

DOI: 10.5455/2320-1770.ijrcog20141205

Research Article

Effects of propoxur on male fertility in wistar rat exposed neonatally

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Received: 15 August 2014

Accepted: 17 September 2014

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ABSTRACT

Background: Propoxur is a carbamate pesticide widely used in crop and foodstuff protection. They are known to cause a wide variety of symptoms in animals.

Methods: Twenty four young male rats were exposed to 0.00, 1.73, 2.60 and 5.20 mg/kg body weight through oral intubation for 90 days.

Results: The testis weight increased significantly ($P < 0.05$) in propoxur-treated animals when compared to the control. The epididymal sperm counts increased not significantly ($P > 0.05$) but the percentage of sperm motility decreased significantly ($P < 0.05$) in propoxur-gavaged males. Histopathological examination revealed the disorganisation of seminiferous epithelia with the loss of germ cells in some gavaged rats. The fertility rate did not vary dose dependently and no significant ($P > 0.05$) change was observed between the control and treated males for the litter size, viability rate and sex-ratio.

Conclusions: Despite the impairment of seminal epithelia and sperm characteristics, male rats orally exposed to the studied doses of propoxur maintained their fertility at the 90th day of treatment.

Keywords: Propoxur, Reproductive organs, Cauda epididymis counts, Cauda epididymis sperm motility, Fertility

INTRODUCTION

Carbamates are a group of pesticides including insecticides, herbicides, acaricide, nematicide and fungicides.¹⁻³

They cause a wide variety of symptoms in mammals by inhibiting the enzyme acetylcholinesterase.^{4,5} This inhibition leads to the accumulation of acetylcholine and then the hyperactivity of sympathetic and parasympathetic nervous systems.^{3,6-9,10} Studies on

chronic exposure to carbamate pesticides reported several effects of unknown origin and suggested that additional mechanisms distinct from acetylcholinesterase are involved.^{3,11} Many biological functions controlled by the autonomous nervous systems are susceptible to be impaired.^{2,12,13}

Propoxur is one of the most important carbamate insecticides. It is used on a variety of insect pests such as chewing and sucking insects, ants, cockroaches, crickets, flies and mosquitoes and may be in control of these in

agricultural or in non-agricultural applications.¹⁴ For the agriculture, it is manufactured to protect cane, cocoa, fruit, grapes, maize, vegetables, rice, cotton, lucerne, forestry and ornamentals. Propoxur is available in several types of formulations including emulsifiable concentrates, wettable powders, baits, aerosol, fumigants and granules. In Cameroon, the formulation commercially named unden is widely used, probably due to its low cost.

Propoxur is a highly toxic compound via the oral route.¹⁴ Its admissible daily dose (dose that can be taken daily by a man of 60 kg throughout the life without any effects) is 0.02 mg/kg.⁵ Applicators who used propoxur regularly showed a pronounced daily fall in whole blood cholinesterase activity.¹⁴ The administration of 18 mg/kg of propoxur resulted to the fall of growth in the mice.⁵ In female rats given high dietary doses of approximately 18 mg/kg/day of propoxur, reduced lactation and litter size were observed.¹⁵ According to the same author, at 25 mg/kg/day administered to pregnant rats, there was a decrease in the number of offspring. Dietary doses of approximately 2.25 mg/kg did not affect fertility, litter size and lactation. In the present study, we led an investigation in order to evaluate the effect of oral administration of this pesticide on some male reproductive parameters in rats exposed early after the birth.

METHODS

Animals

Twenty four male wistar rats of 30 days old and 28 to 32 g of body weight at the start of the essay, and 48 females aged 4 months were produced in animal physiology laboratory of Dschang University/faculty of agronomy and agricultural sciences. Rats were housed in the glass cages at room temperature and 12h-day light/dark cycle, with free access to feed and vitamin-enriched water. The animals used in this study were treated according to ethical principles in animal research.

Pesticide

The propoxur used is a powder commercially named unden 75Ew and was produced by Bayer. The studied doses were 0.00; 1.73; and 5.20 mg/kg body weight.

Essay

The young male rats were distributed into 4 groups of 6 animals. To each group was attributed randomly an experimental dose of propoxur or distilled water. 0.583 ml of distilled water or solution containing propoxur was administered per kilogramme body weight. Males were gavaged daily from post-natal days 30 to 120 and volumes of gavage solution adjusted weekly to body weight. At the end of the treatment period, they were

killed by chloroform overdose. Prior to sacrifice, each male was mated to 2 females during 15 days.

Data collection

Body weight was measured at the end of exposure. The abdomen was then opened and the testes, epididymis, vas deferent, seminal vesicle and prostate were removed and weighed.

The right cauda epididymis was weighed and minced in a known volume of 0.9% NaCl solution (36°C) for sperm motility and concentration evaluation. The motile and non-motile sperms were counted separately in many light microscopic areas. The sperm concentration was obtained using the Thomas haematocytometer.

