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COMPARATIVE ANALYSIS OF CYANIDE CONTENT OF SOME TOBACCO PRODUCTS IN NIGERIA.

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ABSTRACT

The cyanide levels of four commercial brands of cigarettes sold in Nigeria (Benson and Hedges- BH., L & B- LB., Rothmas –RM., St. Moritz – SM); three brands of moist snuff (local preparations – MSI., MS2., MS3), and three brands of dry snuff -DSI., DS2., and DS3, prepared with potassium sesquicarbonate were studied using the methods of Lundquist *et al* (1985) and Hugh (1979). Fresh tobacco leaves (FS) were used as control.

The cyanide levels of the commercial cigarettes were as follows: BH = 0.40±0.02, LB = 0.32 ± 0.02, RM= 0.38 ± 0.02 and SM = 0.35 ± 0.03 μmol/g. On the other hand, the moist stuff brand of tobacco gave the following cyanide concentrations: MSI = 0.42 ± 0.02., MS2 = 0.44 ± 0.10., MS3 = 0.47 ± 0.02μmol/g. Cyanide values for the dry snuff brands were as follows: DSI = 0.42 ± 0.02., DS2 = 0.40 ± 0.01 and DS3= 0.45 ± 0.01μmol/g. On the other hand, a mean (±SD) cyanide value of 0.40 ± 0.02 μmol/g was obtained for fresh tobacco leaves (FS). When the cyanide content of the control was compared with each preparation, there was a significant difference ($P < 0.05$) between the control and all the samples. Also significant differences were observed at $P < 0.05$ between MSI and other moist snuffs and also between DSI and other brands of dry snuff.

KEY WORDS: Cyanide, Tobacco, Cigarettes, Snuff, Piotassium – sesquicarbonate.

INTRODUCTION

Cyanide is a known toxic substance in the whole world. Padwell (1997) reported the use of cyanide in cases of homicide and suicide while Suchard *et al.*, (1998) reported a case of acute cyanide toxicity from ingestion of apricot kernel. Major sources of cyanide poisoning are from food (cassava products) consumption (Uwakwe *et al.*, 1991, Achinewhu *et al.*, 1988) and from tobacco products (Pakhale *et al.*, 1990; Bartecchi, 1995).

Exposure to tobacco products either in public or in working place in considered to be a serious risk to human health (Witschi *et al.*, 1995). Tobacco was introduced in Europe from South America in the 16th Century. The products manufactured from its leaves are used in cigars, cigarettes, snuff pipe and chewing tobacco. (Repace, 1995).

Tobacco leaves are cured, fermented, and aged to develop aroma, before manufacturing them into products that can be smoked, chewed, inhaled or sniffed (Glantz *et al.*, 1995). Snuff (smokeless tobacco) is the powdered form of tobacco used by inhalation. Chemicals and salts are added to boost the alkalinity of the snuff, because the more alkaline the snuff, the more nicotine released (Perez-Trullen, 1995). There are dry and moist snuff brands. Moist snuff is a known carcinogen (Tomar and Henningfield, 1995).

The composition of each tobacco product depends on design, make, manufacturer, and mostly on the natural components of tobacco leaves. Many toxic substances have been reported in tobacco leaves, smoke, and products; these compounds are nicotine, nitrosamines, benzo (α) pyrine, acetaldehyde, hydrogen cyanide, phenols, etc (Kagawa *et al.*, 1990; Pakhale *et al.*, 1990).

Hydrogen cyanide is found not only in the smoke of tobacco products but also in the main products (Philips and Waller, 1991). Pyrolysis of organic materials containing nitrogen compounds such as protein gives rise to cyanogens (Johnson and Kang, 1971). A stick of unburnt cigarette yields around 0.1-0.4mg hydrogen cyanide while the smoke from complete combustion of a stick contains up to 1600 ppm of hydrogen cyanide. (Brunnemann *et al.*,1977).

Hydrogen cyanide is a potent enzyme inhibitor, interfering principally with the respiratory processes of cells. The terminal oxidase in the electron transport chain, is the primary site of inhibition (Passmore and Robson, 1980). In lethal doses, death occurs due to oxygen starvation at the cellular levels. Sublethal doses are characterized by coma, convulsions and other neurological disorders (Philbrick *et al.*, 1971). Local injection of cyanide into the nucleus, locus ceruleus of the rat brain induces respiratory depression (Chavez *et al.*, 1998). Enzymes like ATPase are reduced and depressed by cyanide toxicity (Odunuga and Adenuga, 1997). Central nervous system depression by cyanide was reported by Zhu and Krujevic (1997). Optic neuropathy was also reported by Tucker and Hedges (1993) and costagliola *et al.* (1990).

