Human health hazards of poly aromatic hydrocarbons in Nigerian smokeless tobacco

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A B S T R A C T

Recently we investigated the heavy metal hazards of Nigerian smokeless tobacco products ‘STP’. Since ‘STPs’ are advocated as safer alternatives to cigarettes, the public health implication should be ascertained. This is a risk assessment of poly aromatic hydrocarbons ‘PAHs’ in ‘STPs’ used in Nigeria. Thirty ‘STPs’ from different parts (South East, South West, Niger Delta and North Central) of Nigeria were studied. The 15 PAHs were assayed using gas chromatographic system (6890 series and 6890 plus) equipped with a quadrupole Mass Spectrometer (Agilent 5975 MSD) after ultrasonic extraction of the ‘STPs’ and clean up of the extract. Toxicity equivalent of benzo[a]pyrene concentration (μg/kg) in ‘STPs’ were determined. The daily exposure and the cancer risk associated with exposure to STP were calculated.

Sample A1 (south east) had the highest concentration of PAH 225.84 μg/kg, while sample A3 (North central) had the least PAH concentration of 1.09 μg/kg. ‘STPs’ from South East showed highest levels of PAHs. The total B[a]P TEQ of the ‘STPs’ from the South East ranged from 0.24 to 29.23, South West ranged from 0.94 to 14.55, Niger Delta ranged from 2.28 to 22.88, and North Central ranged from 0.11 to 9.47. The calculated risk estimates for ‘STPs’ from the South East ranged from 5.43 E-05 to 4.50 E-07, South West 2.70 E-05 to 1.74 E-06, Niger Delta 4.30 E-05 to 4.20 E-06, and North Central 1.75 E-05 to 2.08 E-07. Although the calculated risk estimates seem to be within or below the the U.S. EPA cancer risk range of 1 × 10−6–1 × 10−6, the total B[a]P TEQ of the STPs suggest a more indepth risk assessment in animal model to ascertain the safety of PAHs in Nigerian ‘STPs’.

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1. Introduction

Investigation of harmful agents contained in smokeless tobacco is an important step toward the reduction of its adverse public health effects in man. In 2012, the US Food and Drug Administration (FDA) established a list of 93 “harmful and potentially harmful constituents” (HPHCs) for tobacco products [12]. The smokeless tobacco products ‘STPs’ include a variety of tobacco products intended for oral or nasal use. The International Agency for Research on Cancer (IARC) lists 28 carcinogens present in smokeless tobacco [18]. Oral smokeless tobacco use can lead to precancerous oral lesions and other forms of cancer like oral and pancreatic cancer [18]. Smokeless tobacco is also associated with an increased risk of esophagel cancer [5]. In Nigeria, smokeless tobacco is available in dried, cured, and natural forms. In addition to smoking, it can be placed between the cheek and gum (dipped), or sniffed into the nose as finely powdered snuff.

The poly aromatic hydrocarbons (PAHs) are a large group of organic compounds containing two or more aromatic rings [15] and diverse group of carcinogens arising from the incomplete combustion of organic materials including tobacco. The most potent PAH carcinogens are benzo[a]anthracene, benzo[a]pyrene, and dibenz[a,h]anthracene [8].

Nasal snuff inhalation has been reported to be associated with nasal sinus and nasopharyngeal cancer in some parts of Africa [13]. Smokeless tobacco is used in many parts of Nigeria for various reasons such as pleasure, in treating symptoms like nasal congestion, cough, cold, etc., and as tradition in social gatherings in various quantities without the knowledge of its health effects. Recently, there has been advocacy for the use of smokeless tobacco use in many countries including Nigeria as a safer alternative to
cigaret smoking. This promotion may heighten the use of smokeless tobacco.

Unlike some developed countries most smokeless tobacco products ‘STPs’ in Nigeria are not manufactured using the pasteurized tobacco which is claimed to pose a significantly lower risk of cancer but by fire-cured tobacco, among other technologies. Toxicological risk assessment principles have been applied to tobacco products [14]. The information obtained from such a risk assessment has recently been used to propose reductions in toxicants in tobacco products to the regulatory authorities [7]. This study therefore applies a risk assessment methodology developed by some regulatory bodies like the Agency for Toxic Substances and Disease Registry and US Environmental Protection Agency (USEPA) to estimate the cancer risk associated with exposure to most commonly used ‘STP’ in different parts of Nigeria since at the moment there are no regulations of ‘STPs’ in Nigeria. The results of this study are intended to form the basis for scientific discussion on an appropriate regulatory approach for ‘STPs’ [10].

