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Risk Assessment Article

Distribution, Levels, and Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Singed Cattle Hide

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ABSTRACT

Human beings are exposed to polycyclic aromatic hydrocarbons (PAHs) from various occupational, environmental, and dietary sources. The study was carried out in the Cape Coast Metropolis of Ghana to assess the levels of PAHs in treated and untreated cattle hide and the associated health risks thereof. Treated cattle hide (wele) is one of the most well-patronized meat products in Ghana. A total of 90, treated (n = 36), untreated (n = 36), and control (n = 18) cattle hide samples were treated and analyzed using a gas chromatography flame ionization detection (GC/FID) technique. The total PAH concentration in the treated cattle hide ranged from 5.9 μg/kg naphthalene to 719.9 μg/kg benzo[b]fluoranthene. The total PAHs in untreated hide ranged from 57.6 μg/kg naphthalene to 19840.9 μg/kg benzo[b]fluoranthene. The amount of PAHs in the control hide, however, ranged from non-detectable for many of the PAHs to 0.5 μg/kg for fluorene. The carcinogenic risk value associated with the consumption of treated hide in children ranged between 1.0×10^{-3} and 9.4×10^{-3} whereas that of adults ranged between 1.9×10^{-4} and 2.1×10^{-5}. This implies that the continuous consumption of heavily burnt cattle hide may not exempt the consumers from all the possible health cases associated with PAHs.

Key Words: PAHs, singed, cattle hide, risk assessment, wele, worn-out lorry tires.

INTRODUCTION

Treated cattle hide known in Ghana as "wele" is one of the most well-patronized meat products in Ghana. It is prepared for consumption by burning the fresh hide in

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a naked sooty flame of worn-out lorry tires (Essumang *et al*. 2007; Obiri-Danso *et al*. 2008). The burning of the hide is done under uncontrolled fires (*i.e.,* open fires, not regulated) and lacks legislative measures, which is typical for poor households in developing countries like Ghana. The greatest danger posed by the use of the lorry tire is the possibility of a catastrophic fire occurring. Because of the large quantities of petroleum and other chemicals in tires, a burning tire creates thick, black, toxic smoke as well as large discharges of environmental pollutants. Many potential negative environmental and health impacts are normally associated with the burning of tires (Obiri-Danso *et al*. 2008). The burning process may allow the accumulation of compounds present in the soot such as Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated dibenzodioxins, dibenzofurans (PCDDs/Fs) and heavy metals into the edible portions of the meat that may eventually get into the human system when consumed (Essumang *et al*. 2007). The burnt cattle hide is treated (processed) by washing it in water and tenderizing with sodium carbonate or sodium bicarbonate referred to in Ghana as "kanwu." Even though the burnt hide is thoroughly washed before it is displayed on the market for sale, the colour of the consumable hide is completely changed by the soot into pale brown with several black spots.

Contamination of the environment by PAHs is becoming a rising environmental concern. PAHs have a widespread distribution in the environment and the carcinogenicity and mutagenicity of several of these compounds have been proved (Simko 2002; Korenga *et al*. 2001). PAHs have been ranked the seventh most threatening compounds to human health (King *et al*. 2002; ATSDR 2007).

Human exposure to PAHs may come from a wide variety of sources, including occupation (working with coke ovens or in an iron foundry), environment (air pollutants, drinking water, personal habits, *e.g.,* cigarette smoking), medical treatment (coal tar), and diet (broiled and smoked foods) (ATSDR 1995; Lioy *et al*. 1988; WHO 1984; IARC 1983). Humans who are not exposed to PAHs occupationally tend to be exposed to other main PAH sources such as from consumption of meat either grilled or charred as well as PAH-contaminated cereals, flour, bread, and vegetables (Grova *et al*. 2006).

PAHs are known as highly stable contaminants present in many foods (difficult to be broken down in food) (Chung *et al*. 2002). Cooking meat or other foods at high temperatures, which happens during grilling or charring may increase the amount of PAHs in the food. Exposure of meat products directly to smoke could result in higher concentration of PAHs compared to indirect methods in which the smoke used is regulated such as in Liquid Smoke Flavouring where PAHs are partially eliminated by condensation of tars (Roda *et al*. 1999). The highest concentration of PAHs was observed in smoked products immediately after the smoking (Simko 1991) while a decrease in PAHs was observed due to light decomposition and interaction with the compounds present (Simko *et al*. 1991). The adsorbed PAHs on smoked meat products may penetrate into the products where they are protected from light and oxygen, and the concentration then stabilizes after a period of time (Simko *et al*. 1992).

In Ghana, waste (used) lorry tires are used as fuel to remove the hairs from cattle hide (cow hide) at a very high temperature. In view of the fact that tires contain many potentially harmful substances (USFA 1999), singed treatment with scrap tires imposes enormous risk of deposition of toxic elements and compounds into the animal hide, which could significantly compromise meat quality. In this case,

continuous consumption of such potentially contaminated meat product poses a great source of health risk (Costa 2000; Obiri-Danso *et al* 2008). However, other studies have confirmed the presence of PAHs in smoked meat products using wood but that of using waste lorry tires has not been done, hence the need for the study (Djinovic *et al*. 2008; Stumpe-Vīksna *et al*. 2008).

The purpose of this study therefore was to estimate the levels and the distribution of PAHs in processed and unprocessed cattle hide available on the Ghanaian market and to use the results to quantify the toxicological effect of PAHs through risk assessment of cattle hide consumed in Ghana.

