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# Haematologic and Oxidative Stress Effects of Occupational Exposure to Volatile Organic Compounds in *Mus musculus* at Printing Presses in Lagos, Nigeria

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## ABSTRACT

**Background:** Solvents and inks used in printing presses are major sources of occupational exposure to Volatile Organic Compounds (VOCs).

**Objective:** The biological effects of short-term occupational exposure to VOCs were investigated using albino mice, *Mus musculus*.

**Materials and Methods:** Structured questionnaires were administered to printing press workers to assess their knowledge of the potential health effects of VOCs. Hepatic biochemical and haematological indices were evaluated in *Mus musculus* exposed to VOC emissions from two printing presses in Lagos, Nigeria over a period of 45 days following standard methods. Total VOC (TVOC) levels were determined using a VOC Sampler.

**Results:** The questionnaire analyses revealed that printing press workers in Mushin experienced more health symptoms compared to those at Somolu. There were no significant differences ( $p > 0.05$ ) in the hepatic biochemical and haematological indices between mice at control and exposed locations. Average TVOC level was highest at the printing press in Mushin ( $466.33 \pm 130.78 \text{ mg/m}^3$ ) followed by Somolu ( $387.00 \pm 105.15 \text{ mg/m}^3$ ) and control location ( $3.50 \pm 0.71 \text{ mg/m}^3$ ).

**Conclusion:** The results indicate that short-term exposure to VOCs may not be harmful to human health. However, the questionnaire analyses demonstrated that long term exposure may be harmful to human health. Hence, it is recommended that a longer term study be conducted in addition to other biomarkers such as behavioural and physiological indices in order to provide a robust indication of the potential biological effects of these VOCs. The use of personal protection equipment (PPE) and short-term exposure through reduced-time shift regimes are recommended.

**Keywords:** Biological effects, *Mus musculus*, occupational exposure, printing press, volatile organic compounds.

## INTRODUCTION

Volatile Organic Compounds (VOCs) are organic substances with low boiling points and high vapour pressure that are widely used in industry. They are ubiquitous in the environment and represent a significant threat to the health of both humans and animals. A number of VOCs have been identified as important cancer risk factors in the urban environment (1). Occupational exposure to VOCs is especially high in certain professions such as printing which is considered a possible risk source (2, 3). They are components of printing consumables and products like paints, inks, varnishes, adhesives, grease removers and thinners (4). Printing press workers are mainly exposed to these substances during cleaning and plate-changing episodes with organic solvents containing toluene, xylene, methylethylketone, cyclohexane, dichloromethane or tetrachloroethylene. Solvent exposures which are up to ten times that of other tasks can result from these cleaning episodes because they are petrol-based (4, 5).

Though the exposure period during such cleaning activities may be short, the level of daily exposure depends on the number of such clean ups that are conducted.

Most conventional solvents used in the printing industry are VOCs and account for the majority of the 101,537 tons of VOCs emitted per year by the printing industry (6). Exposure levels vary among firms and will depend on the practices of each company. For example, in a Norway study on exposure to organic solvents in offset printing, occupational physicians measured solvent concentrations that ranged from 0.8 ppm to over 100 ppm for isopropyl alcohol, between 0.4 and 4.7 ppm for aliphatic hydrocarbons, from 0.0 to 2.7 ppm for toluene, and between null to 3.0 ppm for other solvents (7). In the case of isopropyl alcohol, neuro-psychiatric symptoms are present at concentrations between 50-100 ppm and an impairment of neuro-psychological performance occurs in exposures up to 100 ppm (8). Furthermore, indoor benzene concentrations of 10 to 50 ppm enhanced oxidative stress through increase in lipid



