

## Effect of Toluene on Male Reproductive Parameters in Wistar Rats

Victoria C. Obinna<sup>1\*</sup> and Gabriel O. Agu<sup>1</sup>

<sup>1</sup>Animal Health and Physiology Unit, Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

### Authors' contributions

This work was carried out in collaboration between both authors. Author VCO designed the experiment, did the work and wrote the manuscript. Author GOA managed the analyses and supervised the work. Both authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** The study assessed the effect of oral exposure of toluene on male reproductive parameters.  
**Methodology:** Twenty rats (190±10g bwt) were randomly assigned to four groups (n=5). Group A (Control) was treated with olive oil (0.5ml), Group B received 31.8mg/kg, Group C received 63.6mg/kg and Group D received 127.2mg/kg. All administrations were by oral gavage for 21 days. Thereafter, the rats were anaesthetized and samples were taken as follows: Blood was used for testosterone assay, caudal epididymides was taken to analyze for sperm count and sperm cell characteristics while testes was weighed and prepared for histomorphology.  
**Results:** No significant difference in the relative testicular weights, epididymal sperm count, sperm viability, motility and morphology relative to control. Marked reduction ( $p = 0.001$ ; group C &  $p = 0.018$ ; group D) in testosterone level occurred in Groups C and D rats treated with 63.6mg/kg and 127.2mg/kg doses of toluene respectively. No anomaly in histoarchitecture of the testes in all the treated rats in comparison with control.  
**Conclusion:** Exposure to toluene by oral route may be noxious to male reproductive functions due to the reduction in testosterone level at higher doses.

**Keywords:** Toluene; male; testosterone; sperm count; reproductive functions.

\*Corresponding author: E-mail: drchiobinna@yahoo.co.uk, victoria\_obinna@uniport.edu.ng;

## 1. INTRODUCTION

The widespread use of dangerous and poisonous organic solvents in industries and chemical preparations is generating much concern, especially in developing world, where they constitute major problem to human, animal and environmental health. Occupational exposure to these organic solvents may be harmful to reproductive health of workers in that they can inflict damage on the genomic materials of their cells as well as induce noxious effects on their sexual function and fertility. Toxic exposures to these solvents can cause direct cell damage in the developing sperm and eggs [1].

Toluene, an organic solvent also known as methylbenzene or phenylmethane [2] is among the most common hazardous sources of environmental pollution. It is broadly used as industrial solvent in manufacturing of automobile fuels, chemical pharmaceuticals and multiple household and commercial products such as ink, glue, paint, rubber, cements and other adhesives [3]. Toluene has emerged as the most commonly abused solvent [4] especially among children and adolescents who deliberately inhale fumes from toluene containing substances such as paints, inks, glues, etc.; in order to get intoxicated and a sensation of euphoria [5] and this abuse may lead to toxicity with increased dose and duration of exposure.

The aim of the study is to investigate if subacute exposure to organic solvents such as toluene has adverse effect on male reproductive parameters using wistar rat as animal model.

## 2. METHODOLOGY

### 2.1 Chemicals and Reagents

Toluene was purchased from Bernaco Enterprises, Nigeria as a clear colourless liquid with pleasant aromatic petroleum odour with CAS No: 108-88-3. The oral LD<sub>50</sub> of Toluene for rats was reported as 636mg/kg [6]. The experimental doses which corresponded to 1/20, 1/10 and 1/5 of the LD<sub>50</sub> were prepared in olive oil purchased from a supermarket.

#### 2.1.1 Animals and treatment

Twenty adult male wistar rats weighing an average of 200g procured from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt,

Nigeria, were used for the study. The animals which were acclimatized for 2 weeks before the study, were fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited) and supplied with clean drinking water all through the study.

The rats were randomly assigned to four groups (Groups A, B, C and D) of five animals each; with group A serving as the control. Control animals (dose = 0mg/kg) received 0.5 ml of olive oil. The rats in groups B, C and D were treated at the doses of 31.8 mg/kg, 63.6mg/kg and 127.2 mg/kg respectively for 21 consecutive days. All treatments were by oral gavage. Animals' weights were measured weekly and the doses adjusted accordingly.