MATERIALS AND METHOD

Sample Collection and Preparation

The cattle hides were sampled from the main slaughter house in the Cape Coast metropolis of Ghana. A total of six (n = 6) pieces of each samples (*i.e.,* treated, untreated, and control) were collected from the slaughter house on a weekly basis for two months (*i.e.,* between December 2007 and February 2008) except the control, which was collected every two weeks. Samples were wrapped in aluminium foil and sent to the laboratory in a black aluminium container. In all, 90 samples were collected for the study comprising 36 treated hide samples (the washed hide that is on sale in our markets), 36 untreated hide samples (freshly burnt hide before washing), and 18 control hide samples (hide, freshly removed from the cow before burning)

The treated cattle hide samples (n = 36), control (n = 18), and untreated (unwashed burnt cattle hide) (n = 36) were sent to the laboratory and were unwrapped from the foil, weighed, and dried on flat aluminium foil in a laboratory oven. The treated hide samples (burnt and washed cattle hide) were dried at 105°C for 8 h a day for two days while the control (fresh hide) and the untreated hide samples were dried at 105°C for 8 h a day for three days due to its oil content (hides with high oil content takes a longer period to dry). The dried samples were cooled for an hour under room temperature, crushed in a porcelain mortar and milled into powder using a laboratory multipurpose miller (Polymix KCH-Universalmühle M20). The homogenized samples were sieved through 600 μm mesh and kept in glass sample bottles for extraction. Dichloromethane was the main solvent used for the extraction of PAHs from the cattle hide samples. The solvents were of analytical grade and those that were not of analytical grade were distilled in glass before use.

Extraction of PAHs from Cattle Hide

The extraction procedure employed for samples in this work is a modification of the method described by Chen and Lin (1997). The modifications had to do with the mode and time of hydrolysis, in this case the reagent [methanolic–potassium hydroxide solution (200:25 v/v)] was added and allowed to stand for about 5 min before the extraction as against the continuous extraction as described by Chen and Lin (1997).

Extraction with Soxtec Unit

About 10 g of each of the homogenized cattle hide samples were weighed and transferred into a 100 mL volumetric flask. Alkaline solution was prepared by dissolving 50 g of potassium hydroxide pellets in a 100 mL volumetric flask and making it to the mark with distilled water (50% alkaline solution). A methanolic–potassium hydroxide solution (200:25 v/v) was then prepared by mixing 200 mL of methanol with 25 mL of the 50% potassium hydroxide solution (8:1 v/v). Addition of this alkaline solution to the meat product was intended to help optimize the removal of accumulated PAHs contained in the lipophilic tissues of the meat through saponification.

Approximately 10 mL of the prepared methanolic–potassium hydroxide solutions (200:25v/v) was added to each of the 10 g samples in the flask, the content was then stirred and allowed to stand for 2 min. The contents of the flasks were quantitatively transferred into Soxhlet extraction thimbles (24.5 mm × 26.0 mm × 60.0 mm) and extracted using a Multiple Soxhlet Extraction unit (SOXTEC SYSTEM HT 1043) for 45 min. The above extraction procedure was repeated for each of the residual samples with another 30 mL of dichloromethane for 30 min. The two extracts were combined in the same flask and concentrated at 30°C to a volume of 2 mL using a rotary evaporator (Rotavapor R-114, BUCHI Water bath B-480).

Alkaline Mixture Separation by Separatory Funnel

About 50 mL of the alkaline concentrated extract mixture was quickly transferred into a 500 mL separatory funnel containing 100 mL of distilled water. The flask was rinsed with 10 mL methanol and twice with 10 mL each of dichloromethane and added to the content of the separatory funnel. This was followed by the addition of 50 mL dichloromethane to make it up to a total of 100 mL volume of dichloromethane-methanol in the separatory funnel. The separatory funnel was then shaken vigorously and allowed to stand for two hours for separation of aqueous and organic layers. The aqueous layer was extracted once with 20 mL of the dichloromethane. The dichloromethane extracts were combined, washed twice with 100 mL distilled water and dried over anhydrous sodium sulphate. The dried extract was poured into a 250 mL flask, concentrated to 2 mL at 30°C using a rotary evaporator (Rotavapor R-114, BUCHI, and water-bath B-480) and kept in 100 mL volumetric flask fitted with aluminium foil for a clean-up. The 2 mL extract was then dried in a decicator for a clean up.

Clean-Up Procedure

About 0.2 g of each cattle hide dried sample extract was purified by using column chromatography. A glass-fitted with chromatographic column of 1.5 cm diameter and 50 cm high was packed with 20 g of silica-gel to a height of 30 cm. Before loading the column, the silica-gel was activated by heating in a laboratory oven for 2 h at 150°C.

The column was conditioned with 30 mL n-hexane. The cattle hide extracts were dissolved in 5 mL of the dichloromethane and applied onto the top of the silica-gel containing the glass-wool. The first 10 mL collected from the column was discarded. The samples were then eluted with 30 mL dichloromethane. The elution

was repeated with 2 × 25 mL dichloromethane. The samples obtained from the column chromatograph were combined and concentrated using the rotary evaporator to 2 mL at 30°C for analysis by U.S. Environmental Protection Agency (USEPA) Method 8100. The above process was repeated for all 90 cattle hide samples.

Recovery Studies

Two recovery study procedures were conducted to test for the efficiency of the extraction system as well as the GC/FID (Gas Chromatography with flame ionization detector) instrument. The first recovery study involves random spiking of the cattle hide samples with four deuterated surrogate standard solutions (D8-Acenaphthylene, D10-Anthracene, *p*-terphenyl-d14 and D12-Benzo[a] pyrene) and extracted the same way as the non-spiked hide samples. The extracted samples were analyzed and the recoveries calculated from the differences in total amounts of PAH standard spiked and the amount realised after the analysis.

The second recovery study involved the use of PAH certified reference material from the U.S. National Institute of Standards and Technology (NIST). About 2.69 g of the reference sample was measured and subjected to the same extraction procedure as applied to all the cattle hide samples. Recoveries were calculated from the differences in PAH certified concentrations and the concentrations obtained after analysis using GC/FID.

