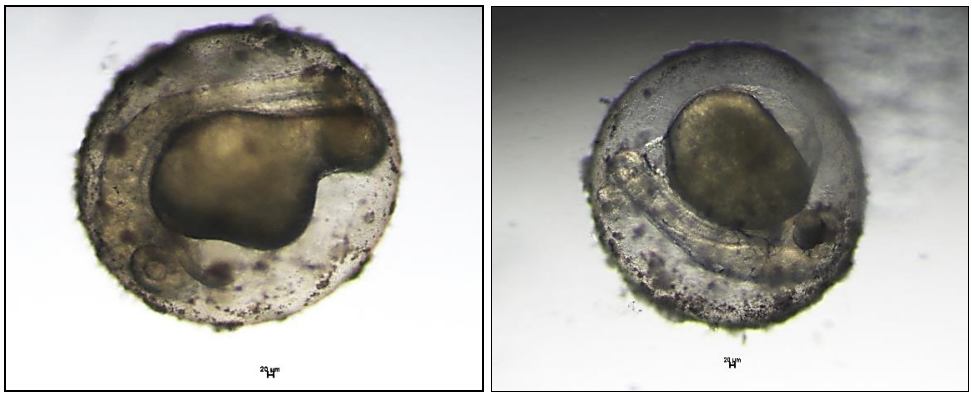
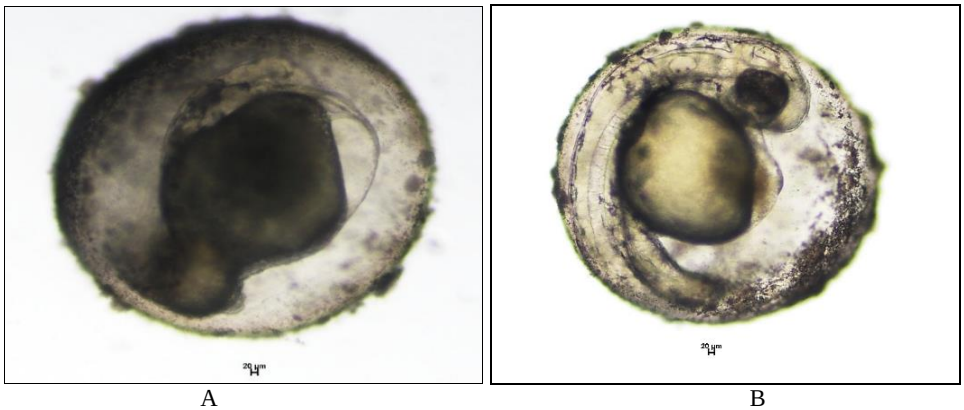


Plate 3.3: Zebrafish embryo (72 hpf) at concretion 14.8 µg/ml showing 1) Oedema of yolk sac and 2) Curl tail



A B

Plate 3.4: Zebrafish embryo (72 hpf) at concretion 16.65 µg/ml showing A: Oedema of pericardium and B: Curl tail



A B

Plate 3.5: Zebrafish embryos (72 hpf) at concentration 55 µg/ml showing A: Complete developmental abnormality of head and tail and delay in hatching, B: yolk sac oedema with hemorrhage and delay in hatching

Conclusion

From all the above research it can be concluded as

- Chlorantraniliprole (CAP) exposure resulted in significant developmental toxicity and endocrine disruption in zebra fish, highlighting its potential risk to Non-target organisms.
- The median lethal concentration (LC_{50}) of Chlorantraniliprole based insecticide (18.5 % w/w SC) was determined to be 17.56 µg/ml, with a clear dose-dependent increase in mortality.

Conflict of Interest: Not available

Financial Support: Not available

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