peroxidation and reduction in reduced glutathione levels in the liver of adult male albino Wistar rats over a period of 14 days (9). Also, significant decreases in erythrocyte count and platelets in the peripheral blood of mice exposed to different doses of VOCs mixture for 10 days were observed (10). According to Djogo *et al.* (5), inhalation of some VOCs may cause irritation of the respiratory tract. Also, environmental emissions of printing solvents may cause ground level ozone formation and some may contribute to stratospheric ozone depletion (5). Occupational health regulations including Occupational Safety and Health Administration (OSHA; 11) and National Institute of Occupational Safety and Health (NIOSH; 12) have been developed with respect to the levels of VOCs in the work environment as a result of documented health hazards associated with exposure to the compounds. Workers in the printing press are commonly exposed to potentially carcinogenic solvents that may cause leukaemia, small cell carcinoma of the lung and other haematopoietic disorders. Also, printing processes such as screen printing, ink jet, rotogravure and flexographic printing utilize solvents which have hepatotoxic risks (13). Exposure may also result in acute effects such as depression of the central nervous system, headache, nausea, dizziness, vomiting, dermatitis, irritation of the eyes, nose, throat and skin (14).

In Nigeria, the National Environmental Standards and Regulations Enforcement Agency (NESREA) have set limits for occupational exposure to specific VOCs such as toluene and xylene in a work environment (15). Though, there is no set limit for Total VOCs (TVOCs), the monitoring and enforcement of compliance to these regulations can only become stringent following monitoring studies of levels and potential biological effects associated with such exposures. The albino mouse (*Mus musculus*) is a model mammalian species utilized for toxicity studies and from which inferences can be made to predict effects of substances on humans. Thus, the objectives of this study were to determine the levels of TVOCs and assess the biochemical and haematological effects of exposure to TVOCs in two printing presses in Lagos, Nigeria using *Mus musculus*.

## MATERIALS AND METHODS

### Study Location

The study was conducted at three locations in Lagos State namely Somolu, Mushin and University of Lagos (UNILAG) Zoological garden. A printing press each was selected at Somolu and Mushin based on the nature and volume of printing activity while the control or reference location was the UNILAG Zoological garden (Figure 1).

### Questionnaire Design and Administration

A structured questionnaire was designed to obtain information such as biodata, nature of printing press and activities, environmental management and health conditions of the press and press workers. A total of 110 questionnaires were administered randomly to print workers in both locations (60 at Mushin and 50 at Somolu).

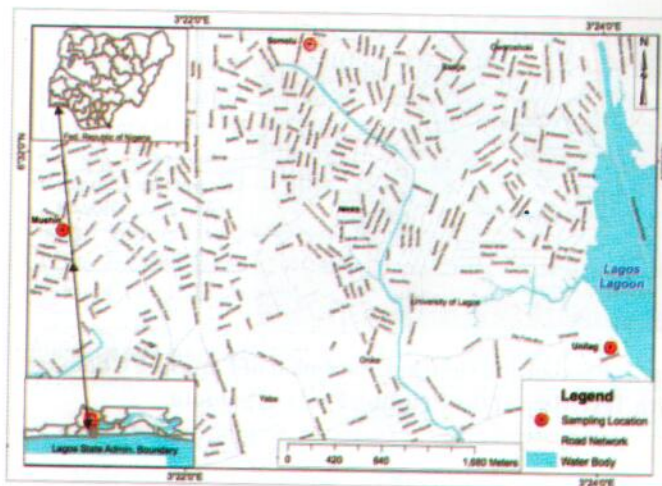


Fig. 1: Map of the Study Area showing Study Locations.

### Printing Presses

The printing press at Somolu was My Jet - Ink Jet Printer (Model EB6C310C046) and Versa Art Printer (Model RS - 640) while the printing press at Mushin was Challenger Printer (Model FY-3208H) and Ink Jet printer (Model 512).

### Test Animals Collection and Experimental Design

Albino mice, *Mus musculus* (Chordata, Mammalia, Rodentia, Muridae) (age: 5 weeks old; body weight: 12-16 g) were purchased from the Zoological garden of the University of Lagos, Nigeria. They were housed in plastic rectangular cages (dimensions: 30 × 43 × 20 cm<sup>3</sup>). The mice were maintained on a constant diet of 60 g of feed per day. Thirty-six mice of both sexes were randomly divided into three groups namely; A, B and C, each consisting of 12 mice each. The mice in groups B and C were exposed to emissions from printing presses at the two study locations (Mushin and Somolu) while mice in group A which served as the control were kept in an emission-free location at the Zoological garden.

