DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SMOKED BUSH MEAT

BY

ABU ABDALLAH (B.Ed CHEMISTRY)

KWAME NKRUMAH UNIVERSTY OF SCIENCE AND TECHNOLOGY (KNUST), KUMASI

JUNE, 2013

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SMOKED BUSH MEAT

By

ABU ABDALLAH (B.Ed CHEMISTRY)

A Thesis Submitted To The School Of Graduate Studies, Kwame Nkrumah University Of Science And Technology, Kumasi, In Partial Fulfillment of The Requirements For The Award of degree of Master of philosophy in Analytical chemistry

COLLEGE OF SCIENCE

Department Of Chemistry

College of Science

June, 2013

DECLARATION

I, Abu Abdallah, hereby declare that this submission is my own research work towards the award of the M.phil. and that, it contains no materials previously published by another person or material which has been accepted or concurrently being used for the award of any other degree in this university or elsewhere, except where acknowledgment has been duly cited in the text and in the references.

ABU ABDALLAH (Student) ---------- ----------

(PG 3906809) Signature Date

CERTIFIED BY:

DR. EVANS ADEI ---------- ----------

(Project supervisor) Signature Date

CERTIFIED BY:

DR. GODFRED DARKO ---------- ----------

(Project Co-supervisor) Signature Date

CERTIFIED BY:

DR. R. B. VOEGBORLO ---------- ----------

(Head of Department) Signature Date

ABSTRACT

Public awareness on the safety of meat and meat products is heightened by the day solely due to reports from most health institutions across the country. The use of all sorts of materials including lorry tyres to prepare bush meat in particular for consumption leaves many residues and contaminants of which polycyclic aromatic hydrocarbons was of major concern. Thus, 12 bush meat samples were obtained from six local producers within the Kumasi Metropolis to investigate the presence and levels of PAHs using Gas Chromatography – Mass Spectrometry (GC-MS). The samples were examined for nine of the most carcinogenic PAHs. The concentrations of naphthalene, 2-methylnaphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene were: 0.210, 0.379, 0.084, 0.329, 0.066, 0.478, 0.0670.153 and 0.095 respectively. PAHs present in the samples were all less than 0.5 µg/kg on the average of the total contamination profile, with phenanthrene and 2-methylnaphthalene occurring with the highest concentrations (maximum contents of 0.920 and 0.527 mg/kg respectively), irrespective of the bush meat type analysed. Smoked antelope meat was potentially more risky, since total PAHs contents for antelope were generally higher as compared to other bush meat samples. Significant however to the findings of this research is that the PAHs levels were far below the European Union recommended levels of 5µg/kg in smoked meat.

ACKNOWLEDGEMENT

After having recognized the Blessings and Mercies of the Almighty Allah (God), my warmest appreciation goes to my supervisor, Dr. Adei for his diverse but immense contribution, corrections, and encouragement. Your words of encouragement kept me going till the final word of this document was written. I am indeed indebted to you. I also wish to thank Dr. Godfred Darko, my co-supervisor, for the technical assistance, the proof reading, the useful comments during the discussion of this script and the numerous advice and more importantly the patience you had for me throughout this research. I am very grateful. To all staff (both teaching and non-teaching) of the Department of Chemistry at the Kwame Nkrumah University of Science and Technology (KNUST), especially the team that granted me interview and recommended my admission, I am indeed thankful. I must acknowledge further the contribution of Mr. Iddrisu Abdul-Mumeen, a friend and a brother who has been very supportive from the preliminary stages of this research to the final write-up. Thank you. I thank finally colleague students of the KNUST in the Department of chemistry. Thank You to all.