2. Materials and methods

Using a market basket protocol thirty brands of smokeless tobacco (dry snuff) purchased in August 2012 from different parts of Nigeria (South East, South West, Niger Delta and North Central) were used in the study. The tobacco used in the present study was fire-cured, fermented, and subsequently processed into a dry, powdered form with less than 10% moisture content packaged and sold in small metal containers.

Glass wares were washed thoroughly with hot detergent solution followed by rinsing with purified water and acetone (analytical grade) respectively. These were finally baked in the oven at 100 °C overnight. To avoid contaminations of smokeless tobacco samples, different glass wares and syringes were used for standards and for solutions extracted from samples.

Extraction of poly aromatic hydrocarbons from the smokeless tobacco samples was done with a sonicator (Ultrasonic bath-Elmasonic S40H) in accordance with US SW-846 Method 3550. Two grams of each of the smokeless tobacco samples was extracted with a 50:50 mixture of acetone and methylene chloride spiked with 1 ml of PAH internal standard and shaken thoroughly for proper mixing before placing in an ultrasonic bath. The concentrations of 16 PAH (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene) were analyzed by gas chromatography (gas chromatograph (GC-FID)) with GC recorder interfaced with a HP.

The EPA-16 PAHs determination was conducted at Jaros Inspection Services Ltd., Port Harcourt, Rivers State, Nigeria using Gas Chromatographic System (6890 series and 6890 plus) equipped with a dual detector (FID-ECD), dual column and TriPlus AS auto-sampler with helium carrier gas and a quadrupole Mass Spectrometer (Agilent 5975 MSD) based on USEPA method 8100 [10]. A 2.00 µl of extracts were injected into the GC port set at column conditions: HP-5 crosslinked PH- ME siloxane, length of 30 m, I.D: 0.25 mm, thickness of 1 µm with helium carrier gas set in the spitless, constant flow mode with 1.2 ml/min flow rate. Other GC–MS operating set-up were done according to the instrument’s method development as specified in the operating instruction manual. Identification and quantification of individual PAHs was based on internal calibration standard containing known concentrations of the 16 PAHs (EPA-16). The specificity of the 16 PAHs sought for in the samples was confirmed by the presence of transition ions (quantifier and qualifier) as shown by their retention times which corresponded to those of their respective standards. The measured peak area ratios of precursor to quantifier ion were in close agreement with those of the standards.

The detection limit (LOD), estimated as three times the background noise (IUPAC criterion), was similar for all analyzed compounds and results were less than 0.015 µg/kg dw for all analytes. The blank values of analytical procedure remained always below the quantification limit (LOQ): 0.05 µg/kg dw, estimated as 10 times.

The potential for cancer effects was consequently estimated by calculating the incremental probability that an individual will develop cancer over a lifetime as a result of chronic exposure to a particular substance (that is, above baseline lifetime risk). Recognizing differences in the route of administration between cigarette (inhalation) and STP constituents (oral absorption or ingestion), the equation used for estimating cancer risk has been adapted as follows [4]:

The cancer potency factor (CPF) is the lifetime cancer risk estimated to result from continuous exposure to a substance at a concentration of 1 mg/kg body weight. The lifetime average daily exposure (ADE) is estimated by adjusting the ADE according to adult body weight (assumed to be 70 kg), the number of years of STP use (assumed to be 30 years) and the average lifetime (assumed to be 70 years).

The equation for estimating the lifetime ADE is the following:

$$\text{Incremental lifetime cancer risk} = \text{ADE}_{\text{lifetime}} \times \text{CPF}$$

$$\text{ADE}_{\text{lifetime}} = \frac{\text{ADE} \times \text{Number of years snuffing}}{\text{Average lifetime}}$$

where $\text{ADE}_{\text{lifetime}} =$ lifetime average daily inhalational exposure (mg/kg body weight/day) and CPF = cancer potency factor (mg/kg body weight/day)$^{-1}$. ADE = average daily exposure, CPF = cancer potency factor also called CSF “Cancer Slope Factor”(mg/kg/day)$^{-1}$; 6.1 (mg/kg/day)$^{-1}$ for B(a)P [10], number of years snuffing assumed to be 30 years, and average lifetime = 70 years [4].

As with the assumptions used in previously published assessments for tobacco products [14,22] the above equation is based on the assumption that 100% of the toxicant is transferred and is thus potentially biologically available, as would be typical for any conservative risk assessment calculation.

