Original Article

Post Exposure Effects of Propoxur (Agricultural Pesticide) on Male Fertility in Wistar Rat

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ABSTRACT

Background: Pesticide toxicity is one of the major environmental health problems for the modern societies, as some of the effects may last long time after exposure. Propoxur is one of the Carbamate pesticide largely used in agriculture in the Western region of Cameroon.

Methods: In order to evaluate the post-exposure effects of propoxur, 48 male rats (12 animals per group) aged 30 d were orally gavaged with 0.00, 1.73, 2.60 and 5.20 $mg.kg^{-1}$ body weight of propoxur for 90 d. At the end of treatment, 6 rats per group were sacrificed and others were followed up for 90 additional days and submitted to a fertility test before sacrifice.

Results: At the end of exposure propoxur significantly increased (P<0.05) the testis weight while it decreased (P<0.05) the cauda epididymal sperm motility in rat. Propoxur treatment and post-treatment exposure showed variable effect on the fertility rate with an increase and decrease at 2.60 and 5.20 $mg.kg^{-1}$, respectively. At the 90_{th} day post treatment, there was a significant decrease (P<0.05) in epididymis weight and sperm count and motility (P<0.05). In addition, post-exposure treatment with propoxur reduced (P<0.05) the pup litter size and the sex ratio at all doses. Histopathological examination revealed a high vacuity of germinal epithelia in treated rats that persisted after post-exposure time.

Conclusion: Propoxur negatively affected male rats' reproductive parameters with more significant adverse effects observed 90 d after the end of exposure.

Keywords: Fertility, Male Rats, Post-Exposure Effects, Propoxur, Reproductive Parameters.

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INTRODUCTION

Carbamates (CRBs) are an important class of pesticides used worldwide in public health, rural and urban settings. They constitute one of the main used pesticide families in agriculture since the ban of long-lasting organochlorine pesticides [1]. CRBs are used in various crops and foods with regard to their large range of actions as insecticides, herbicides, fungicides, nematocides and sprout inhibitors [2]. CRBs pesticides (used in agriculture) can get into human via direct exposure in the farm or through food chain or water. Consequently, CRBs residues have been found in many products intended for human consumption including water, vegetables, and fruits [3-5].

In human system, carbamate half-life does not exceed 8 h [1]. However, the chronic and permanent exposure to these chemicals can lead to serious health disturbance. In male reproductive function, CRBs have been shown to cause deleterious effects on different reproductive outcomes [6]. Propoxur belongs to the carbamate pesticide family. It is one of the most important used both for health and for agricultural purposes on a variety of insect pests such as chewing and sucking insects, ants, cockroaches, crickets, flies, and mosquitoes. In agriculture, it helps to protect cane, cocoa, fruits, grapes, maize, vegetables, rice, cotton, forestry and ornamental plants [7].

Propoxur is available in different formulations including emulsifiable concentrates, wettable powders, aerosol, fumigants, and granules. Studies have demonstrated some adverse effects of propoxur on male reproduction. Administration of this pesticide to adult and young Wistar rats increased the weight of genital organs and decreased the number of spermatozoa per unit tail of epididymis and the sperm motility with

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disorganization of seminiferous epithelia [8, 9]. The most serious deleterious and devastating effects of toxicants including pesticides can occur some long after the end of exposure [10, 11]. However, limited investigations have intended to delineate such effects from propoxur exposure.

The present study evaluated the effects of propoxur on reproductive parameters 90 d after exposure in male rats.

MATERIALS AND METHODS

Animals

Forty eight Wistar male rats and 96 females both of 30 d old breed in Animal Physiology Laboratory of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, Cameroon, were used. They were housed in the glass cages at room temperature with 12 h-d light/dark cycle and had free access to feed and potable water.

They were handled according to ethical guidelines of the Cameroon National Veterinary Laboratory.

Chemicals and Instrumentation

The propoxur with brand named UNDEN 75 Ew was obtained from the phytosanitary division of BAYER. It was a powder formulation and the doses 0.00, 1.73, 2.60 and 5.20 mg $mg.kg^{-1}$ were used for the experiment. These doses were extrapolated from the LD_{50} (89.7 mg/kg) of the pesticide in Wistar male rats [7].

The Mettler PE brand scale of capacity 160 gr and precision 1 mg was used to weigh organs.

