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## Original Research

### Influence of volatile organic solvents' inhalation on activity quotient and biochemical indices of *Mus musculus*

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#### Abstract

The influence of volatile organic solvents (nitrocellulose thinner, gasoline and xylene) on the activity quotient and liver biochemical indices of laboratory mice, *Mus musculus* over a period of 60 days were evaluated. The study entailed the use of a modified nose inhalation exposure method in which the exposure chambers were saturated with the test chemicals for 8 hours daily following which they were transferred to the vapour- free section of the chamber. The estimation of activity quotient by day 60 indicated that mice exposed to xylene were most restless followed by nitrocellulose thinner and gasoline (least restless) with activity quotient values of 0.69, 0.58 and 0.21 respectively. The results of the biochemical indices showed that there was a significant decrease ( $p < 0.05$ ) in the levels of antioxidant enzymes such as reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in exposed mice from day 21 to 60 compared to control. Furthermore, the level of lipid peroxidation indicated by malondialdehyde (MDA) was found to be significantly higher ( $p < 0.05$ ) in exposed mice compared to control. The significance of these findings is that workers (laboratory technicians, painters and gasoline station attendants) who are occupationally exposed to these volatile organic solvents for elongated periods may be at risk of lung related disorders.

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## INTRODUCTION

Volatile Organic Solvents also known as Volatile Organic Compounds (VOCs) are large groups of carbon-based chemicals that easily evaporate at room temperature. Examples include; aromatic compounds (toluene, xylene), ketones (acetone, methylethylketone), alcohols (methanol, ethanol, propanol), acetates (ethylacetate) and chlorinated compounds (methylene chloride, perchloroethylene), etc. They are characterized by a high vapour pressure and easily form vapours at normal temperature and pressure.

VOCs are released into the atmosphere from anthropogenic and biogenic sources. Anthropogenic sources are dominated by the combustion of fossil fuels. Biogenic sources include releases from plant foliage, although the microbial decomposition of organic substances, fresh and marine waters, soils, sediments and geological resources of hydrocarbons

also constitute additional natural sources of this kind of compounds [1]. The atmospheric oxidation of VOCs can produce secondary pollutants such as ground level ozone or peroxy acetyl nitrate [2]. They also produce a great variety of oxidized products such as carbonyl compounds and in fact contribute to the formation of secondary organic aerosol [3-4].

Common symptoms of short-term (acute) exposure to high levels of volatile organic compounds include; eye, nose and throat irritation, headaches, nausea, vomiting, dizziness, etc. Symptoms of Long-term (chronic) exposure to high levels of VOCs include; increased risk of cancer, damage to the liver, kidney and central nervous system. VOCs may be carcinogenic and have been linked to cancer of the lungs, liver and pancreas in laboratory animals. Ethylbenzene also contributes to cell injury at low doses [5] and has been reported in workers after occupational exposure to certain concentrations of Ethylbenzene.

The accurate assessment of the risk to public health posed by VOCs require both the toxicity profile of these compounds on a population-wide basis and the discovery of predictive markers associated with various VOCs. The objectives of this study were therefore to evaluate the influence of VOCs inhalation on the activity quotient (measured in terms of food consumption pattern and body movement per minute) and liver biochemical parameters of laboratory mice (*Mus musculus*) which can serve as early warning signals or biomarkers of exposure to these group of pollutants.

## MATERIALS AND METHODS

**Test animals:** the present study which was conducted in 2010 involved the use of laboratory mice, *Mus musculus* (Chordata, Mammalia, Rodentia, Muridae) (5 weeks old; body weight: 12 – 16g). They were purchased from the Nigerian Institute of Medical Research (NIMR) and kept in plastic rectangular cages (30 x 43 x 20cm<sup>3</sup>) for 14 days to acclimatize to laboratory conditions (Temperature: 29<sup>0</sup>C ± 2<sup>0</sup>C; Relative Humidity: 70% ± 4%) before commencement of the experiments. They were fed with mice feed in pellets purchased from NIMR.

**Test compounds:** *Xylene* - Puriss. p.a. ≥ 99.0 % (GC) was obtained from Sigma Aldrich®, *Gasoline* was obtained from a petroleum products filling station, *Nitrocellulose Thinner* – a commercially available product was obtained from a vendor.

