

## Lorsban-induced changes in haematological parameters, testosterone and thyroxin levels, and semen quality of male rats

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تهدف هذه الدراسة لمعرفة مدى تأثير المبيد الحشري لورسبان على الثوابت الدموية، مستوى هرمون التستوستيرون و التيروكسين و كذا السائل المنوي عند ذكور الفئران. يقدم المبيد عن طريق الفم بجرعات مختلفة (5، 10 و 15 ملغ/كغ/ وزن الحيوان) لمدة ستة أسابيع. تشير النتائج المحصل عليها إلى أن مختلف جرعات المبيد تسبب فقدان ( $P < 0.05$ ) في وزن الجسم. ونقص ( $P < 0.05$ ) في الوزن النسبي للخصية، البربخ والحوصلات المنوية عند الجرعة 15 ملغ/كغ/وزن الجسم. كما لوحظ انخفاض معنوي في محتوى الكريات الحمراء من الهيموغلوبين (MCH) و حجم المعدل الكروي (MCV) عند كل الحيوانات المعاملة مقارنة بالشاهدة. في حين سجل انخفاض غير معنوي في عدد الكريات البيضاء (WBC)، تركيز الهيموغلوبين (Hb)، نسبة الهيماتوكريت (Ht)، متوسط تركيز الكريات الحمراء من الهيموغلوبين (MCHC) و عدد الكريات الحمراء (RBC) عند الحيوانات المعاملة. ومن جهة أخرى لوحظ ارتفاع في عدد الصفائح الدموية (PL) عند الفئران المحقونة بالجرعة العالية. بالمقابل هناك انخفاض ( $p < 0.001$ ) في عدد الخلايا المنوية، الحيوانات المنوية، سرعة انتقالها و كذا في نسبة الإنتاج اليومية للحيوانات المنوية، يرافقها انخفاض في تركيز التستوستيرون في البلازما و ارتفاع تركيز التيروكسين الحر (FT4) مقارنة بالحيوانات الشاهدة. من هذا يتضح أن معاملة الفئران بجرعات أكبر من 15 ملغ/كغ/ وزن الحيوان يسبب خلافا في المؤشرات الدموية و الغذائية مؤدية لانخفاض الخصوبة عند الفئران.

**الكلمات المفتاحية:** الفئران؛ مبيد لورسبان؛ الثوابت الدموية؛ السائل المنوي؛ التستوستيرون؛ التيروكسين.

### Résumé

Cette étude a été effectuée pour examiner les effets toxiques possibles des différentes doses de Lorsban sur les paramètres de sang, les niveaux de testostérone et de thyroxine et la qualité de sperme des rats mâles. Le Lorsban a été administré aux rats par voie orale à des doses de 5, 10 et 15 mg/kg poids corporel/jour pendant 6 semaines. Les résultats obtenus ont prouvé que chacune des trois doses de Lorsban a sensiblement abaissé les gains de poids corporel ( $p < 0,05$ ). Des poids relatifs de vésicules de testicules, epididymal et séminales ont été diminués chez les rats recevant 15 mg/kg seulement ( $p < 0,05$ ). En outre, il y a une diminution en hémoglobine corpusculaire moyenne (MCH) et en volume corpusculaire moyen (MCV) chez tous les animaux d'expérience comparativement aux éléments témoins. Cependant, il n'y avait pas de changements significatifs pour les cellules de sang blanches (WBC), l'hémoglobine (Hb), la valeur d'hématocrite (Ht), la concentration moyenne en hémoglobine de cellules (MCHC) et les globules rouges (RBC) pour des rats qui ont été traités avec le Lorsban. Aussi, il s'est avéré que le Lorsban a causé une augmentation du nombre de thrombocyte (PL) chez les individus traités avec la dose la plus élevée. De même, la dose la plus élevée induit une diminution significative du nombre de spermatozoïdes, du compte de sperme et du taux de passage de sperme avec un affaiblissement observé de production quotidienne de sperme ( $p < 0,001$ ). De telles observations ont été couplées à une réduction des niveaux de testostérone de plasma et une augmentation des niveaux de la thyroxine libre de plasma (FT4). On conclut alors que le traitement avec des doses de 15 mg de Lorsban /kg de poids corporel altère les paramètres hématologiques et endocrinologiques chez les rats, et par conséquent affectera plus tard la fertilité.

