

# Gender Differences in the Haematotoxicity and Weight Changes Associated with Exposure to Gasoline Vapours in Wistar Albino Rats

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## Citation

F Uboh, M Akpanabiatu, P Ebong, I Umoh. *Gender Differences in the Haematotoxicity and Weight Changes Associated with Exposure to Gasoline Vapours in Wistar Albino Rats*. The Internet Journal of Toxicology. 2007 Volume 5 Number 2.

## Abstract

Gender differences in haematotoxicity and weight changes associated with exposure to gasoline vapours (6hrs/day for 20 weeks) in rats was assessed in this study. The results showed that the levels of Hb, PCV and RBC counts in both male and female rats reduced significantly, while the WBC count increased significantly ( $P<0.05$ ) in female and insignificantly ( $P>0.05$ ) in male rats, exposed to gasoline vapours. The comparative percentage decrease in Hb, PCV, RBC were significantly higher ( $P<0.05$ ) in female rats than males. Also, the comparative percentage increase in WBC in females was significantly higher ( $P<0.05$ ) than males. Moreover percentage weight increase was significantly higher in males and lower in females, while percentage growth rate decreased significantly ( $P<0.05$ ) in both male and female rats exposed to gasoline. The results of this study showed that haematotoxicity and weight changes associated with exposure to gasoline vapours are sex-dependent; with females being more vulnerable.

## INTRODUCTION

Liquid gasoline is composed of approximately 150 different hydrocarbons including 60 -70% alkanes, 25 – 35% aromatics, and 5 – 10% alkenes<sup>1</sup>. The liquid gasoline may evaporates or be burnt to release vapours into the environment. These are ubiquitous in the environment and constitute some pollutants in the atmosphere. However, the overall constituents of gasoline fumes depend on the composition of the liquid gasoline, which varies with brand, and storage time<sup>1</sup>. Also, the relative proportion of individual chemical constituents in the liquid and vapour forms of gasoline differs substantially, with the more volatile components disproportionately represented in the vapour<sup>2,3</sup>

According to Wixtrom and Brown<sup>3</sup>, the public is annually exposed to vapours from billions of gallons of unleaded gasoline (UG), and that the major route of exposure of workers to the gasoline fumes is by inhalation during the production and transportation of the UG, and of general public during refueling at service stations. Moreover, it has been reported that after inhalation of equal concentrations of petroleum vapour, lower concentrations of saturated hydrocarbons than unsaturated and aromatics are found in humans and animals blood<sup>4</sup>. Frequent exposures to gasoline vapours or any of the hazardous constituents (particularly

benzene, hexane, tetraethyl lead and xylene) are reported to be haematotoxic<sup>5,6</sup>.

Also, according to Rothman et al.<sup>7</sup>, most haematological parameters (total white blood cell, absolute lymphocytes, platelets, red blood cells counts and haematocrit) were reduced among workers heavily exposed to benzene. The haematotoxic effect of benzene has been reported to involve both bone marrow depression and leukaemogenesis caused by damage to multiple classes of haematopoietic cells with a variety of functions<sup>8</sup>. Also, 2, 5-hexanedione (a toxic metabolite of hexane) has been reported to covalently cross-link red cells membrane proteins and cause damages to the cells<sup>9</sup>. In our previous study, we also observed that inhalation exposure of rats to composite gasoline (Premium Motor Spirit - PMS) vapours may be a potent predisposing factor to anaemic condition<sup>10</sup>. PMS is the blend of gasoline commonly used in Nigeria. However, the sex that is more vulnerable to the haematotoxic effect of these constituents has not been well defined.