DEDICATION

This work is dedicated to my wife Abdul-Rahaman Sahadatu and son Abdul-Hanan T. Abdallah.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACP	Acenaphthalene
ANT	Anthracene
BaA	Benz[a]anthracene
BaP	Benzo[a]pyrene
BbF	Benzo[b]fluoranthene
CHR	Chrysene
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agricultural Organization
FLR	Fluorene
FLT	Fluoranthene
GC-MS	Gas Chromatography mass Spectrometer
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
MFO	Mixed-Function Oxidases
NAP	Naphthalene
PAHs	Polycyclic Aromatic Hydrocarbons

PHE	Phenanthrene
PID	Photo Ionization Detection
Ppb	parts per billion
Ppm	parts per million
PYR	Pyrene
QRA	Quantitative Risk Assessment
QUAD	Quadrupole Analyzer Mass Spectrometer
SFA	Smoke Flavouring Additives
TEF	Toxicity Equivalence Factor
TLC	Thin Layer Chromatography
US	United States
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

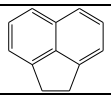
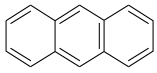
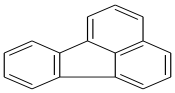
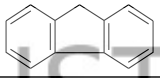
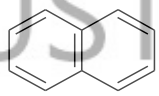
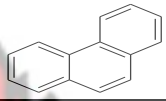
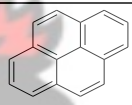
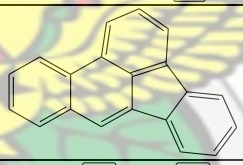
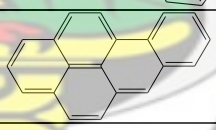
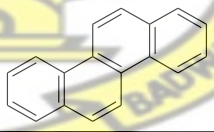
1.1 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental contaminants that emanate from incomplete combustion of fuel or high temperature pyrolysis of fats and oils. It is well known that PAHs occur in curing smoke (Viksna *et al.*, 2008) and that they accumulate on meat products being smoked (Andrée *et al.*, 2010). They have been extensively researched into because of their carcinogenicity and mutagenicity to animals (Anyakora *et al.*, 2006). In 2001, PAHs ranked 9th on the list of most threatening compounds to human health (King *et al.*, 2002).

1.2 Chemical Nature and Toxicity of PAHs

PAHs consist of several hundreds of compounds containing molecules having two or more benzene rings fused together. Some of the PAHs identified are shown in Table 1. The US Environmental Protection Agency (EPA) and the European Union (EU) have PAHs on their list of priority organic pollutants owing to their ubiquitous nature of occurrence, recalcitrance, suspected carcinogenicity and mutagenicity. Polycyclic aromatic hydrocarbons are environmentally persistent due to their relative chemical stability and resistance to biodegradation. Reports have shown that exposure of human body to the environment containing PAHs may induce some fatal diseases such as lung and skin cancers (Chen, 1996).

Table 1: Names and chemical structures of some PAHs

Name	Acronym	Structure	Molecular mass (AMU)
Acenaphthene	ACP		154
Anthracene	ANT		178
Fluoranthene	FLT		202
Fluorene	FLR		166
Naphthalene	NAP		128
Phenanthrene	PHE		178
Pyrene	PYR		202
Benz[a]anthracene	BaA		228
Benzo[b]fluoranthene	BbF		252
Benzo[a]pyrene	BaP		252
Chrysene	CHR		228

Some PAHs congeners such as benzo[a]pyrene and dibenz[a,h]anthracene are both mutagenic and carcinogenic. Haugen *et al*., (1986) postulated that benzo[a]pyrene could be oxidized to benzo[a]pyrene-7, 8-oxide by cytochrome P450 in the body, which in turn is hydrolysed to benzo[a]pyrene-7, 8-diol-9, 10-epoxide by epoxide hydroxylase.

Due to their carcinogenic potential, PAHs have been studied widely in mammals (WHO, 1993) and concern for human population has stimulated interest in knowledge of their distribution as well as accumulation in the environment and in food items. The carcinogenic potential of the PAHs was first elucidated by Percival Pott of St. Bartholomeo's Hospital in London in 1775 (Simko, 2002) when he noted high incidence of cancer of the scrotum among chimney sweepers who had often climbed up inside chimneys to sweep down the soot. Although he deduced correctly that the soot was responsible for the cancer, it was not possible to determine the compounds responsible for such serious tissue damage at that time (Simko, 2002).