**Mots clés :** rats; Lorsban; paramètres hématologiques; qualité de sperme; testostérone; thyroxine.

## Abstract

The present study was carried out to investigate the possible toxic effects of different doses of lorsban on blood parameters, testosterone and thyroxin levels, and semen quality of male rats. Lorsban was administered to rats by oral route at different doses (5, 10 and 15 mg/kg bw/day) for 6-weeks. The obtained results showed that all three doses of Lorsban significantly ( $p < 0.05$ ) lowered the body weight gains. Relative weights of testes, epididymal and seminal vesicles were significantly ( $p < 0.05$ ) decreased in rats receiving 15 mg/kg only. There was also a decrease in mean corpuscular haemoglobin (MCH) and in mean corpuscular volume (MCV) in all experimental animals compared to control ones. While, there were insignificant changes in white blood cells (WBC), haemoglobin (Hb), haematocrit value (Ht), mean cell haemoglobin concentration (MCHC) and red blood cells (RBC) for rats treated with lorsban compared to control group. Meanwhile, it appeared that lorsban caused a rise in thrombocyte (PL) number in individuals treated with the highest dose. Similarly, the highest dose induced a marked ( $p < 0.001$ ) decrease in spermatids number, sperm count and sperm transit rate with an observed impairment of daily sperm production. Such observations were coupled with a reduction in plasma testosterone levels and an increase in plasma free thyroxin (FT4) levels compared to controls. It is, therefore, assumed that treatment with lorsban up to 15 mg/kg bw alters both haematological and endocrinological parameters in rats, and subsequently affects fertility.

**Key words:** Rats; Lorsban; haematological parameters; semen quality; testosterone; thyroxin.

## 1. INTRODUCTION

To meet the needs of an ever-increasing population, a variety of pesticides are widely used in agriculture to combat plagues of diverse crops, increasing productivity and quality of agricultural product. By their nature and their presence in food, water and environment, pesticides are harmful to some forms of life and at certain levels of exposure they may be harmful to humans [1].

Previous studies have shown that exposure to insecticides caused alterations in haematological parameters, and endocrine, reproductive and immune systems. Haratym-Maj [2] has shown that insecticides may cause a mobilization of the haemopoietic system in humans, manifested by a higher level of values of mean numbers of erythrocytes, haemoglobin concentrations and haematocrit as well as leucocytes and monocytes. In addition, growing evidences have also revealed that insecticides can adversely affect endocrine, reproductive and immune systems in both experimental and wildlife animals [3]. A number of studies have also demonstrated that many of these insecticides can be quantified in human reproductive fluids including ovarian follicular fluid and semen [4]. Furthermore, studies conducted on

occupational insecticide workers have shown that exposure to these chemicals caused abortion, stillbirth, male infertility, neonatal deaths, congenital defects and testicular dysfunction [5, 6].

Lorsban is a well known insecticide and its active substances are cypermethrin (20g/l) and chlorpyrifos-ethyl (200g/l). Cypermethrin is extensively used as an ectoparasiticide in animals and as insecticide in crop production and public health programme. At higher doses, cypermethrin can affect the nervous system, decrease growth, increase liver and kidney weights [7]. It has also been shown that cypermethrin induces moderate toxic effects on blood elements and on some of the biochemical functions, including lipoproteins, protein, urea, creatinine, glucose, and total bilirubin in rabbits [8]. In addition, Yousef et al. [9] showed that cypermethrin induced pronounced hazardous effects in several physio-metabolic functions including body weight, feed intake, testosterone levels and reproductive performance of male rabbits.

Chlorpyrifos-ethyl is a broad-spectrum organophosphorus pesticide used as an insecticide to control household pests,

aquatic larvae, mosquitoes, flies, various crop pests in soil and on foliage. It is also used on sheep and cattle for control of ectoparasites [10]. The toxicity of chlorpyrifos is specifically attributed to the inhibition of the enzyme acetylcholinesterase [11]. Previous studies conducted on male rats revealed a decrease in body weight and red blood cell count, increase in platelet count, reduced in serum total protein, albumin and globulin concentrations. A decrease in serum alkaline phosphatase and alanine aminotransferases activities have also been noted [12]. However, some reproductive toxicity studies on chlorpyrifos showed very weak effects on parental reproductive function or no apparent neonatal toxicity in offspring [13,14].