Instrumentation

Levels of PAHs in cattle hide samples were measured using an Agilent 6890N gas chromatograph interfaced with an Agilent 6890N flourimetric detector (FID) operating in a selective split mode. The injection was done manually. A SLB5™-MS fused capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and helium carrier gas at flow rate of 1.5 mL/min were used in the separation. The make-up flow of the helium carrier gas was 20 mL/min, an Air-flow of 300 mL/min and frequency flow of 30 mL/min. The temperature was programmed as follows: oven set-point was 60°C, hold for 2 min, 40°C/min to 170°C, hold for 0.00 min, 10°C/min to 220°C, hold for 0.00 min and 5°C/min to 290 hold for 10 min. Injections of 2 μL were performed in the split mode and the split valve was opened after 2 min. The split ratio was 50:1. Sample peaks were identified based on retention times on target ion chromatograms and in relative abundance of the qualifiers ions selected for each PAH in comparison with PAHs standards.

Calculation of Carcinogenic Risk

Carcinogenic risks are estimated as the incremental probability of an individual developing cancer over a lifetime as a result of exposure to the potential carcinogen. This risk is referred to as the individual excess lifetime cancer risk, IELCR, or just carcinogenic risk. Published values of chemical carcinogenic toxicity (slope factor) are used to calculate risk from the LADD:

$$\text{IELCRij} = \text{SFij LADDij} \tag{1}$$

where IELCRij = individual excess lifetime cancer risk for chemical i, exposure route j [dimensionless], SFij = slope factor for chemical i, exposure route j [mg/kg-d]$^{-1}$, LADDij = lifetime average daily dose for chemical i, exposure route j [mg/kg-d].

This approach to estimating risk is based on the linear low-dose cancer risk model described by the USEPA (1989, 2001), and is considered valid for risks less than 0.01. The model assumes that exposure to any amount of a carcinogen will increase the risk of cancer, that is, there is no safe or threshold dosage. This assumption is fundamentally different from that assumed for non-carcinogens, where a safe "reference dose" exists. Ideally the slope factor used in Eq. (1) should reflect the route of intake (*e.g.*, ingestion, inhalation, or dermal absorption). Unfortunately, toxicological data are not always available for each route (*e.g.*, inhalation data only might be available), and so route-to-route extrapolations must be made. In such cases one sometimes assumes that the slope factor for one unknown intake route is equal to the slope factor for some known route (it is quite common to use the oral slope factor for dermal exposures). Risks are assumed to be additive from multiple chemicals and routes, therefore the total risk is estimated by:

$$\text{IELCRt} = \sum \text{IELCRij} \tag{2}$$

where IELCRt = total individual excess lifetime cancer risk (or, incremental cancer occurrences/individuals exposed).

Calculation of Hazard Index

The potential for non-carcinogenic effects is evaluated by comparing an exposure level greater than the exposure duration (maximum of 70 years) with a reference dose derived for a similar exposure period. This ratio of exposure to toxicity for an individual pathway and chemical is called a hazard quotient. The hazard quotients are usually added across all chemicals and routes to estimate the hazard index. Some, however, will argue that it is more appropriate to only sum the hazard quotients for chemicals that affect the same target organ (*e.g.,* liver or blood).

The non-cancer hazard quotient assumes that there is a level of exposure below, which it is unlikely that even sensitive populations would experience adverse cancer health effects (USEPA 1989, 2001). This reference dose, or *RfD*, is a toxicity value for evaluating noncarcinogenic effects. It has the same units as intake and it is assumed that if the intake is less than the *RfD* (hazard quotient <1) no adverse health affects occur, even if the receptor is exposed to this dose continuously over a lifetime. Two types of *RfDs* are generally used: a subchronic *RfD* for short-term exposures and a chronic *RfD* for long-term exposure. The chemical database in RISC contains the values for chronic *RfDs*. If a subchronic case is being evaluated, it is important to modify the *RfD*. The hazard quotient for an individual chemical and individual route is calculated by:

$$HQij = CADDij/RfDij \tag{3}$$

where $HQij$ = hazard quotient for chemical i, exposure route j[dimensionless], $CADDij$ = chronic daily intake for chemical i, exposure route j[mg/kg-d], $RfDij$ = reference dose for chemical i, exposure route j [mg/kg-d].

The hazard quotients from each chemical and route are then added to obtain the hazard index:

$$HI = \sum \sum HQij \tag{4}$$

where HI = hazard index [dimensionless], HQij = hazard quotient for chemical i, exposure route j [dimensionless].

Statistical Analysis

To understand the PAH contamination with regard to the smoke generated from the waste lorry tires, Levene's Test for equality of variance, correlation studies and regression model analysis of the data were determined using SPSS version 16 software (Tomlinson *et al*. 1980).

RESULTS

Recovery Results

To evaluate the extraction efficiency for the target compounds, recovery studies were carried out using four isotopic PAHs (D8-Acenaphthylene, D10-Anthracene, *p*-terphenyl-d14 and D12-Benzo[a]pyrene). D8-Acenaphthylene-d10 served as a surrogate for four compounds namely, naphthalene, acenaphthylene, acenaphthene, and fluorene. These four compounds have molecular masses close to that of the surrogate (164) and have chemical characteristics similar to that of acenaphthene-d10. D10-Anthracene was used as a surrogate for phenanthrene, anthracene, fluoranthene, and pyrene. Both the molecular masses and structures of these compounds are significantly similar to that of D10-Anthracene. *p*-terphenyl-d14 was used as a surrogate for both chrysene and benzo(a)anthracene. D12-benzo[a]pyrene was used as a surrogate for the six remaining compounds: benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, dibenz(a,h)anthracene, and indeno(1,2,3-cd)pyrene. One similarity that exists among these compounds is the possession of five or six aromatic rings. With this we can propose an approximate recovery for the studied samples as shown in Table 1.

