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# Beta cypermethrin exposure and perinatal reproductive development of female f1 generation of albino rats

Victoria Chinenye Obinna* and Gabriel Ogaba Agu

## Abstract

**Background:** Previous studies have shown that cypermethrin has a teratogenic effect on rat feti born to exposed dam or buck with no information on its effect on their reproductive parameters at maturity. The present study was aimed at evaluating the reproductive effect of perinatal beta cypermethrin (β-cyp) exposure on female F1 generation rat.

**Methods:** Fifteen pregnant animals (day 0 = day of mating, average body-weight = 190 g) were randomly divided into 3 groups. Group A (control) received 0.5 ml olive oil, group B (15 mg/kg β-cyp), and group C (30 mg/kg β-cyp) by oral gavage from gestational day (GD) 1—post natal day (PND) 20. On PND 21, the pups were weaned and bred to 12 weeks of age (maturity). At maturity, 5 females were randomly taken from each group. The estrous cycle was determined for 14 days. Thereafter, the animals were anesthetized and blood was collected for hormonal assay (LH, FSH, estrogen, progesterone) using enzyme immunoassay.

**Results:** β-cyp had no significant effect ($p > 0.05$) on the estrous cycle. There was no significant ($p > 0.05$) change in the serum concentration of the sex hormones except the LH concentration where there was a dose-dependent significant ($p < 0.05$) decrease in treated groups relative to the control.

**Conclusion:** It is therefore concluded that perinatal beta cypermethrin exposure could have a deleterious effect on the reproductive development of female F1 generation rats, even at maturity.

**Keywords:** Beta cypermethrin, pesticide, estrous cycle, perinatal, female hormones, reproduction

## Background

Pesticides may cause reproductive toxicity through several different mechanisms such as direct damage to the structure of cells, interference with biochemical processes necessary for normal cell function, endocrine disruption, and biotransformation resulting in toxic metabolites (Bretveld et al., 2006) A number of studies reported that among women occupationally exposed to pesticides and/or working in the agricultural sector, the risks of spontaneous abortion (Arbuckle, Lin, & Mery, 2001; Arbuckle, Savitz, Mery, & Curtis, 1999; Hemminki, Niemi, Saloniemi, Vainio, & Hemminki, 1980; Nurminen, 1995) and stillbirth (Pastore, Hertz-Picciotto, & Beaumont, 1997; Savitz, Whelan, & Kleckner, 1989) seemed to be significantly increased.

Animal studies on pyrethroid pesticides such as cypermethrin, deltamethrin, and fenvalerate have demonstrated insecticide-related reproductive adverse effects both in male, female, and fetal organisms (Husain, Malaviya, Seth, & Husain, 1992; Malaviya, Husain, Seth, & Husain, 1993; Meeker, Barr, & Hauser, 2008; Oda & El-Maddawy, 2012; Suresh, Bhawna, & Nakuleshwar, 2011). Other studies also showed growth retardation and/or fetal loss due to exposure of pregnant animals to pyrethroids (Abdel-Khalik et al., 1993; Ullah et al., 2006) Pyrethroids have also been reported to cause developmental neurotoxicity (Shafer, Meyer, & Crofton, 2005).

Many studies that considered the teratogenic effect of pyrethroids on rodents limited maternal exposure to pregnancy only (Assayed, Khalaf, & Salem, 2010; Sallam et al.,

* Correspondence: drchiobinna@yahoo.co.uk; victoria_obinna@uniport.edu.ng
Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria

2015; Ullah et al., 2006), but the present work, using beta cypermethrin (β-cyp) as an example, extended the maternal exposure to the period of lactation (PND 0–21) after which the F1 generation was raised to maturity before the collection of samples for analyses. The aim of the present study, therefore, is to evaluate the reproductive development of female F1 generation rats exposed to β-cyp during the perinatal period.

## Methods

### Chemicals and reagents

β-cyp (a mixture of the alpha and theta forms of the insecticide) at 95.8% purity was purchased from Haihang Industry Company, Limited, China, as white to light yellow crystalline powder with CAS No: 52315-07-8 and Batch No: 20140517. The desired doses were prepared in olive oil which was purchased from the supermarket. All other chemicals were of the finest analytical grade.