Anaemia is known to be one of the most dreadful diseases that is widely spread in the world. Causes range from malnutrition, infection, exposure to reactive chemical agents that can interact and destroy the blood cells or depress blood

cells formation. Anaemic condition may be reliably assessed by measuring the levels of haematocrit, haemoglobin and red blood cells in circulation <sup>11,12</sup>. From these reports, it is understood that haemoglobin levels are age –, and sex – dependent. DeMaeyer et al., <sup>11</sup> reported that anaemia is said to be mild if the haemoglobin level is between 10 – 12g/dl, moderate if the level is between 7 – 10g/dl, and severe if the level is below 7g/dl in all ages and sexes in humans. This study aims at assessing the more vulnerable sex to haematotoxic effect associated with exposure of rats to composite PMS vapours.

## **MATERIALS AND METHODS**

### **EXPERIMENTAL ANIMALS**

All animal experiments were carried out in strict compliance with the guidelines of the Institutional Animals Ethics Committee on the care and use of laboratory animals. Thirty two male and female adults Wistar rats weighing 100 – 130g were obtained from the animal house of the College of Medical Sciences, University of Calabar, Calabar, Nigeria, and used for this study. The animals were divided according to sex into four groups (i.e. male test group, male control group, female test group, and female control group) of eight rats each, and were allowed to acclimatize in the experimental animal house for five days before the commencement of the experiment. The animals, housed in stainless steel cages, were fed with the normal rat pellets (Guinea feeds) obtained from Guinea Feeds depot, Calabar, Nigeria. All the rats in both test and control groups have free access to food and water throughout the experimental period.

### **EXPOSURE TO GASOLINE VAPOURS**

A nose-inhalation exposure method earlier described <sup>10,13,14</sup> was modified and used in this study. In this modification, the cages housing the animals in the test groups were placed in 2.835m<sup>3</sup> exposure chambers (2 cages to one chamber), each with two open 1000 cm<sup>3</sup> calibrated beakers containing 500cm<sup>3</sup> of liquid gasoline. The gasoline was allowed to evaporate freely within the exposure chambers at ambient humidity and temperature, and the whole animals in the cages were exposed to the vapours (17.8 2.6 cm<sup>3</sup> hr<sup>-1</sup> kg<sup>-1</sup> m<sup>-3</sup> day<sup>-1</sup>) generated from direct evaporation of the liquid gasoline. The exposure period of 6 hours (9.00 am to 3.00 pm) daily was adopted for 20 weeks. At the end of each day of exposure, the animals were transferred to gasoline vapour –free section of the experimental animal house.

During the exposure period, the initial and final volumes of

the liquid gasoline were respectively noted before and after daily exposure. The daily differences in volume were used to estimate the relative concentrations of the vapour used in this exposure method.

The liquid gasoline (PMS blend) was obtained from Mobil Refueling Station, Marian Road, Calabar, Nigeria.

### **COLLECTION AND ANALYSIS OF BLOOD**

At the end of the exposure period, blood samples were collected by cardiac puncture into heparinised sample bottles. The whole blood samples were used for the determination of the levels of haemoglobin, haematocrit, red blood cells and white blood cells counts. Haemoglobin and haematocrit levels were determined by the methods described by Alexander and Griffiths <sup>15,16</sup>. All absorbance readings for haemoglobin determinations were made using DREL 3000 HACH model spectrophotometer. The total red and white blood cells were counted by microscopic visual identification methods described by Dacie and Lewis <sup>17</sup>.

### **DETERMINATION OF WEIGHT INCREASE AND GROWTH RATE**

Total body weight of each rat was measured using a chemical balance, before and after the experimental period and recorded as initial and final body weight, (i.e., IBW and FBW, respectively). The mean body weight for each group was determined from the measured total body weights. Weight changes were expressed as percentage weight increase and percentage growth rate, where:

Percentage weight increase was calculated from the formula:

**Figure 1**

$$\frac{FBW - IBW}{IBW} \times 100$$

Percentage growth rate was calculated from the formula

**Figure 2**

$$\frac{FBW - IBW}{y} \times 100$$

y

y = Number of days exposed

**STATISTICAL ANALYSES**

Student's t-test was used to evaluate the significance of the differences between the mean values of the respective test groups and the control groups. A significant change was accepted at P<0.05.