We followed a similar extraction method employed by Fritz (1971), who reported about 80% recovery for B[a]P whilst Grimmer and Hildebrandt (1967) obtained recoveries ranging from 87 to 98% for B[a]P, B[b]P, B[a]A, and chrysene.

The recovery of PAHs for the spiked deuterated surrogate standards in the cattle hide was calculated to range from 71% to 119%. Shown in Table 1 are recovery values of the 16 PAHs contained in the randomly spiked samples. Results obtained in Table 2 do not however suggest that this difference in clean-up and recovery had any major effect on the PAH levels determined.

The second recovery study conducted using the NIST reference material showed high recovery PAH values ranging from 65% to 102% with an average PAH recovery value of 83%. The high values obtained could be used to establish the reliability of the extraction system as well as the efficiency of the GC/FID instrument. In fact, the NIST (SP 1, NIST – 1941B) reference material was used to establish the reliability of the extraction system as well as the elution efficiency of the GC/FID instrument

Table 1. Recovery results for the randomly spiked cattle hide samples with deuterated PAH surrogate standard solutions.

Sample	Detection limit (mg/kg)	Recovery (%)
Naphthalene	0.005	93
Acenaphthene	0.005	101
Acenaphthylene	0.005	114
Fluorene	0.005	94
Anthracene	0.004	96
Pyrene	0.005	85
Fluoranthene	0.005	86
Phenanthrene	0.005	97
Benzo (a) anthracene	0.005	91
Chrysene	0.005	119
Benzo (b) fluoranthene	0.005	75
Benzo (a) pyrene	0.005	83
Indeno (1,2,3 - cd) pyrene	0.005	71
Dibenz (a,h) anthracene	0.005	99
Benzo (g,h,i) perylene	0.005	82

since there was no certified reference material for the sample matrix under study at the time of the analysis.

PAH Concentration in Treated Cattle Hide

Shown in Table 2 are the results obtained for the treated cattle hide samples (samples washed after the burning with worn-out tires). Generally, the average weekly PAH concentration in the treated cattle hide ranged from 0.4 μg/kg (naphthalene) to 83.8 μg/kg (anthracene). However, the average weekly concentration of samples in weeks 1 and 3 showed exceptionally high concentrations for benzo[b]fluoranthene (712.0 μg/kg) and anthracene (259.4 μg/kg,). The full data are shown in Table 2.

In most cases, the total weekly PAHs concentration in the treated cattle hide samples did not show any clear deviation from one another, except for week 1 (WTA) that showed the highest total weekly PAH concentration of 1033.7 μg/kg, and the lowest total weekly PAH value (79.1 μg/kg), came from week 5 (E).

PAH Levels in Treated, Untreated, and Control Cattle Hide

The rationale behind the analysis of the freshly burnt hide (untreated) directly from the slaughter house was to evaluate how much PAHs have been introduced to the fresh hide by way of burning in the sooty flame from the waste lorry tires as well as the pyrolyzed dripping fat and also to estimate how much of this amount has been removed by the treatment process (washing).

The mean PAH concentration in treated, untreated and control cattle hide samples over the 6 weeks period of sample collection is shown in Table 3.

Table 2. Mean PAH concentration in treated cattle hide (μg/kg).

PAH	WTA	WTB	WTC	WTD	WTE	WTF	Average	*SD*	Variance
Naphthalene	1.0	0.4	0.5	0.7	1.4	1.9	0.98	0.60	0.36
Acenaphthene	8.2	3.2	3.5	2.5	1.2	1.5	3.35	2.54	6.47
Acenaphthylene	1.0	1.2	1.5	1.4	0.8	1.8	1.28	0.35	0.12
Anthracene	259.4	25.1	169.8	62.6	38.8	83.9	106.6	90.6	8206.1
Benzo (a) anthracene	9.1	10.5	5.4	5.6	1.1	9.7	6.89	3.55	12.61
Benzo (a) pyrene	16.2	22.4	10.9	34.7	17.5	24.8	21.09	8.27	68.46
Benzo(b) fluoranthene	712.0	1.2	1.6	3.1	1.3	0.7	119.9	290	84114
Benzo (g,h,i) perylene	6.6	2.4	1.4	5.4	1.6	13.2	5.10	4.50	20.26
Benzo (k) fluoranthene	2.5	0.7	nd	5.4	0.7	1.1	1.73	1.98	3.91
Chrysene	4.2	2.0	5.5	5.3	2.2	9.7	4.79	2.83	7.98
Dibenz(a,h) anthracene	4.2	3.5	4.1	5.3	5.1	2.3	4.08	1.13	1.27
Fluoranthene	1.1	1.0	1.3	1.0	1.0	14.2	3.25	5.38	28.93
Fluorene	3.5	4.4	4.5	3.9	4.0	3.1	3.89	0.50	0.25
Indeno(1,2,3-cd) pyrene	1.8	nd	0.2	4.1	0.5	6.4	2.17	2.56	6.57
Phenanthrene	1.1	1.9	1.2	1.1	1.6	1.6	1.41	0.34	0.16
Pyrene	1.9	1.2	2.5	0.7	0.5	26.8	5.58	10.44	108.9

WTA–WTF = Treated cattle hide, A to F = week 1 to week 6, nd = Below Detection of 0.001 μg/kg

Table 3. Mean of means of PAH concentrations of treated, untreated, and control cattle hide samples (μg/kg).