Study Design

The male rats were distributed into 4 groups of 12 animals each namely 3 experimental and 1 control groups. The experimental groups were orally administered propoxur at doses of 1.73, 2.60 and 5.20 $mg.kg^{-1}$ of body weight respectively, while the control group received 1 $ml.kg^{-1}$ of distilled water. The animals were gavaged daily from post-natal d 30 to 120 and administered volumes were adjusted weekly to body weight. At the end of the treatment period, 6 animals out of 12 per group were sacrificed while the other 6 animals were followed for additional 90 d to evaluate any post exposure outcome. Fifteen-days to the sacrifice, each male was housed with 2 virgin and untreated females to evaluate the animal fertility.

Data Collection

At the end of the treatment and post exposure follow-up, the animal body weight was recorded, animals were sacrificed, and blood was collected. The organs (testes, epididymis, seminal vesicle, vas deferent, and prostate) were dissected out and weighed.

The right cauda epididymis was weighed and minced in 100 ml of 0.9% NaCl solution (36 °C) for evaluation of sperm motility and concentration. Briefly, a drop of the prepared solution was placed on a slide and then observed under the light microscope at magnification 400. The motile and non-motile sperms were counted separately in many light microscopic areas. The sperm concentration was estimated using the Thoma's haematocytom-eter.

The left testis was submitted to a histopathology analysis. Briefly, the entire testis was fixed in Bouin's fluid, washed, dehydrated in alcohol baths of ascending grade, clarified in xylene immersion, hardened in paraffin, sectioned and stained with haematoxylin and eosin. The tissue sections were observed under a light microscope (400X magnification) for qualitative and quantitative analysis in the seminiferous tubules and intertubular space.

Fertility rate in the male rats was calculated based on the number of males, which procreated per group. The obtained pups were examined for the litter size, viability and male/female ratio.

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Differences between groups were assessed using one-way ANOVA followed by Duncan's test at 5% significance. All analyses were performed using the SPSS (ver. 20.0, Chicago, IL, USA).

RESULTS

Reproductive Organs Weights

The administration of different doses of propoxur to 30 d old male for 90 d increased testes weight but did not significantly (P>0.05) affect other reproductive organ weight (Table 1). Ninety days after the end of the treatment, there was a significant decrease (P<0.05) in the epididymis weight in animals treated with 1.73 and 5.20 $mg.kg^{-1}$ of the pesticide. The other organs did not show any post-exposure effect, though a decreased tendency was noted on the weights of testis and seminal vesicles of animals treated with the pesticide.

Sperm Concentration and Motility

The number of sperms per cauda and per gram epididymis was not significantly increased by

propoxur at the 90th day of treatment but the number of sperms per cauda epididymis significantly decreased (P<0.05) in exposed animal after the post-exposure follow-up (Table 2). In addition, the number of sperms per gram was comparable among treatments after post-exposure time. The sperm motility was significantly reduced by propoxur treatment at doses of 1.73 and 5.20 mg.kg^{-1} and this effect remained throughout the post-exposure period as compared to the control animals.

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Male Reproductive Performances

Treatment of young male rats with 1.73 and 5.20 mg.kg^{-1} of propoxur significantly increased (P<0.05) the animal fertility rate while it decreased it at 2.60 mg.kg^{-1} as compared to the control group (Table 3). However, after 90 d post-exposure, this parameter showed variable activity with decrease and increase effect at 2.60 and 5.20 mg.kg^{-1}, respectively. Pup litter size did not vary after treatment but significantly reduced (P<0.05) at all pesticides doses after post-exposure follow-up. Pup viability was affected neither by the treatment nor after the post-exposure time. Treatment with 2.60 mg.kg^{-1} of propoxur increased the pup sex ratio, while 5.20 mg.kg^{-1} decreased it. However, all chemical doses decreased (P<0.05) the animal male/female ratio after post-exposure period.

Histological Analysis of Rat Testis

The only damage noticed on the 90th day of treatment with propoxur was few cells vacuolization in the interstitial compartment (Fig. 1B, 1C and 1D). These abnormalities not only persisted 90 d after the end of exposure but were even more accentuated, mainly in the terminal part of the tubule, causing its vacuity in most sections (Fig. 2C et 2D).

Table 1. Reproductive organ weights of different groups of rats.