Some previous studies have showed the toxicity of cypermethrin or chlorpyrifos alone on haematological parameters, reproductive performance and hormone levels. Nevertheless, a little is known on the effect of the combination of the two active forms of the pesticide. In spite of lorsban is a widely used insecticide, but to our knowledge there are not enough published data showing the effects of this compound on various haematological parameters and reproductive performance of male rats. Therefore, the present study aimed to investigate the haematological and reproductive toxicity of lorsban in male rats.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Animals

Lorsban (22% EC) is a mixture of two insecticides, cypermethrin (20g/l) and chlorpyrifos-ethyl (200g/l). Cypermethrin [( $\alpha$ -cyano-3-phenoxybenzyl 3 (2-2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate), (C<sub>22</sub> H<sub>19</sub> Cl<sub>2</sub>NO<sub>3</sub>)] is a synthetic pyrethroid. Chlorpyrifos-ethyl [O, O,-diethyl O-(3,5,6-trichloro-

2pyridinyl) phosphorothioate (C<sub>9</sub>H<sub>11</sub>Cl<sub>3</sub>NO<sub>3</sub>PS)] [7].

Male wistar rats of 4 months of age were used in the present study, with a mean body weight of 350g. Rats were kept singly in plastic cages under standardized animal house conditions (25-28°C, 12L:12D schedule of light and dark, relative humidity (60 ± 5 %), they received a standard pellet feed and water *ad libitum*. The animals were randomly divided into four groups each of 8 rats.

### 2.2 Lorsban pesticide administration

Lorsban was initially diluted in water. Each animal received daily 1ml of the pesticide throughout the six weeks experimental period. Animals of groups 2,3 and 4 received respectively doses of 5 mg/kg/bw, 10 mg/kg/bw and 15 mg/kg/bw, while those of group 1 served as control. The dose was adjusted weekly according to the average body weight of the rats.

### 2.3 Body and sex organ weights

Body weight was recorded weekly throughout the study period. At the end of the treatment period, animals were sacrificed and the male reproductive organs (testes, epididymis and seminal vesicles) were quickly removed and weighed individually and then relative organ weights were calculated.

### 2.4 Haematological analysis

At the end of the experimental period, blood samples were collected by decapitation and placed immediately on ice. EDTA was used as an anticoagulant for determination of selected haematological parameters. Red blood cell (RBC) counts, white blood cell (WBC), haematocrit value (packed cells volume; PCV) and haemoglobin (Hb) level, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean

corpuscular volume (MCV) were measured using automatic ABACUS counter (Abacus haematology analyzer DIATRON Messtechnik Ges.m.b.H. A-1141 Wien, Ameisgasse 49-51/2. AUSTRIA).

**2.5 Sperm and spermatid number**

The left testis and epididymis from each rat were excised and cleared off the attached and connective tissue and weighed. After removal of tunica albuginea the testis was minced with scissors and homogenized in 10 ml 0.9% NaCl containing 0.5 % Triton X-100; the homogenate was mixed using vortex mixer. The number of homogenization-resistant spermatids was counted in haemocytometer (Mallassez) chamber. Daily sperm production (DSP) was calculated by dividing the number of homogenization-resistant spermatid by 6.1 [15,16].

The cauda epididymis was cut into small pieces by a disposable blade in 10 ml of 0.9% NaCl containing 0.5 % Triton X-100 and homogenized and spermatozoa were counted as described above. The epididymal sperm transit rate was estimated for each male rat by dividing the epididymal sperm number by the daily sperm production [17].

**2.6 Hormones analysis**

Blood samples were centrifuged at 2500 rpm for 15 min and plasma was stored at – 20°C for later analyses. Plasma testosterone and free thyroxin (FT4) concentrations were measured using an ELISA kit, purchased from DRG diagnostics, GmbH, Germany.

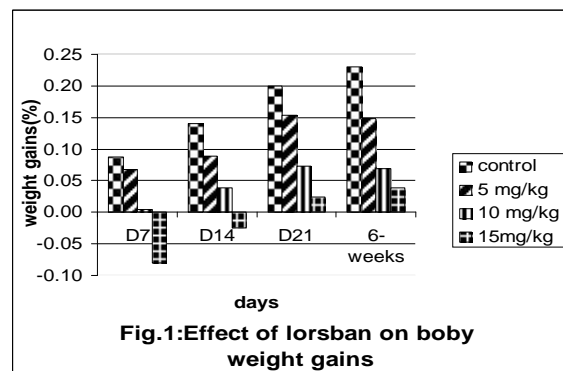
**2.7 Statistical analysis**

Data were given as arithmetic means ± SEM. Statistical evaluation of the data was performed by Student’s t-test and the level of significance was chosen as p<0.05, p<0.01 and p<0.001.