PAH	MW	Mean of treated cattle	Mean of untreated cattle hide	Mean of the control cattle hide
Naphthalene	128	1.0	9.6	nd
Acenaphthene	154	3.4	57.2	0.01
Acenaphthylene	152	1.3	67.0	0.3
Anthracene	178	106.6	3190.2	0.2
Benzo (a) anthracene	228	6.9	393.7	nd
Benzo (a) pyrene	252	21.1	215.7	nd
Benzo(b) fluoranthene	252	120.0	3306.8	nd
Benzo (g,h,i) perylene	276	5.1	40.8	nd
Benzo (k) fluoranthene	252	1.7	34.5	nd
Chrysene	228	4.8	174.3	nd
Dibenz(a,h) anthracene	278	4.1	40.8	nd
Fluoranthene	202	3.3	112.3	nd
Fluorene	166	3.9	75.7	0.55
Indeno(1,2,3-cd) pyrene	276	2.2	30.6	nd
Phenanthrene	178	1.4	23.5	0.46
Pyrene	202	5.6	108.9	nd

nd = Below Detection of 0.001 μg/kg.

DISCUSSION

PAH in Treated Cattle Hide

Almost all the 16 U.S. promulgated PAHs were detected in various concentrations in the study except for benzo[k]flouranthene and indeno[1,2,3-cd]pyrene, which were not detected in week 3 and week 2 samples. The total PAHs concentration estimated as the sum of 16 PAH concentrations over the 6 weeks of sample collection showed benzo[b]fluoranthene as having the highest concentration (719.9 μg/kg), followed by anthracene (639.5 μg/kg), benzo[a]pyrene (126.5 μg/kg), benzo[a]anthracene (41.4 μg/kg), pyrene (33.5 μg/kg), benzo[g,h,i]perylene (30.6 μg/kg), chrysene (28.8 μg/kg), dibenz[a,h]anthracene (24.5 μg/kg), fluorene (23.3 μg/kg), acenaphthene (20.1 μg/kg), fluoranthene (19.5 μg/kg), indeno[1,2,3-cd]pyrene (13.0 μg/kg), benzo[k]fluoranthene (10.4 μg/kg), phenanthrene (8.5 μg/kg), and acenaphthylene (7.5 μg/kg), with the lowest being naphthalene (5.9 μg/kg). The mean PAH concentrations in treated cattle hide are given in Table 2.

Similar but generally lower results were reported by Chen and Lin (1997), who used wood to smoke dark meat in which they found anthracene as having the highest concentration of 122.4 μg/kg and the lowest concentration of 5.1 μg/kg for indeno[1,2,3-cd]pyrene for the 16 priority PAHs. They also reported the total carcinogenic PAHs concentration to have increased from 18.7 μg/kg to 52.6 μg/kg during wood smoking of duck meat between 0.5 to 3 h. In this work, the total concentration of carcinogenic PAHs ranged from the lowest value of 13.0 μg/kg

for indeno[1,2,3-cd]pyrene to the highest of 719.9 μg/kg benzo[b]fluoranthene, showing absolutely very high concentration levels of carcinogenic PAHs using waste lorry tires for the smoke generation,.

Simon *et al*. (1969) also observed in their studies that benzo[a]pyrene concentration increased from 4 to 13 μg/kg during wood smoking of Frankfurt sausage for 5–10 min. The benzo[a]pyrene concentration in the treated hide in this study showed an increase from 10.9 μg/kg to 34.7 μg/kg. Comparing the level of benzo[a]pyrene from the two results in the same matrix, it can be stated that results from this study showed relatively higher PAH concentration for the use of waste lorry tires. Toth and Blaas (1972) and Potthast (1979) observed that the carcinogenic PAH levels in traditionally (wood) smoked meat products can be 2–10 times higher than those in other meat products by pan-frying, or radiation, as in electric broiling and baking. Afolabi *et al.* (1983), who worked on different traditional wood smoked meat samples, observed that increasing smoking temperature also increased PAH concentrations.

PAH levels in the present study were much higher than those reported in the above papers, possibly because of the nature of the smoke (sooty flame from worn-out lorry tires). The wide variations in PAH concentrations can be directly related to the smoking conditions, the temperature of combustion, the degree of smoking, the time of smoking and the fat content of products (Malanoski *et al*. 1968; Gomaa *et al*. 1993). As stated earlier, the West German meat regulation body has stated that the edible portion of smoked meat products should contain no more than 1μg/kg of benzo[*a*]pyrene. This strict standard, according to Toth and Blass (1972) was introduced in an attempt to rid the market of black smoked products that were containing high levels of benzo[a]pyrene up to 55 μg/kg. This German standard seems to be stricter than the one set by FAO/WHO (WHO 1987), which recommended that a benzo[a]pyrene maximum limit in meat products should not exceed 10 μg/kg.

Considering the level of benzo[a]pyrene in the treated hide, the total concentration over the 6-weeks sampling period gave 126.5 μg/kg benzo[a]pyrene. Comparing the level of benzo[a]pyrene values from this work with the standard values of FAO/WHO, Germany's and a few published ones, it can be stated that the PAH levels in the treated hide may be unfit for human consumption. Also, by these standards and from comparisons made from published works, the treated cattle hide may be deemed undesirable since their relative PAH contents are high enough to impact negative health effects on consumers.

PAH Levels in Treated, Untreated, and Control Cattle Hide

It was estimated from this work that the average PAH concentrations of the untreated hide is between 10 to 57 times higher than the treated hide concentrations. This result as contained is more than likely to impact as was anticipated since the method of treatment of the untreated hide causes thick soot containing high levels of PAHs that adhere to the surface of the burnt hide. Five non-carcinogenic PAHs (acenaphthene, acenaphthylene, anthracene, fluorene, and phenanthrene) were detected in the control sample at very low concentration of 0.01μg/kg, 0.3 μg/kg, 0.2 μg/kg, 0.6 μg/kg, 0.5 μg/kg, respectively. The presence of these PAHs in the

control sample (fresh hide) could be due to feeding of the animal on plants containing deposited PAH particles or drinking water sources containing low levels of dissolved PAHs.

Naphthalene had the lowest total PAH concentration in both treated and untreated hide samples as 5.9 μg/kg and 57.6 μg/kg with benzo[b]fluoranthene recording the highest in both products as 719.9 μg/kg and 19841.0 μg/kg, respectively.