Organs	Experiment period	Dose (mg.kg^{-1})			
		0.00	**1.73**	**2.60**	**5.20**
Testis	End of exposure	0.27 ± 0.06^{a}	0.31 ± 0.02^{ab}	0.39 ± 0.11^{b}	0.32 ± 0.59^{ab}
	90 d PE	0.35 ± 0.11^{a}	0.27 ± 0.10^{a}	0.40 ± 0.26^{a}	0.29 ± 0.07^{a}
Epididymis	End of exposure	0.12 ± 0.01^{a}	0.12 ± 0.02^{a}	0.12 ± 0.02^{a}	0.12 ± 0.01^{a}
	90 d PE	0.13 ± 0.02^{a}	0.09 ± 0.01^{b}	0.10 ± 0.03^{a}	0.08 ± 0.02^{b}
Vas deferent	End of exposure	0.04 ± 0.00^{a}	0.05 ± 0.01^{a}	0.04 ± 0.00^{a}	0.04 ± 0.01^{a}
	90 d PE	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.00^{a}	0.03 ± 0.01^{a}
Seminal vesicle	End of exposure	0.31 ± 0.09^{a}	0.33 ± 0.07^{a}	0.26 ± 0.09^{a}	0.32 ± 0.07^{a}
	90 d PE	0.27 ± 0.11^{a}	0.19 ± 0.09^{a}	0.24 ± 0.04^{a}	0.15 ± 0.12^{a}
Prostate	End of exposure	0.09 ± 0.01^{a}	0.09 ± 0.02^{a}	0.08 ± 0.02^{a}	0.08 ± 0.01^{a}
	90 d PE	0.10 ± 0.03^{a}	0.06 ± 0.04^{a}	0.09 ± 0.03^{a}	0.08 ± 0.01^{a}

Data are in mean ± standard deviation of 6 observations. a,b: Within the same line, numbers with the same letters are not significantly (P>0.05) different; for the same parameter different letters mean significant difference (P<0.05). PE: post exposure.

Table 2. The cauda epididymal sperm concentration and motility of different animal groups.

Sperm characteristics	Experiment period	Dose (mg.kg^{-1})			
		0.00	**1.73**	**2.60**	**5.20**
Number/cauda (x10^{6})	End of exposure	7.51 ± 5.97^{a}	4.25 ± 3.29^{a}	9.56 ± 6.16^{a}	8.64 ± 4.11^{a}
	90 d PE	8.62 ± 5.81^{a}	1.25 ± 0.94^{b}	5.25 ± 3.01^{ab}	2.86 ± 1.84^{ab}
Number/gram (x10^{6})	End of exposure	54.67 ± 37.36^{a}	35.65 ± 28.90^{a}	75.72 ± 43.69^{a}	81.25 ± 33.22^{a}
	90 d PE	52.24 ± 28.91^{a}	14.64 ± 8.23^{a}	38.93 ± 20.91^{a}	26.66 ± 24.39^{a}
Motility (%)	End of exposure	64.19 ± 9.10^{a}	43.99 ± 4.21^{b}	57.04 ± 7.22^{a}	44.09 ± 12.37^{b}
	90 d PE	75.93 ± 13.55^{b}	69.16±14.21^{ab}	77.31 ± 11.36^{b}	56.07 ± 10.09^{a}

Data are in mean ± standard deviation of 6 observations. a,b: Within the same line, numbers with the same letters are not significantly (P>0.05) different; for the same parameter different letters mean significant difference (P<0.05). PE: post exposure.

Table 3. Reproductive performances of different animal groups.

Parameters	Experiment period	Dose (mg.kg^{-1})			
		0.00	1.73	2.60	5.20
Fertility rate (%)	End of exposure	33.33 ± 0.52^{b}	100.00 ± 0.00^{d}	20.00 ± 0.45^{a}	83.00 ± 0.41^{c}
	90 d PE	50.00 ± 0.58^{b}	50.00 ± 0.58^{b}	33.00 ± 0.58^{a}	60.00 ±0.55^{c}
Litter size	End of exposure	6.50 ± 0.71^{a}	6.50 ± 3.70^{a}	6.00 ± 1.00^{a}	4.67 ± 3.21^{a}
	90 d PE	8.00 ± 2.00^{a}	4.00 ± 1.00^{b}	5.33 ± 0.58^{ab}	5.67 ± 1.53^{ab}
Viability rate (%)	End of exposure	92.86 ± 10.11^{a}	72.73 ± 48.67^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	90 d PE	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	86.67 ± 11.55^{a}	80.95 ± 32.98^{a}
Male/female Sex ratio (%)	End of exposure	61.90 ± 6.73^{ab}	50.12 ± 11.96^{ab}	83.33 ± 2.01^{b}	35.71 ± 31.13^{a}
	90 d PE	75.00 ± 5.00^{c}	25.00 ± 5.00^{a}	36.67 ± 11.55^{ab}	47.62 ± 4.12^{b}

Data are in mean ± standard deviation of 6 observations. a,b: Within the same line, numbers with the same letters are not significantly (P>0.05) different; for the same parameter different letters mean significant difference (P<0.05). PE: post exposure.