**3. RESULTS**

**3.1 Body weight and relative reproductive organs weight**

As shown in Figure 1, there has been decrease (-8 %) in body weight of animals treated with 15 mg/kg of lorsban, compared to control rats (+8 %) during the first experimental week, while there has been a slight increase in body weight of the same animals at the end of the experimental period of about 3.9%. Yet, body weights gain was less pronounced in the other treated animals compared to the control ones. In addition, there were significant (p<0.05) reduction in testes, epididymis and seminal vesicles weights only in rats treated with the highest dose compared to control rats (Table 1).



**Figure 1.** Body weight and relative reproductive organs weight

**Table 1.** Effect of lorsban on relative weights of reproductive organ (testes, epididymis and seminal vesicles) after 6-weeks treatment period.

Relative weight	Experimental groups			
	Control	5 mg/kg	10 mg/kg	15 mg/kg
Testes (%)	0.47 ± 0.029	0.48 ± 0.015	0.49 ± 0.023	0.39 ± 0.025*
Epididymis (%)	0.16 ± 0.018	0.15 ± 0.014	0.15 ± 0.009	0.13 ± 0.01*
Seminal vesicle (%)	0.064 ± 0.011	0.062 ± 0.007	0.058 ± 0.005	0.051 ± 0.003*

Values are given as mean ± SE. Statistically significant different (\*p<0.05) from the control group.

### 3.2 Haematological parameters

Changes in blood parameters in both control and treated groups are summarized in Table 2. It seems that treatment with lorsban did not alter white blood cells (WBC), haemoglobin (Hb), haematocrit (Ht), mean cell haemoglobin concentration (MCHC) and in red blood cell count (RBC). On the other hand, there was significant decrease in mean corpuscular haemoglobin (MCH) and in mean corpuscular volume (MCV) in the experiment animals compared to the control at any doses. Meanwhile lorsban caused increase in thrombocyte (PL) number in group treated with the highest dose of lorsban.

**Table 2.** Changes in haematological parameters of male rats orally administered lorsban for 6 weeks.

Parameters	Experimental groups			
	Control	5 mg/kg	10mg/kg	15mg/kg
RBC( $10^{12}/l$ )	9.0±0.32	9.0 ±0.52	8.7 ±0.74	8.0±0.52*
HB (g/dl)	15± 0.36	15 ± 0.66	14±0.87	13± 0.57*
Ht (%)	50±1.47	51±2.13	48 ±2.71	47± 1.57*
WBC( $10^9/l$ )	7.6±0.96	8.5 ±0.79	8.1 ± 0.96	8.7±0.97*
PL ( $10^9/l$ )	603±53	644± 89	649±73	664 ± 38*
MCV (fl)	58±1.16	55±2.18*	53 ±2.31**	52±1.76**
MCH (pg)	17±0.50	16±0.99*	15±0.67*	13±0.51**
MCHC(g/dl)	30±0.59	29±1.17	30±0.48	28±0.49*

Values are given as mean ± standard error. Statistically significant different (\*P<0.05, \*\*P<0.01) from the control group.

### 3.3 Semen parameters

Spermatid number and sperm counts, daily sperm production and sperm transit rate are presented in Table 3. The observed low testis and epididymis weights in group treated with lorsban at highest dose (15mg/kg) were accompanied by the reduction in testicular spermatids count and sperm count from caudal epididymis as well as daily sperm production (p<0.001). In addition, the results showed a decline in sperm counts at dose 10 mg/kg bw (P<0.01). Moreover, an increase in sperm

transit rate (p<0.05) at the highest dose was recorded.

**Table 3.** Effect of 6 weeks oral administration of lorsban on spermatids number, sperm count, daily sperm production and sperm transit rate of adult male rats.

