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## **Some Biochemical and Haematological Effects Associated with Chronic Inhalation of Crude Acetylene in Rabbits**

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The effects of chronic exposure to crude acetylene on some biochemical and haematological indices were studied in New Zealand White rabbits exposed to inhalation of 58,000 ppm crude acetylene for 10 min at 12 h intervals for 3 weeks. The treatment provoked significant elevation of aspartate and alanine transaminases in plasma of test rabbits relative to controls ( $p < 0.001$ ;  $p < 0.005$ ). In addition, catalase activity was significantly depressed in the heart, liver and kidney tissues ( $p < 0.005$ ), while superoxide dismutase was significantly raised in heart tissue ( $p < 0.008$ ). Packed cell volume was significantly lower in rabbits exposed to crude acetylene ( $p < 0.05$ ), while marginal decreases were seen in the other haematological parameters investigated. These results suggest that unlike pure industrial acetylene, crude acetylene may have deleterious effects on vital organs and blood constituents. Thus the use of *in situ* generated acetylene by welders may be hazardous to health.

**Key words:** Crude acetylene, PCV, tissue lesions, welders

## INTRODUCTION

Acetylene is a colourless, highly inflammable garlic-like smelling gas that forms explosive mixtures with air over a wide concentration range<sup>[1,2]</sup>. When mixed with oxygen, it is used in oxyacetylene torches for welding<sup>[3]</sup>. A plethora of data from toxicity studies of possible organ damage from exposure to acetylene and its surrogate methylacetylene strongly suggest that even at very high doses (up to 800,000 ppm), these gases do not bring about organ lesions nor do they affect biochemical, haematological or mutagenic parameters<sup>[4-9]</sup>. However these studies were based on inhalation of pure industrial acetylene. Consequently they do not reflect the typical work environment of the artisan welders in underdeveloped countries who are constrained by paucity of funds to rely solely on crude acetylene generated *in situ* from the reaction between calcium carbide and water. Available evidence show that crude acetylene produced from calcium carbide contains hazardous contaminants such as phosphine and arsine<sup>[10]</sup>.

In Nigeria, the services of roadside welders are in great demand, necessitating daily consumption of thousands of tones of calcium carbide. Since these welders do not operate with relevant protective gear, they may be constantly exposed to crude acetylene inhalation.

This study was therefore carried out to investigate some biochemical and haematological effects that may be associated with chronic exposure to crude acetylene, the form preferred by roadside welders in Nigeria.

## MATERIALS AND METHODS

**Animals and acetylene exposure:** Eight New Zealand White rabbits aged about three months were obtained from a breeder in Benin City. The animals were housed singly in clean metal hutches and acclimatized on growers' mash (Bendel Feed and Flour Mills Ltd, Ewu, Nigeria) for 2 weeks prior to the experiment. They were subsequently assigned randomly to 2 groups each of 4 rabbits. Members of one group (treated group) were then subjected to inhalation of 58,000 ppm crude acetylene for 10 min at 12 h intervals for 3 weeks. For this purpose, acetylene was generated *in situ* by addition of 12 g of calcium carbide to 100 mL of distilled water in a 500 mL conical flask bearing an air-tight rubber bung and delivery tube through which the ensuing acetylene was passed into an airtight 80 L inhalation chamber housing the rabbits. In order to ensure rapid completion of the reaction and attainment of the desired acetylene concentration within the inhalation chamber, the CaC<sub>2</sub> was ground to

powder in a dry hand mortar prior to use. Preliminary trials showed that the reaction was much slower with CaC<sub>2</sub> lumps. Members of the untreated group (controls) were placed in a similar inhalation chamber for the same period but without acetylene exposure. After each treatment, the rabbits in each group were returned to their individual hutches. Throughout the duration of the experiment, both groups were maintained on growers' mash and drinking water *ad libitum*.

The volume of acetylene liberated from the complete reaction of 12 g of calcium carbide at STP was converted to its corresponding value at 300 K i.e. 27°C (the internal temperature of the inhalation chamber) and expressed in ppm.