**RESULTS AND DISCUSSION**

The results of this study on the effect of exposure to gasoline vapour on some haematological indices (Hb, PCV, RBC and WBC) and total body weight in female and male Wistar albino rats are shown in Tables 1 and 2, as well as fig. 1.

**Figure 3**

Table 1: Effect of gasoline vapours inhalation on some haematological indices in male and female rats

Group	Hb(g/dl)	PCV (%)	RBC (cells mm <sup>-3</sup> )	WBC(cells mm <sup>-3</sup> )
Mc	12.3 ± 0.3	48.0 ± 1.3	7.69 x 10 <sup>6</sup> ± 261.2	4.392 x 10 <sup>3</sup> ± 551.0
Mt	9.6 ± 0.6*	45.0 ± 0.6*	6.55 x 10 <sup>6</sup> ± 363.1*	5.059 x 10 <sup>3</sup> ± 741.0*
Fc	12.5 ± 0.8	45.7 ± 1.1	6.90 x 10 <sup>6</sup> ± 193.5	3.483 x 10 <sup>3</sup> ± 879.3
Ft	9.5 ± 0.7*	41.8 ± 0.9*	5.35 x 10 <sup>6</sup> ± 456.2*	4.858 x 10 <sup>3</sup> ± 611.4*

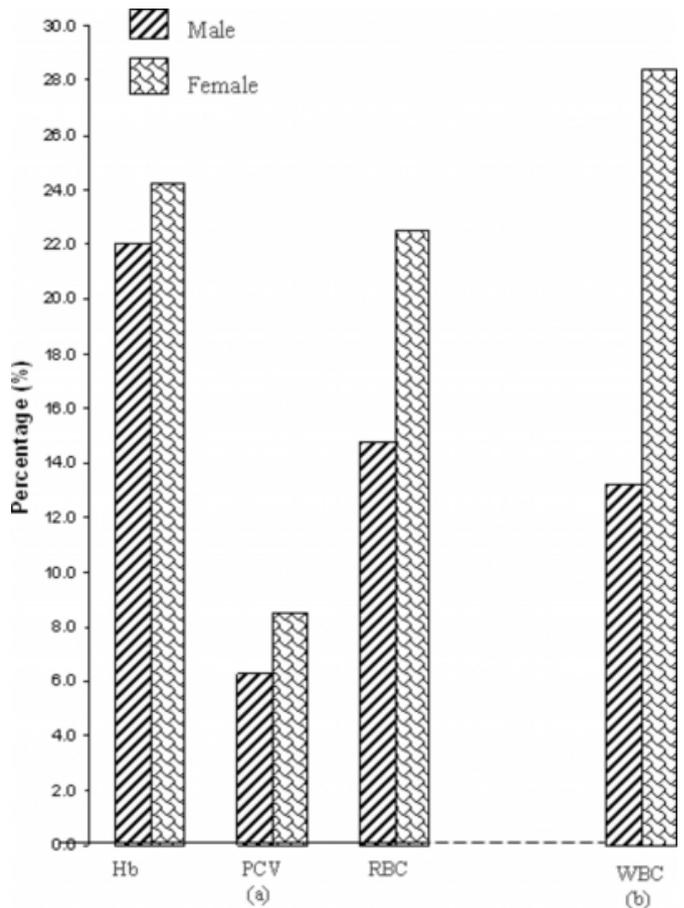
Values are presented as mean ± SEM, n = 8, \*\* P<0.05 compared with the respective controls. Mc = Male control, Mt= Male test, Fc = Female control, Ft = Female test.

