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Toxicological Impacts of Lambda-Cyhalothrin on Chick Embryo Morphogenesis

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ABSTRACT

Purpose: The negative consequences of prenatal Lambda-cyhalothrin (LCH) exposure have been well documented in clinical and experimental trials. This study presents an overview of research undertaken on chick embryos to investigate the numerous negative implications of embryonic exposure to LCH.

Methods: There were two groups each having sixty eggs named as control group (C), with Lambda-Cyhalothrin (LCH) group. Each egg was injected with 0.1mL of 5% DMSO solution in extra corn oil in control group and in (LCH) group each egg was injected with 0.1mL of 5% DMSO solution + 0.01mg/kg LCH solution in extra corn oil on zero day of incubation. For further development eggs were placed in an automatic incubator. The eggs were taken out of incubator on day 14 of incubation. Embryo were separated into dishes using brushes. Embryos were immersed in Bouin's fluid for four hours following recoveries. After that embryos were preserved in 70% alcohol to be examined for various anomalies. Photographs of selected embryos were captured and saved.

Results: By examining the embryos in each group, following morphological defects were found. Embryonic cataract, reduced beak (anathia) and eye opening. Haemorrhagic patches on the body, muscular dystrophy, various hindlimb deformities like torted shanks, focomelia and absent or improperly formed digits were also seen. The morphometric study also revealed a substantial difference between the control and LCH groups in several body characteristics such as biparietal distance, fronto-occipital lengths, eye circumference, beak lengths, and various appendicular lengths.

Conclusions: The findings of this study conclude that lambda-cyhalothrin insecticide exhibits embryotoxic and teratogenic effects in chick embryos even at relatively low doses.

Keywords: Pyrethroids, Lambda-cyhalothrin, toxicity, teratogenicity, chick

INTRODUCTION

Lambda-cyhalothrin is a highly effective, photostable type-II pyrethroid¹ extensively used for insect pest control in various crops, cotton plantations, and vegetable production.² It disrupts nerve conduction by delaying sodium channel closure in neuronal membranes, affecting calcium and chloride channels, ultimately leading to muscle paralysis and insect death.³

The widespread application of LCH poses significant toxicity risks to non-target species, including birds,

mammals, and aquatic organisms.⁴ This chemical demonstrates embryotoxicity even at low doses and is more persistent and toxic.⁵ Exposure can cause skin, eye, and nervous system disorders, with prolonged contact linked to cancer.⁶ Its extensive use in agriculture and public health leads to environmental harm and human poisoning.⁷ LCH further induce metabolic dysfunction, tissue damage, and embryotoxicity, with developing embryos being particularly susceptible.⁸

This insecticide is also linked to genotoxicity, chromosomal abnormalities, and reproductive dysfunction in rats.⁹ Prenatal exposure to lambda-cyhalothrin leads to reduced birth weight, fat accumulation, metabolic disruptions, weight loss, and increased embryonic mortality.¹⁰

In poultry, the LD50 of lambda-cyhalothrin in adult chicks is 228.5mg/kg, with dose-dependent effects causing teratogenic deformities, skeletal abnormalities, and hepatic toxicity, including condensed nuclei in liver cells.¹¹ Although research on its impact on birds is limited, lambda-cyhalothrin may affect avian reproduction, necessitating further study using chick embryos as a model for vertebrate development.¹²

Although research on its impact on birds is limited, lambda-cyhalothrin may affect avian reproduction, necessitating further study using chick embryos as a model for vertebrate development.¹² As poultry serves as a primary global source of eggs and meat, it is particularly vulnerable to pesticide residues in feed, emphasizing the need for regulated pesticide usage.¹³ A study conducted by the European Food Safety Authority finds that maximum residue level for LCH at the level of 0.02 mg/kg in poultry eggs¹⁴. The buildup of these residues in eggs and meat not only endangers consumers health but also increases risks of developmental defects in avian species.¹⁵

By selecting a dose of 0.01 mg/kg, we aimed to mimic a chronic low-dose exposure scenario, which birds might realistically encounter in pesticide-treated habitats. This sublethal dose is below the acute LD50 values reported for many bird species, thus allowing the investigation of potential behavioral, physiological, or reproductive effects that might occur under repeated environmental exposure without causing immediate mortality. The chosen dose ensures ecological relevance and provides insights into the long-term risks posed by lambda-cyhalothrin to avian and human population.

