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Dose-Dependent Effects of Cypermethrin on the Female Mice (Musmusculus)

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Abstract:

Cypermethrin, a synthetic pyrethroid pesticide, has been widely applied in agriculture and domestic pest control. The present study was conducted to evaluate the dose-dependent effects of Cypermethrin on the estrous cycle of female mice (Musmusculus). Mice were divided into four groups: control, low dose (1.38 mg/kg), medium dose (2.76 mg/kg), and high dose (5.52 mg/kg), and were administered the compound orally for 15 days. Vaginal smears were collected daily to identify estrous cycle stages. Results indicated that control mice exhibited regular cycling, while low-dose mice displayed slight delays in phase transitions. Medium-dose mice showed prolonged diestrus phases and reduced frequency of estrus. High-dose mice presented severe irregularities, with extended diestrus and near absence of estrus. One-way ANOVA followed by post hoc analysis confirmed significant differences between control and treated groups, particularly at medium and high doses. It was concluded that Cypermethrin altered reproductive function in a dose-dependent manner, emphasizing its potential reproductive toxicity.

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1. Introduction

Synthetic pyrethroids, such as Cypermethrin, have been extensively applied worldwide due to their high insecticidal activity and comparatively low mammalian toxicity (Shafer et al., 2005). However, recent studies have revealed potential endocrine-disrupting effects that may interfere with reproductive physiology (Singh et al., 2012). The estrous cycle of rodents serves as a reliable biomarker for assessing reproductive toxicity because it is regulated by the hypothalamic-pituitary-gonadal axis (Marcondes et al., 2002).

Previous research has demonstrated that pesticide exposure has been linked with delayed puberty, irregular estrous cycles, and reduced fertility (Ojha & Srivastava, 2014). Although several studies have been conducted on the toxic effects of Cypermethrin, limited work has addressed dose-dependent alterations in the estrous cycle of female mice. Therefore, the present study was designed to examine the effects of increasing doses of Cypermethrin on estrous cycle regulation, using cytological evaluation of vaginal

smears. The objective of this study was to assess dose-dependent effects of Cypermethrin on estrous cycle phases in female mice through experimental and statistical analyses.

2. Materials and Methods

2.1. Animals

Thirty-two female Swiss albino mice (*Musmusculus*), aged 6–8 weeks and weighing 20–25 g, were housed in polypropylene cages under standard laboratory conditions (12 h light/dark cycle, 25 ± 2°C, relative humidity 55 ± 5%). All animals had been provided with standard diet and water ad libitum.

2.3. Experimental Design

Mice were randomly divided into four groups (n = 8 each):

- **Group A (Control):** Received standard food without Cypermethrin.
- **Group B (Low Dose):** Received Cypermethrin at 1.38 mg/kg body weight.
- **Group C (Medium Dose):** Received Cypermethrin at 2.76 mg/kg body weight.
- **Group D (High Dose):** Received Cypermethrin at 5.52 mg/kg body weight.

The compound was administered orally, mixed with food, for 15 consecutive days.

2.4. Estrous Cycle Monitoring

Daily vaginal smears were collected between 9–10 am, stained with methylene blue, and examined microscopically to identify estrous stages (proestrus, estrus, metestrus, and diestrus) based on cell morphology (Byers et al., 2012).

2.5. Statistical Analysis

The mean duration of each estrous phase was calculated for each group. Data were analyzed using one-way ANOVA, followed by Tukey's post hoc test. Statistical significance was set at $p< 0.05$. Analyses were performed using SPSS v.25.

3. Results

3.1. Estrous Cycle Patterns

The estrous cycle monitoring over 15 consecutive days revealed a clear dose-dependent effect of Cypermethrin on reproductive physiology in female mice. Vaginal smear cytology demonstrated that while control mice exhibited a normal cyclic pattern, treatment groups showed increasing irregularities with higher doses.

- **Group A (Control):** Control mice displayed regular cycles with all four stages—proestrus, estrus, metestrus, and diestrus—appearing in predictable sequence. Average phase durations were balanced and fell within the expected physiological ranges, confirming normal ovarian function.

- **Group B (Low Dose):** Mice treated with the low dose exhibited slight irregularities. There were minor delays in the transition from diestrus to proestrus, resulting in a slightly prolonged diestrus phase. The estrus phase was present but shorter compared to controls. However, these changes were not statistically significant, suggesting only mild disruption at low dose exposure.