Parameters	Experimental groups			
	Control	5mg/kg	10mg/kg	15mg/kg
Spermatids number ( $\times 10^6$ )	219±33	201±27	190±37	107±30***
Sperm count( $\times 10^6$ )	139±2.1	132±32.8	100±21.6*	82±10.4**
Daily sperm production( $\times 10^6$ )	44±5.4	41±4.5	39±6.1	26±4.8***
Sperm transit rate (days)	4.07±0.57	4.2±0.98	4.4±0.53	4.9±0.42*

Results are expressed as mean ± standard error, statistically significant different (\*P<0.05; \*\*P<0.01; \*\*\*P<0.001) from the control group.

### 3.4 Hormone levels

Changes in serum levels of testosterone and free thyroxin (FT4) following Lorsban administrated presented in Table 4. The concentration of serum testosterone was extensively decreased (p<0.01) only in rats treated with the highest dose (15 mg/kg bw). However, the level of FT4 was significant (p<0.05) increase mainly in the same group of animals.

**Table 4.** Effect of 6 weeks oral administration of lorsban on plasma testosterone and thyroxin levels of adult male rats.

Hormone concentration	Experimental groups			
	Control	5mg/kg	10mg/kg	15mg/kg
FT4 (pmol/l)	11.88±1.39	12.21±1.55	12.57±0.90	14. 24±0.90*
Testosterone (nmol/l)	13.07 ±1.19	12.82±1.24	12.54±1.97	7.33±1.03**

Results are expressed as mean ± standard error, \*p<0.05; \*\*p<0.01.

## 4. DISCUSSION

In the present study there was a significant decrease in body weight gains of rats treated with lorsban at all doses

compared to control animals mainly by a decrease in both food and water consumption (data not presented). Aldana et al. [18] reported a statistically significant decrease in body weight after administration of 300 mg/kg cypermethrin to male rats for 7 days. Furthermore, body weight gain and water intake were reduced in male rats ingested 2.13 mg/animal/ day abamectin [19].

In the present study, rats treated with lorsban showed insignificant changes in erythrocyte count, haematocrit value and haemoglobin content compared to the control group. This result is consistent with the result reported in literature that organophosphates insecticides caused changes of some haematological and biochemical parameters in experimental animals [20]. The main haematological response of male rats to the sub-chronic exposure to lorsban pesticide was a significant decrease in MCV and MCH in any treatment and an increase in thrombocyte (PL) number (Table 2) in the group treated with the higher dose which may be due to microcytic anemia and thrombocytosis. Similarly, Kalender et al. [21] observed increase of thrombocytes in rats treated with 10 mg/kg, per day diazinon for 7 weeks, whereas Ratnasooriya et al. [22] observed a reduction in MCV in male rats treated with the pyrethroid Lambda cyalothrin at 100 mg/kg /bw for 7 days. Also, Yousef et al. [8] reported that cypermethrin caused alterations in the haematological parameters of rabbits.

According to the results reported in this work, the high dose of lorsban has adverse effects on reproductive parameters. In recent years, there has been increasing concern regarding potential adverse effects of various environmental contaminants designed as endocrine disruptors/hormonally active agents. These concerns have originated in part, from observations of developmental and reproductive disorders in wildlife

populations exposed to wide range of synthetic chemicals such as pesticides that have been released into the environment in large amount since the World War II. Subsequently, several incidents in wildlife population strongly correlated the decreased reproductive capacity with specific industrial chemicals [6].

The present data showed that, there was a significant reduction ( $p < 0.05$ ) in all reproductive organ weights (testes, epididymis and seminal vesicles) at the highest dose (15 mg/kg/ bw) of lorsban. These findings are in accordance with Latchoumycandane et al. [23] who reported that the administration of methoxychlor for 7 days in adult rats caused a reduction in the weights of the epididymis, seminal vesicles, and ventral prostate. Also, Yousef et al. [9] found that rabbits gavaged with 24 mg/kg/bw of cypermethrin showed a reduction in body weight and in relative weight of testes and epididymis, and serum testosterone concentrations.

In deltamethrin-treated animals, the absolute and relative weights of sex accessory organs (ventral prostate and seminal vesicle) appeared to decrease in a dose-related manner. Also, testicular and epididymal absolute weights were significantly reduced in male offspring rats exposed to the highest dose of deltamethrin (4.0 mg/kg) when compared to control animals [24]. In addition, relative and absolute ovaries and the absolute seminal vesicles weights in F0 rats, and the weights of testes and ventral prostate of F1 were decreased at 100mg/kg/bw chlorpyrifos-methyl [25].