### Animals and treatment

Fifteen mature female albino rats weighing an average of 190 g procured from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria, were used for the study. The rats were acclimatized for 2 weeks before commencing the study. They were fed ad libitum with commercially sourced feed (Top Feeds Nigeria Limited) and supplied with clean drinking water all through the study.

Following acclimatization, one female was paired with a male in a cage overnight. Mating was confirmed the following day by the presence of sperm cells in vaginal smear or presence of vaginal plug and designated gestational day (GD) 0. They were then grouped into 3, each group housed in a cage. Group A (control) received 0.5 ml olive oil, group B (15 mg/kg β-cyp), and group C (30 mg/kg β-cyp, by oral gavage from gestational day (GD) 1—post natal day (PND) 20. On PND 21, the rat pups were weaned and bred to 12 weeks in order to attain maturity. At maturity, 5 females were randomly taken from each group and the estrous cycle was determined for 14 days starting at the estrus phase.

### Sample collection

The vaginal smear was collected daily in the morning using the pipette smear technique. The tip of a dropping pipette containing a few drops of normal saline (0.9% NaCl) was inserted into each rat's vagina. The fluid was used to flush cells from the vaginal lining after which the resulting suspension was placed on a clean glass slide and examined under a light microscope. The phases of the estrous cycle were confirmed depending on the different characteristic cells.

Thereafter, the animals were anesthetized and blood samples collected from the retro-orbital plexuses using the microhaematocrit capillary tube. The collected blood was allowed to stand for 30–45 min in order to coagulate and then centrifuged for 15 min at 3000 revs/min to obtain the serum for hormone analysis. The serum was then tipped into a separate vial, placed in microcentrifuge tubes, capped, and stored at − 20 °C until analysis. The serum was later subjected to hormonal assay to assess the LH, FSH, estrogen, and progesterone levels.

### Statistical analysis

Statistical analysis was done using SPSS 21. All values were expressed as mean ± SEM, and data were analyzed by one-way ANOVA followed by the Tukey post test. The significance level was set at $p < 0.05$.

## Results

Figure 1 summarizes the effect of perinatal β-cyp exposure on the estrous cycle of female F1 generation rats at sexual maturity. The result shows that there was no significant ($p > 0.05$) effect on proestrus, estrus, metestrus, and diestrus phases of the estrous cycle when compared with the control. However, a dose-dependent non-significant ($p > 0.05$) decrease and increase were observed in the estrus and diestrus phases, respectively, in relation to the control.

There was no significant ($p > 0.05$) difference in the mean serum concentrations of estrogen, progesterone, and FSH in all the test groups when compared with the control (Figs. 2, 3 and 4). However, a dose-dependent non-significant ($p > 0.05$) decrease was observed in the mean estrogen level relative to the control (Fig. 2). Also from the result, a dose-dependent significant ($p < 0.05$) decrease occurred in the mean LH level in relation to the control (Fig. 5).

## Discussion

Pesticides affecting reproduction may act on selected stages, targeting the prenatal stage and the pre-pubertal stage or the adult, thereby resulting in developmental impairments and/or damage to the reproductive organs and/or impaired fertility (Malik, Aggarwal, Kalpana, & Gupta, 2011). These chemicals can be transmitted prenatally to the developing fetus or postnatally from breast milk to the suckling. Exposures via breast milk can be significant, particularly when the mother has substantial constant exposures or has accumulated a remarkably high body burden of persistent chemicals (Anderson & Wolff, 2000). It has been reported that neonatal exposure to pyrethroids may induce developmental toxicity in adults (Malik et al., 2011).

In our earlier studies, β-cyp, an example of pyrethroid, was found to alter the biochemical (Obinna & Kagbo, 2018a) and hematological (Obinna & Kagbo, 2018b) profiles of rats' offspring exposed during the perinatal period but had no significant effect on the