The exposure chambers housing the test mice were kept in the printing room (Figures 2 and 3) where the printing presses were kept and printing activities occurred 6 days per week. In order to simulate occupational exposure to VOCs in the printing presses, the test animals were exposed to VOCs during printing activities via inhalation. An exposure period of 9 h (9 am to 5 pm) daily was adopted after which the mice were left in the printing room without printing activities till the next work day. The exposure duration was 45 days. This study followed the principles in the Declaration of Helsinki on the humane treatment of animals used in research (<http://www.wma.net/en/30publications/10policies/a18/>). Also, the care and use of the animals were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (16).

### Assessment of Weight Changes in Control and Exposed Mice

The average weight of mice at the control (University of Lagos Zoology Garden) and exposed locations (printing presses at Mushin and Somolu) were ascertained using a





Exposure Chamber with Test Mice at the Printing Press in Somolu.

**Fig. 2: Photograph showing Placement of Exposure Chamber with Test Mice at the Printing Press in Somolu.**



Exposure Chamber with Test Mice at the Printing Press in Mushin.

**Fig. 3: Photograph showing Placement of Exposure Chamber with Test Mice at the Printing Press in Mushin.**

Binatone weighing balance (Model: ks-7020) at days 0, 15, 30 and 45. The results were expressed in grams (g).

#### Evaluation of Biochemical Indices

At 45 days post-exposure, 12 mice (6 per sex) were randomly selected from each exposed group (B and C) and 4 mice (2 per sex) from the control group (A). The mice were sacrificed; their livers were excised, rinsed with distilled water, stored in specimen bottles and transported in ice-packed coolers to the Biochemistry Laboratory of the University of Lagos for analysis. At the laboratory, the livers were washed in pre-chilled 1.15% KCl solution, blotted and weighed. Afterwards, they were homogenized with 0.1 M phosphate buffer (pH 7.2). The homogenate was centrifuged at 2500 rpm for 15 min and the supernatant was decanted and stored at -20°C until analysis. Malondialdehyde (MDA) – an index of lipid peroxidation, reduced glutathione (GSH) – a biomolecule, and antioxidant enzymes - superoxide dismutase (SOD), catalase (CAT) and glutathione-s-transferase (GST) were determined as described by Sogbanmu and Otitoloju (17).

Briefly, the concentration of protein in each sample was determined using the Biuret method (18) and Bovine Serum Albumin (BSA) as standard. Serial dilutions of stock BSA solutions were made by using varying concentrations. Biuret reagent was added to each diluted protein standard solution (stock BSA) and the mixture was allowed to stand at room temperature for 30 min before reading. The absorbance of the solution was read at 540 nm and a graph of absorbance against BSA concentration (mg/ml) was then plotted. Suitable dilutions of the test sample were made with distilled water. This was done to reduce the level of protein in the post mitochondria fraction (PMF) to the sensitivity range of the Biuret method. 1 ml of diluted samples (i.e. 0.1 ml of sample with 0.9 ml of distilled water to make 1 in 10 dilution) was taken and the process for protein determination as described above was repeated. The absorbance was read at 540 nm against a blank containing 1 ml of distilled and 4 ml of Biuret reagent. The protein content of samples was extrapolated from the protein standard curve to get the actual amount of protein in the sample.

For the lipid peroxidation assay, the levels of homogenized MDA were estimated by thiobarbituric acid reaction assay (TBARS) using the method of Yagi (19) in which MDA is measured spectrophotometrically at absorbance levels of 535 nm to assay the extent of lipid peroxidation in the sample. The results were expressed in MDA units/mg protein.

The GSH content of the liver as non-protein sulphhydryls was determined according to the methods of Sedlak and Lindsay (20) and the absorbance was read at 412 nm. The results were expressed as units of GSH / mg protein.