This study on chick embryos aims to assess the effects of prenatal exposure to lambda-cyhalothrin, offering insights into safer pesticide management and evaluating its teratogenic risks for both poultry production and human health.

MATERIAL AND METHODS

Eggs and Groups

A total of 120 freshly fertilized *Gallus domesticus* (common chick) eggs were obtained from various villages near Sargodha city. The eggs were first washed with boiled and cooled lukewarm water, air-dried, and then disinfected using cotton soaked in 70% alcohol to eliminate contamination. They were randomly divided into two groups of 60 eggs each: a control group (C) without any treatment and a Lambda-Cyhalothrin

group (LCH) exposed to Lambda-Cyhalothrin. The eggs were then incubated in an automatic incubator under standard hen egg incubation conditions for further development after administering the respective doses.

Dose preparation and Intoxication

Toxicant preparation

Lambda-cyhalothrin ($C_{23}H_{19}ClF_3NO_3$, CAS No. 91465-08-6) is a synthetic pyrethroid insecticide, commercially formulated as Karate 5EC (Syngenta, USA), with an oral LD50 of 228.5 mg/kg in adult chickens (Pinakin et al., 2011; Alrawe et al., 2022).

Lambda-cyhalothrin stock solution (1 mg/kg) preparation

By dissolving 0.0037g of lambda-cyhalothrin (LCH) in 10mL of solvent (5%DMSO solution in corn oil) 1mg/kg stock solution of lambda-cyhalothrin (LCH) was obtained.

Desire dose preparation of lambda-cyhalothrin (0.01mg/kg)

The required dose was prepared by using dilution method. To get 0.01mg/kg concentration of lambda-cyhalothrin required volume of LCH stock solution (1mg/kg) in the given volume of solvent was calculated by using succeeding formula:

$$C_1 V_1 = C_2 V_2$$

Dose group

- **Control group**

Each egg was injected with 0.1mL of a 5% DMSO solution dissolved in corn oil.

- **Lambda-cyhalothrin group**

In this group, each egg was injected with 0.1mL of a 5% DMSO solution combined with 0.01mg/kg LCH solution in corn oil.

Dose administration

Before administering the dose, the eggs were cleaned with cotton swabs soaked in 70% alcohol and allowed to air dry. A small window was carefully created in the center of the eggshell on the lateral side using a 1mL disposable syringe. To facilitate this process, a drop of HCl was applied to soften the eggshell with the help of a sterile syringe.

Prior to inoculation, the eggs were positioned horizontally to ensure the embryo moved to the top, preventing any damage during dose administration. The respective doses were injected on day 0 of incubation into the center of the egg yolk using a 1mL syringe through the lateral side. After treatment, each egg was immediately sealed with molten wax to prevent contamination. Finally, before being placed in the incubator, each egg was weighed using an electronic balance.

Incubation

For further development, the treated eggs were placed in an automatic incubator set at a temperature of $37 \pm 0.5^\circ\text{C}$ with 60% humidity and an automatic egg rotation every 6 hours. To maintain the required humidity levels, water was manually added on daily basis. As above stated, vital conditions are critical for chick care and effective hatching, they were continuously monitored. On day 4, eggs showing no embryonic development or signs of growth retardation were identified through candling and subsequently discarded.

Recoveries

On day 14 of incubation, the eggs were removed from the incubator. The embryos were carefully extracted by opening the broader end of the eggs using forceps and scissors. After piercing the eggshell, the contents were transferred into a Petri dish containing saline water. The embryos were then carefully separated using brushes. Following recovery, the embryos were immersed in Bouin's fluid for four hours. Subsequently, they were preserved in 70% alcohol for examination of potential anomalies. Photographs of selected embryos were taken and documented for further analysis.

Photography and morphological studies

A 16MP camera in super-macro mode was used to capture images of selected 14-day-old embryos from each group for terato-morphogenic analysis. The images were then processed and cropped using COREL PHOTO-PAINT11 to evaluate morphological malformations and highlight teratological abnormalities.