Other disease effects of cyanide toxicity include reduction of red blood cell glutathione (Costagliolia *et al.*, 1990); venous blood arteriolization and multiple organ failure (Martin – Bermudez and Maestre – Romero, 1997). There are also many reports of the effect of cyanide on vitamine B12, C and E metabolism (Oku *et al.*, 1991; Girand *et al.*, 1995).

There is need therefore, for effective markers of tobacco exposure. Smoked tobacco is made up of particulate and gas phases. Cortinine, in form of nicotine has been used as a marker of particulate phase while carbon monoxide is used for gaseous phase (Vasey, 1981).

Carbon monoxide and cortinine have not been so effective as markers of tobacco exposure. Carbon monoxide has a disadvantage of having short half-life of 4 hours (Rawbone, 1981) and thus disappears in the blood very early. It is therefore only used as a maker of

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recent smoke exposure. Holiday *et al.* (1995) showed that cortinine levels in some clinical trails and smoking cessation was found not to be remarkably different in both smokeless and smoking tobacco users.

Thiocyanate (SCN) on the other hand has a longer half-life of about 10-14 days compared to carbon monoxide and can therefore reflect a long time exposure of tobacco products. (Rawbone, 1981). An added advantage of thiocyanate as an index of tobacco exposure is that carbon monoxide is not absorbed from the buccal mucosa or conducting pathways of the respiratory tract, whereas the level of thiocyanate, derived from a water-soluble smoke component (hydrogen cyanide) is increased by absorption from buccal mucosa and may thus be of significance.

We deemed it necessary therefore to survey the levels of hydrogen cyanide in some tobacco products in order to enlighten the users, and the manufacturers on the levels of cyanide in these products. The aim is to highlight the possible dangers a smoker or inhaler may be subjected to based on the cyanide level in the tobacco.

MATERIALS AND METHOD

Sample Treatment

The four brands of cigarette, Benson and Hedges (BH), L&B (LB), Rothmas (RM) and St. Moritz (SM) were bought in a nearby commercial shop in Port Harcourt, Nigeria. The wraps and the filters were removed.

Fresh tobacco leaves (FS) were bought from a market in Port Harcourt, fermented, dried in the sun and blended into fine powder. Three brands of moist snuff (MSI, MS2 and MS3) were prepared by mixing the dried sample (10g) with 1,2, and 3mls of distilled water and allowing to stay over night. Three brands of dry snuff – DS1, DS2 and DS3 were each prepared by blending the dry snuff with 10, 20, and .30mg of potassium sesquicarbonate per 100g of the sample. Fresh tobacco leave samples were used as control.

Determination Of Cyanide Content

The cyanide content in the tobacco was determined using the methods described by Hugh (1979),Lundquist *et al.* (1985), Uwakwe *et al.* (1991) and. Exactly 1g of each tobacco product was placed in a large side-arm bottle. 4ml of 0.1N sodium hydroxide was added. The two bottles were connected to each other and to a nitrogen tank. Into the bottle containing the sample, 5ml of 1M sulphuric acid was added and the container was closed immediately. The liberated hydrogen cyanide in the sample was transferred to the NaOH trap by aeration with Nitrogen gas at a flow rate of 0.5L/min.; after 45 minutes aeration, the NaOH bottle was removed and 1ml of 0.04M picric acid was added. The solution was shaken, allowed to stand for 10-15 minutes in the dark and measured spectrophotometrically at 515nm. Hydrogen cyanide concentration was extrapolated from a hydrogen cyanide standard curve. Triplicate analysis was done.

Statistical Analysis

Data were represented as mean ± S.E.M. Results obtained were analyzed using students t-test. The value of $P < 0.05$ was considered as significant.