The present results indicated that lorsban at dose 15 mg/kg/bw caused a significant ( $p < 0.01$ ) decrease in testis spermatid number, epididymal sperm count and daily sperm production compared with the control animals. The observed decrease in semen characteristics could be explained by the fact that lorsban acted directly on the

testes and affected the androgen biosynthesis pathway which regulates the weight, size and secretory function of testes, epididymes, seminal vesicles, ventral prostate and vas deferens. In accordance with these results, sub-lethal doses of organophosphate pesticides lead to alterations in reproductive performance in birds and mammals [26]. There are some possible mechanisms for the antigonadal action of organophosphates, they may exert a direct inhibitory action on the testis and affect androgen biosynthesis pathways, affect the pituitary gland, causing changes in gonadotrophin concentrations and thus subsequent spermatogenic impairment or they may change the concentration of neurotransmitters [27]. Also, some authors noted a reduction in epididymal sperm counts, testicular sperm counts and daily sperm production by many pesticides. Results conducted by Yousef et al. [8] showed that treating rabbits with the pyrethroid cypermethrin caused a significant decline in ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm per ejaculate and packed sperm volume, increased the numbers of abnormal and dead sperm.

It is clear that there is a relationship between the production of sperm, the level of testosterone and the leydig cells. The fall in serum testosterone levels in lorsban exposed rats is consistent with previous data showing alteration in leydig cells [28]. Methoxychlor decreased serum concentration of testosterone [30]. The study of Banbino and Hsueti [31] suggested an interaction of pesticides with hypothalamo-pituitary gonadal axis controlling spermatogenesis and may also interact directly with sertoli or leydig cells responsible for testicular production of proteins involved in the transport and the production of testosterone.

The reduction in serum testosterone concentrations (Table 4) is in agreement with the findings conducted by Elbetieha et

al. [31] and Yousef et al. [9] who found that serum level of testosterone, follicle-stimulating hormone and luteinizing hormone were significantly reduced in male rats and rabbits exposed to cypermethrin. Jeong et al. [25] reported that chlopyriphos-methyl (10 or 100mg/kg/bw) induced suppression of estrogen, androgen and T4 in dose-dependency when exposed during prenatal and postnatal period until 13 weeks old in F1 male rats. Also, treatment of male rats from postnatal day 22 to 48 with atrazine (50mg/kg/bw) reduced both serum and intratesticular testosterone concentrations by approximately 50% and LH-stimulated hormone in cultured leydig cells suggests that atrazine inhibits testosterone production rather than increasing catabolism [32]. These effects on testosterone were attributed to the fall in serum LH, since LH serves as a normal stimulus for the secretion of this steroid from the testicular Lydig cells.

There is growing evidence that environmental chemicals can disrupt endocrine systems. Most evidence originates from studies on reproductive organs. However, there is also suspicion that thyroid homeostasis may be disrupted. There are few studies existing on the effects of pesticides on the thyroid function. DDT exposure of birds decreased T4 [33]. In contrast, our results showed an increase in serum FT4 level in group treated with the highest dose (15mg/kg/bw). This effect may be due to the interaction between the pesticide and thyroxine binding protein causing abnormal binding protein which involved in an increase in the level of FT4. The increase of FT4 being in accordance with reported data by Calvert et al. [35] which showed an increment in the levels of FT4 in 278 workers employed in the manufacture of 2,4,5,-trichlorophenol contaminated with dioxin (TCDD). Hagmar [35] reviewed 13 studies (among them 6 in neonates and infants) and showed contradictory data on the increase, decrease or no change of FT4, TT3 and TSH levels.

## 5. CONCLUSION

From the obtained results, the sub-chronic exposure to the low doses of Lorsban (5 and 10 mg/kg/day) did not show harmful effects on haematological and semen characteristics, and the levels on testosterone and thyroxin while, the high dose of lorsban (15 mg/kg/day) showed toxic effects on blood indices and fertility of male rats. Therefore, it is recommended that there must be great care when using any insecticide to control insects.

### Abbreviations:

MCH : mean corpuscular haemoglobin;  
 MCV : mean corpuscular volume;  
 WBC : white blood cells;  
 Hb : haemoglobin;  
 Ht : haematocrit value;  
 MCHC : mean cell haemoglobin concentration;  
 RBC : red blood cells;  
 PL : thrombocyte;  
 FT4 : plasma free thyroxin levels.

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