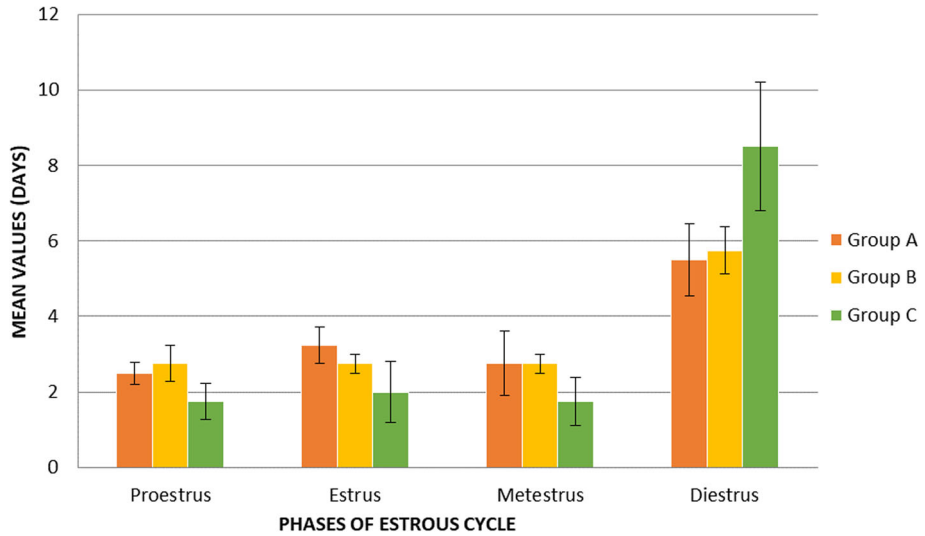


**Fig. 1** Effect of perinatal β-cyp exposure on the estrous cycle of female F1 generation rats at sexual maturity. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p > 0.05$). Groups A, B, and C represent the control (given 0.5 ml olive oil), 15 mg/kg β-cyp-treated rats, and 30 mg/kg β-cyp-treated rats, respectively

reproductive development of male F1 generation at sexual maturity (Obinna & Agu, 2018). The present study, which evaluated the reproductive development of sexually matured female F1 generation rats exposed to β-cyp during the perinatal period, found that the estrous cycle was not significantly altered. However, a minor decrease in the estrus phase and also a slight increase in the diestrus phase were observed. Also, of all the female serum sex hormones assessed, only the serum LH concentration showed a significant decrease, although a slight decline in serum estrogen concentration was observed. Both reductions occurred in a dose-dependent manner.

Generally, the estrous cycle is classified into four phases namely the proestrus phase, the period of rapid follicular growth which precedes the onset of oestrus; the estrus phase, the heat period when ovulation occurs and is usually seen as the fertile period since mating is allowed only in this phase; the metestrus phase, the period of early corpus luteum development; and the diestrus phase, the period of mature corpus luteum activity, which usually ends with the regression of the corpus luteum. These four phases are also grouped into two, the follicular phase and the luteal phase with proestrus and estrus as part of the follicular phase, and metestrus and diestrus as part of the luteal phase.

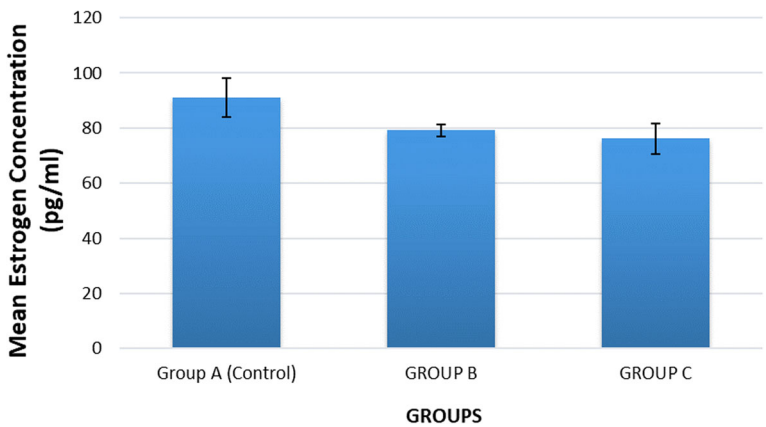


**Fig. 2** Effect of perinatal β-cyp exposure on serum estrogen concentration of female F1 generation rats at sexual maturity. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p > 0.05$). Groups A, B, and C represent the control (given 0.5 ml olive oil), 15 mg/kg β-cyp-treated rats, and 30 mg/kg β-cyp-treated rats, respectively