RESULTS

The levels of cyanide obtained from all the samples are presented in Table 1.0

A range of 0.32 ±0.017- 0.40 ± 0.019 µmol/g (8.88±0.46 – 10.80± 0.051 mg/g) for cigarette; 0.42 ± 0.017 – 0.47 ±

Table 1.0: Cyanide Levels of Some Tobacco Products

Sample	BH	LB	RM	SM	MSI	MS2	MS3	DS1	DS2	DS3	FS
Cyanide Levels (µmol/g)	0.40 ± .019[a]	0.32 ± 0.017[a]	0.35 ± 0.015[a]	0.35 ± 0.030[a]	0.42 ± 0.017[a]	0.44 ± 0.100[ab]	0.47 ± 0.020[ab]	0.42 ± 0.017[a]	0.40 ± 0.010[ac]	0.45 ± 0.010[ac]	0.40 ± 0.020

Remarks: Values represents mean ± S.E.M. of triplicate analysis of each sample.

a = Significant values of $P < 0.05$ when FS was compared with each sample.

b = Significant values of $P < 0.05$ when MSI was compared with MS2 and MS3

c = Significant values of $P < 0.05$ when DSI was compared with DS2 and DS3.

0.020 µmol/g (11.34 ± 0.46 –12.69 ± mg/g) for moist snuff and 0.40 ± 0.01 –0.45 ± 0.01 µmol/g (10.80± 0.27 – 12.15 ±0.27 mg/g) for dry snuff was obtained. At $P < 0.05$, all the sample showed significant difference compared with the control. When MSI was compared with MS2 and MS3, a significant difference ($P<0.05$) was observed with both MS2 and MS3 i.e increasing the moisture in snuff increased the cyanide content. Thus MSI with lower content of moisture gave cyanide level of 0.42 ± 0.017 µmol/g while MS3 with a higher moisture content gave cyanide level of 0.47 ± 0.02 µmol/g. Also dry snuff showed low levels (0.42±0.017-0.45 ± 0.010 µmol/g) of cyanide than moist snuff (0.42 ± 0.017 – 0.47 ± 0.020 µmol/g).

Potassium sesquicarbonate increased cyanide content from a level of 0.42 ± 0.017 to 0.45 ± 0.010 (i.e 11.34 ± 0.46 – 12.15 ± 0.54 mg/g). A significant difference occurred at $P < 0.05$ as level of potassium sequicarbonate was increased.

Generally, the result showed a low level of cyanide in western cigarette than in local preparations whether moist or dry.

DISCUSSION

The results obtained thus far from this work indicate a high cyanide level in crude preparations of tobacco products like snuff and fresh leaves than in western products like cigarette. This is in line with the findings of Pakhale *et al.* (1990) who reported that locally manufactured Indian cigarettes contain more cyanide in the mainstream smoke than in western products. Cyanide level of Indian local cigarette was 688 – 904 microgram/gram (25.58-33.48 mg/g) while a level of 366 – 638 microgram/gram (13.56-23.63mg/g) was found in western cigarettes.

The cyanide content of tobacco produced therefore depends on the manufacturer's specification. Debethizy and Borgerding (1990) showed that when tobacco is manufactured through heating rather than burning, the tar, hydrogen cyanide , nicotine, carbon monoxide, phenolic compounds, benzo (a) pyrene, and nitrosamine contents were reduced with degrees of reduction ranging from 10-100 folds.

The cyanide content of cigarette relates to other toxic components and thus cyanide levels found in this work is an indication of the amount of these toxic components. Woodward and Tunstall-Pedoe, (1992) found a correlationship between tar content and cyanide level while Vogt *et al.* (1977) found a correlationship between cyanide level and carbon monoxide. He found a correlation coefficient of 0.571 between them.

Brunnemann *et al.* (1977), observed that there is a relationship between the amount of cyanide in unburnt and the smoke of the burnt cigarette, which also depends on the composition of the cigarette. These workers reported a cyanide level of 395, 160 and 520 µg/g in cigarette, cigar and little cigar respectively. Hence, the amount of cyanide found in the tobacco products in this study could be an index of hydrogen cyanide, tar and carbon monoxide in the smoke when these products are completely burnt.

In smokeless tobacco, much report on cyanide levels is lacking. However, Holiday *et al.*, 1995 used thiocyanate level to differentiate between exclusive smokers and smokeless tobacco users. They found a higher value of 13.915 ± 63.7mg/L for exclusive smokers and 11.52 ± 16.9 mg/L for smokeless users. In the present study, the cyanide level in the western cigarettes (8.88 ± 0.46 – 10.80 ± 0.51 mg/g) is lower than those found in the smokeless tobacco i.e moist snuff of 11.34 ± 0.46 – 12.69 ± 0.54 mg/g. The method of preparation therefore must have a tendency of reducing the cyanide level in western products than in local products. The fact that moist snuff has a high cyanide and possibly also higher carcinogen level was confirmed by Tomar and Henningfield (1995).