### 2.2 Sample Collection and Analyses

On day 22, the animals were weighed and anesthetized. Blood samples were collected by cardiac puncture into the sterile plain bottles for testosterone assay. The blood samples were allowed to stand for 30-45 mins in order to coagulate and then centrifuged for 15 mins at 3000 rev/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 the hormonal assay for assessment of Testosterone levels using Accu-bind ELISA kits (Testosterone Test System Product Code: 3725-300) from Monobind Inc. Lake Forest, CA 92630, USA. The testes were excised, cleared of adhering tissues and weighed independently. The epididymides were harvested and the caudal part was used to determine the epididymal sperm count and characteristics. Testicular samples were fixed in Bouin's solution, and then processed for histomorphological examination as described by Lillie [7]. The testicular sections were embedded in paraffin, sectioned at 4-5µm and stained by Haematoxylin and Eosin blue.

The relative organ (testicular) weight was estimated individually as follows:

$$\text{Relative Testicular weight} = \frac{\text{Actual Testicular weight (g)}}{\text{Body weight (g)}} \times 100\%$$

### 2.3 Statistical Analyses

Statistical analyses were done using SPSS 21. All values were expressed as mean ± SEM, and

data were analyzed by one-way ANOVA followed by the Tukey post hoc test. The significance level was set at  $P < .05$ .

### 3. RESULTS

#### 3.1 Mean Relative Testicular Weights

No significant variation ( $P = 0.09, 0.41$  and  $0.32$ ) was observed in the average relative testicular weights of toluene treated rats in comparison with control (Fig. 1)

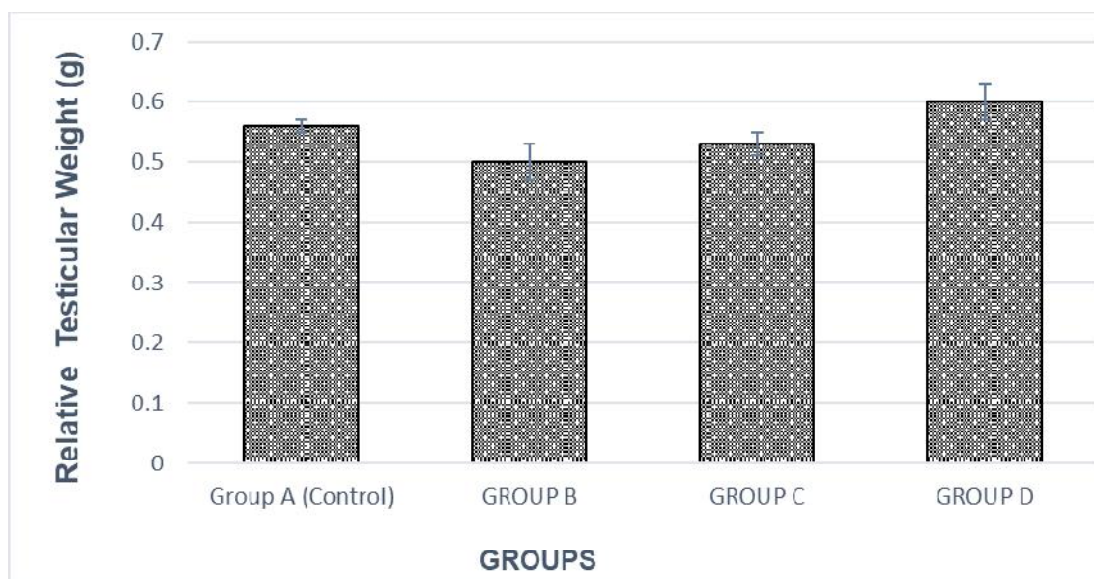
#### 3.2 Testosterone Level

Fig. 2 shows the result of varied toluene doses on serum testosterone concentration. At the dose of 31.8mg/kg, toluene did not make any

significant difference ( $P = 0.59$ ) on testosterone concentration. However, at the doses of 63.6 and 127.2 mg/kg, there was a marked reduction ( $P = 0.001$  and  $P = 0.018$  respectively) in testosterone level relative to the control.

#### 3.3 Epididymal Sperm Count and Sperm Cell Characteristics

Toluene evoked a non-significant decrease ( $P = 0.93$  and  $0.31$ ) in epididymal sperm count at higher doses in relation with the control (Fig. 3). Insignificant difference in the sperm motility, viability and morphology was recorded in toluene treated rats in comparison with control (Figs. 4 and 5).