**Experimental design:** forty mice of both sexes were randomly divided into four groups namely; A, B, C and D, each consisting of ten mice. The mice in groups B, C, and D were exposed to gasoline, nitrocellulose thinner and xylene vapours respectively in exposure chambers (a special cage constructed to suit the experimental design) while those in group A (control group) were kept in the vapour-free section of the chamber. A modified nose inhalation exposure method was adopted. In this exposure method, the cages housing the test animals were kept in an exposure chamber saturated with the three test chemicals for 8 hours after which they were transferred to the vapour-free section of the chamber. Saturation of the exposure chamber was done by allowing the three chemicals in tin containers highly perforated at the upper end, to evaporate and fill the chambers at ambient temperature and humidity. The test animals were wholly exposed to the vapours evaporating from the container during the exposure period. An exposure period of 8 hours (9am to 4pm) daily was adopted for 60 days.

### Activity quotient studies

**Feed consumption pattern:** 110g of feed were fed to

the mice every two days. Feed consumption pattern was measured and recorded through out the experimental period. The average was calculated after 21 days, 45 days and 60 days.

**Body movement per minute:** was measured for control and exposed mice.

Activity quotient was then determined as follows:

$$\text{Activity Quotient (AQ)} = \frac{\text{Body Movement Per Minute}}{\text{Quantity of Feed Consumed}}$$

**Preparation of tissue homogenates:** two mice were randomly selected from each group and sacrificed after being paralysed. Their livers were excised and stored in plain bottles after draining in Potassium chloride. Specimens were stored in ice packs prior to transportation to the Biochemistry laboratory for analysis.

### Biochemical indices assay

**Reduced glutathione (GSH) assay:** The GSH content of liver tissue as non-protein sulphhydryls was estimated according to the method described by [6]. The absorbance was read at 412nm.

**Superoxide dismutase (SOD) assay:** SOD enzyme activity was determined according to the method by [7]. The SOD enzyme assay determined the difference between superoxide anion decomposition and production i.e. its ability to inhibit the autooxidation of epinephrine. Enzyme activity was monitored at absorbance level of 450 nm. Concentrations are expressed as SOD Unit/mg protein or U/mg.

**Catalase (CAT) assay:** Serum catalase activity was determined according to the method of Beers and Sizer as described by [8]. The decrease in absorbance at 240nm due to the decomposition of Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was measured in a UV recording spectrophotometer. The results were expressed in CAT units/mg protein or U/mg.

**Lipid peroxidation assay:** the levels of homogenized tissue malondialdehyde (MDA), as an index of lipid peroxidation were determined by thiobarbituric acid reaction (TBARS Assay) using the method of [9].

**Statistical analysis:** to test the null hypothesis that there was no difference between

means for the various treatments and control, results were appropriately subjected to

one-way analysis of variance (ANOVA) using SPSS® Version 16. Further analysis of

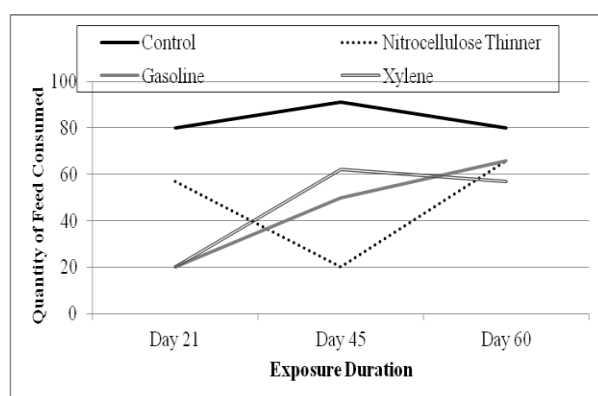
associations by Duncan test was carried out where there was a significant difference at

5% ( $P < 0.05$ ) level of significance.

## RESULTS

### Activity quotient studies

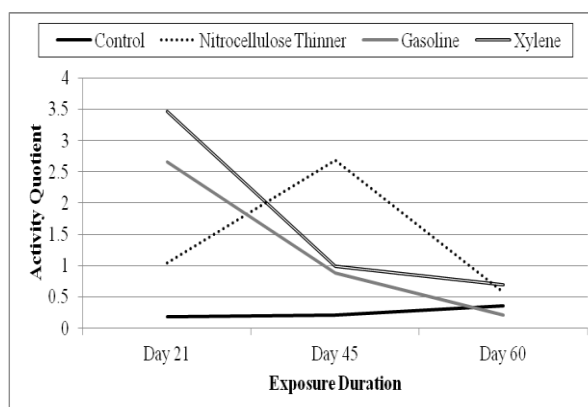
**Feed consumption pattern:** the results indicated that the exposed mice fed less than the control at day 21. Feeding improved after the 21<sup>st</sup> day of exposure through to the 60<sup>th</sup> day in all the exposed mice except for mice exposed to nitrocellulose thinner which showed decreased feed consumption at day 45 and then improved through to day 60 (Figure 1).