Superoxide dismutase enzyme activity was evaluated by its inability to inhibit the auto-oxidation of epinephrine which was determined by an increase in absorbance at 450 nm according to the method of Sun and Zigma (21). The enzyme activity was expressed as SOD units/mg protein where one unit is defined as the amount of enzyme required to inhibit 50% epinephrine reduction per minute and per milligram of protein at 25°C and pH 7.8.

Catalase activity was determined according to the method of Aebi (22) by measuring the decrease in absorbance at 240 nm as a result of the decomposition of hydrogen peroxide ( $H_2O_2$ ) in an ultraviolet recording spectrophotometer. The results were expressed in CAT units/mg protein where one unit is the amount of enzyme that hydrolyses 1  $\mu$ M of  $H_2O_2$  per minute and per milligram of protein at 30°C and pH 8.0.

The GST enzyme activity measured the formation of a conjugate between 1 Mm GSH and 1 Mm 1-chloro-2,4-dinitrobenzene (CDNB) by monitoring at absorbance level of 340 nm according to the methods of Habig *et al.* (23). The results were expressed as GST units/mg protein.

#### Assessment of Haematological Indices

At 45 days post-exposure, 12 mice (6 per sex) were randomly selected from each exposed group (B and C) and 4 mice (2 per sex) from the control group (A). Five hundred microliters of blood was obtained through the eyes of each mouse using a heparinized haematocrit tube and transferred into bottles containing ethylenediaminetetraacetic acid (EDTA) as



anticoagulant (24). Haematological parameters such as white blood cells (WBC), platelets (PLT), red blood cells (RBC) and packed cell volume (PCV) were estimated using an electronic coulter counter (ADVIA-TM 60) according to Otitolaju *et al.* (25). Also, derived haematological indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae according to Jain and Schalm (26), as cited by Ololade and Oginni (27):  
 $MCV \text{ in femtolitres (fL), i.e. } (\times 10^{-15} \text{ L}) = PCV / RBC \times 1000$   
 $MCH \text{ in picograms (pg), i.e. } (\times 10^{-12} \text{ g}) = Hb / RBC \times 10$   
 $MCHC = Hb \text{ in } 100 \text{ mg blood} / PCV \times 100$

#### Measurement of Total Volatile Organic Compounds Level at Study Locations

The TVOCs level in the air at the study locations was determined with the aid of a VOC sampler, AEROQUAL (Model: series 500). The sampling points and time of sampling at each study locations are shown in Table 1. The results were expressed in milligram per cubic metre ( $\text{mg}/\text{m}^3$ ).

**Table 1: Sampling Points and Time for Total Volatile Organic Compounds Measurements at Study Locations**

Location	Sampling Points	Time
Somolu	Production room (without extractor on)	10:30 am
	Production room (with extractor on)	
	Reception (about 10 m distance)	
	Corridor/balcony of office	
	Corridor/stairway entrance (close to extractor outlet)	
Mushin	Production room (without extractor on)	1:20 pm
	Production room (close to extractor)	
	Production room (farthest from extractor)	
	Outside (front corridor)	
	Outside (behind extractor)	
Control	Laboratory room	3:20 pm
	Garden area	

#### Statistical Analysis

The results were expressed as mean  $\pm$  standard deviation or standard error. One-way analysis of variance (ANOVA) followed by Duncan post-hoc test was utilized to check for significant difference between treatment means and control at  $p < 0.05$ . All statistical tests were conducted using SPSS 20.0 version.

## RESULTS

#### Questionnaire Administration

The results of the questionnaires administration showed that majority of the respondents at the Mushin and Somolu locations had worked less than 5 years in the printing press, worked 6 days a week, had 5-10 workers, ran print jobs daily

and had extractors in their printing presses. The most common health symptoms experienced by the printing press workers at both study locations were headaches, dizziness and tiredness. However, at the Mushin location, workers also experienced eye and nose irritation (Table 2).