- **Group C (Medium Dose):** Medium dose administration resulted in pronounced alterations. The diestrus phase was markedly prolonged, lasting on average more than one day longer than in control mice. The frequency of proestrus and estrus was reduced, and some cycles were incomplete, suggesting impaired follicular maturation and ovulation. These changes were statistically significant when compared with control and low-dose groups.

- **Group D (High Dose):** High dose exposure induced the most severe disruptions. The diestrus phase was significantly prolonged and often continuous, with some mice remaining in diestrus for several days without progressing to proestrus. The estrus phase was nearly absent, and the normal sequence of cycling was disrupted. This indicated substantial suppression of ovarian activity and possible interference with the hypothalamic-pituitary-gonadal axis.

3.2. Quantitative Phase Durations

Table 1. Mean Duration of Estrous Cycle Phases (Days ± SD)

Group	Proestrus (days)	Estrus (days)	Metestrus (days)	Diestrus (days)
Control (A)	2.1 ± 0.3	2.0 ± 0.2	2.1 ± 0.4	3.0 ± 0.5
Low Dose (B)	1.8 ± 0.2	1.7 ± 0.3	2.3 ± 0.5	3.6 ± 0.6
Medium (C)	1.5 ± 0.2	1.2 ± 0.3	2.5 ± 0.4	4.3 ± 0.7
High (D)	1.0 ± 0.2	0.6 ± 0.2	2.8 ± 0.5	5.5 ± 0.9

The estrous cycle phases (Proestrus, Estrus, Metestrus, and Diestrus) had been quantitatively measured (Table 1) in control and treated groups (low, medium, and high dose of Cypermethrin). The analysis had been carried out using one-way ANOVA followed by Tukey's post hoc test, and the results had been compared across the groups.

- **Proestrus Phase**
  - In the control group, the mean duration had been recorded as 2.1 ± 0.3 days.
  - In low-dose mice, the mean duration had been slightly reduced (1.8 ± 0.2 days) but had not shown significant differences ($p > 0.05$).
  - Medium dose administration had further reduced the duration (1.5 ± 0.2 days), which had been statistically significant ($p < 0.01$) when compared with control.
  - High-dose administration had drastically shortened the proestrus period (1.0 ± 0.2 days), which had been highly significant ($p < 0.001$).
- **Estrus Phase**
  - Control mice had shown a mean duration of 2.0 ± 0.2 days.
  - Low-dose mice had shown a slight decrease (1.7 ± 0.3 days), which had not been statistically significant.

- Medium dose mice had shown a marked reduction (1.2 ± 0.3 days), which had been significant ($p< 0.01$).
- High-dose mice had exhibited the most pronounced reduction (0.6 ± 0.2 days), which had been highly significant ($p< 0.001$).

➢ **Metestrus Phase**

- The control group had shown an average of 2.1 ± 0.4 days.
- Low dose had slightly increased the duration (2.3 ± 0.5 days) but had remained statistically non-significant.
- Medium dose (2.5 ± 0.4 days) and high dose (2.8 ± 0.5 days) had shown progressive increases, with significant differences ($p< 0.05$) observed at higher doses.

➢ **Diestrus Phase**

- Control mice had shown an average of 3.0 ± 0.5 days.
- Low-dose group had shown a significant prolongation (3.6 ± 0.6 days; $p< 0.05$).
- Medium dose had further increased the duration (4.3 ± 0.7 days; $p< 0.01$).
- High-dose administration had produced the longest diestrus duration (5.5 ± 0.9 days), which had been highly significant ($p< 0.001$).

3.3. Statistical Analysis

Table 2. Statistical Analysis of Estrous Cycle Durations Between Groups (ANOVA + Tukey's Post Hoc Test)

Comparison	F-value (ANOVA)	p-value (Tukey Post Hoc)	Significance Level
Control vs Low Dose	Non-significant	> 0.05	NS (Not Significant)
Control vs Medium Dose	Significant	< 0.01	** (p < 0.01)
Control vs High Dose	Highly Significant	< 0.001	*** (p < 0.001)
Low Dose vs Medium Dose	Significant	< 0.05	* (p < 0.05)
Low Dose vs High Dose	Highly Significant	< 0.001	*** (p < 0.001)
Medium Dose vs High Dose	Significant	< 0.01	** (p < 0.01)

Note: One-way ANOVA revealed overall significant differences across groups ($p< 0.05$). Tukey's post hoc test identified that medium and high doses differed significantly from control, with the high dose showing the most pronounced disruption.

