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# Toxicological effect of inhalation exposure to nitrocellulose paint thinner fumes (FIAB®, ABRO® and SPRINT®) in wistar albino rats

***Patrick-Iwuanyanwu K. C., Okon E. A., Areh N. W. and Wegwu M. O.**

*Department of Biochemistry (Toxicology unit) University of Port Harcourt, P.M.B 5323, Choba, Rivers State, Nigeria*

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**ABSTRACT**

*The toxicological effect of inhalation exposure to nitrocellulose paint thinners were evaluated. Wistar albino rats were placed in exposure chambers and allowed to inhale fumes from three different commercial brands of nitrocellulose paint thinners (FIAB®, ABRO® and SPRINT®) commonly used by professional painters in the Niger Delta Area of Nigeria while the control group was kept in a fume-free chamber. The exposure lasted for 4 h every day for a period of 28 days. Serum L-alanine aminotransferase (L-ALT), L-aspartate aminotransferase (L-AST), alkaline phosphatase (ALP), total bilirubin, direct bilirubin and urea levels of rats exposed to nitrocellulose paint thinner fumes increased significantly ($p \leq 0.05$) when compared with control. However, there was no significant difference in the level of cholesterol of exposed rats as compared with control. Packed cell volume (PCV), haemoglobin (Hb) and white blood cells (WBC) level of exposed rats increased significantly ($p \leq 0.05$) when compared with control. Histological examination of the liver tissues of control group showed normal architecture whereas hepatocytes of groups exposed to nitrocellulose paint thinner fumes were characterized by severe fatty change, inflammation of the cells around the portal tract and dilated sinusoids. The results suggest that continuous exposure to paint thinner fumes may be toxic and capable of causing multiple organ toxicity.*

**Key words**: Inhalation exposure, nitrocellulose paint thinner, biochemical parameters, haematological parameters, histological examinations

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## INTRODUCTION

Nitrocellulose paint thinner is a solvent used to thin oil-based paints and also to clean excess paint from work surfaces or application tools. It is highly volatile, hazardous and flammable. It gives out fumes that are harmful if inhaled [1].Commercially, paint thinner is usually a name for mineral spirits. Although the exact composition of paint thinners are usually a trade secret, the liquid and solvent can be very hazardous and pose a strong toxicity effect particularly from toluene, which contain as much as 15 percent of benzene [2]. Inhalation of fumes emitted from paint thinners causes headache, dizziness, tiredness, drowsiness, somnolence and intolerance of alcohol. Others include emotional instability, loss of appetite, nausea, epigastric oppression and abdominal colic [2,3]. The constituents of nitrocellulose thinner have been detected in household and workplace air [4,5]. There is increasing concern about involuntary exposure of industrial workers to this organic solvent especially professional auto and furniture painters [6,7]. Toxicity can occur from acute unintentional or deliberate inhalation of excessive amount of paint thinner fumes, ingestion or transdermal absorption which results in serious multi-organ toxicity and death [1,8]. Daily exposure to high concentrations of paint thinner has been observed to reduce luteinizing hormone

secretion from the anterior pituitary which, in turn, inhibits testosterone production [9]. It has also been reported to cause a decrease in weight of epididymis and spermatic count in rats [10]. Abuse of paint thinner among young sniffers has become a social and public health problem (11,12]. Solvent abuse has been reported to cause metabolic disturbances, pathological changes in various tissues and sudden deaths [13].Occupational exposure to constituents of paint thinners such as benzene, toluene and styrene-butadiene have a significant decrease in circulating erythrocytes, haemoglobin, platelets, total white blood cells, and absolute numbers of lymphocytes and neutrophils [14,15]. The objective of this study was to investigate the toxicity effects of the inhalation exposure to nitrocellulose paint thinner fumes in Wistar albino rats.

## MATERIALS AND METHODS

Three different commercial brands of Nitrocellulose paint thinners namely ABRO®, SPRINT® and FIAB® were purchased from Choba, Mile 3 and Alakahia markets respectively in Rivers State, Nigeria.

### Animals

A total of 32 Wistar albino rats weighing between 120-180g, obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt, Nigeria were used in this study. They were randomly selected into 4 groups (Control, FIAB®, ABRO® and SPRINT® respectively) of eight animals each. All experimental animals were housed in stainless steel cages in a well-ventilated Animal House and kept under standard environmental condition of 12/12 h light/dark cycle at room temperature of 25$^{o}$C and had access to feed and water *ad libitum*. They were allowed to acclimatize for 7 days to laboratory conditions before the experiment. The experiment was performed in accordance with the guidelines established by the European Community for the Care and Use of Laboratory Animals and were approved by Institutional Animal Ethical Committee (IAEC). The test groups were exposed to FIAB®, ABRO® and SPRINT® nitrocellulose paint thinner fumes respectively, while the control group was kept in a section of the animal house free from nitrocellulose paint thinner fumes (Table 1).