It can be observed from the table that significant amounts of PAHs were lost due to washing of the untreated hide. However, the detected PAHs in the treated hide after the treatment process (washing) was still higher than the FAO/WHO and IARC set guidelines for monitoring PAH levels in the edible portions of meat products. Shown in Figure 1 is a graphical comparison of the mean PAH levels of treated, untreated, and control hide samples. Anthracene and benzo[a]flouranthene recorded the highest PAH concentration peak heights. However, the level of PAHs measured in this work especially that for benzo[a]pyrene has suggested the fact that the recent cancer occurrence in Ghana (Adams 2005) may be as a result of exposure to high concentrations of PAHs during cooking, baking, and perhaps from consumption of heavily smoked meat among others (not confirmed). On the average, a consumer eats a minimum of about 150 g of cattle hide (wele) at a meal (Essumang *et al.* 2007) thereby ingesting various levels of PAHs. There is therefore an urgent need for education on the dangers associated with the consumption of this cattle hide product. The levels of PAHs of treated, untreated, and control hide samples are compared in Figure 1.

Statistical Evaluation

Levene's Test for equality of variance conducted revealed that there is a significant difference between the treated and untreated cattle hide samples analyzed. It also

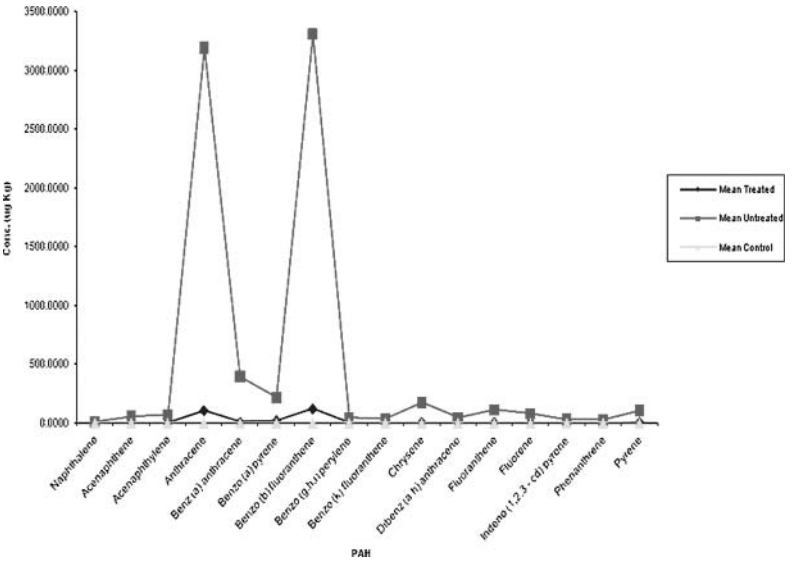

Figure 1. Comparison of treated, untreated, and control hide samples.

indicated that PAHs contamination of the meat product by traditional smoking method is more, and correlation analysis conducted also revealed that there were significant positive and strong correlation relationships between PAHs of the hide samples.

Correlation studies of the treated and the untreated cattle hide indicate a combination of strong, weak positive and inverse associations. This result is expected since the PAHs in the meat might have come from two different sources (*i.e.*, pyrolyzed oil and the burning of the lorry tires).

A regression model analysis conducted on levels of PAHs in the treated hide shows that benzo[b]fluoranthene, Chrysene and anthracene contributed about 99.2% towards the total PAHs concentration in the treated cattle hide and are therefore used in the assessment of PAH risk impact on human health.

Carcinogenic and Non-Carcinogenic PAHs

PAHs have been classified as carcinogenic and non-carcinogenic compounds. The mean concentration values for treated cattle hide samples showed naphthalene with the lowest value of the non-carcinogenic PAHs to be 5.9 μg/kg and anthracene being 639.5 μg/kg with the highest PAH value.

Shown in Figure 2 is a comparison between total carcinogenic PAHs with that of non-carcinogenic PAHs. The total non-carcinogenic PAH concentrations are generally lower in the studied samples with an exception of anthracene. The low values of the non-carcinogenic PAHs recorded were attributed to a possible degradation by heat, oxygen, and light during storage and in the extraction process since

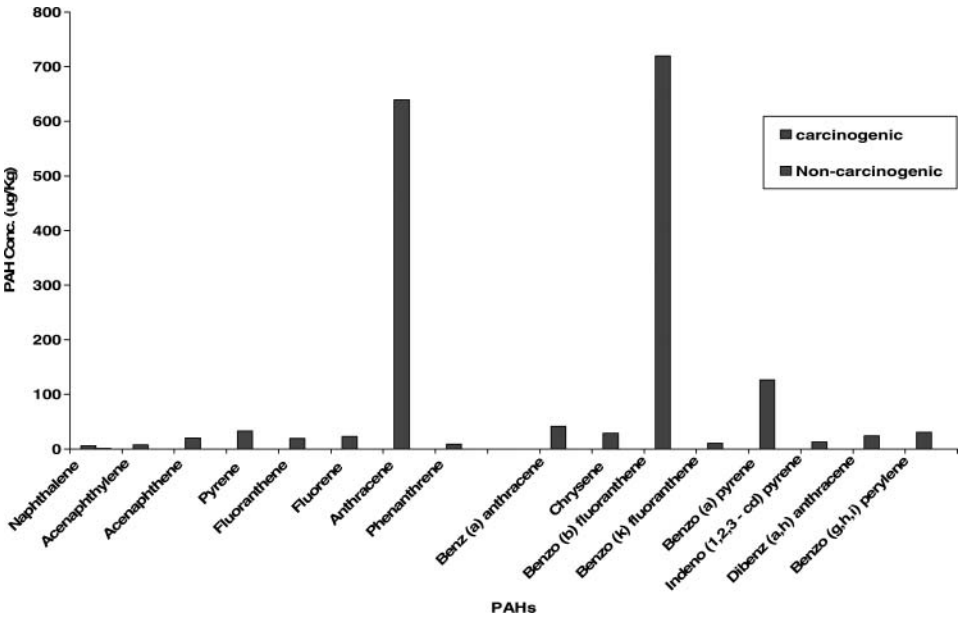

Figure 2. Carcinogenic and non-carcinogenic PAHs in treated cattle hide compared.

most PAHs with low molecular weight ratios are lost under such conditions. The carcinogenic PAHs are relatively higher with benzo[b]fluoranthene having the highest average PAH concentration of 719.9 μg/kg.