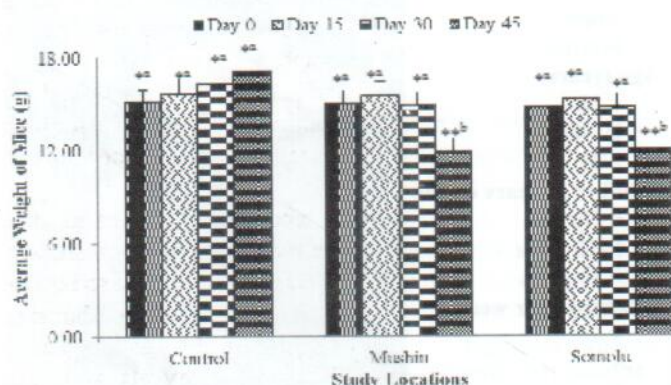
**Table 2: Responses of Printing Press Workers at Mushin and Somolu to Questionnaires**

Biodata and work-related Questions	% Responses					
	Mushin	Somolu				
Gender						
Male	100	100				
Female	0	0				
Marital status						
Single	72	92				
Married	28	8				
Age (years)						
< 20	42	40				
20–40	33	50				
> 40	25	10				
Number of years on the job						
< 5 years	38	64				
5–10 years	35	24				
> 10 years	27	12				
Workdays per week						
< 5	33	20				
6	49	70				
7	18	10				
Number of workers						
< 5	33	40				
5 to 10	50	48				
> 10	17	12				
Frequency of jobs						
Daily	83	84				
Weekly	17	16				
Ventilation system						
Extractor	60	76				
Air conditioner	25	20				
Window	15	4				
Symptoms	Yes	No	Indifferent			
	Mushin	Somolu	Mushin	Somolu	Mushin	Somolu
Headaches	88	84	12	14	0	2
Dizziness	70	76	22	16	8	8
Eye irritation	50	40	42	36	8	24
Nose irritation	87	20	13	70	0	10
Tiredness	55	72	38	16	7	12
Fast heart rate	3	8	92	84	5	8
Painful tingling in body	0	4	90	88	0	8
Irritation without reason	8	6	82	94	10	0
Concentration problems	17	0	83	96	0	4
Short memory	13	8	82	90	5	2
Perspiration	0	0	97	96	3	4
Coordination problems	3	0	93	96	3	4
Tightness of chest	0	4	97	90	3	6



### Weight Changes in Mice at the Control and VOCs Exposed Locations

The evaluation of weight changes in mice at the control and VOCs exposed location given the same amount of food showed that there was a gradual increase in the average weight of mice at the control site from  $15.10 \pm 0.80$  g at day 0 to  $17.10 \pm 0.80$  g at day 45. Mice at the printing press in Mushin experienced a reduction in average weight from  $15.00 \pm 0.80$  g at day 0 to  $11.90 \pm 0.80$  g by day 45. There was a decrease in average weight of mice at the printing press in Somolu from  $14.80 \pm 0.80$  g at day 0 to  $12.00 \pm 0.50$  g by day 45. There was a significant ( $p < 0.05$ ) decrease in the average weight of mice at Mushin and Somolu printing presses by day 45 compared to control. There was a significant difference ( $p < 0.05$ ) between the average weights of mice at the exposed locations compared to those at the control location (Figure 4).



**Fig. 4: Average Weight Changes of Control and Exposed Albino Mice Over a Period of 40 Days.** Dissimilar Alphabet Letters in Superscript shows Significant Difference ( $P < 0.05$ ) in Weight Changes of Mice Between the Days for each Location. \*\*Shows Significant Difference ( $P < 0.05$ ) In Weight Changes Of Mice Across The Study Locations.