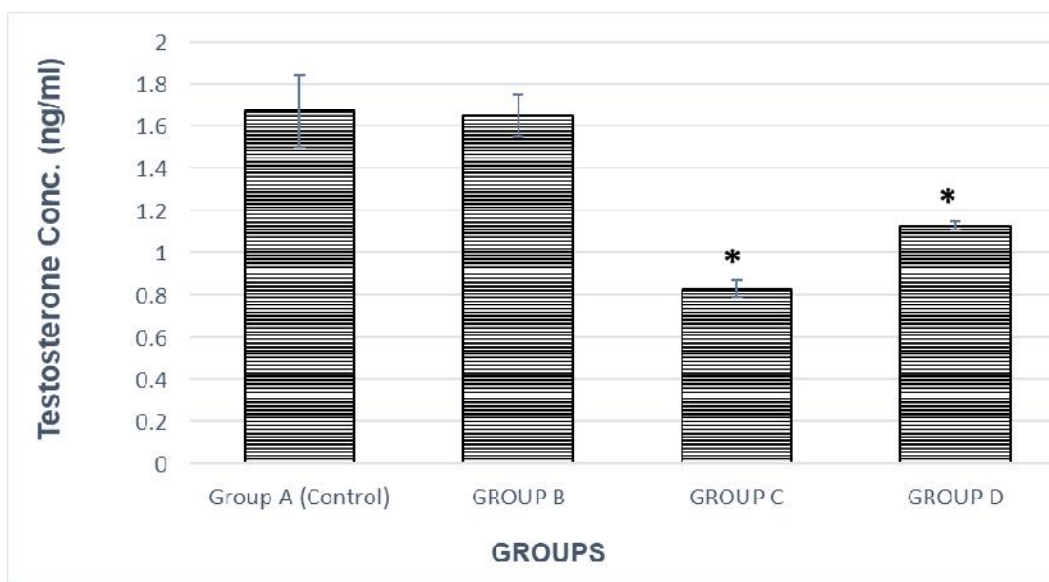


**Fig. 1. Effect of Toluene on Relative testicular weight of rats treated for 21 days. Results are given as mean  $\pm$  SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ( $p > 0.05$ ). Groups A, B, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively.**

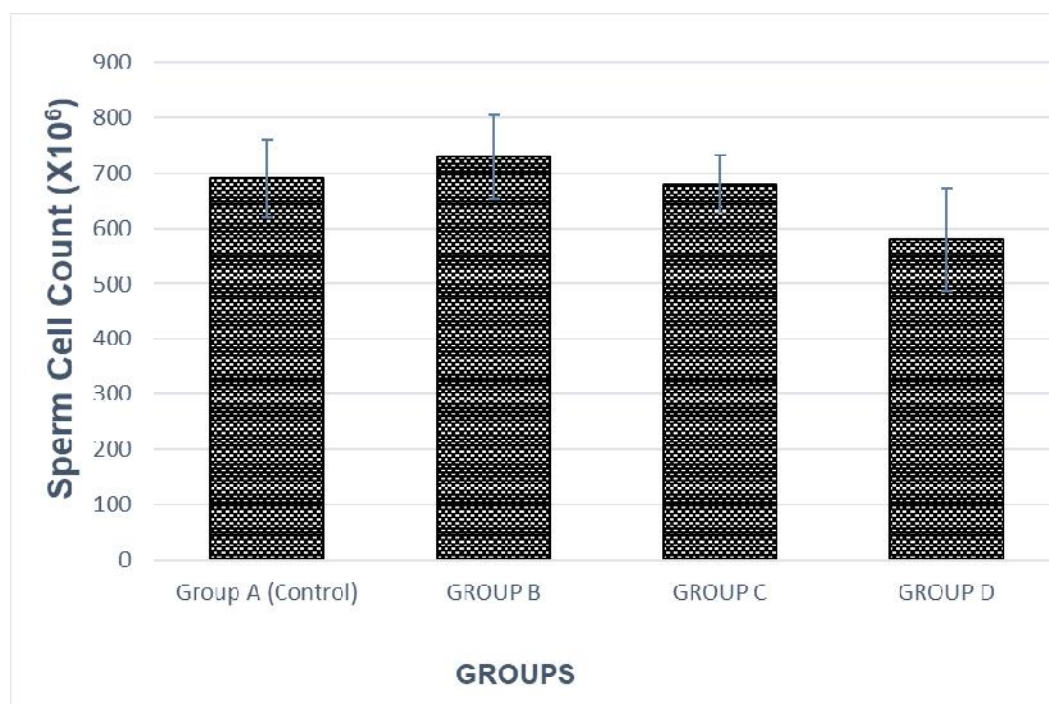
**Table 1. Effect of Toluene on Sperm cell characteristics of rats treated for 21 days**