The total carcinogenic PAHs in smoked meat calculated from this study were somewhat different from those reported in commercial meat products as indicated by some of the researchers mentioned above in that the carcinogenic PAHs have higher concentration than the non-carcinogenic ones. This difference may be attributed to the fact that some PAHs are susceptible to oxygen, heat, and light degradations and might have caused the reduction in the PAHs with low molecular weight (Chen and Lin 1997; USEPA 1989).

Risk Assessment of PAHs in the Treated Hide

In this carcinogenic risk assessment study, the formation and concentrations of PAHs formed during processing were investigated for the heavily burnt cattle hide, a meat product prepared by burning fresh hide in a sooty flame of waste lorry tires and consumed by people in Ghana. This investigation seems to present one among the most peculiar and worst ways to prepare singed PAH containing foods. The risk assessment was conducted on seven individual PAH concentrations by employing Central Tendency Exposure (CTE), in accordance with USEPA's *Risk Assessment Guidance for Superfund* (RAGS). The risk value was estimated for lifetime of 70 years for adults and up to 2 years for children. Shown in Table 4 are the results of carcinogenic PAH risk assessment for the ingestion route in humans. Dibenz[a,h]anthracene, benzo[b]flouranthene, benzo[a]anthracene, benzo[a]pyrene and benzo[b]flouranthene recorded the highest risk value for adults and children in the treated cattle hide samples. The total PAH carcinogenic risk values for ingestion of PAHs from eating treated cattle hide in adults are 2.10×10^{-5} and in children are 1.0×10^{-3}. The result implies that at least about 2 adults out of 100,000 adults may suffer from cancer related disease in their lifetime and at least 1 out of 1000 children may also suffer from cancer-related disease in their lifetime through the eating of smoked treated cattle hide. The summary of PAH

Table 4. Summary of PAH carcinogenic risk for treated and untreated cattle hide.

	Ingestion of PAH (treated cattle hide)		Ingestion of PAH (untreated cattle hide)	
PAHs	Adult	Child	Adult	Child
Benzo[a]anthracene	5.2×10^{-7}	2.5×10^{-5}	2.1×10^{-5}	1.0×10^{-3}
Benzo[a]pyrene	8.9×10^{-6}	4.4×10^{-4}	6.9×10^{-5}	3.4×10^{-3}
Benzo[b]flouranthene	1.0×10^{-5}	5.1×10^{-4}	9.4×10^{-5}	4.7×10^{-3}
Benzo[k]flouranthene	9.6×10^{-9}	4.7×10^{-7}	9.8×10^{-8}	4.9×10^{-6}
Chrysene	3.8×10^{-9}	1.8×10^{-7}	9.2×10^{-8}	4.5×10^{-6}
Dibenz[a,h]anthracene	8.8×10^{-7}	4.4×10^{-5}	6.4×10^{-6}	3.2×10^{-4}
Indeno[1,2,3-cd]pyrene	1.5×10^{-7}	7.7×10^{-6}	4.9×10^{-7}	2.4×10^{-5}
Total	2.1×10^{-5}	1.0×10^{-3}	1.9×10^{-4}	9.4×10^{-3}

Table 5. Summary of hazard quotients for treated and untreated cattle hide.

	Ingestion of PAH in treated hide		Ingestion of PAH in untreated hide	
PAHs	Adult	Child	Adult	Child
Acenaphthene	5.2×10^{-4}	1.1×10^{-3}	5.5×10^{-3}	1.1×10^{-2}
Anthracene	1.4×10^{-3}	2.9×10^{-3}	2.5×10^{-2}	5.1×10^{-2}
Fluoranthene	2.3×10^{-4}	4.8×10^{-4}	3.4×10^{-3}	7.0×10^{-3}
Fluorene	4.2×10^{-4}	8.6×10^{-4}	7.3×10^{-3}	1.5×10^{-2}
Pyrene	5.7×10^{-4}	1.2×10^{-3}	4.4×10^{-3}	9.1×10^{-3}
Total	3.9×10^{-3}	8.2×10^{-3}	5.0×10^{-2}	1.0×10^{-1}

carcinogenic risk for treated hide that is the one consumed in Ghana are given in Table 4.

The risk assessment conducted on the concentrations of samples from this work saw some of the risk values higher than the health based guideline level (10^{-5}), indicating high health risk to humans exposed to PAHs through the ingestion of PAHs from treated cattle hide. It was therefore considered that PAH exposure through the consumption of cattle hide may be a major health hazard. Results obtained from this work also shows that regular consumers of smoked hide stand a high risk of possible cancer-related diseases and hence, appropriate steps need to be taken to mitigate the health burden of these compounds on individuals exposed to them. It should however, be noted that the untreated hide is not eaten directly but had to go through cleaning processes (washing) to attain the consumable state known as the treated hide.

Hazard assessment was also conducted by using hazard quotients for treated and untreated hide as shown in Table 5. The total PAH hazard quotients for ingestion of PAHs from eating treated hide in adults is 3.9×10^{-3} and in children is 8.2×10^{-3}. The hazard quotients indicate that at least 4 out of 1000 adults may suffer from non-cancer related illnesses in their lifetime through consumption of treated smoked hide. For children, the incident of non-cancer-related diseases are, about 8 out of 1000 for treated hide (USEPA 1995). Contained in Table 5 is the summary of hazard quotients for treated and untreated cattle hide.