### Biochemical Studies

The hepatic biochemical indices analysis in mice collected from the VOCs exposed and control locations at day 45 revealed that malondialdehyde levels ranged from  $0.17 \pm 0.01$  U/mg protein in the control mice to  $0.24 \pm 0.04$  U/mg protein in mice at Mushin. Reduced glutathione levels ranged from  $0.50 \pm 0.02$  U/mg protein in mice at Mushin to  $0.80 \pm 0.06$  U/mg protein in control mice. Glutathione-s-transferase concentrations ranged from  $0.94 \pm 0.02$  U/mg protein in mice at Somolu to  $1.00 \pm 0.03$  U/mg protein in control mice. Superoxide dismutase levels ranged from  $3.15 \pm 0.14$  U/mg protein in mice at Somolu to  $3.35 \pm 0.55$  U/mg protein in control mice. Catalase levels ranged from  $15.74 \pm 0.93$  U/mg protein in mice at Mushin to  $18.14 \pm 0.95$  U/mg protein in mice at Somolu. Although there were no significant differences ( $p > 0.05$ ) in the oxidative stress marker (malondialdehyde) between the exposed and the control, it was observed that the levels were slightly higher in the exposed mice. Furthermore, there was a general slight reduction in antioxidants level in the exposed mice compared to the control, though not statistically significant (Figure 5).



**Fig. 5: Hepatic Biochemical Indices in Control and VOCs Exposed Mice in Printing Presses at Day 45.** MDA: Malondialdehyde (an Index of Lipid Peroxidation), GSH: Reduced Glutathione, GST: Glutathione-S-Transferase, SOD: Superoxide Dismutase, CAT: Catalase. Antioxidant Enzyme Levels, GSH and MDA are Expressed in U/Mg Protein, where Unit (U) =  $\mu\text{mol/ml/min}$ .

### Haematological Studies

The evaluation of haematological indices in the test mice after 45 days exposure showed that the concentrations of WBCs (Mushin –  $8.42 \pm 1.27 \times 10^9/\text{L}$ ; Somolu –  $10.31 \pm 0.89 \times 10^9/\text{L}$ ), RBCs (Mushin –  $8.37 \pm 0.56 \times 10^{12}/\text{L}$ ; Somolu –  $7.26 \pm 0.73 \times 10^{12}/\text{L}$ ) and MCH (Mushin –  $13.28 \pm 0.18$  pg; Somolu –  $13.43 \pm 0.26$  pg) were lower in mice at the exposed locations compared to control (WBC –  $11.20 \pm 1.49 \times 10^9/\text{L}$ ; RBC –  $8.65 \pm 0.42 \times 10^{12}/\text{L}$ ; MCH –  $14.56 \pm 1.27$  pg) (Figure 6).

However, the levels of platelets (Mushin –  $139.55 \pm 34.81 \times 10^9/\text{L}$ ; Somolu –  $81.37 \pm 12.63 \times 10^9/\text{L}$ ), haemoglobin (Mushin –  $12.35 \pm 0.38$  g/dl; Somolu –  $11.93 \pm 1.15$  g/dl) and MCHC (Mushin –  $29.90 \pm 0.24$  g/dl; Somolu –  $30.35 \pm 0.55$  g/dl) were higher in mice at the exposed locations compared to control (Platelets –  $72.10 \pm 6.23 \times 10^9/\text{L}$ ; Haemoglobin –  $12.33 \pm 0.97$  g/dl; MCHC –  $25.88 \pm 3.35$  g/dl) (Figure 6).

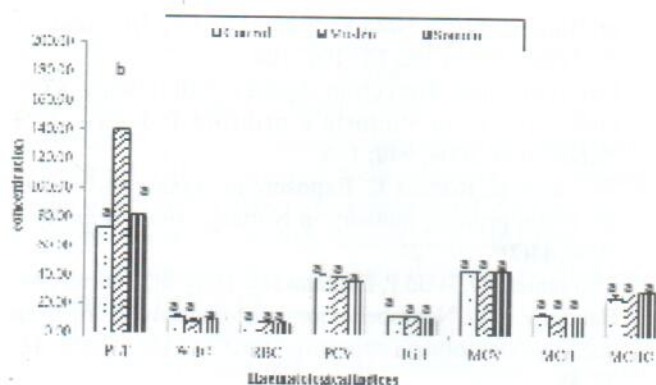
Furthermore, the PCV value was highest in mice exposed at the printing press in Mushin ( $39.22 \pm 1.55$  %) followed by control ( $38.66 \pm 2.51$  %) and Somolu ( $35.80 \pm 3.09$  %). The MCV value was highest in test mice at Mushin ( $44.62 \pm 0.76$  fl) followed by control and Mushin ( $44.50 \pm 0.87$  fl and  $44.50 \pm 1.11$  fl respectively) (Figure 6).