Morphometry

After removal from the fixative, each embryo was individually weighed using an electronic balance. To prevent dehydration, the embryos were placed in Petri plates containing a small amount of fixative. Morphological malformations were examined in embryos from each group. Various morphological measurements, including crown-rump length, fronto-occipital length, biparietal distance, eye width and

length, as well as forelimb and hindlimb lengths, were recorded for each embryo using a Vernier caliper (ensuring no zero error). All collected data were preserved for further statistical analysis.

Statistical analysis

After data collection, statistical analysis was conducted using an independent sample t-test with a significance level set at $p \leq 0.05$. The analysis was performed using IBM SPSS Statistics 20 software.

RESULTS

Results after morphology

Embryos in the control group exhibited all typical morphological traits, including the development of hind and forelimbs, properly formed eyes and beak, normal down growth, and standard body size. However, after treatment with lambda-cyhalothrin, a noticeable reduction in average body size was observed compared to the control group. The most frequently noted abnormalities included embryonic cataracts, incomplete down development, reduced beak size (anathia), and impaired eye opening. Additionally, several other anomalies were identified, such as a crooked spine, hemorrhagic patches on the body, and muscular dystrophy. Various hindlimb deformities, including tortorted shanks, phocomelia, and absent or malformed digits, were also observed. Some forelimb anomalies, such as amelia and meromelia, were also present (Figure 1).

Morphometric results

The mean length of the crown ramp was used as a covariate in the statistical analysis of the embryo's weight, Eye, beak brachium and middle finger lengths, using the t-test method, findings showed a highly significant difference ($p \leq 0.000$) between LCH and control groups. The mean length of the crown-ramp, eye width, circumference of eye, anti-brachium and thumb lengths revealed high significance ($p \leq 0.03$). While for fronto-occipital length, biparietal distance, shank length, lower length, index and little finger lengths reveal significant difference ($p \leq 0.05$) (Table. 1)

Table 1 The table shows all additional values of morphometric measurements for relevant group

Morphological variables	Mean + SEM	
	Control	LCH
Mean embryo weight (g)***	8.26±0.03	2.74±0.25
Mean crown-ramp length (mm)**	56.24±0.72	30.97±7.8
Mean fronto-occipital length (mm)*	17.66±0.88	11.00±0.32
Mean biparietal distance (mm)*	8.16±2.05	9.14±0.28
Mean width of eye (mm)**	11.63±0.58	8.72±0.20
Mean length of eye (mm)***	10.33±0.42	8.20±0.12
Mean eye circumference (mm)**	28.19±1.19	21.72±0.59
Mean beak length (upper) (mm)***	8.01±0.48	6.53±0.26
Mean beak length (lower) (mm)**	8.67±0.99	5.11±0.58
Mean anti-brachium length (left) (mm)*	6.38±1.60	5.20±0.22
Mean anti-brachium length (right)(mm)**	7.38±0.52	4.28±0.21
Mean brachium length (left) (mm)**	7.05±0.28	6.14±0.12
Mean brachium length (right) (mm)***	7.59±0.25	6.13±0.13
Mean shank length (left) (mm)*	8.33±0.52	6.79±0.11
Mean shank length (right) (mm)*	9.06±0.39	6.44±0.21
Mean thumb length (left) (mm) **	4.16±0.37	2.46±0.18
Mean thumb length (right) (mm) **	4.22±0.36	2.44±0.18
Mean middle finger length (left) (mm) ***	6.98±.46	5.01±.18
Mean middle finger length (right) (mm) **	6.98±0.38	5.82±0.12
Mean index finger length (left) (mm)**	6.48±0.53	6.48±0.19
Mean index finger length (right) (mm) *	6.34±0.55	3.67±0.22
Mean little finger length (left) (mm)*	6.12±0.10	4.43±0.31
Mean little finger length (right) (mm) *	6.17±0.06	4.64±0.43
Mean lower leg length (left)(mm) *	15.46±0.39	12.07±0.08
Mean lower leg length (right)(mm) *	16.14±0.09	11.26±0.37

