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## Effects of Acute and Chronic Propoxur Insecticide Exposure on the Hypothalamic-pituitary-testicular axis in Rat

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### ABSTRACT

Propoxur is a carbamate insecticide widely used both in indoor and outdoor place to control insects. This present work was conducted to study the effect of acute and chronic exposure of propoxur (PPX) on hormonal and histological changes in the rat Hypothalamic-pituitary testicular axis. The control (group 1) received physiological saline, group 2, received PPX by inhalation every other day for one month while group 3, received PPX by inhalation every other day for two months. A significant decrease in the level of testosterone (TT) and increase in follicular stimulating hormone (FSH) and luteinizing hormone (LH) were observed in PPX treated groups. Histological studies of the PPX treated rat hypothalamus, pituitary gland and testis revealed variable degrees of degenerative changes in the seminiferous tubules up to total cellular destruction. There was no reversal of destruction 30 days after withdrawal of the insecticide, indicating a persistent effect. The above-mentioned findings suggest that acute and chronic exposure to PPX insecticide has an obvious deleterious effect on rat Hypothalamic-pituitary testicular axis. Therefore, inhalation of such insecticide should be limited with special care in handling to limit or minimize its hazards.

**Keywords:** carbamate, propoxur, testosterone, FSH, LH, testes

## INTRODUCTION

Propoxur (2-Isoproxy-phenyl-N-methylcarbamate) (Baygon®) is a carbamate insecticide with anticholinesterase activity. Carbamate poisoning was postulated as a result of inhalation of sprays or contamination of crops or food <sup>1</sup>. As a broad-spectrum insecticide, it is one of the most frequently used insecticides. The widespread use of carbamate insecticides, like propoxur, for agricultural and household use has raised concerns as a result of their potential toxic effects, especially on the male reproductive system <sup>2</sup>.

PPX shows no embryotoxic or teratogenic effects in rats or rabbits at doses up to 400 mg/kg in the diet or 16 mg/kg body weight per day by gavage, respectively. In a 3-generation reproduction study in rats at dose levels of 5 or 10 mg/kg body weight per day, PPX did not have a negative effect on fertility, gestation or lactation indices and there were no treatment-related gross abnormalities <sup>3</sup>.

Behavioral and neurochemical changes were noted after chronic exposure to low concentration of carbamates <sup>4</sup>. After chronic exposure it was discovered to be incorporated in the hair of rabbits <sup>5</sup>. Repeated doses of PPX were found to bring about inhibition of B6-dependent kyurinine hydrolase (KH) and kyurinine aminotransferase (KATE) activities in mouse liver <sup>6</sup>. Recently it was discovered that virtually all carbamates inhibit 17-p-estradiol and progesterone activity in human breast and endometrial cancer cells *in vitro* <sup>3</sup>. Also, gonadal toxicity has been reported in male rats after chronic exposure to mancoze a fungicide of ethylene bisdithio-carbamate group <sup>7</sup> and the fungicide benomyl and its primary metabolite, carbendazim <sup>8-10</sup>. Splenotoxic injury induced by PPX has also been reported in rats after single or repeated oral administration <sup>11</sup>. However, androgens are known to be of prime importance for normal spermatogenesis, and histopathological changes of the testes are considered to be an indicator for testicular dysfunction <sup>12</sup>. This study evaluated the

effect of exposure to PPX on rat testis using hormonal and histopathological techniques.

## MATERIALS AND METHODS

Ethical approval was obtained from the University of Ilorin Ethical Committee. Twenty-one (21) adult male Wistar rats weighing between 150 and 200 g were housed under standard laboratory conditions and allowed free access to food and water *ad libitum*. The rats were divided randomly into three groups comprising of seven animals each. Group one served as control and received normal saline (0.1 ml/day) orally for a period of two months. The second group received 1 PPM of PPX (PPX as pure powder, from Bayer Pharmaceuticals) dissolved in saline. The third group was given 2 PPM for a period of two months and then left for one month after insecticide withdrawal. At the end of treatments, blood samples were taken from the animals through the apex of the heart and serum was separated and kept at -20°C for subsequent biochemical studies. The animals were then sacrificed by cervical dislocation and the testes were removed for histopathological studies.