The testis was fixed in Bouin's fluid, and then washed, dehydrated in alcohol bath of ascending grade, clarified in xylene immersion, embedded in paraffin, sectioned at 5µm and stained with haematoxylin and eosin. The tissue sections were observed under a light microscope (400x) for qualitative and quantitative changes in the seminiferous tubules and intertubular space.

Fertility rate was calculated on the basis of the number of males which procreate per lot. Litter was examined for size, viability and sex-ratio.

Statistical analysis

Data were expressed in mean \pm SEM. Statistical analysis was done by ANOVA and Duncan test at 5%.

RESULTS

Reproductive organs weight

The testis weight (Table 1) was higher in propoxur-treated rats as compared to the control, with a significant ($P < 0.05$) difference in 2.60 mg/kg-gavaged animals. The weights of the epididymis, vas deferent, seminal vesicles and prostate were very closed among treatments.

Cauda epididymal sperm concentration and mobility

The epididymal sperm counts (Table 2) were comparables ($P > 0.05$) among dose groups. On the other hand, the percentage of motile spermatozoa decreased significantly ($P < 0.05$) in 1.73-and 5.20 mg/kg-treated rats when compared to the control group.

Male reproductive performances

Fertility rate was significantly ($P < 0.05$) the highest in male rats receiving 1.73 mg/kg, followed by the 5.20 mg/kg treated group and the control. No significant ($P > 0.05$) difference was observed between control and gavaged animals for the litter size, viability rate and sex ratio (Table 3).

Table 1: Effects of propoxur on the male reproductive organs weight in rat.

| Organs | Doses (mg.kg ⁻¹ day ⁻¹) | | | |
|-----------------|------------------------------------------------|---------------------------|--------------------------|---------------------------|
| | 0.00 (control) (n=6) | 1.73 (n=6) | 2.60 (n=6) | 5.20 (n=6) |
| Testis | 0.27 ± 0.06 ^a | 0.31 ± 0.02 ^{ab} | 0.39 ± 0.11 ^b | 0.32 ± 0.59 ^{ab} |
| Epididymis | 0.12 ± 0.01 ^a | 0.12 ± 0.02 ^a | 0.12 ± 0.02 ^a | 0.12 ± 0.01 ^a |
| Vas deferent | 0.04 ± 0.00 ^a | 0.05 ± 0.01 ^a | 0.04 ± 0.00 ^a | 0.04 ± 0.01 ^a |
| Seminal vesicle | 0.31 ± 0.09 ^a | 0.33 ± 0.07 ^a | 0.26 ± 0.09 ^a | 0.32 ± 0.07 ^a |
| Prostate | 0.09 ± 0.01 ^a | 0.09 ± 0.02 ^a | 0.08 ± 0.02 ^a | 0.08 ± 0.01 ^a |

a,b: Within the same line, numbers with the same letters are not significantly (P >0.05) different
n: Number of observations

Table 2: Influence of propoxur on the cauda epididymal sperm concentration and motility.

| Cauda epididymal sperm characteristics | Doses (mg.kg ⁻¹ day ⁻¹) | | | |
|----------------------------------------|------------------------------------------------|----------------------------|----------------------------|----------------------------|
| | 0.00 (control) (n=6) | 1.73 (n=6) | 2.60 (n=6) | 5.20 (n=6) |
| Number/cauda (x10 ⁶) | 7.51 ± 5.97 ^a | 4.25 ± 3.29 ^a | 9.56 ± 6.16 ^a | 8.64 ± 4.11 ^a |
| Number/gram (x10 ⁶) | 54.67 ± 37.36 ^a | 35.65 ± 28.90 ^a | 75.72 ± 43.69 ^a | 81.25 ± 33.22 ^a |
| Motility (%) | 64.19 ± 9.10 ^a | 43.99 ± 4.21 ^b | 57.04 ± 7.22 ^a | 44.09 ± 12.37 ^b |

a,b: Within the same line, numbers with the same letters are not significantly (P >0.05) different
n: Number of observations

Table 3: Influence of propoxur on the reproductive performances in rats.

| Parameters | Doses (mg.kg ⁻¹ day ⁻¹) | | | |
|--------------------|------------------------------------------------|-----------------------------|----------------------------|----------------------------|
| | 0.00 (control) (n=6) | 1.73 (n=6) | 2.60 (n=6) | 5.20 (n=6) |
| Fertility rate | 0.33 ± 0.52 ^{ab} | 1.00 ± 0.00 ^c | 0.20 ± 0.45 ^a | 0.83 ± 0.41 ^{bc} |
| Litter size | 6.50 ± 0.71 ^a | 6.50 ± 3.70 ^a | 6.00 ± 1.00 ^a | 4.67 ± 3.21 ^a |
| Viability rate (%) | 92.86 ± 10.11 ^a | 72.73 ± 48.67 ^a | 100.00 ± 0.00 ^a | 100.00 ± 0.00 ^a |
| Sex-ratio | 61.90 ± 6.73 ^{ab} | 50.12 ± 11.96 ^{ab} | 83.33 ± 2.01 ^b | 35.71 ± 31.13 ^a |

a,b: Within the same line, numbers with the same letters are not significantly (P >0.05) different
n: Number of observations