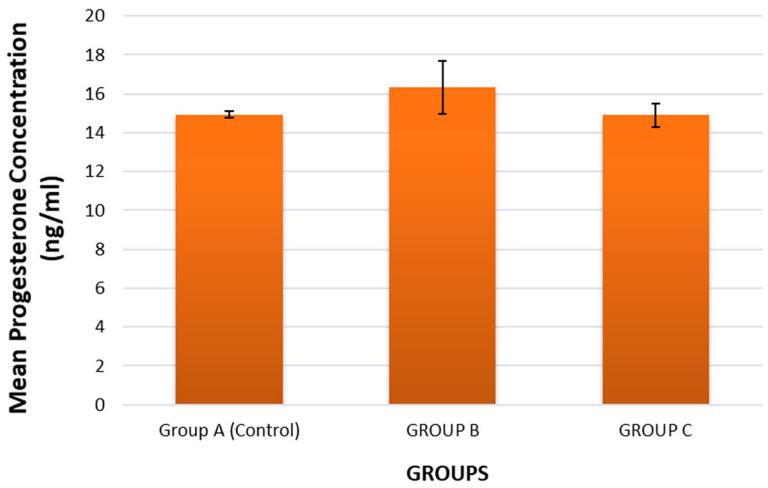


**Fig. 3** Effect of perinatal β-cyp exposure on serum progesterone concentration of female F1 generation rats at sexual maturity. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p > 0.05$). Groups A, B, and C represent the control (given 0.5 ml olive oil), 15 mg/kg β-cyp-treated rats, and 30 mg/kg β-cyp-treated rats, respectively

The estrous cycle is synchronized by the female sex hormones since all the phases of the estrous cycle occur as a result of the cyclical increase and decrease of these hormones. Correlating the estrous cycle and the female hormonal system, it is known that during the follicular growth and maturation which occurs at proestrus, there is a rise in FSH concentration which triggers the development and maturation of follicles in animals. According to Verma, Tyagi, & Agarwal (2015), the FSH released in response to GnRH secretion from the hypothalamus initiates the ovarian follicular development and the theca cells of these follicles synthesize androgens which are converted to estrogen in the granulosa cells by the enzyme, aromatase. As the level of estrogen increases, inhibition of FSH occurs with an attendant stimulation of LH release (Sejian, Meenambigai, Chandirasegaram, & Naqvi, 2010). The increased blood estrogen level triggers the shutdown of the FSH and also the release of LH. At estrus, the residual peripheral FSH and the pre-ovulatory LH surge brings about the rapid swelling of the follicle few hours to days before ovulation depending on the animal species. This interplay of hormones with their effects causes ovulation. The LH also stimulates the formation of corpus luteum from the ruptured follicle (Hall, 2011) and this occurs at metestrus. The cells of the corpus luteum secrete the ovarian hormones—progesterone, estrogen, and inhibin. The increase in the concentrations of these hormones inhibits the production of LH

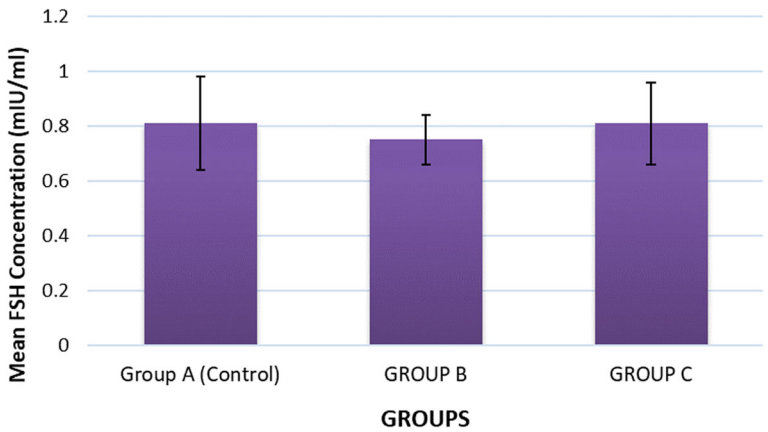


**Fig. 4** Effect of perinatal β-cyp exposure on serum FSH concentration of female F1 generation rats at sexual maturity. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p > 0.05$). Groups A, B, and C represent the control (given 0.5 ml olive oil), 15 mg/kg β-cyp-treated rats, and 30 mg/kg β-cyp-treated rats, respectively