## HORMONAL ASSAY:

The serum levels of LH, FSH and testosterone were analyzed using an enzyme-linked immunosorbent assay (ELISA) kit (Shanghai crystat Day Biotech Co. LTD, Shanghai, China for LH & FSH and IBL International GMBH Flughafenstrasse Hamburg, Germany for testosterone) according to the manufacturer's guidelines

## Histological analysis

Routine histological processing was performed using the hematoxylin and eosin staining procedure. After the testes had been properly fixed in Bouin's solution, the testes were then dehydrated with ascending grades of alcohol, cleared in xylene, and infiltrated into molten paraffin wax before being embedded in molten paraffin wax to form a

tissue block. The rotary microtome was used to section the paraffin block containing the tissue at a thickness of 4 µm. After floating in a water bath at 40 ° C, the sections were then transferred to a glass slide and stained with hematoxylin and eosin stains. The slides were examined under a light microscope (magnification 200x).

### Photomicrography

The slides were mounted on the microscope with the cover slip facing the objective lenses and secured on the stage using the stage clips. The coarse and fine adjustment knobs were used to focus and resolve the specific region of the tissue for viewing. An Amscope camera was inserted into one of the ocular lenses and connected to a laptop. The micrographs were captured on the laptop using the Amscope software application at magnifications of X200. The histological sections were examined to observe any changes in the structure of the testes.

### Statistical analysis

All quantitative analyses were done using GraphPad (version 8) software and presented as mean and standard error of mean to show statistical significance and comparison between each group using the analysis of variance (ANOVA). Comparison was done between all experimental groups and differences were determined using  $p < 0.05$ , giving credence to the statistical significance of the comparisons.

### RESULTS

From Table 1, the result shows that animals that received acute inhalation of PPX at 1PPM and 2PPM respectively had a significant reduction in the level of testosterone, and increase in FSH and LH when compared to the control. Hence, the administration of PPX for a period of 30 and 60 days produced a significant decrease in testosterone and increase in FSH and LH levels (Table 1).

**Table 1:** Effect of PPX insecticide on Testosterone, Follicle Stimulating Hormone and Luteinizing Hormone

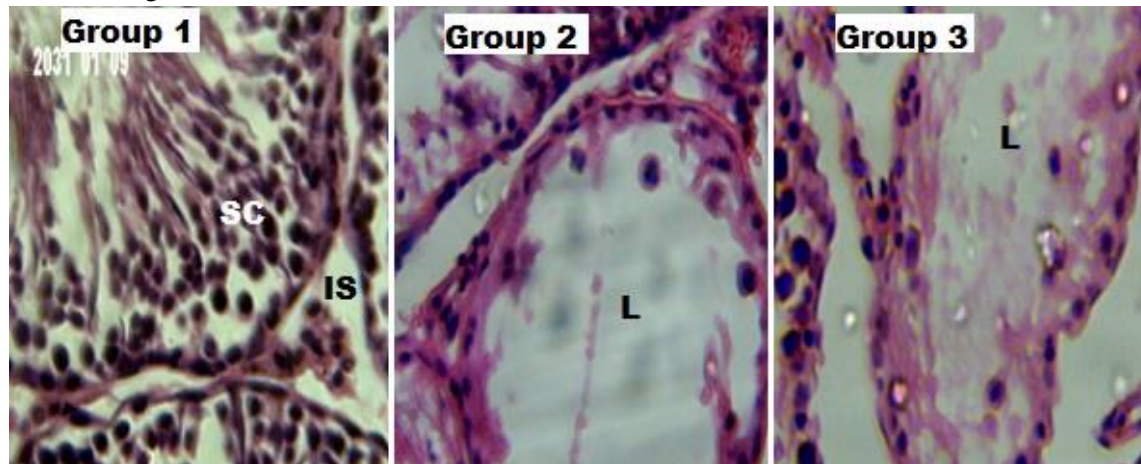
Group n=7	Group 1 DW	Group 2 C <sub>P8</sub>	Group 3 CP <sub>16</sub>
LH (mIU)	6.30±0.54	8.76±0.11*	9.00±0.23**
FSH (mIU)	4.60±0.28	5.44±0.36*	6.50±0.37**
TT (ng)	5.32±0.24	1.34±0.04	1.65±0.02

. \*  $P < 0.01$  \*\*  $P < 0.001$  DW: Distilled Water, P<sub>8</sub>: Propoxur (8wks), P<sub>16</sub>: Propoxur (16wks), CP<sub>16</sub>: *Cissus Populnea* (16wks); PPX – propoxur, LH- luteinizing hormone, FSH –Follicle stimulating hormone, TT – total testosterone.