From the reaction equation,



Twelve gram CaC<sub>2</sub> liberate 0.1875 moles of acetylene. This is equivalent to 4.2 L at STP or 4.6 L at 300 K. Since the volume of the inhalation chamber was 80 L, the concentration of acetylene in ppm (i.e. cc per million cc)

$$= 4600/0.08 \approx 58,000 \text{ ppm}$$

$$[1000,000 \text{ cc} = 1 \text{ million cc, therefore } 80,000 \text{ cc (80 L)} = 0.08 \text{ million cc}]$$

At the end of the inhalation experiment, the rabbits were anaesthetized with pentobarbitone sodium (40 mg kg<sup>-1</sup>, intraperitoneal) and their marginal ear veins were cannulated for blood sample collection. 4.5 mL blood sample obtained from each rabbit was added to 0.5 mL of 3.8% sodium citrate solution and gently mixed. Plasma was obtained by centrifuging the blood samples at 2000 g for 10 min followed by decantation. The rabbits were subsequently sacrificed and their hearts, livers and kidneys were rapidly excised. Sections of these organs were taken up in sample vials for enzyme analyses. All analyses were performed within a few hours of sample collection.

**Determination of haematological indices:** White Blood Cell Count (WBC), Platelet Count (PC) and Packed Cell Volume (PCV) were estimated by use of an automated blood sample analyzer (QBC Autoread Plus, UK)<sup>[11]</sup>. Blood samples were pipetted into QBC capillary tubes and spun in a parafuge centrifuge (Becton Dickson, UK) for 5 min. The samples were thereafter placed one at a time in the autoread analyzer and values read off.

Relative Whole Blood Viscosity (RWBV) and the Relative Plasma Viscosity (RPV) were evaluated using the method first described by Reid and Ugwu<sup>[12]</sup>. A graduated syringe (1 mL Gillette) was fitted with a hypodermic

needle (21G, 0.8 x 40 mm, Nr 2). One milliliter of whole blood or plasma was drawn and the plunger of the syringe was carefully removed after turning the needle point-down and holding it in place with a retort stand so that samples could drop under gravity. Using a stopwatch, the time taken for the content of the syringe to be emptied was taken and divided by the time taken by 1 mL of distilled water under the same experimental conditions. RWBV and RPV values were calculated viz.,

$$RWBV = Tb/Tw \text{ and } RPV = Tp/Tw$$

where, Tb, Tp and Tw are the time of flow of whole blood, plasma and distilled water, respectively.

Erythrocyte Sedimentation Rate (ESR) was determined by the method described by Dacie and Lewis<sup>[13]</sup>. Blood samples were introduced into ESR tubes up to the zero mark and the tubes were then placed on the Westergren rack and allowed to stand undisturbed on the bench. Readings were taken after 1 h.

**Biochemical assays:** Superoxide dismutase, SOD was assayed colorimetrically by monitoring the inhibitory effect of the enzyme on the autoxidation of epinephrine<sup>[14]</sup>. Catalase activity was assayed in terms of the first order rate constant for the decomposition of hydrogen peroxide by the tissue extract<sup>[15]</sup>. Alkaline phosphatase, ALP was estimated colorimetrically by monitoring the rate of liberation of paranitrophenol from the synthetic substrate paranitrophenyl phosphate<sup>[16]</sup>. Aspartate transaminase, AST and alanine transaminase ALT were assayed colorimetrically as described by Bergemeyer and Bernt<sup>[17]</sup>.

**Statistics analysis:** All data are presented as the Mean±SEM and n represents the number of rats from which samples were taken. Comparisons were made between test and control groups by use of the Student's t-test (GraphPad Prism software, UK) and differences were regarded as significant with p<0.05.

## RESULTS

Table 1 shows that there were no significant differences in feed intake and weight gains between the two groups, although weight gain in rabbits given crude acetylene was slightly lower. The acetylene did not lead to loss of fur nor were there any noticeable eye and skin lesions.