**Table 1: Experimental design**

| Group | Treatment and Exposure | Duration | No. of rats |
|---|---|---|---|
| 1 | normal feeds+ water only | 28 days | 8 |
| 2 | normal feed+ water + FIAB® fumes | 28 days | 8 |
| 3 | normal feed+ water + ABRO® fumes | 28 days | 8 |
| 4 | normal feed+ water + SPRINT® fumes | 28 days | 8 |

### Exposure to Nitrocellulose paint thinner fumes

The method reported by [16] Uboh *et al* (2005) was adopted but with slight modifications. Briefly, the animal cages housing the test groups were placed in exposure chambers measuring 150 cm x 90 cm x 210 cm. Two highly perforated 1000ml cans containing 500 ml of FIAB® paint thinner were placed in the exposure chamber and the animals were allowed to inhale the fumes evaporating from the cans. The same procedure was adopted for the ABRO® and SPRINT® fumes respectively. The exposure lasted for 4 h every day for a period of 28 days. The exposure time was between 8:00am to 12 noon, after which the animals were transferred to fumes-free section of the experimental animal house.

### Sample Collection

At the end of the experimental period, the animals were anaesthetized with chloroform in an anaesthetic chamber 24 h after the last exposure. They animals were sacrificed using cervical dislocation method. Blood samples were obtained by cardiac puncture from each rat by means of a 2ml hypodermic syringe and needle. The blood samples were introduced into clean dry bottles (EDTA bottles) for haematological parameters while heparinized tubes were used to collect blood for biochemical estimation. The heparinized blood was centrifuged within 5 min of collection at 2500 rpm for 10 minutes. Serum was collected into a clean dry sample container. The levels of biochemical parameters were estimated using the Humazym MUV-test kits. Bilirubin was analyzed by colorimetric method as described by [17]Randox Bilirubin Manual (2003).The white blood cells (WBC) and the differentials were estimated using the improved Neubauer counting chambers as described by [18]. The haemoglobin (Hb) concentration was determined by the Cyameth-haemoglobin method while the Packed Cell Volume (PCV) was determined by the micro method also as described by [18].

**Histopathological study**

A portion of the liver of all the rat groups was fixed in 10 % buffered neutral formalin for 48 h followed by bovine solution for 6 h and then processed for paraffin embedding. By using a microtome, sections of 5 µm thickness were taken, processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin [19] and subjected to histopathological examination.

**Statistical analysis**

Data obtained from the studies are expressed as the mean value ± standard error of mean (S.E.M). Differences among mean of control and exposed groups were determined using statistical package for social scientist (SPSS 11.0). A probability level of less than or equal to 5% ($p \leq 0.05$) was considered significant

## RESULTS

The results of the effect of inhalation exposure of rats to nitrocellulose paint thinner fumes (FIAB®, ABRO® and SPRINT®) on biochemical parameters are shown in Table 2. The activities of the three most prominent marker enzymes, Aspartate aminotransaminase (AST), alkaline phosphatase (ALP) and Alanine aminotransaminase (ALT) were significantly ($p \leq 0.05$) increased in rats exposed to paint thinner fumes when compared with the control. The activities of AST, ALP and ALT were observed to be highest in rats exposed to ABRO® fumes. The levels of total bilirubin, direct bilirubin and urea also increased significantly ($p \leq 0.05$) when compared with control (Table 2). However, there was no significant ($p \leq 0.05$) difference in the level of cholesterol in rats exposed to nitrocellulose paint thinner fumes when compared with control. The results of the effects of inhalation exposure to nitrocellulose paint thinners on haematological parameters in rats are presented in Table 3. The results showed that packed cell volume (PCV), haemoglobin (Hb) and white blood cells (WBC) levels obtained from the exposed rats were significantly ($p \leq 0.05$) increased when compared with control. The results of the histological examination are shown in figures 1-4. Histological examination of control rats showed normal architecture (Fig. 1). However, the hepatocytes of rats exposed to ABRO®, FIAB® and SPRINT® fumes respectively were characterized by severe fatty change (Figs. 2 and 3), moderate inflammation of the cells around the portal tract (Fig. 3), dilated sinusoids, mild fatty change and mild inflammation of the cells surrounding the portal tract (Fig. 4) respectively.

