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# EFFECT OF INHALATION EXPOSURE TO KEROSINE AND PETROL-FUMES ON SOME ANAEMIA-DIAGNOSTIC INDICES IN RATS.

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**ABSTRACT**

Changes in total body weight, some anaemia-diagnostic indices (haematocrit or packed cell volume (PCV), haemoglobin (Hb) and total serum protein) were determined in rats (*Wistar* albino strain) after 2 weeks of 4 hours daily inhalation exposure to ungraded concentrations of kerosene and petrol fumes. The results obtained for those rats exposed to petrol and kerosene fumes showed a significant decrease ($P<0.05$) in all the indices assessed, when respectively compared with the results obtained for the control rats. The total serum proteins, haemoglobin and haematocrit levels were observed to decrease by 39.2, 43.2 and 28.9 percents, respectively, in those rats exposed to petrol fumes, and by 40.9, 46.9 and 38.0 percents, respectively, in those rats exposed to kerosene fumes when compared, respectively, with the control. From the result s obtained for changes in total body weight, it was observed that the percentage weight increase and growth rate of 4.6 and 22.2 percents, for rats exposed to petrol fumes, and -2.83 and -13.89 percents, respectively, for rats exposed to kerosene fumes, were significantly lower ($P<0.05$), compared, respectively, with 24.0 and 112.5 percents obtained for the control rats. The decrease in PCV, Hb and total serum protein, as well as weight loss and growth retardation reported in this work was observed to be more severe in rats exposed to kerosene fumes than those exposed to petrol fumes. The observations made from this study indicate that kerosene fumes inhalation is comparatively, more hazardous than petrol fumes inhalation in causation of anaemia in rats. And that inhalation of kerosene and petrol fumes may dispose the subject to anemic condition.

**KEYWORDS:** Anaemia, Kerosene fumes, Petrol-fumes, Total body weight, Total serum protein.

## INTRODUCTION

Petroleum fumes, obtained from evaporation or combustion of petroleum products/fractions constitute some components of petroleum pollutants in the air (Environmental Health Criteria 20, 1982). Most petroleum fractions contain aliphatic, aromatic and a variety of branched, saturated and unsaturated hydrocarbons (Klaassen, 1990). These fumes are ubiquitous in the environment, and the commonest sites of contact include refineries, oil fields, petrol stations, petrochemical industries, motor mechanical workshops and traffic-congested areas where direct inhalation of the vapour is common. The most affected population are those occupationally exposed, automobile owners and users, those residing in traffic-congested areas as well as users of kerosene stoes and lantern. However, reports indicate that chronically exposed individuals are the oil drillers, refinery workers, petrochemical workers, petrol station attendants and motor mechanics (Yardley Jones *et al.*, 1991; Smith *et al.*, 1993; Carballo *et al.*, 1994; Ong *et al.*, 1994; Anderson *et al.*, 1995; Raoble and Wong, 1996). It has been demonstrated that after inhalation of equal concentrations more saturated hydrocarbons than unsaturated aromatic hydrocarbons are found in human and animal blood (Dahl *et al.*, 1988; Eide, 1990; Zahlsen *et al.*, 1990; 1992; 1993).

Some of the petroleum products' constituents, such as tetraethyl lead, benzene, xylene and the alkanes, have been reported to be haematotoxic (d'Azevedo *et al.*, 1996; Ross, 1996; Rothman *et al.*, 1996; Synder and Hedli, 1996). Also, most haematologic parameters (total white blood cells, absolute lymphocyte count, platelets, red blood cells and haematocrit) have been reported to be lower among workers heavily exposed to benzene (Rothman *et al.*, 1996). According to Synder and Hedli (1996), benzene toxicity involves both bone marrow depression and leukemogenesis caused by damage to multiple classes of haematopoietic cell and a variety of haematopoietic functions. Carbon disulphide and 2, 5-hexanedione (a toxic metabolite of hexane) have been reported to covalently cross-link red cells and axonal membrane proteins, such as gamma ($\gamma$) - diketones (Amarnath *et al.*, 1991; Genter *et al.*, 1987; Valentine *et al.*, 1991; 1992; 1993). These observations indicate that 2,5-hexanedione and carbon disulphide toxicity, mediated by protein cross-linking, may cause damages to the affected cells. Hence, the combined toxicity effect of benzene, hexane, carbon disulphide and other petroleum products' constituents may form potent predisposing factor to aplastic and haemolytic anaemia, following frequent exposures.