CONCLUSIONS

Results from previous and the present study confirm the presence of PAHs in untreated and treated smoked meat products. However, this study has shown much higher PAH levels when worn-out lorry tire are used for the singing process, which suggests that the practice of burning with worn-out lorry tires (wood) as a source of energy for treating meat products may be a source of contamination. Analysis of PAH concentrations revealed that all the 16 listed PAHs were well distributed in both the treated and the untreated hide samples at very elevated levels (Djinovic *et al* 2008; Stumpe-Vīksna *et al*. 2008; Costa 2000; Obiri-Danso *et al*. 2008).

Risk assessment conducted on the concentrations of samples from this work also saw risk values higher than the health based guideline level (10^{-5}), indicating high

health risk to humans exposed to PAHs through the ingestion of treated or untreated cattle hide.

Consumer evaluation in Ghana would always demonstrate distinct preference for treated cattle hide (wele) due to its affordability, ability to provide a consumer the benefit in terms of mouth feel (chewable), and the power of being used as an alternative cheap meat resource. The majority of people would always hasten to its consumption and are therefore confronted with the associated adverse effects and health consequences. This therefore requires extensive education on the proper way of treating the cattle hide to avert any possible health hazards.

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APPENDIX

Table A1. Mean PAH concentrations of treated cattle hide (μg/Kg).

PAH	WTA	WTB	WTC	WTD	WTE	WTF	Avg	SD	Variance
NA	1.0298	0.3815	0.4732	0.6687	1.3742	1.9431	0.9784	0.5996	0.3595
AC	8.1914	3.1888	3.5024	2.5143	1.166	1.5128	3.3460	2.5429	6.4664
Acyl	1.0434	1.1481	1.464	1.3462	0.7777	1.7891	1.2614	0.3524	0.1242
An	259.388	25.0632	169.77	62.5633	38.8314	83.8918	106.5846	90.5874	8206.0693
B[a]A	9.1025	10.4657	5.3636	5.6311	1.097	9.7008	6.8935	3.5512	12.6110
B[a]P	16.1686	22.4431	10.9081	34.7290	17.4924	24.7799	21.0869	8.2741	68.4605
B[b]F	711.985	1.1682	1.5927	3.0780	1.3344	0.7091	119.9779	290.0242	84114.0240
Bg,h,i	6.6329	2.4138	1.3823	5.4071	1.5619	13.1816	5.0966	4.5014	20.2623
B[k]F	2.5267	0.6464	ND	5.3801	0.6858	1.1336	1.7288	1.9784	3.9142
Chy	4.1631	2.0037	5.4618	5.2746	2.1453	9.6971	4.7909	2.8255	7.9833
D[a,h]A	4.1850	3.4709	4.1110	5.3340	5.1095	2.2558	4.0777	1.1263	1.2686
Fl	1.0964	0.9729	1.2622	0.9499	1.0006	14.2293	3.2519	5.3790	28.9340
F	3.5289	4.3528	4.4465	3.8725	3.98	3.1291	3.8850	0.4983	0.2483
Ind.	1.7706	ND	0.2391	4.0898	0.5095	6.3929	2.1670	2.5641	6.5745
Phe	1.0461	1.9266	1.1889	1.1347	1.5762	1.5776	1.4084	0.3405	0.1159
Py	1.8638	1.1570	2.5006	0.6800	0.4478	26.8281	5.5796	10.4373	108.9374

Table A2. Weekly PAH Mean concentrations in untreated cattle hides (μg/Kg).

PAH	WUA	WUB	WUC	WUD	WUE	WUF	Avg	SD	Variance
NA	22.5930	13.2273	5.9302	2.1246	2.3239	11.4286	9.6046	7.8436	61.5219
AC	36.8215	102.41	57.5047	51.386	61.9575	32.9974	57.1796	24.9059	620.3035
Acyl	33.2101	176.571	31.499	107.93	28.3979	24.5577	67.0276	62.2489	3874.9227
An	1285.285	11644.70	1115.247	3241.7	1259.768	594.6249	3190.2225	4239.7861	17975786.4886
B[a]A	1063.966	615.226	111.3563	143.69	318.99	109.1067	393.7225	381.3471	145425.6344
B[a]P	119.9300	376.016	57.7798	550.0100	120.9684	69.6703	215.7298	200.9361	40375.2994
B[b]F	720.2507	10948.1	2382.661	3618.0000	624.4087	1547.506	3306.8244	3906.9408	15264186.4424
Bg,h,i	35.3721	96.0221	25.0951	57.5210	17.1093	13.5786	40.7830	31.3286	981.4827
B[k]F	36.779	65.0011	12.4797	66.5120	13.4837	12.9202	34.5292	25.8941	670.5042
Chy	90.1776	537.568	95.0213	242.2400	58.5484	22.178	174.2886	193.1219	37296.0874
D[a,h]A	47.7009	25.3609	35.8642	71.3630	49.7194	14.4621	40.7451	20.0882	403.5364
Fl	296.9158	124.324	46.0277	13.2270	29.2978	164.0925	112.3142	107.7096	11601.3630
F	44.9366	138.785	72.258	80.6370	39.4267	78.0532	75.6827	35.4503	1256.7221
Ind.	45.5246	47.6003	29.6431	13.8590	31.0785	15.7084	30.5689	14.2473	202.9854
Phe	13.8798	73.4131	15.5201	15.9350	13.9914	8.2817	23.5036	24.6040	605.3556
Py	88.7331	154.025	81.3066	25.465	75.0649	228.6335	108.8713	71.6194	5129.3436

Plate 1. Burning of fresh cattle hide in a naked sooty flame of worn out lorry tyres.

Plate 2. Burning of fresh cattle hide in a naked flame of worn out lorry tyres.