There was no statistical difference ( $p > 0.05$ ) between the haematological indices in the exposed and control mice except for PLT count which was significantly higher ( $p < 0.05$ ) in the blood of test mice at Mushin compared to control and Somolu (Figure 6).

### Assessment of TVOCs Level at Printing Presses

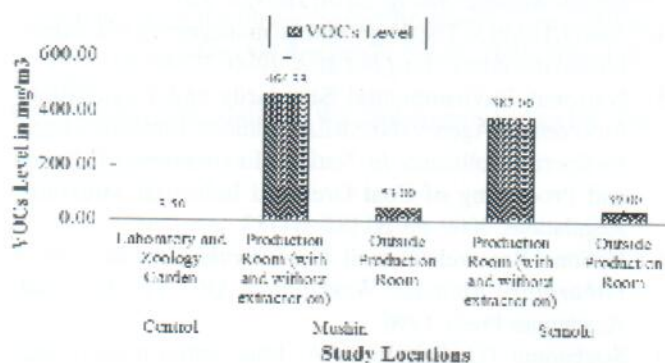
The assessment of the level of TVOCs at the two printing presses revealed that TVOCs level was highest at Mushin (range:  $24 - 612$   $\text{mg/m}^3$ ; mean:  $466.33 \pm 130.78$   $\text{mg/m}^3$  (in the production room) and  $53.00 \pm 49.37$   $\text{mg/m}^3$  (outside the production room)) followed by Somolu (range:  $30 - 463$   $\text{mg/m}^3$ ; mean:  $387.00 \pm 105.15$   $\text{mg/m}^3$  (in the production room) and  $39.00 \pm 12.73$   $\text{mg/m}^3$  (outside the production room)). The highest levels





**Fig. 6: Haematological Indices in Control and VOCs Exposed Mice in Printing Presses at Day 45.** N=6. Results are expressed as mean  $\pm$  standard error. Dissimilar superscript letters represent significant difference ( $p < 0.05$ ) between study locations. PLT – Platelets ( $\times 10^{10}/L$ ), WBC – White Blood Cells ( $\times 10^7/L$ ), RBC – Red Blood Cells ( $\times 10^{12}/L$ ), PCV – Packed Cell Volume (%), HGB – Haemoglobin ( $\times 10^7 \mu l$ ), MCV – Mean Corpuscular Volume (fl), MCH – Mean Corpuscular Haemoglobin (pg), MCHC – Mean Corpuscular Haemoglobin Concentration (g/dl).

of TVOCs measured in this study were observed in the production rooms of the two printing presses assessed. The TVOCs level was lowest at the control site (range: 3 - 4  $mg/m^3$ ; mean:  $3.50 \pm 0.71 mg/m^3$ ) (Figure 7).



**Fig. 7: Volatile Organic Compounds Level at Study Locations.**

## DISCUSSION

The biological effects associated with occupational exposure to VOCs have necessitated their assessment in workplaces in order to ascertain the health status of occupationally exposed persons and formulate policies or recommendations for their safeguard. In this study, the information gleaned from respondents who were mainly printing press workers revealed that there were signs of ill-health common to workers from the 2 printing presses particularly headaches, dizziness, eye and nose irritations. These responses agree with the findings of Chung-Yen *et al.* (28) who reported associations between high TVOC levels in offices and upper respiratory symptoms (stuffy nose, sneezing and dry throat), eye dryness and irritation, lower respiratory symptom (difficulty

in breathing), skin dryness, tiredness and dizziness. According to Molhave (29), a level of VOCs higher than  $3.0 mg/m^3$  in a non-industrial indoor environment may cause discomfort and ill-health. Long term exposures to low concentrations of VOCs in ambient air at or higher than set limits may lead to liver or kidney effects (5). Thus, though the higher percentage of printing press workers in this study have worked less than 5 years, additional years on the job with exposure to the increasing VOC concentrations may lead to major ill-health outcomes in the workers (30).