**Table 2: Effect of Nitrocellulose paint thinner fumes on biochemical parameters in rats after 28 days of inhalation exposure**

| Groups | AST (U/L) | ALP (U/L) | ALT (U/L) | Total Bilirubin (mg/dl) | Direct Bilirubin (mg/dl) | Cholesterol (mg/dl) | Urea (mg/dl) |
|---|---|---|---|---|---|---|---|
| Control | 7.33±0.88$^a$ | 15.67±1.20$^a$ | 6.33±0.67$^a$ | 13.17± 1.76$^a$ | 6.50± 0.84$^a$ | 2.40± 0.10$^a$ | 5.37± 0.15$^a$ |
| FIAB | 13.67±0.32$^b$ | 25.67±0.33$^b$ | 14.67±0.33$^b$ | 29.32± 0.84$^b$ | 14.30± 0.60$^a$ | 2.50± 0.26$^a$ | 6.97± 0.43$^a$ |
| ABRO | 18.50±1.53$^c$ | 30.00±1.76$^b$ | 18.0±0.58$^c$ | 30.63± 0.88$^b$ | 15.17± 0.75$^b$ | 2.20± 0.26$^a$ | 10.57 ±2.24$^b$ |
| SPRINT | 15.67±1.20$^b$ | 28.00±1.00$^b$ | 15.33±1.20$^b$ | 35.67 ±3.85$^b$ | 16.90 ±1.55$^b$ | 2.60± 0.17$^a$ | 15.30± 3.32$^b$ |

*Values are expressed as mean ±SEM (n=8). Values wi th different superscripts (a-c) are considered to be significantly different ($p \leq 0.05$)*

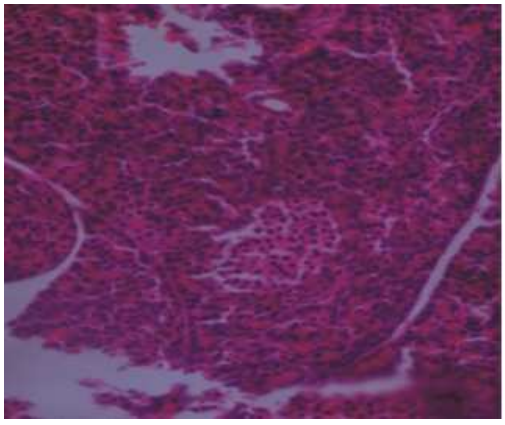

**Figure 1: A section of the rat liver showing normal architecture of control rats**

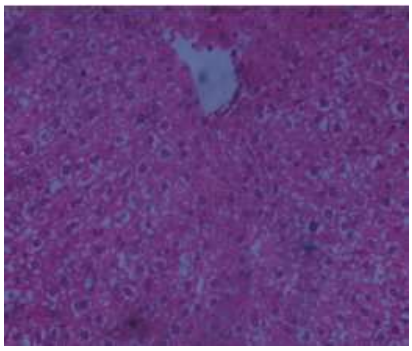

**Figure 2: A section of the liver of rats exposed to ABRO® paint thinner fumes showing severe fatty change and moderate inflammation of the cells around the portal tract**

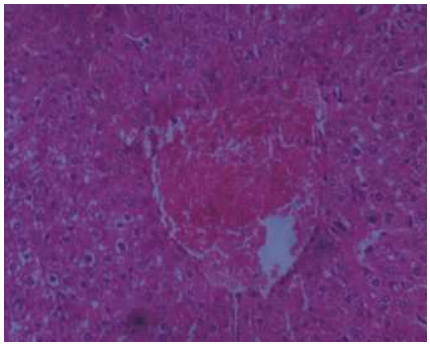

**Figure 3: A section of the liver of the rat exposed to FIAB® paint thinner fumes showing severe fatty change.**

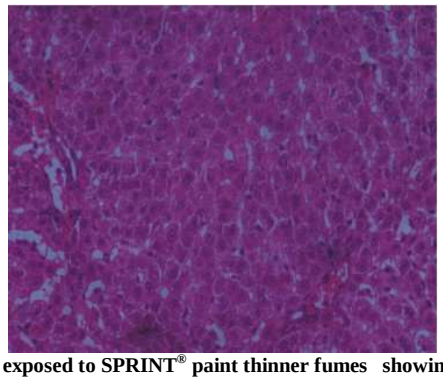

**Figure 4: A section of the liver of the rat exposed to SPRINT® paint thinner fumes showing sinusoids dilated, mild fatty change and mild inflammation of the cells surrounding the portal tract.**