Potassium sesquicarbonate is always added to local snuffs. There is a local believe that this salt increases the nicotine assimilation because of its alkalinity. This should not be encouraged since the cyanide level was observed in this work to be increased as the concentration of the salt is increased.

The lethal dose of cyanide is believed to be about 60mg per day in adult man (Oyenuga and Amazigo, 1957). This was confirmed by Seiger, 1975 who reported that the half lethal dose of cyanide was 30 – 35mg. Exposure to approximately 0.5mg/L hydrogen cyanide for 4 weeks caused slight endothelial oedema in the aoetic intimal tissue of rabbits (Hugod and Astrup, 1981). The air concentrations of HCN exposure was calculated to correlate to the human smoking condition. They found out that a rabbit inhaling 0.5mg/L of HCN per hour per kilogram body weight is equivalent to the amount a human will inhale after smoking three to six sticks of cigarette in an hour.

The findings of this work suggest that people should reduce the rate of both cigarette and smokeless tobacco use. If 0.5mg/dl of cyanide caused damage to the heart, then 8.9 – 125mg/g for both smoked and smokeless tobacco products studied in this work should be considered a toxic level. Another report showed that 200mg/L of HCN in rat reduced the specific activity of ATPase by 50% when compared with control (Odenuga and Adenuga, 1997) while 108-216 mg HCN induced reductions of both synaptic efficacy and postsynaptic excitability. (Zhu and Krujevic, 1997).

In conclusion therefore, the cyanide levels of all the tobacco products estimated is considered high. Snuff, whether moist or dry may pose a danger to health. Potassium sesquicarbonate does not reduce cyanide concentration. Thus, it's a very dangerous component of tobacco, since it increases not only nicotine but also cyanide.

REFERENCE:

Achinewhu, S. C., Barber, L. I and Ijeoma, I. O., 1998. Physicochemical properties and garification of selected Cassava cultivars in Rivers State. Nigeria, Plant Foods Human Nutr 52(2):133-140

Bartecchi, C. E:, Mackenzie, T. D. and Schier, R. W. 1995. The global tobacco epidemic. Scientific. Am. 272(5) : 44-51.

Brunemann, K. D., YuL and Hoffmann, D., 1977. Cyanide level present in Tobacco smoke. J. Anal. Toxicol:1,38.

Chavez, J. C., Pichiule , P. Haxhiu M.A. and Lamanna J. C., 1998. Local Injection of NaCN into the nucleus locus ceruleus of the rat brain induces respiratory depression. Adv. Expt. Med. Biol. 454:481-465.

Costagliola, C, Cotticelli, M and Menzione, M. (1990). Red cell reduced glutathione and tobacco smoke induced optic neuropathy. Metab. Pediatr. Syst. Ophth. 13(2-4): 96-98.

Debethizy, J. D. and Borgerding, M. F., 1990. Chemical and Biological Studies of Cigarette and tobacco. J. Clin. Pharmacology 30(8): 755-763.

Glantz, S. A., Barnes, D. E., Bero, L., Hanura, P., and Slade, P., 1995. Looking through the keyhole of tobacco industry. JAMA , 274(3): 219-224

Girand, D. W., Martin, H. D. and Driskell, 1995. Plasma and dietary vitamin C and E levels of tobacco chewers, smokers and nonusers. J. AM. Diet. Assoc. 95(7): 798-800.

Greenhalgh, R. A., 1981. Smoking and Arterial Disease. Pitman press. Bath. Great Britain.

Holiday, D. B., McLarty, J. W., Yanagihara, R. H., Riley; I. and Shephard, S. B., 1995. Two Biochemical makers effectively used to separate smokeless tobacco users from smokers and nonusers. South Med. J. 88(11): 1107-1113.

Hugh, J. W., 1979. Estimation of hydrogen cyanide released from Cassava by organic solvents. Expt. Agric 15: 395-399.

Hugod, C. and Astrup P., 1981. Studies of Coronary and Aortic intimal morphology in rabbits, exposed to gas phase components. In smoking and arterial disease Edited by Greenhalzh, R. M. (1981). Pitman press. Great Britain. P. 89-94.

Johnson, W. R. and Kang, J. C., 1971. Pyrolsis of organic Materials. J. Org. chem.. 36, 189.

Kagawa, J., Nakadate, T. and Ishihara – Y., 1990. Constituents of Tobacco smoke and their biological effects. Kokyu To. Junken. 38(1): 11-16.