In 1920, Japanese workers discovered that painting extracts of soot on the skin of mice caused tumours of the skin (Simko, 2002). In 1929, the first pure chemical carcinogen dibenz(ah)anthracene was isolated from soot extract at the Chester Beatty Research Institute (Kennaway). It has been proved that on the basis of wide epidemiological and statistical analysis, cigarette was a prime cause of lung cancer (Simko, 2002). Careful analysis of the smoke and tar obtained from cigarettes showed, that it contained many carcinogenic PAHs, from which Benzo[a]pyrene was assessed as the most potent (Simko, 2002).

1.3 Sources of PAHs

Both natural and anthropogenic sources contribute to PAHs levels in the environment (Anyakora *et al.*, 2007). Apart from crude oil and petroleum based products which have been found to contribute high amounts of PAHs into the environment, other sources include natural fires, volcanic eruptions, thermal geological reactions, industrial processes, burning and combustion of fossil fuels, exhaust fumes from vehicles, tobacco-smoke, waste incineration, domestic heating using wood, coal and

mineral oil (Grova *et al.*, 2002; Anyakora *et al*., 2007), as well as aluminium production (Wenzl *et al.*, 2006).

Processing of bush meat for consumption mostly involve treatment of the meat with smoke. Smoking is a processing technique in which meat is exposed directly to wood smoke which may be generated by a variety of methods (Guillen *et al.*, 1997). It has been well documented that the various processing methods such as smoking can induce formation of PAHs in processed foods. Smoked products have traditionally received special attention because considerable amounts of PAH have been detected (Gomaa *et al.*, 1993; Karl & Leinemann, 1996; Larsson, Pyysalo & Sauri, 1988). Several processing methods, including smoking, grilling and roasting, have been reported to induce formation of PAHs in foods. Of the various types of foods investigated, processed meat products were found to contain high amount of PAHs (Chen 1996). Thus, the formation of PAHs during processing of foods poses a potential health hazard to humans.

1.4 Possible Human Health Hazards of PAH

An issue that has not been given the needed attention in the Ghanaian market is how bush meat is processed for consumption and its implication on the health of the consuming public. A number of factors related to the smoking process affect the composition of smoke as well as the PAHs uptake in the products, with the combustion temperature being critical (Simko, 2005; Garcia-Falcón and Simal-Gándara, 2005; Roseiro et al., 2011). There is very little or no chemical investigation into the use of smoke in the processing of the bush meat which is a common practice in Ghana.

It has been reported (Simko, *et al*., 1992; Gomaa, *et al*., 1993) that the application of liquid smoke flavouring can considerably reduce the amount of PAHs. To reduce the risk caused by formation of PAHs during smoking, some processors use liquid smoke flavouring instead. Foodstuffs such as meat and fish and some types of cheese have been smoked in many countries for centuries. Originally, the purpose was to preserve the food; partly by reducing the moisture content and partly through the transfer of anti-microbiological components, such as aldehydes and phenols, form the smoke to the food.

Now smoking is primarily used to achieve the characteristic taste and appearance of smoked food, with preservation playing a minor role. Nevertheless, smoking can still influence the shelf life of food because components of the smoke may inhibit growth of some microorganisms. Smoking food items in uncontrolled processing conditions, characteristic for traditional smoking process, results in high levels of PAHs (Alonge, 1987, 1988; Afolabi *et al*., 1983; Simko, 2002).

Food smoking belongs to one of the oldest food processing technologies which mankind has used for at least 10,000 years.

Smoking started to be widely used not only for special organoleptic profiles of smoked products, but also for the inactivating effect of smoke (and heat) on enzymes and microorganisms (Chen, 1997). Today smoking technology uses mainly the special effects of various sensory active components (phenol derivatives, carbonyls organic acids and their esters, lactones, pyrazines, pyrols and furan derivatives) contained in smoke for aromatization of meat products to make food with a specific organoleptic profile, widely demanded on the market.

The exact mechanism of PAHs formation in food processing or cooking is not precisely known. However, it is generally considered that incomplete combustion is involved (SFC, 2002; Philips, 1999; Bartle, 1991). Formation of PAHs occurs through pyrolysis of fat at temperatures of above 200 ºC (SFC, 2002) and is favoured at a temperature range of 500 ºC-900 ºC especially above 700 ºC (Bartle, 1991). More PAHs is formed at higher cooking temperatures (Knize, *et al*., 1999). Pyrolysis of other organic matter such as proteins and carbohydrates might be involved (Knize, *et al*., 1999), but the greatest concentrations of PAHs have been shown to arise from fat pyrolysis (Bartle, 1991).