**Figure 1.** Feed Consumption Pattern in Control and Exposed Mice over a period of 60 days

**Body movement per minute:** the results revealed that body movement per minute in mice exposed to nitrocellulose thinner decreased steadily from 60.00 at day 21 to 53.75 at day 45 and 38.50 by day 60. Mice exposed to xylene also had decreased body movement from 69.25 at day 21 to 61.75 at day 45 and 39.50 at day 60. The lowest decline in body movement was observed in mice exposed to gasoline as it reduced from 53.25 at day 21 to 44.00 at day 45 and 14.00 at day 60 (Table 1).

Activity quotient (ratio of body movement to quantity of feed consumed) in control mice increased monophasically throughout the study period. On the other hand, activity quotient in mice exposed to nitrocellulose thinner and xylene decreased monophasically from days 21 through 60, while, activity quotient of mice exposed to gasoline showed biphasic effects, i. e. increase in activity from day 21 to 45 followed by a decrease from day 45 to 60 (Figure 2).



**Figure 2.** Activity Quotient of Control and Exposed Mice over a period of 60 days

### Biochemical indices assay

**GSH:** the results showed that GSH activity was significantly ( $p < 0.05$ ) higher in control mice than levels in exposed mice. Generally, GSH values reduced the most in mice exposed to xylene (8.64, 5.88, 3.77 U/mg pro) followed by nitrocellulose thinner (16.48, 17.18, 13.06 U/mg pro) and then gasoline (22.51, 11.66, 9.60 U/mg pro) on days 21, 45 and 60 respectively (Table 2).

**SOD:** the results revealed that SOD activity was significantly ( $p < 0.05$ ) higher in control mice (299.69 U/mg pro) compared to levels in exposed mice. Generally, SOD values decreased the most in mice exposed to nitrocellulose thinner (123.92, 116.33, 68.28 U/mg pro) followed by xylene (141.61, 121.39, 93.57 U/mg pro) and then gasoline (269.34, 194.74, 165.65 U/mg pro) at days 21, 45 and 60 respectively (Table 3).

**CAT:** the results showed that CAT activity was significantly ( $p < 0.05$ ) higher in control mice (2004.63 U/mg pro) compared to levels in exposed animals. Generally, CAT values declined the most in mice exposed to nitrocellulose thinner (828.92, 778.17, 456.75 U/mg pro) followed by xylene (947.33, 812.0, 625.90 U/mg pro) and then gasoline (1801.63, 1302.59, 1108.05 U/mg pro) at days 21, 45 and 60 respectively (Table 4).

**Lipid peroxidation:** the results showed that the level of MDA in the liver of the exposed mice decreased significantly ( $p < 0.05$ ) in control mice (0.40 U/mg pro). Values gradually increased in mice exposed to nitrocellulose thinner (1.45, 1.84, 2.50 U/mg pro) and gasoline (1.43, 2.30, 4.21 U/mg pro) but the reverse was the case in mice exposed to xylene (6.33, 4.12, 2.17 U/mg pro) following exposure over 60 days (Table 5).

**Table 1.** Body Movement Per Minute (Mean  $\pm$  SD) of Experimental Mice over a period of 60 days

Days	Control	Thinner	Gasoline	Xylene
Day 21	<sup>a</sup> 14.75 $\pm$ 4.35	<sup>de</sup> 60.00 $\pm$ 15.01	<sup>cde</sup> 53.25 $\pm$ 3.30	<sup>e</sup> 69.25 $\pm$ 15.67
Day 45	<sup>a</sup> 18.75 $\pm$ 5.32	<sup>cde</sup> 53.75 $\pm$ 18.89	<sup>bcd</sup> 44.00 $\pm$ 3.37	<sup>de</sup> 61.75 $\pm$ 24.43
Day 60	<sup>ab</sup> 29.00 $\pm$ 13.64	<sup>bc</sup> 38.50 $\pm$ 8.66	<sup>a</sup> 14.00 $\pm$ 5.77	<sup>bc</sup> 39.50 $\pm$ 13.53

Values with dissimilar letters (a, b, c, d, e) are significantly different ( $p < 0.05$ ) from each other