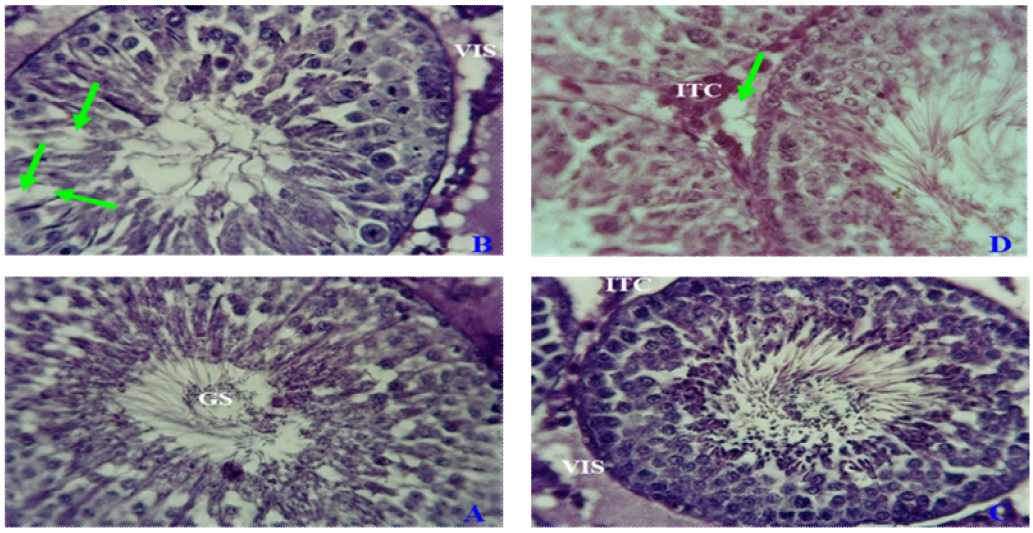

Figure 1. Histological sections of rat testis after 90 d of porpoxur treatment

A : control vehicle, B : 1.73 mg.kg^{-1}, C : 2.60 mg.kg^{-1} , D : 5.20 mg.kg^{-1} . GS: lumen of the seminiferous tubule, ITC: interstitial cells, VIS: vacuolization in the interstitial space. → : Vacuole.

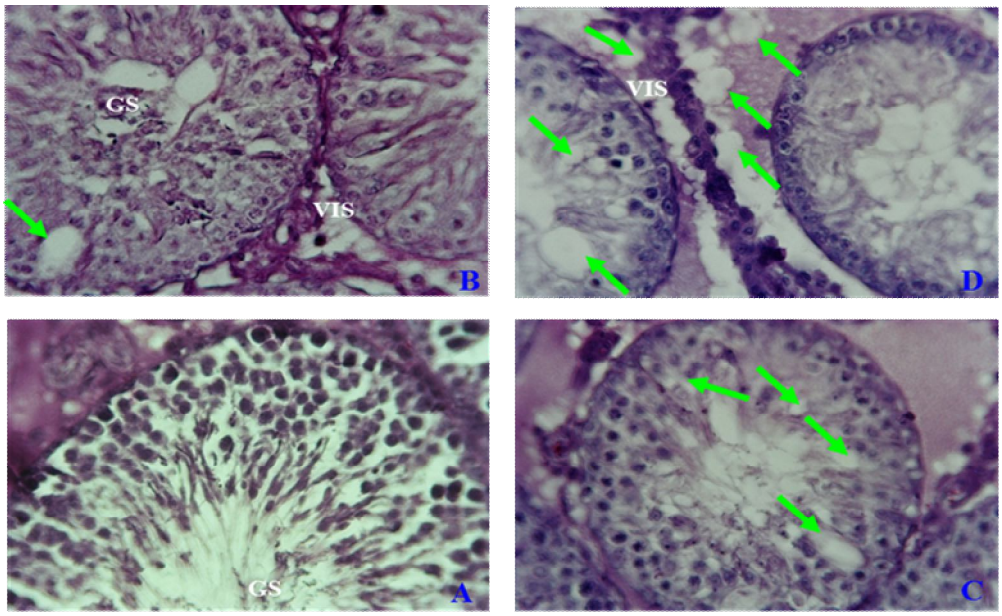

Figure 2. Histological sections of rat testis 90 d after propoxur treatment.

A : control vehicle, B : 1.73 mg.kg^{-1}, C : 2.60 mg.kg^{-1} , D : 5.20 mg.kg^{-1} . GS: lumen of the seminiferous tubule, ITC: interstitial cells, VIS: vacuolization in the interstitial space. → : Vacuole.