When food is in direct contact with a flame, pyrolysis of fat in the meat generates PAHs (Philips, 1999). Alternatively, the melted fat from food dripping on to the heat source generates PAHs and the PAHs will in turn be deposited on the meat surface as the smoke rises (SCF, 2002; Philips, 1999). Another possible mechanism for the formation of PAHs is the incomplete combustion of the fuel itself. Incomplete combustion of charcoal generates PAHs which are brought, on to the surface of the food and are adsorbed (Wu *et al*., 1997). A host of factors affecting PAHs formation has been identified which include; distance of food from the heat source (Philips,1999; Nawrot et al.,1999; Knize *et al*,1999); fat content of the food (Knize *et al*, 1999); duration of cooking (Nawrot *et al*.,1999); temperature used; whether melted fat is allowed to drop on to the heat source (SCF, 2002; Nawrot *et al*.,1999) and type of fuel used (SCF, 2002).

The direct exposure of meat products to smoke brings about formation of higher concentrations of PAHs in meat as compared to indirect methods. Concentration of PAHs in processed meat decreases with time due to photo-degradation and interactions that take place in the meat. However, PAHs also penetrate into the

smoked products, where they are protected from light and oxygen and after sometime, their concentrations stabilize at a constant level.

A relatively new alternative to smoking is the use of smoke flavoring additives (SFA). The first SFA was developed from smoke condensate by the Kansas pharmacist Wright (Simko, 2002). Nowadays, SFAs are being produced and applied widely in innumerable variations of taste and odour in solid and liquid states. In fact, the chemical composition for both modifications is identical however, the SFA has much lower PAH load.

Lijinsky and his coworkers were the first to observe the formation of carcinogenic PAHs in charcoal grilled and smoked foods (Chen, 1997). The authors also postulated that during grilling the melted fat which drips on the hot charcoal is pyrolysed under high temperature, and the PAHs formed in the smoke are then deposited on the meat surface as the smoke rises. In addition, the authors also found that fatty meats yield grilled products with higher levels of PAHs than lean meat (Chen, 1997). These findings were further supported by Toth and Blaas (1972), In general, the higher grilling temperature, the greater the formation of PAHs. In a review dealing with the formation of PAHs in meat products during grilling, Fretheim (1983), concluded that the best ways to prevent formation of PAHs were; avoid excessive heating, keep melted fat away from heat source and use lean meat for grilling. It has been well established that roasting can accelerate PAHs formation, and the amount formed depends upon time and temperature (Chen, 1997). Lawrence and Weber (1984) determined PAHs in Canadian samples of milk powder and found that the direct heating can induce higher amount of formation of carcinogenic PAHs. In recent study Chen and Lin (1997) reported that eleven PAHs were detected after roasting duck breast at 200 ^{0}C for 40 min, and in most cases the amounts of PAHs increased along

increasing roasting time. The formation of PAHs during roasting may be due to some food components, such as fatty acid, triglyceride and cholesterol, which under high temperature heating may degrade to form PAHs.

This age-old technique of smoking brings desired flavours, improves colour and appearance, and has a tenderizing action and preserves foods. Karl and Leinemann (1996), asserted that traditional direct smoking, in which the smoke is generated in the same chamber where the product is processed, exposes it to higher PAH content than indirect smoking, which uses a separate chamber for smoke generation. They indicated in their research that in indirect smoking, it is possible to lower PAH load by passing the smoke through filters and washers and even cool the smoke before it comes in contact with the food. In developed countries, computerized smoking chambers with external smoke generators and temperature control systems have generally replaced the direct smoking systems using traditional kilns. In the developing world, and more so Ghana, traditional direct smoking systems are almost exclusively used even today. The most common method of smoking of meat or fish in Ghana uses wire gauze on steel drums fuelled by wood.