Groups	Spermatozoa Morphology (%)			Sperm cell Parameters (%)		
	Normal	Abnormal	Active	Sluggish	Dead	Viability
A	76.00 $\pm$ 3.67	24.00 $\pm$ 3.67	71.00 $\pm$ 5.10	10.00 $\pm$ 1.58	19.00 $\pm$ 4.00	72.00 $\pm$ 4.06
B	77.00 $\pm$ 3.74	23.00 $\pm$ 3.74	76.00 $\pm$ 3.67	10.00 $\pm$ 1.58	14.00 $\pm$ 2.45	80.00 $\pm$ 3.54
C	72.00 $\pm$ 2.55	31.00 $\pm$ 1.87	67.00 $\pm$ 2.55	12.00 $\pm$ 1.22	21.00 $\pm$ 2.45	74.00 $\pm$ 1.87
D	72.00 $\pm$ 5.39	28.00 $\pm$ 5.39	72.00 $\pm$ 7.35	9.00 $\pm$ 1.00	19.00 $\pm$ 6.78	70.00 $\pm$ 3.53

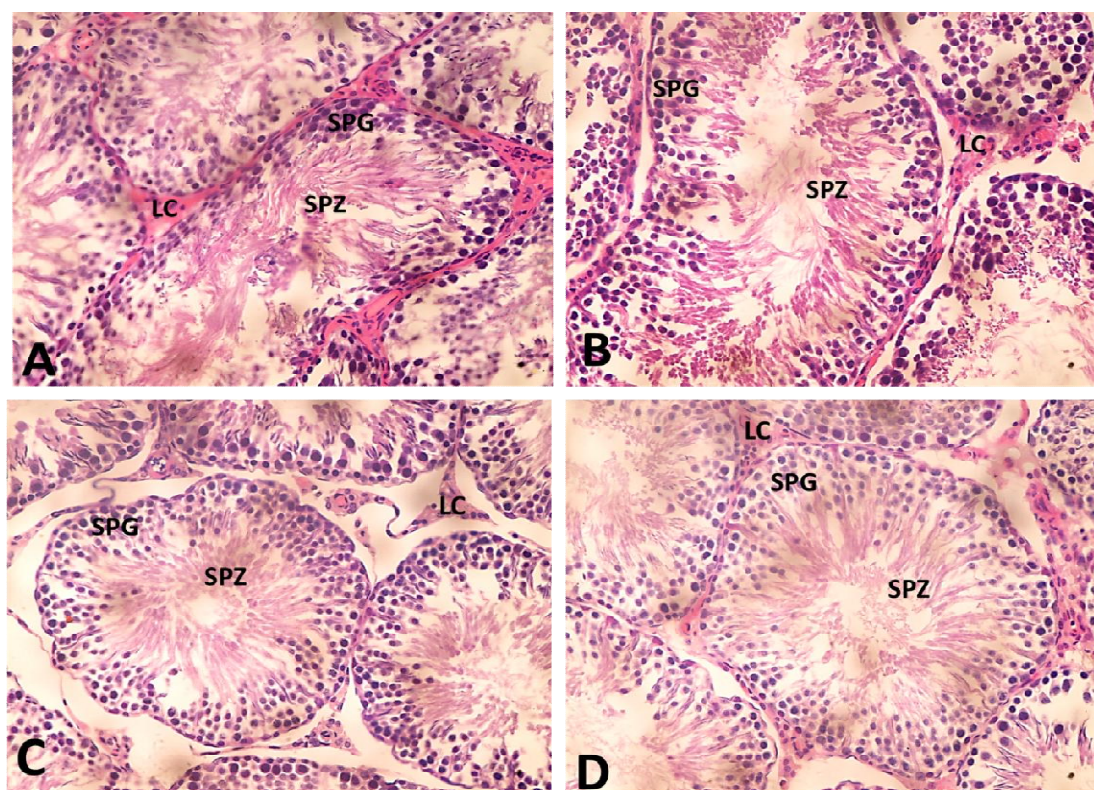
Results are given as mean  $\pm$  SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ( $p > 0.05$ ). Groups A, B, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively.