A significant decrease in the average weight of exposed mice at the two printing presses, especially by day 45, was observed in this study. This is consistent with the report of Abubakar *et al.* (31) who observed a significant reduction in weight gain in Sprague-Dawley rats exposed to petrol vapours. Similarly, weight loss in male rats exposed to gasoline vapours have been reported by Uboh *et al.* (32). A probable reason for the observed weight loss in the exposed mice could be an interference with the growth signalling pathways which can lead to growth retardation or weight loss (33). Also, a loss or decrease in appetite due to interaction of the VOCs with cellular and molecular mechanisms that stimulate the appetite for food could result in weight loss as observed in the exposed mice in this study.

The evaluation of hepatic biochemical indices in mice exposed to VOCs in printing presses via inhalation showed that there was no significant indication of oxidative stress due to VOCs exposure. Similar results were observed by Abubakar *et al.* (31) who reported a lack of significant changes in oxidative markers in the erythrocytes of Sprague-Dawley rats exposed to petrol fumes. On the contrary, statistically significant levels of reactive oxygen species (ROS) and MDA were observed in the blood of workers occupationally exposed to benzene in India (34). The observed slightly high levels of MDA and low levels of antioxidants suggest that a longer term exposure might result in a statistically significant reduction in antioxidants level and high levels of lipid peroxidation in the VOCs-exposed mice.

Haematological indices can provide indication of haematopoietic responses to VOCs exposure. The changes observed in the haematological indices such as the lower levels of WBC, MCH and RBCs and higher levels of PLTs in the exposed mice compared to control agrees with the findings of Hyeon-Yeong *et al.* (35) with similar observations on exposure of male Fischer 344 rats to sub-chronic levels of 1,3-dichloro-2-propanol (1,3-DCP) gas/fumes. Similarly, significant increase in haemoglobin and decrease in WBC has been observed in workers occupationally exposed to VOCs (benzene and carbon monoxide) with longer exposure periods (30). The causes of elevated PLT count or thrombocytosis as observed in this study may be attributed to inflammation, anaemia, iron deficiency amongst others (36). These symptoms could lead to irritation and bleeding from the nose as observed in the responses of printing press workers in this study. The largely insignificant differences between the biological effects observed in test and control mice may be attributed to the duration of the study, nature and timing of printing activities which would result in exposure to particularly high levels of VOCs and design of the experimental chamber.



A TVOC level  $< 200 \mu\text{g}/\text{m}^3$  has no effect on health or comfort while levels between 200 to  $3000 \mu\text{g}/\text{m}^3$  cause irritation and possible discomfort (37). The average TVOCs levels measured at the two printing presses were above  $200 \mu\text{g}/\text{m}^3$ , particularly in their production room, which prove that adverse health outcomes such as irritation and discomfort are bound to occur. Although, similar studies measuring TVOC levels in printing presses have reported concentrations ranging from 1897–3742  $\mu\text{g}/\text{m}^3$ , the concentrations were observed for a period of 8 h and included episodes to particularly high exposures to printing VOCs (38).

## CONCLUSION

The human health effects of occupational exposure to VOCs in printing press have been presented in this study. The responses from printing press workers, especially pertaining to the health symptoms they experience, may be slightly related to the results of the biochemical, haematological and TVOCs levels investigated. These symptoms, such as fatigue, dizziness, headaches and irritation, also referred to as 'sick building syndrome' (SBS), are experienced while workers are within a building or office or in this case, printing press. There is a need for more frequent studies or biomonitoring, inclusion of more printing presses conducting various printing operations and longer term studies. This will aid the accumulation of baseline data that will probably lead to the setting of guidelines or limits for TVOCs emissions in the printing industry in Nigeria. Furthermore, it is recommended that other multiple biomarkers be assessed in this category of occupationally exposed persons in order to provide a more holistic data and information on the health risks posed to them.

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## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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