It has been established that raw meat (from animals) does not contain appreciable levels of carcinogenic PAHs and no accumulation along the food chain has been observed for these contaminants in animal fat tissue (European Food Safety Authority, EFSA 2008). Nevertheless, the presence of PAHs in Vertebrate fish has been observed. According to most authors, due to their ability to rapidly metabolize PAHs, fish generally contain very low PAH concentration; even when they come from heavily contaminated areas (Van der Oost *et al.*, 2003*).*

1.5 Bush meat in Ghana

Bush meat is an important component of household food security and income in West and Central Africa. Bush meat, the meat of wild animals, is one of the most valuable tropical forest products after timber. It is an important food source, consumed in both rural and urban areas, and can make a significant contribution to the cash income of rural households living in extreme poverty. In Ghana, bush meat is widely available alongside domestic meat and fish. Whilst consumers prefer the taste of bush meat, domestic meat and fish are less expensive and are more widely eaten. Bush meat is eaten throughout the year, but its high price means that it tends to be purchased in only small quantities. Its consumption peaks during festivals and holidays. Bush meat is mostly sold processed (i.e. dressed and smoked): smoked meat has a longer shelf life, but is nearly twice as expensive. Overall, the monthly volume of bush meat sold by retailers is 15,859 kg with a retail value of US$48,000, (Falconer, 1992) subject to seasonal variation. If additional sources of bush meat are incorporated (from informal sales, gifts, and personal captures), total bush meat consumption in a Ghanaian city can be estimated at 21,410 kg per month, or about 0.01 kg per person per day (Guy *et al.*, 2004).

1.6 Indicator of PAHs in Smoked Meat

Taking account of the situation regarding the presence of PAHs in smoked food and problems to assess and interpret correctly the variable concentrations of individual carcinogens with different biological effect benzo[a]pyrene has been chosen as the general indicator of total PAHs present in smoked foods. A maximum acceptable concentration of 1 $ngkg^{-1}$ benzo[a]pyrene in smoked foods has been set in force since 1973 even in spite of the fact that benzo[a]pyrene constitutes only between 1 and 20%

of the total carcinogenic PAHs. Later other countries such as Austria, Czech Republic, Switzerland, Italy and the Slovak Republic, have also adopted a specification, which requires that the concentration of benzo[a]pyrene should not exceed a limit of 10 $ngkg^{-1}$.

Different approaches have been proposed for risk characterization of the PAH mixture in food, the most popular being the use of benzo[a]pyrene as a marker and the Toxic Equivalency Factor (TEF) approach. Based on an examination of PAH profiles in food and on evaluation of a carcinogenicity study of two coal tar mixtures in mice, both the Scientific Committee of Food Additives (JECFA) 2005, suggested that benzo[a]pyrene should be used as a marker of the occurrence and effect of PAHs in food. However, latter the TEF approach was reassessed and it was deemed as not scientifically valid because of the lack of data from oral carcinogenicity studies on individual PAHs, their different modes of action and the evidence of poor predictivity of the carcinogenic potency of PAH mixtures based on the currently proposed TEF values (EFSA, 2008; SCF, 2002).

In 2005, the European commission introduced for benzo[a]pyrene (chosen as a marker of the occurrence and carcinogenic potency of the entire class of carcinogenic and genotoxic PAHs) a maximum level of 5 μgkg^{-1} in smoked fish and meat (European Commission 2005). Before the introduction of this regulation, lower legal limits for benzo[a]pyrene existed in a number of European Union Member States such as 2 μgkg^{-1} for Belgium and 1 μgkg^{-1} for Germany (Wenzl *et al.*, 2006).

Smoked and grilled foods may contribute significantly to PAH dietary intake if such foods are part of the usual diet. Long term consumption of traditionally smoked products could be responsible for the higher incidence of liver and stomach cancer in

Ghana. There is limited or virtually no information on the levels of PAHs in smoked fish and meat in Ghana. Very limited studies on the monitoring of PAHs in the environment are available. Most studies have focused on other forms of pollution and degradation, but not on PAHs. Some few works on PAHs include those of Gilbert *et al*., (2006); Esumang *et al*., (2009); Joyce *et al.*, (2010).

1.7 Problem Statement

Ghana is one of the countries in the tropical zone endowed with forest reserves with various species of wild animals inhabiting in these reserves. The Ashanti Region located at the central part of the country is harboring about 60% of the nation's forest reserves. This undoubtedly makes the region and Ghana one of the major consumers of bush meat since a larger percentage of the wild species reside in these forest reserves.