AST and ALT activities were significantly higher in the plasma of the treated rabbits when compared with controls (p<0.001; p<0.005) (Table 2). Crude acetylene

Table 1: Feed intake, weight gains and physical appearance of rabbits in both groups after 3 weeks

	Treated	Control
Feed intake [g/rabbit/day]	137±12	137±7
Weight gain [g/rabbit]	171±23	188±15
Physical appearance		
Fur	Normal	Normal
Skin lesion	Abs	Abs
Eye lesion	Abs	Abs

[Values are Mean±SEM, n = 4]. Abs = absent

Table 2: Plasma levels of AST, ALT and ALP of the rabbits after 3 weeks exposure to crude acetylene

	AST (Units/L)	ALT (Units/L)	ALP (Units/L)
Treated	51.46±0.93*	26.95±2.38**	76.59±22.21
Control	11.19±0.40	8.77±0.24	69.35±12.63

(Values are Mean±SEM) \*p<0.0001 and \*\*p<0.005 compared to corresponding control values (n = 4 per group)

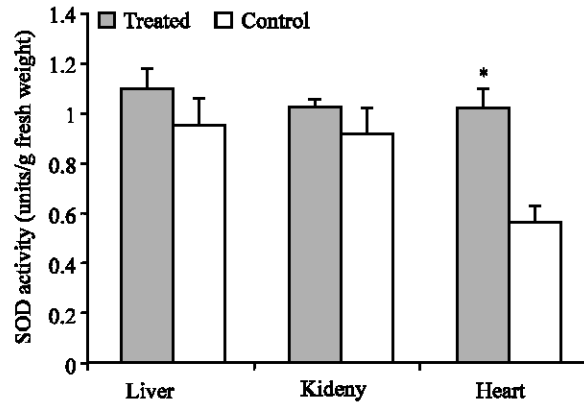


Fig. 1: SOD activities in liver, kidney and heart of rabbits after 3 weeks exposure to crude acetylene. (\* p<0.008, n = 4 per group)

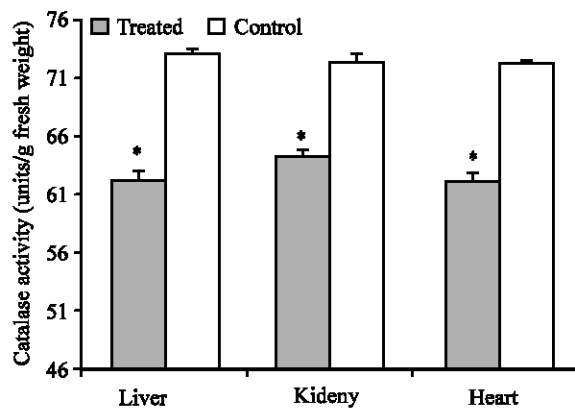


Fig 2: Catalase activities in liver, kidney and heart of rabbits after 3 weeks exposure to crude acetylene (\* p<0.005, n = 4 per group)

inhalation significantly reduced PCV (p<0.05), while decreases in the levels of other hematological parameters were not statistically significant (Table 3).

**Table 3: Changes in the values of some haematological parameters in rabbits after 3-week exposure to crude acetylene gas**

	WBC ( $\times 10^3/\text{mL}$ )	PCV (%)	PC ( $\times 10^3/\text{mL}$ )	ESR (mm/h)	RWBV	RPV
Treated	3240.00 $\pm$ 186.00	33.00 $\pm$ 1.96*	111.30 $\pm$ 5.15	4.00 $\pm$ 0.58	3.53 $\pm$ 0.13	1.65 $\pm$ 0.03
Control	3360.00 $\pm$ 132.70	39.33 $\pm$ 0.44	130.00 $\pm$ 10.00	3.25 $\pm$ 0.25	4.18 $\pm$ 0.31	1.63 $\pm$ 0.05

(Values are Mean $\pm$ SEM) \*p<0.05 compared to the corresponding control value (n = 4 per group)

Figure 1 and 2 show crude acetylene-induced changes in some tissue profiles of SOD and catalase, respectively. While SOD was elevated in all the tissues studied, the increase was significant only in heart muscle ( $p < 0.008$ ) (Fig. 1). On the other hand, catalase activity was significantly decreased in the liver, kidney and heart tissues of the treated group when compared to controls ( $p < 0.005$ ) (Fig. 2).