*: P≤ 0.05, **: P≤ 0.001, ***: P≤ 0.0001

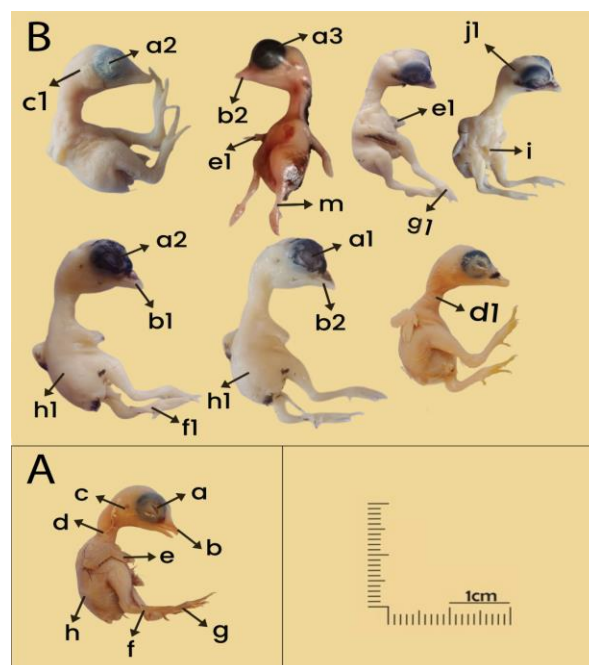


Figure 1. **A:** Control, **B:** LCH a: Normal eye formation with eye lids in embryo, **a1:** Wide open eye and embryonic cataract, **a2:** Eye without eye lids, **a3:** Protruding eye, **b:** Normal beak, **b1:** Reduced lower beak, **b2:** Reduced beak, **c:** Normal external auditory meatus, **c1:** External auditory meatus is absent, **d:** Normal neck, **d1:** Twisted neck, **e:** normal fore limbs, **e1:** Reduced/rudimentary fore limbs (Meromelia), **f:** Normal hind limbs, **f1:** short hind limbs with torted shank, **g:** Normal digits of hind limbs, **g1:** Reduced hind limb digits (amelia), **h:** normal down feather development, **h1:** Reduced or no down development, **i:** Hemorrhagia, **j:** Anterior neuropore opening, **m:** Muscular dystrophy

DISCUSSION

The teratogenic effects of in-Ovo lambda-cyhalothrin (LCH) exposure were assessed using chick embryos as a model, with comparisons made to the control group. This study revealed that, similar to many insecticides, LCH exhibits embryotoxic and teratogenic properties in chick embryos. The observed morphological abnormalities in this study are strongly supported by various micrometric parameters analyzed, aligning with previous research findings.¹⁴⁻¹⁷

Due to lipophilic nature of LCH is readily absorbed by cellular membranes, allowing it to penetrate the insect's cuticle rapidly.¹⁸ Once inside, it disrupts nerve conduction, ultimately leading to paralysis and death by impairing the insect's ability to regulate muscle function.¹⁹

Studies in both human and animal models have demonstrated that pesticides exert their toxic effects primarily through oxidative stress induction. The teratogenic potential of lambda-cyhalothrin (LCH) is primarily attributed to the presence of a fluoride halogen. This fluoride-containing pesticide induces oxidative stress by generating reactive oxygen species (ROS). Furthermore, LCH biochemically disrupts mitochondrial respiration and contributes to oxidative damage.²⁰ Oxidative stress, in turn also interfering with normal embryonic development.²¹

In this study exposure led to embryonic death, growth retardation, and a range of developmental abnormalities, including embryonic cataracts, impaired limb development, delayed overall growth, reduced crown-rump length, smaller head circumference, decreased weight, and diminished muscle growth. Additionally, LCH significantly affected the development of claws and down feathers. Our findings suggest that LCH exposure may have considerably reduced protein synthesis and deposition in embryonic tissue.

This heightened susceptibility is attributed to the significantly higher metabolic rate of differentiating tissues and organs in developing embryos compared to adults, making them more prone to oxidative damage.²²

Nonetheless, the study is constrained by a limited sample size of 120 fertilized chick eggs, which may influence the broader applicability of the results.

Future research studies using larger sample size suggested to overcome this constraint and ensure more reliable and generalizable results.

CONCLUSIONS

Based on the findings of our study, it can be concluded that lambda-cyhalothrin exhibits embryotoxic and teratogenic effects in chick embryos

even at a relatively low dose (0.01 mg/kg). Therefore, its use should be restricted. Given its potential risks, we do not recommend the application of this insecticide, and its usage should be strictly limited in both household and agricultural settings.

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CONFLICT OF INTERESTS

"None"

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