Unfortunately, however, the obsolete technologies used in processing the meat are very likely to induce PAHs in the meat. Smoking, grilling and other traditional methods of processing meat using combustion fumes could introduce high levels of PAH into the food. Processed meat (bush meat) using smoke from burnt car tyres is raising serious concern (Obiri-Danso *et al.*, 2008) because of the adverse effects it may have on consumers. Smoke from burnt car tyres could contribute high levels of PAHs in processed meat (bush meat) possibly due to high temperatures, oxidation, physical deposition of the possible PAH containing soot, and or due to incomplete combustion.

No or very little systematic clinical studies have been carried out to investigate the levels of contamination of smoked bush meat eaten in the country. Yet the bush meat industry is a major contributor to the nutritional requirements of the majority of

Ghanaians. This research therefore seeks to investigate the levels of PAHs in smoked bush meat consumed in the country.

1.8 Objectives of the Study

The main objective is to determine the levels of PAH contamination of smoked bush meat.

The specific objectives are:

- To assess the levels of PAH in smoked bush meat.
- To compare these levels to the WHO/EU standard maximum level of PAH in food safe for consumption.
- Make recommendations to inform policy decision on the consumption of smoked bush meat

1.9 Justification

Smoked bush meat is served as delicacy in several parts of Africa including Ghana. Processing of bush meat includes singeing off the hair of animals in flames by various substances such as wood mixed with spent engine oil, plastics mixed with refuse or discarded car tyres. These materials could easily generate PAHs to contaminate the meat. This presents a significant health hazard to consumers and therefore the need to investigate the levels of PAHs in bushmeat in order to assess their suitability for human consumption. Up to date no systematic studies have been carried out on the levels of PAH in bushmeat consumed in the country. This research will therefore serve as the baseline for further possible research. It will also form the basis for informed policies to be made on the processing technologies and consumption of bushmeat in the country.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Role of animal protein in nutritional requirement of man

Meat plays a very important role in the nutritional needs of humans especially in developing countries where meat has the potential to improve diets which are often based on a few food crops that provide vitamins, minerals and proteins. The daily protein intake of a person should be 1g per kilogram body weight, and it is desirable that 50% of this intake be animal protein. This recommended level is largely exceeded in developed countries with an average intake of animal protein of 5 g per day per person (FAO, 1990).

Vitamin B_{12} which occurs in animal products that include meat, fish, poultry and milk is essential to protect nerve cells and for the formation of blood cells in bone narrow. Niacin, riboflavin and vitamin B_6 are also found in significant quantities in red meats. Red meats are abundant sources of iron, an element which is required by the body to build and maintain blood haemoglobin, which carries oxygen to body cells. Red meat is also a rich source of zinc, an essential trace element that contributes to tissue development, growth and wound healing.

Meats are an excellent source of protein, a fundamental component of all living cells. Fresh and processed meats contain a high quality or complete protein with all essential amino acids in good quantity (Taylor and Bogart, 1973).

2.2 The exploitation methods of bush meat for food

There are different methods recognized in the exploitation of bush meat for consumption. Some farmers hunt to feed themselves and their families and it is

therefore a means to supplement their dietary protein. Dasman (1976) explained that an enormous loss of animal species is attributed to hunting. There are either full or part time hunters whose hunting is limited by law to kill certain categories of species, age, sex and number of animals. Most of the hunters live in the rural areas and sell their meat there, which then gets to the urban areas at high prices.

Organized hunting parties or traditional tribes who are seldom licensed often kill large numbers of bush meat systematically. Such parties could travel long distances, sometimes across international frontiers. They often sell their meat secretly in rural areas.

Dasman (1976) mentioned sport hunting as another form of wild life exploitation but explained that it is not presently an important activity in most countries as compared to the commercial and subsistence activities. For sport hunting, hunters are allowed to enter a game production reserve during the open season to hunt freely after paying a levy. The captured animals are subsequently weighed and paid for by the sport hunter.