Anaemia, one of the most widespread diseases in the world, may be reliably diagnosed by measuring the levels of haematocrit and haemoglobin in the body. The levels of haemoglobin below which a person is said to be anaemic, according to WHO (1996), are given as: ≤11g/dl for children between 6 months to 4 years, ≤11.5g/dl for children between 5 to 11 years, ≤12g/dl for children between 12 to 14 years, ≤12g/dl for women and adolescent girls (non-pregnant), ≤11g/dl for pregnant women, and ≤13g/dl for men and adolescent boys.

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Table 1: Effect of petrol and kerosene fumes inhalation on some anaemia-diagnostic indices in rats.

| Group | PCV (%) | Hb (g/100ml | TSP(g/100ml) | IBW(g) | FBW(g) |
|---|---|---|---|---|---|
| Control | 38.12±2.21 | 9.16±0.16 | 4.87±0.22 | 84.25 ±10.40 | 104.50 ± 12.98 |
| Petrol fumes | 27.12±2.00* | 5.20±0.06* | 2.96±0.18* | 87.88±6.83* | 91.88 ±7.57* |
| Kerosene fumes | 23.63±1.60* | 4.86±0.17* | 2.88±0.18* | 8 8.25±11.02* | 85.75 ± 7.08* |

Data are presented as x ± SEM. n = 8. *P<0.05 compared with control.
TSP = Total serum protein;
IBW = Initial body weight; FBW = Final body weight.

Also, DeMaeyer (1989) defined anaemia in all ages and sexes as: 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.

Since a greater percentage of the populace is directly or indirectly exposed to petroleum fumes through inhalation, this study was carried out to determine and establish the haematological risk of such exposure in rats as the experimental models.

## MATERIALS AND METHODS

### Experimental Animals

Weanling Wistar albino rats weighing 83-108g were obtained from the animal house of the College of Medical Sciences, University of Calabar, Calabar, Nigeria. The animals, randomly divided into three groups (two test and one control) of 8 rats each, were allowed to acclimatized in the experimental animal house for 5 days before the experiments began. The animals, housed in stainless steel cages (45 x 25 x 30cm) were fed with normal rat chow (Guinea feeds product) purchased from High Quality Livestock feeds store, Calabar, Nigeria. All the test and control animals had free access to food and tap water throughout the experimental period.

### Exposures

The animals in the test groups were exposed to petrol and kerosene-fumes respectively, while those in the control group were kept in fumes-free section of the experimental animal house. Inhalation mode of exposure was employed. In this inhalation study, the cages housing the test animals were placed in exposure chambers (100 x 75 x 200cm) saturated with the respective petroleum fraction for 4 hours after which they were transferred to fumes-free section of the animal house daily. The exposure chamber was saturated with the fumes by allowing the kerosene and petrol fractions, in four 1 litre cans (with 0.5 litres of the fraction each) highly perforated at the upper end to allow the fractions to evaporate and fill the chamber at ambient temperature and humidity. This was done 2 hours before and throughout the exposure period. Petrol and kerosene were obtained from Mobil filling station, Calabar, Nigeria. The test animals were allowed to inhale the respective fumes evaporating from the cans during the exposure period. The exposure duration of 4 hours daily was adopted for 2 weeks (14 days), and was performed during day time (9.00am - 1.00pm).

The animals were killed after their respective weights were taken at the end of 2 weeks exposure period. Blood specimen were collected for experimental analyses.

### Collection and Preparation of Blood Specimen for Analyses

Blood samples were obtained by cardiac puncture and divided into two sets of screw-cap (one plain and the other heparinised) sample bottles. One set of samples in the heparinised bottles were used for PCV and Hb determinations, while the specimen in the plain bottles were allowed to clot and the serum extracted after spinning with MSE model (England) centrifuge at 2000 rpm for 5 minutes. The serum samples were used to analyse for the total serum protein.

PCV and Hb levels were determined by the methods described by Alexander and Griffiths (1993 a, b), while the total serum protein level was determined by Biuret method as described by Doninger *et al.* (1972).

All absorbances were read using DREL 3000 HACH model spectrophotometer.