DISCUSSION

Health disorders resulting from exposure to environmental toxicants can be observed after a very short term or long after exposure. Though the first category can be disastrous and even lethal, the second is more serious due to its latency and its more generalized effect affecting different system of the organism [10-12]. Long-term exposure to pesticides has been associated with diverse alterations including nerve dysfunctions, infertility, birth defects, endocrine disruption, neurological disorders, and cancers [12, 13]. Many of these long-term effects of pesticides could also be attributed to the endocrine disruptive potential of this category of chemicals [14].

In Cameroon, agriculture represents one of the major sources of income for the increasing population. This agriculture therefore largely uses pesticides among which CRBs such as propoxur [15, 16]. Though CRBs have a relatively short lifetime, their constant use can pose serious health concerns for the farmers and consumers [7, 17]. In fact, propoxur residues have been found in water samples intended for human consumption [18]. This pesticide is also categorized as an endocrine disruption chemical and studies have pointed the link between pesticide exposures and male dysfunctions [15, 19]. The evaluation of propoxur on male reproductive endpoints could, therefore, provide more insights on the mechanism of the toxicity of this pesticide.

Ninety days after exposure, propoxur (5.20 mg/kg) significantly decreased the epididymis weight as compared to the control group and animals at the end of the treatment. These points out a certain latent toxicity effect of propoxur on this reproductive organ. The epididymis is a highly convoluted duct/tube and, for most species, can be divided into distinct anatomical regions namely caput, corpus, and cauda. The epididymis plays several important roles including sperm maturation, sperm transport, sperm storage, and sperm protection [20, 21]. It has also been considered as a target for certain toxicants including pesticides with some having latent effects [22, 23]. Such adverse effect of propoxur could have consequences on sperm parameters and animal fertility. Interestingly, propoxur post-exposure observation showed decrease in epididymal sperm count and motility. Pesticides including propoxur have shown to increase oxidative stress through induction of compounds such as H_2O_2 [24]. Sperm cells are particularly vulnerable to oxidative stress-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFAs) and their cytoplasm contains low concentrations of scavenging enzymes [25, 26]. Therefore, the decrease in sperm count and motility could be due to a certain testicular oxidative stress induced by propoxur. In addition, carbamate pesticides reduce acetylcholinesterase activity, thus, block nerve impulses [27]. Hence, this effect may alter the release of pituitary hormones, namely FSH and LH leading to the reduction of sperm production in the testis. This hypothesis could explain the decrease in sperm density recorded in treated rats compared to the control. Moreover, this might have led to the reduction of epididymal weight as indicated [23]. The decrease of sperm count and motility has also been observed male rats after post-treatment time with a pesticide chlorpyriphos-ethyl [28]. Such observations point out a certain latent and/or persistent effect of pesticide side effect on male reproductive function.

The final physiological outcomes of any chemical are seen in term parameters such as fertility rate, litter size, viability rate and male/female sex ratio. In the present study, propoxur post exposure showed decreased fertility rate, litter size, and sex ratio. These could be seen as consequences of the negative effects of the pesticide on key reproductive organs such as the epididymis as shown in the present study. In fact, the fewer the number of sperms and the lower the motility, the lower the fertility of animals because limited spermatozoa would be available for impregnation of females [29, 30]. In spermatozoa, y-cells are more susceptible to different types of alterations (from internal or external origin) than x-cells [31]. This could explain the decreased male/female sex ratio noted in pups obtained from propoxur post exposed rats. Even though not significant, the dose-dependent trend observed in decreased pup viability is worth to be noted. In fact, the low sperm motility in pesticide-exposed animals may also due to morphological alteration of sperm cells leading the reducing viability of pups as noted in this study [32, 33]. Alteration of sperm parameters is consistent with the histological analysis of the testicular section that showed the undifferentiated germinal cells liberated in the lumen of the seminiferous tubules.

Compared to the 90 d treated animals, the additional 90 d post-exposure rats displayed more

serious alteration of the litter size, sex ratio, and pup viability. This illustrates a certain latent effect of propoxur that seems to be more harmful long time after the end of exposure. Therefore, the population exposed to such chemical could experience more reproductive adverse health effects long after the last exposure.

CONCLUSION

Propoxur administration to male rats moderately affects their reproductive functions at the end of treatment period while it shows more significant adverse effects 90 d after exposure. These findings suggest a certain contribution of propoxur in the infertility of the population exposed to such a chemical.

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