### DISCUSSION

The Acetylene Panel of the American Chemistry Council has validated some existing data relating to acute inhalation toxicology of acetylene and methyl acetylene in mammals<sup>[4]</sup>. These data support LC<sub>50</sub> values of >100,000, 800,000 and 850,000 ppm for rats, rabbits and dogs, respectively. Preliminary inhalation studies with crude acetylene in our laboratory revealed that a dose of 580,000 ppm (calculated from reaction stoichiometry) resulted in anaesthesia and death of over 50% of the rabbits used. However when this dose was reduced to 58,000 ppm i.e. one-tenth of previous value, the animals tolerated it without any observable adverse effect.

In this study, the use of crude acetylene arose from the need to mimic the operational environment of artisan welders so that any observed toxic effects could easily be related to their occupation. Increases in plasma levels of AST and ALT serve as reliable indices of assessment of damage to the parenchymatous cells of the heart and liver, respectively<sup>[17,18]</sup>. Thus the observed significant increases in the activities of these enzymes are pointers to crude acetylene-induced lesions in heart and liver tissues. This is supported by the pronounced depression in catalase activities in heart, liver and kidney, which suggests that the deleterious effects of crude acetylene may occur through a mechanism involving oxidative stress. SOD and catalase are among a battery of biological antioxidant systems that protect membranes from oxidative damage by reactive oxygen species<sup>[19-21]</sup>. It is likely that the observed elevation of tissue SOD, especially in heart, may be an initial adaptive response to cope with the toxic effects of crude acetylene. Similar initial adaptive responses have been reported in experimental animals exposed to cadmium<sup>[22,23]</sup>. The significant decrease in PCV may have contributed to the tissue damage induced by crude acetylene. Decreases in PCV are known to compromise vital organ functions through hypoxia<sup>[23]</sup>.

Hypoxia in turn impairs membrane integrity and leads to loss of tissue enzymes to the extracellular fluid<sup>[18,24]</sup>. Consequently, it seems that the observed increases in plasma AST and ALT may be the result of tissue damage by either oxidative stress or hypoxia or both. Since ALP and most of the haematological parameters were decreased, though not significantly, it is possible that these decreases may become significant at higher doses of crude acetylene exposure and prolonged inhalation periods.

To date, studies on acetylene toxicity in experimental animals have revealed unequivocally that the pure gas is of very low acute toxicity. For instance, the LC<sub>50</sub> of the gas in rats is 850,000 ppm<sup>[25]</sup>. In addition, repeated exposure of rats, mice, rabbits, dogs and guinea pigs to pure acetylene at concentrations up to 800,000 ppm revealed no evidence of vital organ damage<sup>[4]</sup>. Moreover results from several epidemiological studies have shown that acetylene is not a risk factor in mutagenesis<sup>[6-9]</sup>. On the other hand the few human fatalities of acetylene inhalation so far reported were attributed to toxic effects of phosphine and arsine contaminants released during the industrial production of the gas from calcium carbide<sup>[10]</sup>. Phosphine is the active principle of aluminum phosphide widely employed as a rodenticide<sup>[26]</sup>. Unlike acetylene, numerous toxic effects of phosphine have been reported. These include decreases in red blood cell, haemoglobin and haematocrit<sup>[27,28]</sup>; histotoxic damage to gastrointestinal tract, lungs, liver and kidney<sup>[26,29]</sup>; as well as elevation of hepatic enzymes in serum<sup>[29]</sup>. Although the levels of phosphine and arsine in the crude acetylene were not measured, it is known that these gases are regular contaminants of crude acetylene<sup>[10,30]</sup>. Consequently, it seems very reasonable to infer that the deleterious effects of crude acetylene seen in the present study are attributable to its noxious contaminants rather than acetylene itself.

In conclusion, this is probably the first study dealing with some biochemical and haematological effects of crude acetylene inhalation in an experimental animal model. If animal-to-man extrapolation is permitted, present results suggested that chronic inhalation of crude acetylene may pose serious health hazards and also underscores the need to discourage the practice of *in situ* generation of acetylene by welders especially in under developed countries.

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