2.1. Toxic equivalent B[a]P concentration

The concentrations of carcinogenic PAHs, expressed as B[a]P equivalent were calculated using the model developed by [6]

$$\text{TEQ} = \sum (\text{PAH}_i \times \text{TEF}_i)$$

TEQ = toxicity equivalent of a mixture, $\text{TEF}_i$ = toxic equivalency factor, i.e., relative potency (as based on carcinogenicity) to benzo(a) pyrene, PAH$_i$ = concentration of PAH congener $i$, and $\text{TEF}_i$ = toxic equivalent.

2.2. Daily Exposure

Due to the health risk associated with exposure to carcinogenic PAH, in the present study the average daily exposure based on the use of STP was calculated by a modification of the equation ATSDR [1].

$$\text{Dose} = \frac{\text{Concentration} \times \text{Intake Rate} \times \text{Exposure Factor}}{\text{Body weight}}$$

$$\text{Body weight}$$
Dose = estimated exposure dose (mg/kg/day), concentration = contaminant concentration (mg/kg), intake rate = intake rate of STP (assuming 10 pouches per day, 1 g/pouch) 50 g [21], exposure factor = exposure factor (6 times a week per year = 312/365 unitless), conversion factor = conversion factor (10^-6 mg/mg), and body weight = body weight (assumed to be 60 kg).

3. Results

The result for the PAH content of the STPs are shown in Table 1. The STPs are ordered by different regions within the country from which they were collected. All the 30 samples of STPs analyzed had varying degrees of PAH contamination. No sample from the four regions contained all the 15 individual PAHs measured. Sample A1 (South East) had the highest concentration of total PAH contamination of 225.84 µg/kg, while sample A3 (North Central) had the lowest total PAH concentration of 1.09 µg/kg. STP samples collected from south east showed higher levels of total PAH contamination than the samples from other regions. The highest concentration across the four different regions occurred in A1(South East), A21(South West), A17(South South), and A6(North Central) with 225.84, 40.53, 58.23, and 28.6 µg/kg, respectively (Table 1).

The toxicity equivalent (TEQ) of benzo[a]pyrene concentration(µg/kg) in smokeless tobacco product from different regions of Nigeria is shown on Table 2. The total B[a]P TEQ of the STP from the South East ranged from 0.24 to 29.23, South West ranged from 0.94 to 14.55, Niger Delta ranged from 2.28 to 22.88 and North Central ranged from 0.11 to 9.47. STP A30 has the highest B[a]P concentrations from the South East of 9.61, whereas the B[a]P concentrations from South West, Niger Delta and North Central were 9.88, 9.35 and 7.4, respectively Table 2.

Table 3 shows the daily exposure concentrations to carcinogenic PAHs in smokeless tobacco products from different parts of Nigeria. Based on the result of the analysis, the cancer risk associated with exposure to STP was calculated and presented in Table 4. The calculated risk estimates for STP from the South East ranged from 4.50 E-07 to 5.43 E-05, South West 1.74 E-06 to 2.70 E-05, South South 4.20 E-06 to 4.30 E-05, and North Central 2.08 E-07 to 1.75 E-05.

4. Discussion

In this study, we have quantified the levels of 15 PAHs and carried out a detailed risk assessment of a wide range of smokeless tobaccos used in Nigeria. The present study may be one of the most extensive surveys of PAHs in STPs published to date from sub Sahara Africa. The PAHs marked as carcinogenic by the Scientific Committee on Food (SCF), for which further investigation of the relative levels in consumables is required are: benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene chrysene,cyclopenta[c,d]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]-pyrene, dibenzo[a,l]pyrene, dibenzo[a,f]pyrene, indeno[1,2,3-cd]pyrene, 5 benzo[j]fluoranthene, cyclopenta[c,d]pyrene, 5-methylchrysene [11] This study has
shown measurable levels of some of these PAHs. Some PAHs are both carcinogenic and mutagenic. It is however feared that, these category of PAHs that have not been found to be carcinogenic may be active as synergists that increase the carcinogenicity of other PAHs [19]. Human exposure to single PAHs does not occur since PAHs are always encountered as complex mixtures. This peculiarity of exposure to complex mixture of varying composition constitute the challenge in human risk assessment.