**Table 2.** GSH values (U/mg pro) in experimental mice over a period of 60 days

VOCs	Control	Day 21	Day 45	Day 60
Nitrocellulose Thinner	<sup>g</sup> 53.31 $\pm$ 3.86	<sup>e</sup> 16.48 $\pm$ 0.71	<sup>e</sup> 17.18 $\pm$ 0.79	<sup>d</sup> 13.06 $\pm$ 0.63
Gasoline	<sup>g</sup> 53.31 $\pm$ 3.86	<sup>f</sup> 22.51 $\pm$ 0.38	<sup>cd</sup> 11.66 $\pm$ 0.48	<sup>bc</sup> 9.60 $\pm$ 0.31
Xylene	<sup>g</sup> 53.31 $\pm$ 3.86	<sup>b</sup> 8.64 $\pm$ 0.09	<sup>a</sup> 5.88 $\pm$ 0.40	<sup>a</sup> 3.77 $\pm$ 0.60

Values with dissimilar letters (a, b, c, d, e, f, g) are significantly different ( $p < 0.05$ ) from each other

**Table 3.** SOD values (U/mg pro) in experimental mice over a period of 60 days

VOCs	Control	Day 21	Day 45	Day 60
Nitrocellulose Thinner	<sup>h</sup> 299.69 $\pm$ 11.38	<sup>c</sup> 123.92 $\pm$ 9.54	<sup>c</sup> 116.33 $\pm$ 5.80	<sup>a</sup> 68.28 $\pm$ 3.80
Gasoline	<sup>h</sup> 299.69 $\pm$ 11.38	<sup>g</sup> 269.34 $\pm$ 3.79	<sup>f</sup> 194.74 $\pm$ 5.80	<sup>e</sup> 165.65 $\pm$ 3.80
Xylene	<sup>h</sup> 299.69 $\pm$ 11.38	<sup>d</sup> 141.61 $\pm$ 4.38	<sup>c</sup> 121.39 $\pm$ 3.80	<sup>b</sup> 93.57 $\pm$ 2.19

Values with dissimilar letters (a, b, c, d, e, f, g, h) are significantly different ( $p < 0.05$ ) from each other

**Table 4.** CAT values (U/mg pro) in experimental mice over a period of 60 days

VOCs	Control	Day 21	Day 45	Day 60
Nitrocellulose Thinner	<sup>h</sup> 2004.63 $\pm$ 76.13	<sup>c</sup> 828.92 $\pm$ 63.86	<sup>c</sup> 778.17 $\pm$ 38.76	<sup>a</sup> 456.75 $\pm$ 25.38
Gasoline	<sup>h</sup> 2004.63 $\pm$ 76.13	<sup>g</sup> 1801.63 $\pm$ 25.38	<sup>f</sup> 1302.59 $\pm$ 38.76	<sup>e</sup> 1108.05 $\pm$ 29.30
Xylene	<sup>h</sup> 2004.63 $\pm$ 76.13	<sup>d</sup> 947.33 $\pm$ 29.30	<sup>c</sup> 812.00 $\pm$ 25.38	<sup>b</sup> 625.90 $\pm$ 14.65

Values with dissimilar letters (a, b, c, d, e, f, g, h) are significantly different ( $p < 0.05$ ) from each other

**Table 5.** MDA values (U/mg pro) in experimental mice over a period of 60 days

VOCs	Control	Day 21	Day 45	Day 60
Nitrocellulose Thinner	<sup>a</sup> 0.40 $\pm$ 0.04	<sup>b</sup> 1.45 $\pm$ 0.03	<sup>c</sup> 1.84 $\pm$ 0.03	<sup>a</sup> 2.50 $\pm$ 0.02
Gasoline	<sup>a</sup> 0.40 $\pm$ 0.04	<sup>b</sup> 1.43 $\pm$ 0.04	<sup>e</sup> 2.30 $\pm$ 0.03	<sup>g</sup> 4.21 $\pm$ 0.07
Xylene	<sup>a</sup> 0.40 $\pm$ 0.04	<sup>h</sup> 6.33 $\pm$ 0.06	<sup>f</sup> 4.12 $\pm$ 0.03	<sup>d</sup> 2.17 $\pm$ 0.06

Values with dissimilar letters (a, b, c, d, e, f, g, h) are significantly different ( $p < 0.05$ ) from each other

## DISCUSSION

This study investigated the influence of volatile organic solvents (nitrocellulose thinner, gasoline and xylene) on the activity quotient and liver biochemical indices of laboratory mice, *Mus musculus* over a period of 60 days. The findings from this study clearly indicate that the three volatile organic solvents; nitrocellulose thinner, xylene and gasoline induced toxicological effects though sublethal on *Mus musculus* ranging from subtle physiological changes to biochemical effects over an exposure period of 60 days. Xylene was found to be the most toxic followed by nitrocellulose thinner and gasoline when exposed to laboratory mice, indicating that these compounds show vertebrate toxicity. The high potency of xylene agrees with the findings of [10] who stated that water soluble fraction of xylene was more toxic than other monocyclic aromatic compounds. The higher toxicity of xylene and its volatility is attributed to the fact that it is more stable and has a relatively longer lifespan compared to nitrocellulose thinner and petrol [11].