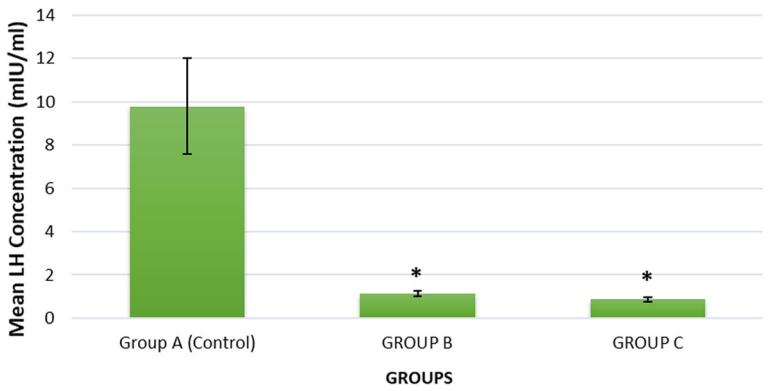


**Fig. 5** Effect of perinatal beta cypermethrin exposure on serum LH concentration of female F1 generation rats at sexual maturity. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). *Indicates a significant difference at $p < 0.05$. Groups A, B, and C represent the control (given 0.5 ml olive oil), 15 mg/kg β-cyp-treated rats, and 30 mg/kg β-cyp-treated rats, respectively

and FSH at diestrus. In the absence of fertilization, the corpus luteum regresses and becomes involuted which effect markedly reduces the secretion of these ovarian hormones, thereby resulting in positive feedback on the anterior pituitary to stimulate the secretion of FSH. As the FSH level begins to rise, new ovarian follicular growth is initiated at proestrus for a new cycle to commence.

Therefore, the pre-ovulatory surge of LH is a prerequisite for ovulation which is known to occur at the estrus phase. According to Hall (2011), if the pre-ovulatory surge of LH is not of sufficient magnitude, ovulation will not occur. Therefore, the diminished level of LH may have negatively affected ovulation giving rise to a decrease in the estrus phase with a consequent increase in the diestrus phase. The dose-dependent decrease and increase in estrus and diestrus phases respectively observed in the test groups agrees with the findings of Obinna & Kagbo (2017) who associated this disruption in the estrous cycle by β-cyp to short supply of LH. Similarly, the decrease in the serum LH concentration may be attributed to the effect of the pesticide on the release of LH which may possibly disrupt ovulation. This decrease in LH may be attributed to the high affinity of β-cyp to pituitary hormones. This finding indicates that perinatal exposure of β-cyp affects the cyclicity of the rats which resulted in persistent diestrus phase with a decrease in the estrus phase, an aspect of the follicular phase. A persistent decrease in the estrus phase could lead to infertility since it is the only period in the cycle when mating is allowed.

From earlier studies, cypermethrin has been implicated as endocrine disruptors in male and female animals (Li, Pan, Hu, Li, & Xu, 2013; Obinna & Agu, 2016; Obinna & Kagbo, 2017). It is rather not surprising that this pyrethroid, which had the ability to traverse the placenta to the fetus, could cause harm to the developing female offspring which manifested at sexual maturity.

## Conclusion

Based on the findings of this study, it is concluded that perinatal beta cypermethrin exposure could have a deleterious effect on the reproductive development of female F1 generation rats, even at maturity.

### Abbreviations

ANOVA: Analysis of variance; FSH: Follicle stimulating hormone; GD: Gestational day; LH: Luteinizing hormone; NaCl: Sodium chloride; PND: Post natal day; β-cyp: Beta cypermethrin

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### Authors' contributions

VCO designed and carried out the study, performed the statistical analysis, and wrote the manuscript. Author GOA supervised the study, managed the analyses of the study, and proofread the manuscript. Both Authors read and approved the final manuscript.

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### Availability of data and materials

Not applicable

### Ethics approval

The rats for the study were humanely handled in accordance with the Ethics and Regulation guiding the use of research animals as approved by the University.

### Consent for publication

Not applicable

### Competing interests

The authors declare that they have no competing interests.

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## Publisher's Note