Lundquist, D., Hans, R. and Sorbo, B., 1985. Determination of Cyanide in whole blood crythrocytes and plasma. Clin. Chem. 31(4): 591-595.

Martin – Bermudez. R. and Maestre – Romero, A ,1997. Venous blood arteriolization and multiple organ failure after cyanide poisoning. 23 (12): 1286.

Odenuga, O. O. and Adenuga, G. A., 1997. Sodium Nitrate alone protects the brain Ca^{2+} - ATPase against potassium cyanide induced neurotoxicity in rats. Biosci. Rep. 17 (6): 543-546.

Oku, H., Fukushima, K., Miyata, M., Wakakura, M. and Ishikawa, S., 1991. Cyanide with vitamin B12 deficiency as the course of experimental tobacco amblyopia Nippon-Ganka –Gakkai – Zasshi, 95(2): 158-164.

Oyenuga, V. A. and Amazigo, E. O., 1957. A note on the hydrocynic acid content of Cassava. W. Afri. J. Biol. Appl. Chem. 1:39-43.

Padwell, A., 1997. Cyanide Poisoning - case studies of one homicide and two suicides. Clin. Chem. 43(9): 1595-1600.

Pakhale, S.S., Jayant, K. and Bhide, S. U., 1990. Chemical analysis of smoke of Indian cigarettes, bidis and other indigenous forms of smoking –levels of hydrogen cyanide and benzo (a) pyrene. Indian J. Chest. Dis. Allied. Sci. 32 (2): 75-81.

Passmore, R. and Robson, J. S., 1980. A. companion to medical studies, Vol. 2, 2nd Coln., Blackwell Sci. Pub. Pp. 40-49.

Perez – Trullen, A., 1995. The contents of tobacco smoke. Pharmacology of nicotine. Arch. Brnceneumol; 31(3): 101-108.

Philbrick, O. J., Hill, C. C. and Alexander, J. C., 1971. Physiological and biochemical changes associated with Linamarin administration to rats. Toxicol. Appl. Pharmacology. 42: 539-551.

Philips, G. F. and Walker, R. E., 1991. Yields of tar and other smoke components from U.K. cigarettes. Food Chem. Toxicol. 28(7): 469 –471.

Rawbone, R. G., 1981. The value of non-invasive methods for evaluating exposure to tobacco smoke. In smoking and Arterial Disease Pitman press. Great Britain by Greenhalgh, R. M. Page 64-73.

Repace, J. C., 1995. Tobacco, history, and the AMA comment. Lancet 346(8970): 261.

Seigler, D. S., 1975. Isolation and Characterization of naturally occurring Cyanogenic Compounds. Phytochemistry 14: 9.

Suchard, J. R., Wallace, K. L., and Gerkin, R. D., 1998. Acute cyanide toxicity caused by apricot kernel ingestion. Ann Emerg-Med. 32(6): 742-744.

Tomar, S. L. and Henningfield, J. E., 1995. Additional evidence implicating moist snuff as a potent carcinogen. J. Natl. cancer Inst. 87 (24): 1824.

Tucker, K. and Hedges, T. R., 1993. Food shortages and an epidemic of optic and peripheral neuropathy in Cuba. Nutr. Rev. 51(12): 349-357.

Uwakwe, A. A.; Monanu, M. O. and Anosike, E. O., 1991. Whole blood cyanide levels of mainly dietary origin. Plant foods for Human Nutrition 41: 117-124.

Vasey, C. J., 1981. Thiocyanate and Cigarette consumption in smoking and Arterial Disease Edited by Greenhalzh, R. M. (1981). Pitman Press Great Britain. Pp. 107-117.

Vogt., T. M., Selvin, S., Widdowson, G. and Hulley, S. B.,1977. Gas phase markers of tobacco smoke exposure. Amer. J. Public Health, 67, 545.

Witschi, H.; Pinkerton, K. E.; Coggins, C. R., Peen, A. and Gori, G. B., 1995. Environmental facts and societal issues. Fundam. Appl. Toxicol. 24(1): 3-12.

Woodward, M. and Tunstall – Pedoe, H., 1992. Do smokers of lower tar cigarettes consume lower amounts of smoke components? Bar. J. Addict. 87(6): 921-928.

Zhu, P. J. and Krujevic – K. 1997. Adenosine release mediates cyanide – induced suppression of CA1 neuronal Activity. J. Neurosci 17(7): 2355-2364.