The high level of restlessness or hyperactivity observed in the exposed mice compared to control at day 21 indicates physiological stress which resulted in a gradual decline in activity from days 45 to 60. High level of restlessness would inevitably imply low attention span and hence poor appetite for food. The mice exposed to xylene showed more restlessness, an observation which also agrees with findings of [12] which stated that motor coordination in mice exposed to the xylene was disrupted in a concentration-dependent manner. Also, the biphasic activity exhibited by mice exposed to nitrocellulose thinner fumes agrees with the result of [13] who reported biphasic effects, i.e., increase in locomotor activity followed by decreases in albino Swiss-Webster mice exposed to vapours/fumes of 1,1,1-Trichloroethane.

The activity of antioxidant enzymes may be enhanced or inhibited under chemical stress depending on the intensity, nature and the duration of the stress applied, as well as, the susceptibility of the exposed species. Glutathione-S-Transferase (GST) is a cytosolic or microsomal enzyme that catalyses the conjugation of GSH with oxidative products, such as 4-hydroxyalkenals (membrane peroxides) and/or base propanals, resulting from DNA oxidative degradation [14]. Therefore, it plays an important role in protecting tissues from oxidative stress [15-16]. Thus, the decrease in GSH levels indicates that the biomolecule was actively protecting the tissue from oxidative stress induced by the three VOCs. Impairment in antioxidant enzymes will produce an imbalance between pro and antioxidant systems causing the formation of toxic hydroxyl radicals, with direct consequences on the cell integrity and cell functions itself [17].

In this study, the activity of the enzyme SOD was inhibited in the liver of the albino mice exposed to the test chemicals. SOD is known to provide cytoprotection against free radicals induced damage by converting superoxide radicals generated in peroxisomes and mitochondria to hydrogen peroxides. The hydrogen peroxide is then removed from the system by the enzyme CAT, which converts it to water and molecular oxygen. The inhibition of the enzyme SOD by the test chemicals will, therefore, lead to increased oxidative stress in the liver tissues as a result of the damaging activities of the superoxide radicals. Furthermore, the inhibition of the enzyme SOD expectedly resulted in a reduction in the activity of CAT, due to a decrease in hydrogen peroxide generation from SOD activities. This indeed proved to be the case in this study as there was a significant reduction in CAT activity in the exposed mice. Similar observation of a decrease in CAT activity following an inhibition of the activity of SOD has been reported by [18].

Exposure of *M. musculus* to xylene, nitrocellulose thinner and gasoline was found to cause an increase in level of malondialdehyde indicative of oxidative damage in the liver of exposed mice compared to control. This result is in agreement with findings of [19-21] who reported an increase in lipid peroxides (LPO) in tissues of animals exposed to petroleum hydrocarbons. The increase in LPO is due to an inhibitory effect on mitochondrial electron transport system leading to stimulation in the production of intracellular reactive oxygen species (ROS) [22]. Elevated ROS level in tissues leads to cellular damage when the rate of its generation surpasses the rate of its decomposition by antioxidant defense systems. The measurement of lipid peroxide levels in plant and animal tissues exposed to different pollutants have been recognized as reliable early warning signal of exposure to environmental stress and integrated to environmental monitoring programmes [18, 20, 23].

The inhibition of enzymatic antioxidant defense in this study was found to be correlated with an increase in oxidative damage as reflected by the increased level of MDA in liver tissues of exposed mice. The use of biochemical responses as biomarkers during environmental monitoring programmes is derived from the basis that a toxic effect manifests itself at the sub-cellular level before it becomes apparent at higher levels of biological organization. The observed inhibition of the antioxidants defense enzymes; SOD, CAT and GSH in conjunction with an increase in MDA levels in the liver tissues of test animals exposed to VOCs can therefore serve as a good battery of biomarkers for early detection especially in people working in areas like gasoline filling stations and depots, spray painting workshop and laboratory

scientists. There is a need for sensitisation and awareness programmes on the occupational health effects posed by these volatile organic solvents, as well as, the need for exposed workers to be adequately protected by using appropriate personal protection equipment (PPE) and reducing exposure through reduced-time shift regimes.

**Ethics:** the studies were carried out in accordance with the University of Lagos Ethics Committee guidelines for experiments with whole animals.

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