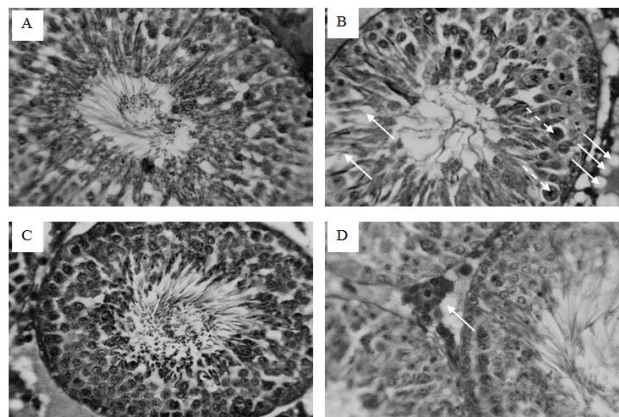


Figure 1: Testis histological sections of rat at 90th day of exposure to propoxur.

A: control; B: rats gavaged with 1.73 mg/kg; C: rats treated with 2.60 mg/kg; D: rats treated with 5.20 mg/kg body weight. Note the integrity of tubules in control (A) and highest dose groups (C, D), the presence of vacuoles (whole arrow) and degenerating germ cells (interrupted arrow) in the lowest dose-gavaged rats (B). Magnification: 400x

Histopathology

The testis histological sections (Figure 1) reveal the existence of degenerating germ cells and vacuoles in the epithelia of a few numbers of seminiferous tubules and in intertubular spaces in propoxur-treated rats (picture B). The variation of the pathologies degree was not dose dependant; most of the seminiferous tubules were not affected even in the highest dose groups (picture C and D).

DISCUSSION

The increase of testes weight in propoxur-treated males is the contrary of a previous result showing no significant change after 90 days of gavage with 100 mg/kg of carbaryl, a carbamate pesticide.¹⁶ The increase of the testis weight observed in the present study could be attributed to food consumption since a parallelism has been observed between both parameters. Indeed, it is reported that mammals insufficiently fed during prepubertal period do not present well developed gonads at adulthood.¹⁷

As the functioning of the accessory sex organs is under the control of the gonads,¹⁷ it is not clear why the weight of epididymis, vas deferent, seminal vesicles and prostate remained unchanged with increasing dose of propoxur while the testis weight increased. An identical result has been obtained by treating prepubertal rats with up to 10.50 mg.kg⁻¹ chlorpyrifos-ethyl.¹⁸ The oral administration of 100mg/kg carbaryl also resulted in comparability of the weight of these glands.¹⁶ However, the weight of the accessory sex glands decreased significantly in rats gavaged with a dose of carbofuran (carbamate) as small as 0.8mg/kg body weight.¹⁹

The disorganisation of the seminal epithelium, the presence of a great rate of vacuoles at *ad-luminal* location and the position of necrosing germ cells indicate that there has had loss of germ cells and that the loss of those cells begins with precocious spermiation, before progressively affects less differentiated cell types.

Sperm count remained unchanged and sperm motility rate lowered with increasing dose of propoxur. Other carbamate pesticides such as carbofuran and carbaryl orally administered to rats led to a decline in epididymal sperm count and percentage of sperm motility.^{16,19,20} The statistical comparability of the cauda epididymal sperm count in the present study was foreseeable given that no dose related significant difference was noted for the epididymal weight. Also, according to an author, the weight of the epididymis could be related to its sperm content.²¹

The decline of sperm motility percentage in propoxur-treated rats is imputable to the cytotoxic effect of propoxur and precocious spermiation as evidenced by the presence of vacuoles and degenerating germ cells in the epithelia of seminiferous tubules.

The lack of significant difference among mortality rates could be attributed to the similarity of the litter size in different dose groups.

CONCLUSION

Propoxur impaired the seminal epithelia and sperm characteristics. However, despite those effects, male rats orally exposed to the studied doses maintained their fertility at the 90th day of treatment.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the institutional animal ethics committee

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DOI: 10.5455/2320-1770.ijrcog20141205

Cite this article as: Kenfack A, Ngoula F, Chombong JK, Vemo NB, Ndukum JA, Ngouateu OB, Zeukeng GMZ, Tsambou AMM, Nain TP, Guiekep JAN, BMama B, Tchoumboué J, Kamtchouing P. Effects of propoxur on male fertility in wistar rat exposed neonatally. *Int J Reprod Contracept Obstet Gynecol* 2014;3:898-902.