From Table 1, the Hb, PCV levels and RBC counts in the male rats exposed to gasoline vapour, i.e., male test rats (9.6 ± 0.6g/dl, 45.0 ± 0.6% and 6.55 x 10<sup>6</sup> ± 363.1 cells mm<sup>-3</sup>, respectively) were significantly lower (P<0.05), while the WBC counts (5.059 x 10<sup>3</sup> ± 741.0 cells mm<sup>-3</sup>) was significantly higher (P<0.05), compared respectively with the Hb, PCV levels and RBC counts (12.3 ± 0.3g/dl, 48.0 ± 1.3% and 7.69 x 10<sup>6</sup> ± 261.2 cells mm<sup>-3</sup>, respectively) and WBC counts (4.392 x 10<sup>3</sup> ± 551.0 cells mm<sup>-3</sup>) obtained for the male control rats. Also, the Hb, PCV levels and RBC counts in the female rats exposed to gasoline vapour, i.e., female test rats (9.5 ± 0.7g/dl, 41.8 ± 0.9% and 5.35 x 10<sup>6</sup> ± 456.2 cells mm<sup>-3</sup>, respectively) decreased significantly (P<0.05), while the level of WBC (4.858 x 10<sup>3</sup> ± 611.4 cells mm<sup>-3</sup>) was increased significantly (P<0.05), compared with

the Hb, PCV, RBC levels and WBC counts in the female control rats (12.5 ± 0.8g/dl, 45.7 ± 1.1%, 6.90 x 10<sup>6</sup> ± 193.5 cells mm<sup>-3</sup> and 3.483 x 10<sup>3</sup> ± 879.3 cells mm<sup>-3</sup>, respectively).

**Figure 4**

Figure 1: Gender-differences in the effect of gasoline vapours on (a) percentage decrease in Hb, PCV and RBC, and (b) percentage increase in WBC, levels.



These results showed that the Hb, PCV levels and RBC counts decreased by 22.0±1.3, 6.3± 0.9 and 14.8± 1.5 percents, respectively in male rats, and by 24.2 ±2.1, 8.5± 1.2 and 22.5 ±1.8 percents, respectively in female rats; whereas WBC counts increased by 13.2±1.6 percents in male rats, and 28.3±2.3 percents in female rats, following exposure to gasoline vapour (figure 1). The decrease in the Hb, PCV levels and RBC counts, as well as the increase in WBC counts following exposure to gasoline vapour is observed to be sex-dependent, with the females being more vulnerable.

**Figure 5**

Table 2: Effect of gasoline vapours inhalation on the total body weights of male and female rats

Group	IBW(g)	FBW (g)	%WI	%GR
Mc	124.83 ± 7.67	243.83 ± 17.45	95.3 ± 3.8	85.0 ± 3.6
Mt	105.53 ± 14.56*	219.78 ± 19.49*	108.3 ± 5.3*	81.6 ± 4.9*
Fc	127.08 ± 5.62	187.08 ± 10.72	47.2 ± 2.4	42.9 ± 2.2
Ft	128.00 ± 11.99*	181.75 ± 15.53*	42.0 ± 3.1*	38.4 ± 2.8*

Values are presented as mean ± SEM, n=8, \*\*P<0.05 compared with the respective controls. Mc = Male control, Mt = Male test, Fc = Female control, Ft = Female test, IBW = Initial body weight, FBW = Final body weight, % WI = Percentage weight increase, % GR =Percentage growth rate.

Table 2 shows the results of the effect of exposure to gasoline vapour on the total body weights of male and female Wistar albino rats. From these results, the initial body weights of male control, female control, male test and female test rats (124.83 ± 7.67, 127.08 ± 5.62, 105.53 ± 14.56 and 128.00 ± 11.99g, respectively) were respectively compared with their final body weights (243.83 ± 17.45, 187.08 ± 10.72, 219.78 ± 19.49g and 181.75 ± 15.53g, respectively) to obtain percentage weight increase of 95.3 ± 3.8, 47.2 ± 2.4, 108.3 ± 5.3 and 42.0 ± 3.1 percents, respectively, and percentage growth rates of 85.0 ± 3.6, 42.9 ± 2.2, 81.6 ± 4.9 and 38.4 ± 2.8 percents respectively. These results indicated that exposure to gasoline vapour may results in a significant increase (p<0.05) in the percentage weight increase in male rats, decrease (p<0.05) in the percentage weight increase in female rats, and decrease (p<0.05) in the percentage growth rate in both male and female rats. The observed effect of exposure of rats to gasoline vapour on the percentage weight increase and growth rate suggests that the toxicity effect, i.e. weight loss and growth depression, associated with of the constituents of gasoline vapour may be sex-dependent, with females being more adversely affected.