Although not all PAHs are considered carcinogenic (e.g., pyrene), the U.S. EPA has confirmed that the following benzo(a)anthracene, benzo(a) pyrene, benzo(b) fluoranthene, benzo(k) fluoranthene, chrysene, dibenz(a,h)anthracene, and indeno[1,2,3-c,d]pyrene are probable human carcinogens [2]. Similarly the National Toxicology Program (NTP) of the United States Public Health Service opined that 15 individual PAHs to be “reasonably anticipated to be human carcinogens” [20]. Also the International Agency for Research on Cancer (IARC) upgraded its overall evaluation of benzo(a) pyrene to Group 1 (carcinogenic to humans) in 2010 [17]. Benzo[a]pyrene presence in smokeless tobaccos has been a focus of concern in public health as a result of some recent surveys [24,26]. A single PAH (e.g., benzo[a]-pyrene) may not always be used as a surrogate for all PAHs in drawing conclusions for the other PAHs, especially those with only two or three aromatic rings [9]. Benzo[a]pyrene is just one of many carcinogenic PAHs in smokeless tobacco. Moreover, some PAHs act as promoters for the carcinogenicity of other PAHs, without necessarily being carcinogenic themselves [16]. A more accurate risk assessment of other PAHs in smokeless tobacco will help evaluate the potential public health concerns in users of smokeless tobacco. Although benzo[a]pyrene can and has been used as a broad marker for overall PAH content, more accurate evaluations can be obtained with expanded analysis for each of the individual PAHs [9]. Notwithstanding, STP samples from various part of Nigeria contain worrisome levels of B[a]P. The cancer potency in the samples varied substantially and the concentrations ranges from 0.11 to 29.23 μg/kg. The high TEF value observed in sample A1 may be due to the high total individual PAH concentration. Sample A3 which has the lowest B[a]P/TQ value was observed to have only few individual carcinogenic PAH contributing to the total PAH concentration. The varying concentrations of B[a]P/TQ equivalent is due to the carcinogenic PAH contribution to total PAH concentration. Fire curing of tobacco may explain the high levels of BaP (a product of combustion) in some of the STPs [23,25]. The present study suggests that BaP concentrations of some of the STP were at levels that do meet the criteria for an ‘acceptable’ cancer risk. BaP is an avoidable constituent of STPs, therefore it should be eliminated starting with an enforceable regulatory limit as suggested by the WHO’s tobacco regulation study group [27].

The maximum value from the South East is 5.43 E-05, but remained within the range advocated by USEPA (E-06–E-04). The maximum value of cancer risk calculated for south west, south south and North Central all fell within the USEPA risk limit. The minimum risk limit of South East and North Central (4.50 E-07 and 2.08 E-07) were either within or below the benchmark for acceptable risk by USEPA. Excess cancer risk is expressed as a portion of the population that may be affected by a carcinogen during a lifetime of exposure. An estimated risk of 1 x 10^-6 predicts the probability of one additional cancer, over background, in an exposed population of one million. The estimated cancer risk from exposure to PAHs

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**Table 2**

Toxicity equivalent quotient (TEQ) of benzo[a]pyrene concentration (μg/kg) in smokeless tobacco product carcinogenic PAHs.