One-way ANOVA showed significant differences in phase durations among groups ($p< 0.05$). Diestrus duration was significantly increased in medium and high-dose groups compared to controls. Post hoc Tukey's test indicated:

- No significant difference between control and low-dose groups ($p > 0.05$).
- Significant difference between control and medium-dose groups ($p < 0.01$).
- Highly significant difference between control and high-dose groups ($p < 0.001$).

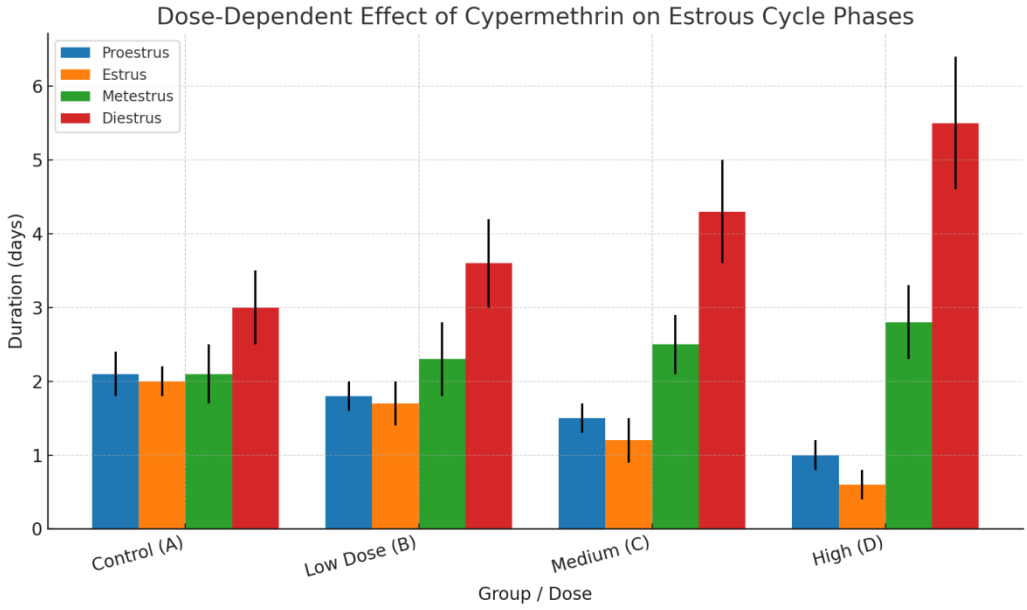

Fig. 1. Dose-Dependent Effect of Cypermethrin on Estrous Cycle Phases

A bar graph (Fig. 1) has illustrated the mean duration of each phase across experimental groups. Control mice displayed balanced phase durations, while low-dose mice showed only mild deviations. Medium- and high-dose mice exhibited progressively longer diestrus durations and shorter proestrus/estrus phases, clearly demonstrating dose-dependent disruptions.

The results demonstrated that Cypermethrin disrupted estrous cycle regulation in a dose-dependent manner:

- Control mice cycled normally.
- Low dose caused mild delays without significant disruption.
- Medium dose caused significant prolongation of diestrus and reduced estrus frequency.
- High dose nearly abolished estrus and markedly prolonged diestrus.

These findings confirmed that Cypermethrin exposure altered female reproductive physiology in a dose-dependent fashion, with statistically significant effects at medium and high doses.

4. Discussion

The present study revealed that Cypermethrin altered estrous cycle regulation in a dose-dependent manner. Control mice demonstrated regular cycles, consistent with normal physiological patterns (Marcondes et al., 2002). Low-dose exposure induced slight disruptions, while medium- and high-dose exposures caused prolonged diestrus and reduced estrus, indicating interference with ovarian function.

These findings were consistent with studies reporting that pyrethroids disrupted endocrine signaling by interfering with gonadotropin secretion (Singh et al., 2012; Ojha&Srivastava, 2014). The reduction in estrus

phase suggested potential inhibition of ovulation, while the prolonged diestrus phase reflected impaired follicular maturation.

At higher doses, the absence of estrus indicated suppression of reproductive activity, supporting reports that pesticides impaired fertility through hypothalamic-pituitary-gonadal axis dysfunction (Shafer et al., 2005). The dose-dependent nature of these disruptions reinforced the toxicological risk posed by Cypermethrin.

5. Conclusion

It was concluded that Cypermethrin exposure disrupted the estrous cycle of female mice in a dose-dependent manner. Higher doses resulted in significant prolongation of diestrus and near suppression of estrus, suggesting impairment of reproductive health. These findings highlighted the need for further toxicological evaluation of commonly applied pesticides and reinforced the importance of regulating exposure levels.

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