**Table 3: Effect of Nitrocellulose paint thinner fumes on haematological parameters in rats after 28 days of inhalation exposure**

| GROUPS | PCV (%) | HB (g/dl) | WBC (cell mm/cm$^3$) |
|---|---|---|---|
| CONTROL | 27.25±1.11$^a$ | 8.43±0.29$^a$ | 5200±70.71$^a$ |
| FIAB | 28.50±1.71$^b$ | 8.81±0.59$^b$ | 5300±108.01$^b$ |
| ABRO | 29.50±0.87$^b$ | 8.95±0.41$^b$ | 5375±25.00$^b$ |
| SPRINT | 30.75±0.85$^c$ | 9.43±0.26$^c$ | 5425±62.92$^c$ |

*Values are expressed as mean ±SEM (n=8). Values wi th different superscripts (a-c) are considered to be significantly different (p≤0.05)*

## DISCUSSION

Biochemical and haematological indices have often been used to assess the state of health of a given organism exposed to toxicants in a particular environment. The results of this present study showed that the inhalation exposure of experimental rats to three different brands of nitrocellulose paint thinner fumes significantly increased

the activities of serum marker enzymes. These marker enzymes are cytoplasmic in origin and are often released into circulation after cellular damage [20]. However, the increase in serum marker enzymes observed in the present study suggest that the exposure of rats to nitrocellulose paint thinner fumes may be toxic and was able to reach the liver and induce a detectable damage within the study period. An increase in the activity of AST has been reported in $CCl_4$-induced toxicity in rats (21,22,23] and also in rats fed diets contaminated with kerosene and petrol [24]. This increase may be due to the abnormal dynamic properties of cellular membranes following exposure to hydrocarbon fractions present in nitrocellulose paint thinners. The elevation of ALT as observed in this study is indicative of liver injury [25]. The marker enzyme, ALT might have leaked from damaged cells due to increased permeability of the hepatocellular membrane due to necrosis, indicating organ dysfunction [26]. The result of the present study is also in agreement with the findings of [27], who reported that the rise in the enzyme AST is usually accompanied by an elevation in the level of ALT, which plays a vital role in the conversion of amino acids to keto acids. These observable changes in the cell membrane may be attributed to the reactive free radicals species from the metabolism of hydrocarbons which are the major constituents of nitrocellulose paint thinners. The increase in the level of ALP in the rats exposed to these nitrocellulose paint thinners imply that damage may have occurred to liver cells, since the activity of this enzyme in the serum is reported to be increased in the case of liver damage [28]. Alkaline phosphatase (ALP) is involved in the transport of metabolites across the cell membranes, protein synthesis, and synthesis of certain enzymes, secretory activities and glycogen metabolism. The increase in this enzyme activity may be due to a disturbance in the transport of metabolites or alteration in the synthesis of certain enzymes as in other hepatotoxic conditions [29].

The elevation of total and direct bilirubin of exposed rats is indicative of obstruction in the excretion of bile [30]. Bilirubin, an endogenous organic anion binds reversibly to albumin and it is transported to the liver, conjugates with glucoronic acid and excreted in the bile. Therefore hepatobiliary disease is indicated when conjugated fraction of total bilirubin exceeds the upper limit of normal, even if the total serum bilirubin is normal or near normal [31]. The elevation of urea observed in rats exposed to nitrocellulose paint thinners may be an indication of renal dysfunction. Since in renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance [32].

The increase levels of haematological parameters observed in this study in all the exposed groups may be due to increase absorption of iron. The rise in packed cell volume (PCV) and haemoglobin (HB) levels have been reported in rats exposed to toluene, a major constituent of nitrocellulose paint thinners to cause dehydration suggesting that toluene inhalation may reduce the volume of plasma water causing a relative increase in red blood cells (RBCs), which concentrates the RBCs in a condition called haematoconcentration [10]. According to [33], an elevated PCV may be attributed to an increase in blood cells, a condition called polycythaemia. This may be secondary to a decreased amount of oxygen, called hypoxia, or the result of a proliferation of blood forming cells in the bone marrow. An elevated white blood cell count is an important criterion to detect various health problems, such as blood disorders, bone marrow diseases and inflammatory diseases, Dehydration and haemoconcentration can also lead to an increase in white blood count. Furthermore, the increased WBC count indicates that the nitrocellulose paint thinners to an extent affected the defense mechanism of treated rats [34].

Histological examination of the liver tissues of the experimental rats indicated that the frequent exposure to nitrocellulose paint thinner fumes affects the structural integrity of the liver cells. This implies that the liver is one of the major target organs of the paint thinner fumes-induced injury. However, the results of the study indicate that the rats in the control group were not under stress.

## CONCLUSION

In conclusion, the cumulative oxidative damage is therefore likely to be one of the underlying mechanisms responsible for the hepatotoxic and haematoxic effects of nitrocellulose paint thinner fumes, as observed in this study.

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