| Region       | B[a]P | B[a]A | B[a]+k| CHRY | B[a],k|TEQ |
|--------------|------|------|-------|------|------|-----|
| **South East** |      |      |       |      |      |     |
| A1           | 5.62 | 0.69 | 1.74  | 0.12 | 19.69| 1.36|
| A2           | ND   | ND   | ND    | ND   | 4.01 | ND  |
| A4           | ND   | 1.7  | 1.2   | 0.08 | ND   | ND  |
| A8           | ND   | ND   | 0.19  | 0.02 | 2.44 | ND  |
| A9           | 2.27 | ND   | 0.55  | 0.053| ND   | ND  |
| A12          | ND   | 0.17 | 0.75  | 0.05 | ND   | 0.97|
| A14          | 5.10 | 1.52 | 1.7   | ND   | 13.1 | 1.5 |
| A18          | 1.91 | 0.57 | 0.6   | 0.05 | 2.6  | 0.39|
| A20          | 5.91 | 1.52 | 0.75  | 0.02 | 1.23 | 0.47|
| A22          | 4.21 | 0.63 | 1.16  | 0.03 | 3.73 | 0.72|
| A25          | ND   | 0.32 | 0.32  | ND   | 4.43 | 0.42|
| A27          | ND   | ND   | 0.15  | 0.08 | 0.01 | ND  |
| A28          | 0.99 | 0.05 | 0.74  | 0.06 | 1.17 | 0.15|
| A29          | 0.94 | 0.17 | 0.07  | 0.003| 2.77 | 0.23|
| A30          | 9.61 | 0.06 | 0.93  | 0.12 | 6.98 | 0.41|
| **South West** |      |      |       |      |      |     |
| A5           | 0.68 | ND   | 0.41  | 0.014| ND   | ND  |
| A7           | 3.96 | ND   | 0.04  | 0.052| ND   | ND  |
| A10          | ND   | ND   | ND    | 0.063| 0.65 | 0.23|
| A11          | 5.13 | ND   | 0.003 | ND   | ND   | ND  |
| A21          | 9.88 | ND   | 0.15  | 0.013| 3.91 | 0.57|
| A23          | 9.1  | ND   | ND    | 0.09 | 2.01 | ND  |
| **South South** |      |      |       |      |      |     |
| A15          | 4.32 | ND   | 0.10  | 0.001| ND   | ND  |
| A16          | 0.73 | 0.25 | 0.28  | 0.043| ND   | 0.98|
| A17          | ND   | ND   | 0.21  | ND   | 22.67| ND  |
| A24          | 1.64 | 0.52 | 0.33  | 0.003| 2.77 | 0.92|
| A26          | 9.35 | 0.01 | 0.25  | 0.008| 6.37 | 0.23|
| **North Central** |      |      |       |      |      |     |
| A3           | ND   | ND   | ND    | ND   | 0.01 | 0.102|
| A6           | 7.4  | ND   | ND    | 0.002| 1.99 | 0.17|

TEF: PAHs toxic equivalency factor with respect to B[a]P (Nisbet and LaGoy, 1992).
Table 3
Daily exposure concentration to carcinogenic PAH from smokeless tobacco products.

| Samples       | South East | South West | Niger Delta | North Central |
|---------------|------------|------------|-------------|---------------|
| Samples       |            |            |             |               |
| South East    |            |            |             |               |
| A1            | 2.08 E-05  | 2.86 E-06  | 4.30 E-05   | 1.75 E-05     |
| A2            | 2.86 E-06  | 3.28 E-07  | 4.20 E-06   | 2.08 E-07     |
| A4            | 2.12 E-06  | 2.19 E-06  | 1.93 E-05   | 8.90 E-06     |
| South West    | 7.84 E-07  | 2.92 E-06  |             |               |
| A5            | 7.84 E-07  | 2.92 E-06  |             |               |
| A10           | 6.69 E-07  | 1.71 E-07  |             |               |
| A11           | 3.65 E-06  | 1.04 E-05  |             |               |
| A23           | 7.98 E-06  | 1.14 E-05  |             |               |
| South South   | 3.15 E-06  | 1.62 E-06  |             |               |
| A15           | 3.15 E-06  | 1.62 E-06  |             |               |
| A16           | 1.63 E-05  | 4.40 E-06  |             |               |
| A24           | 1.14 E-05  | 4.40 E-06  |             |               |
| A26           |             | 1.14 E-05  |             |               |
| North Central | 7.84 E-08  | 6.75 E-06  |             |               |
| A3            | 7.84 E-08  | 6.75 E-06  |             |               |
| A6            |             |            |             |               |

Table 4
Cancer risk resulting from exposure to smokeless tobacco products in Nigeria.

| Range       | South East | South West | Niger Delta | North Central |
|-------------|------------|------------|-------------|---------------|
| Max         | 5.43 E-05  | 2.70 E-05  | 4.30 E-05   | 1.75 E-05     |
| Min         | 4.50 E-07  | 1.74 E-06  | 4.20 E-06   | 2.08 E-07     |
| Mean        | 1.40 E-05  | 1.14 E-05  | 1.93 E-05   | 8.90 E-06     |

arising from use of STP were calculated to be within or below the U.S. EPA cancer risk range of $1 \times 10^{-4}$–$1 \times 10^{-6}$ [3].

4.1. Study limitations

The limited number of ‘STPs’ and ‘STP’ constituents included in the calculations in this investigation is considered conservative estimates of actual cancer risk. The true bioavailability of PAHs in ‘STPs’ may have been underestimated or overestimated, given that bioavailability may differ by compound and that, over time, users may increase the intensity of use and correspondingly increase their exposure to these constituents.

5. Conclusions

The total B[e]P TEQ of the STPs in this study is of public health concern. Although this study suggests addressing the safety of many smokeless tobaccos in the Nigerian market, we recommend that further indepth risk assessments of these smokeless tobaccos in vivo.