Histology of the testes of control rats (Group 1) showed normal seminiferous tubules (ST). Each tubule was surrounded by an outer flattened adventitial cell and an inner basement membrane. The interstitial connective tissue showed fibroblasts, blood vessels and interstitial cells (IC) of Leydig. The seminiferous tubules appeared uniform in size and shape. They were lined by regularly arranged rows of spermatogenic cells (SC) in different stages of maturation. In Group 2, the seminiferous tubules of 1 PPM group displayed variable grades of degenerative changes varied from

cloudy swelling up to complete cellular destruction. Most of the seminiferous tubules showed disorganization with sloughing of their cells in the lumina. Some tubules exhibited proportional spermatogenic hypoplasia with marked thinning of the spermatogenic cell rows. Primary spermatocytes revealed areas of swelling or even ballooning with ill-defined nuclear membrane. Their nuclei appeared eccentric. In Group 3, the spermatids of the 2 PPM group showed the highest degree of destruction among the spermatogenic cells (SC); most of the cells were swollen with ill-defined

membrane and dispersion of the chromatin and nucleoli (Figure 1).



**Figure 1:** The seminiferous tubules of experimental rats showing normal microarchitecture of the testis, including the spermatogenic cells (SC) and interstitium (IS) within which are the interstitial cells, in the control (Group 1), while the treated Groups 2 and 3 presented with increased luminal diameter and scanty spermatogenic cells.

## DISCUSSION

The chronic exposure of carbamate insecticides is an occupational hazard in the agricultural industry<sup>11</sup>. Spermatogenesis solely depends on testosterone production by Leydig cells in response to FSH and LH stimulation. The FSH increases Sertoli cell synthesis of an androgen binding protein, a requirement for maintenance of high concentration of testosterone. LH stimulates testosterone production by the interstitial cells of the testis<sup>7</sup>.

This study observed that acute inhalation of PPX at 2PPM resulted in a significant reduction in the level of testosterone, and increase in FSH and LH. Similar results were obtained from organophosphorus compound after chronic exposure to rats for a period of 7 weeks<sup>14</sup>. The explanation for these hormonal disturbances is due to an alteration in neuroendocrine-reproductive axis. It was reported that the administration of dimethoate (organophosphorous compound) for 65 consecutive days to male rats caused suppression of testicular function which was dose-related<sup>15</sup>. A significant reduction in testosterone level, and the

number of motile sperms with an increase in the percentage of dead sperms was also observed. They explained the decrease in testicular function by a direct toxic effect of the insecticide on testicular tissue. Similar results were obtained by other investigators after chronic administration of carbendazim (a metabolite of benomyl) to male rats resulting in dramatic reduction in sperm production, elevation in serum LH, FSH and changes in human chorionic gonadotrophins (hCG)<sup>16</sup>. The histological observation of the seminiferous tubules of the rats exposed to inhalation of PPX at 1 PPM and 2 PPM respectively, displayed variable grades of degenerative changes. The infertility produced by carbendazim was attributed to a direct toxic effect of the insecticide on testicular function, an alteration in the internal endocrine environment or a direct effect on the central nervous system (CNS). Furthermore, another study noted that the chronic exposure of male rats to mancozeb (30 days) produced a significant testicular dysfunction as indicated by a marked reduction in testosterone level and sperm count<sup>17</sup>.

However, in the above-mentioned studies it seems that the hormonal changes observed in our study, after subchronic exposure to *propoxur*, is in favor of a direct toxic effect of the insecticide or possibly through an alteration in the neuroendocrine environment. Testicular atrophy and degenerative changes of the seminiferous tubules have been reported in experimental animals with variant of insecticides<sup>18,19</sup>. Our histopathological results are in agreement with another study that observed that the administration of benomyl and its primary metabolite carbendazim at moderate to low dosage induced sloughing of germ cells and abnormal development of the head of elongating spermatids<sup>10</sup>.

### Conclusion

The persistent alteration in the hormonal profile as well as the histopathological changes in the testis after discontinuation of PPX indicates a long-lasting reproductive damage. Since humans are exposed to carbamates from various environmental sources, a public health education is needed to raise an awareness about the hazards and to minimize their harmful side effects.

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