### Determination of Total Body Weights

Total body weights were determined using

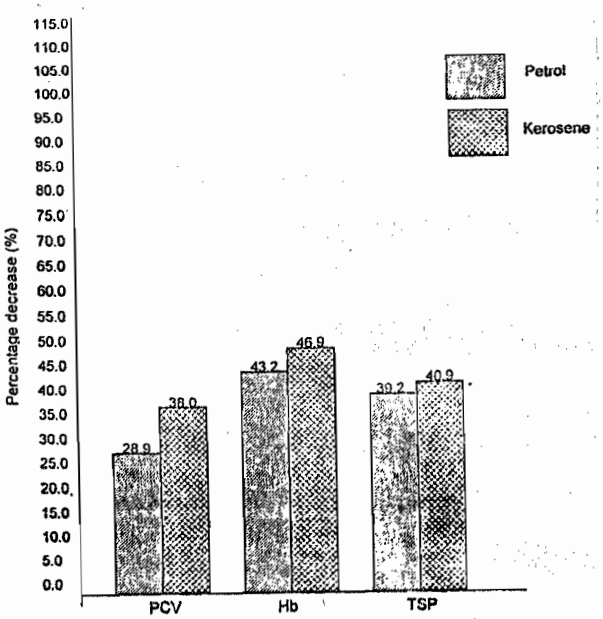


Figure 1: Percentage decrease in some haematological indices (PCV and Hb) and total serum protein (TSP), following inhalation exposure to petrol and kerosene fumes in rats.

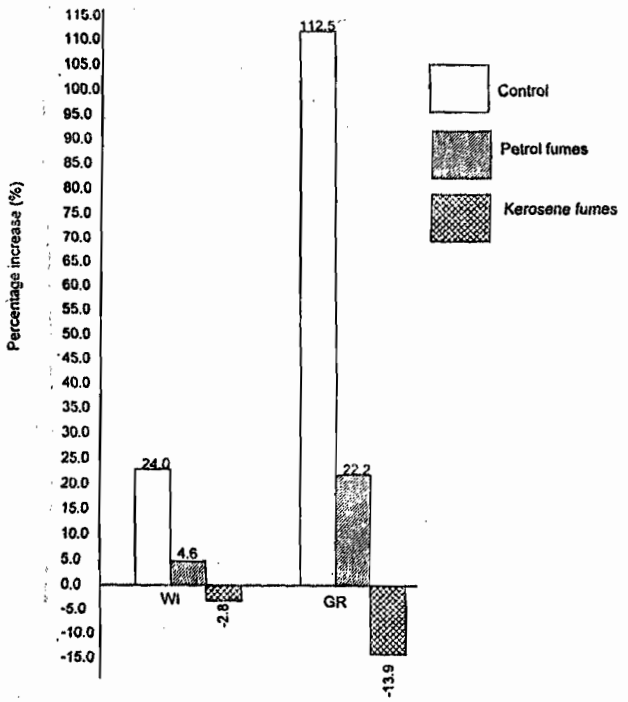


**Figure 2:** Percentage weight increase (WI) ad growth rate (GR) following inhalation exposure to petrol and kerosene fumes in rats.

chemical balance before and after experimental period (as initial and final body weights respectively), and the mean body weight for each group calculated. Weight changes were expressed as percentage weight increase and percentage growth rate, where:

(i) Percentage weight increase was calculated from the formula:

$$\frac{\text{Final body weight - Initial body weight}}{\text{Initial body weight}} \times 100$$

(ii) Percentage growth rate was calculated from the formula:

$$\frac{\text{Final body weight - Initial body weight}}{\text{Number of days exposed}} \times 100$$