**Fig. 2.** Effect of Toluene on Testosterone concentration of rats treated for 21 days. Results are given as mean  $\pm$  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, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively.



**Fig. 3.** Effect of Toluene on Sperm cell count of rats treated for 21 days. Results are given as mean  $\pm$  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, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively



**Plate 1. Photomicrographs of testicular sections of rats from Control and treated groups after 21 days of treatment, stained with H&E ( $\times 400$ ). Groups A, B, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively. No abnormality in the testes of treated rats when compared with the control. The interstitial spaces contain the Leydig cells (LC), the seminiferous tubules are filled with spermatogenic cells (SPG) and the lumen contains spermatozoa (SPZ).**

### 3.4 Histomorphological Findings

Photomicrographs of testicular sections of rats from toluene treated groups are not different from those of the control. No obvious abnormality was observed (Plate 1).

## 4. DISCUSSION

Occupational solvent exposure may elicit adverse effects on male and female reproductive systems depending on the dose and duration of exposure. According to Lindbohm & Sallmén [1], these adverse effects may manifest as alterations in sex hormone levels, diminished libido and potency, menstrual disorders, premature menopause, delayed menarche, ovarian dysfunction, impairment of semen quality, and reduced male and female fertility.

From this study, exposure to toluene had no significant effect on the relative testicular weight as well as the viability, motility and morphology of sperms from exposed male wistar rats for 21 days. Although, the testosterone concentration was significantly decreased at higher doses of toluene, a non-significant reduction in epididymal sperm cell count was recorded at the higher doses.

The decrease in testosterone level as recorded in this study suggests that toluene likely exerted its effect on the Leydig cells and/or the hypothalamus-pituitary-gonadal axis by inhibiting the secretion of the hormones or altering their regulation. This reduction in testosterone level may be associated with the hampering of the enzymes / the pathway necessary for its production or the mimicking of the hormone [8] by toluene which is known to be an endocrine disruptor [9]. Endocrine disrupting chemicals

(EDCs) are compounds that alter the normal functioning of the endocrine system of both animals and humans [10]. They act through several mechanisms such as mimicking the action of a natural hormone by acting as an agonist, preventing a hormone from binding to the receptor by acting as an antagonist as well as disrupting the production / regulation of hormones or the transport of a hormone within a biological system.

The reduction in serum testosterone level can have adverse effect on spermatogenesis as reflected in the non-significant decline in epididymal sperm cell count, since spermatogenesis depends on testicular testosterone. However, the decrease in epididymal sperm cell count though not significant could be due to the short duration of exposure (21 days) which may be too short to cause marked effect on spermatogenesis. This assertion is supported by the non-significant effect on relative testicular weight and the absence of distortion or any form of abnormality in the histoarchitecture of testicular sections obtained from the toluene treated rats.

The result of this study is in agreement with the study by Ono et al. [11] who reported that exposure of seven-week-old male Sprague-Dawley rats to toluene vapor inhalation (0, 4000, or 6000 ppm; 2 h/day) daily for 5 weeks produced no exposure-related changes in the testicular weight and spermatogenesis within testes. However, the study is in contrast with this present study in the aspect of non-significant change in testosterone level and suppressed epididymal sperm count, sperm motility and sperm quality. Our result disagrees with the work of Djemil et al. [12] which demonstrated that treatment of male rabbits with toluene or xylene solvents at 50, 100 and 150 ppm by oral gavage for 24 days caused decreased sperm concentration, motility, speed and vitality as well as cellular malformation within seminiferous tubules and epithelial cells especially in groups treated with the highest dose of toluene or xylene. However, the study and the present study reported reduction in testosterone concentration. The reason for the disparity in the results from the two studies and the present study may likely be associated with the doses and duration of exposure as well as the pharmacokinetics and pharmacodynamics of toluene toxicity in the different animal models used - rats and rabbits.

## 5. CONCLUSION

From our findings in this study, it is therefore concluded that oral administration of toluene as used in this study may be toxic to male reproductive functions due to the decline in testosterone level at higher doses.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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