In this study, changes in the HB, PCV Level, RBC, WBC counts and total body weights, following exposure of male and female rats to gasoline vapour, were used to assess whether the haematotoxic and weight loss effects earlier reported for PMS in rats<sup>10</sup>, are sex-dependent. It is clear that UG composition is highly variable between manufacturers and over time<sup>2</sup>. Hence, the health risk associated with exposure to gasoline vapour may also vary with blend and storage period. Various health hazards associated with exposure to the vapours from different blends of gasoline used in the United States has been extensively studied<sup>18,19,20</sup>. For instance, API 91 – 01 blend of UG vapour has been

reported to increase the incidence of liver tumours in a chronic bioassay in female mouse<sup>20</sup>. Also, inhalation of PS – 6 UG and MTBE blends has been reported to be hepatocarcinogenic in female mice, only at high dosage<sup>18,20,21</sup>. Moreso, the haematotoxic effect earlier reported in our study<sup>10</sup>, indicates that inhalation exposure to PMS blend of gasoline vapour may be a predisposing factor to anaemia.

Anaemia, one of the most widely spread diseases in the world, is known to has multifactorial causes<sup>5,8,21,22,23,24</sup>. It is generally known that iron, folate and vitamin B<sub>12</sub> deficiencies, malnutrition and infections are the leading causes of anaemia<sup>22,23</sup>. However, our previous study indicated that the roles of chemical agents (such as composite constituents of kerosene and gasoline fumes) may be equally important in the causation of anaemia<sup>10</sup>. Also, d'Azevedo et al.,<sup>5</sup> and Rothman et al.,<sup>7</sup> reported the haematotoxic effect of inhalation exposure to benzene and xylene in rats, and humans occupationally exposed.

The results of this study therefore showed that gasoline fumes contain such chemical agents which when inhaled in appreciable concentration can interact with specific tissues in the body and cause reduction in the Hb and PCV levels, RBC counts and total body weight; and increase in the WBC counts. These observation agree with the results reported for workers heavily exposed to benzene and rats exposed to benzene and xylene by inhalation<sup>5,6,7,8</sup>. According to these reports, benzene toxicity causes both bone marrow depression and leukaemogenesis, damaging multiple classes of haematopoietic cells and a variety of haematopoietic cells functions. Similar effect on weight increase has been reported for female mice<sup>23</sup>. Although the specific mechanism(s) through which gasoline vapours exhibits haematotoxic effect is (are) not clearly understood, it is believed that it may be as a result of bone marrow depression, with reduction in the rate of formation and development of erythrocytes committed stem cells as reported for benzene toxicity<sup>8</sup>, or increased destruction of the red blood cells, as reported for carbon disulphide toxicity<sup>9</sup>. The various constituents of the gasoline vapours might have been metabolized to various reactive species which can selectively interact with the red blood cells membrane proteins and damage the cells. Moreover, the increase in the WBC counts following exposure to gasoline vapours, observed in this study, may be one of the mechanisms devised to defend the body against the toxicity effects of the vapours constituents.

From the results of this study, it is interesting to observe that the haematotoxic effect, weight loss and growth depression associated with gasoline vapours are sex-dependent in rat, and that females are more vulnerable to the effects. The specific molecular mechanism(s) of this sex-dependent toxicity effects is (are) not clear. However, with reference to the result of our recent findings<sup>14</sup>, the interplay of the differences in the actions and functions of sex hormonal secretions may be assumed to be implicated. In conclusion, the female gender of rats is observed to be more susceptible to the haematotoxic effect, weight loss, and growth depression associated with exposure to gasoline vapours.

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