**Statistical Analysis**

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

## RESULTS

The results of the study on the effect of inhalation exposure of rats to petrol and kerosene fumes on some anaemia-diagnosting indices (Hb, PCV, total serum protein and total body weight, i.e., IBW and FBW) are shown Table 1, Figures 1and 2. The values of Hb, PCV, total serum proteins, IBW and FBW obtained for rats exposed to petrol fumes (5.20 ± 0.06g/100ml, 27.12 ± 2.00%, 2.96 ± 0.18g/100ml, 87.88 ± 6.83g and 91.88 ± 7.57g, respectively) and kerosene fumes (4.86 ± 0.17g/100ml, 23.63 ± 1.60%, 2.88 ± 0.18/100ml, 88.25 ± 11.02g and 85.75 ± 7.08g, respectively) were observed to be significantly lower ($P<0.05$), compared respectively with the values obtained for the rats in the control group (9.16 ± 0.16g/100ml, 38.12 ± 2.21%, 4.87 ±0.22g/100ml, 84.25 ±10.40g and 104.50 ±12.98g, respectively). These results show that the levels of Hb, PCV and total serum protein decreased by 43.2, 28.9 and 39.2 percents, respectively, in rats exposed to petrol fumes; and by 46.9, 38.0 and 40.9 percents, respectively, in rats exposed to kerosene fumes, when compared with the values for the control group (figure1).

From figure 2, the results also show that the mean percentage weight increase and growth rate obtained for rats exposed to petrol fumes (4.6 and 22.2 percents, respectively), and rats exposed to kerosene fumes (-2.8 and -13.9 percents, respectively) were significantly lower ($P<0.05$), compared, respectively, with the values obtained for rats in the control group (24.0 and 112.5 percents, respectively). The observed effect of inhalation exposure of rats to petrol and kerosene fumes on total body weight, indicates weight loss and growth retardation. However, the hazardous effects were observed to be more severe in rats exposed to kerosene fumes than those exposed to petrol fumes.

## DISCUSSION

Anaemia, reported to be one of the most widespread diseases in the world, has multifactorial causes (d'Azevedo *et al.*, 1996; Ross, 1996; Rothman *et al.*, 1996; Synder and Hedli, 1996; Akpanabiatu *et al.*, 1998; Topley, 1998). Although it is generally observed that iron, folate and vitamin $B_{12}$ deficiencies, as well as infections are the leading causes of anaemia (Topley, 1998). The results of this study indicate that the role of chemical agents (chemical constituents of kerosene and petrol fumes) may be equally important in causation of anaemia.

Lower levels of Hb and PCV are reported in this study following inhalation exposure to kerosene and petrol fumes in rats. These results strongly correlate those reported by d'Azevedo *et al.* (1996) and Rothman *et al.* (1996) for human and animal subjects exposed to such chemical agents as benzene and xylene. The decrease in the levels of Hb and PCV observed in this study may be as a result of bone marrow depression, which reduces the rate of red cells synthesis, as reported for benzene toxicity (Synder and Hedli, 1996); or increased destruction of the red cells, as reported for carbon disulphide toxicity (Amarnath *et al.*, 1991; Valentine *et al.*, 1992, 1993). There is a clear indication, from this study, that kerosene and petrol fumes contain such chemical agents which when inhaled at appreciable concentration, can reduce the levels of Hb and PCV in the body, and that kerosene fumes constituents are more potent in this action than petrol fumes' constituents. However, the specific mechanism(s) by which the inhaled kerosene and petrol fumes reduce Hb and PCV levels is (are) not clear. Low level of total serum protein was also observed in this study. The observed low serum protein, following kerosene and petrol fumes inhalation, corroborates the reported low Hb level and also supports the anaemia-inducing effect of kerosene and petrol fumes. The

present data shows a significant positive correlation between whole blood haemoglobin concentrations and haematocrit levels prevalent in anaemic condition (Topley, 1998).

Lower percentage weight increase and growth rate have also been observed in this \study following inhalation exposure of rats to kerosene and petrol fumes. These lower percentage weight increase and growth rate are consistent with those reported for different species after oral and dermal exposures to various crude oils (Feuston and Mackerer, 1996; Feuston *et al.*, 1997), and for lead toxicity (Hammond *et al.*, 1990, 1993; Hammond and Succop, 1995). Although the mechanism(s) leading to the low understood, the observation gives a clear indication that frequent inhalation of these fumes may cause weight loss and growth retardation in rats. Weight loss has been reported as a common feature associated with anaemia (Passmore and Eastwood, 1986). Hence, it is clear from the result of this study that kerosene and petrol fumes' constituents may be considered among the predisposing factors to anaemia in rats.

In conclusion, this work suggests that frequent exposure to kerosene and petrol fumes may lead to anaemia and that the predisposing effect of kerosene fumes constituents is comparatively more severe than that of petrol fumes constituents in rats.

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