2.3 Distribution channels of bush meat

The starting point of the general distribution of bushmeat is the hunter. Most of them reside in the rural areas and either sell the fresh meat locally for consumption in the rural areas or give it to their wives for preservation and subsequently to the village collector. The hunter can also sell directly to the village collector who will carry out the smoking and preservation process himself/herself. Alternatively, the hunter's wife may sell fresh carcasses to the road side seller who will smoke it and also sell. Otherwise, the roadside seller may have to buy from the village collector. The urban

retailer usually buys from the village collector or roadside seller in the smoked or dried form.

2.4 The bush meat trade

Bush meat is valued throughout West Africa as a source of income and food. Throughout West Africa, greater quantities of bushmeat are consumed during the rainy season than the dry season (Hladik, 1987). For many rural male farmers, hunting is an important activity during the lean agricultural periods. Falconer (1992) reported that data collected over a ten-year period in Kumasi, Ghana suggested that bush meat sales are being maintained though there are seasonal fluctuations. The demand falls during the early rainy season when fish as an alternate source of protein is plentiful and cheap. In June and July, the market is flooded with meat as hunters strive to bring in as much as possible before the closed season when hunting is banned and trade slows down. Asibey (1965) analyzed Ghana's market price trends for bush meat from 1956-1963. He found out that prices had increased by 25% in seven years.

2.5 Value of bush meat

Bush meat is widely used in Ghana and sometimes as the main source of food. Nimo (1994) purports that out of the 200 consumers interviewed one hundred and ninety five (97.5%) used wild life as food. Some believe bush meat taste good, is cheap and easily available from the consumer's point of view. Also it had been used for food since the days of their forefathers and had been handed down from generation to generation.

In all, 44 animals were catalogued as being used as food, and these ranged from mammals, reptiles, and rodent to birds. The wild animals that were frequently utilized were antelopes, grass cutters, rabbits, rats, bush-tail porcupines and bush fowls.

Nimo (1994) further indicated that eighty four (84) respondents (42%) either used or were aware of wild life being used for traditional, ritual or medicinal purposes. Various parts of wild animals were used in a variety of ways, ranging from the use of skins for making multi-purpose drums and stools, other parts for making powerful potent medicines for all kinds of ailments including spiritual healing and fortification. According to 62 respondents (31%) they derived recreational value from the use of wild life. This value was realized through being entertained by monkeys, parrots and captured young wild animals. Fifty eight respondents (19%) either knew or were aware of the wildlife trade in the Juabeng District. Items involved were skins of antelopes and snakes, polished or painted shells of snails and tortoise and brightly coloured feathers used for various purposes. Nimo (1994) reported his findings on the use of specific parts of wild life for medicinal and other purposes and those are presented in Table 2.

Table 2: Medicinal/traditional/ritual value of wildlife and their products in the New Juabeng District

Type of Animal	Part (s) used	Purpose
Antelope	Skin	For making drums
Monkey	Skin	For some kinds of chief stool
Squirrel	Stomach content	For treating wounds and safe delivery
African Civet	Anal secretion, faeces	treat convulsion, prevent boils in the armpit
Tortoise	Whole	Juju for vanishing into thin air
Ground Squirrels	Penis and testicles	Juju against those chasing married women
Rat	Head	for pregnant women
Rat	Bone	For crawling children and rituals for twins
Bush-tailed porcupine	whole Shell	For women seeking pregnancy stops bed wetting
Snail	Legs	Burnt for multipurpose use For pregnancy

Nimo, 1994

2.6 Economic value of bush meat

Bush meat is by far the most expensive of all meats in many countries (Asibey, 1981). According to him, often the demand and cost of bush meat are increasing much more rapidly than prices for domestic meat. For example, an analysis of market prices in Accra, Ghana, revealed that in the period 1980-1986,bushmeat prices increased eight-fold whole those for beef increased six fold. Ntiamoah-Badu (1987) reported that in many parts of Africa, the high demand for and cost of bush meat compared to other forms of animal protein has created a situation where the hunters find it more profitable to sell their catch (rather than eat it) and buy fish which is much cheaper for their families.

In Nigeria the demand for bush meat has been increasing (Mba,1983) an increase of 159% since 1975 was reported and it has been estimated that the value of bush meat to the economy is 3.6 billion naira (about 370,000 dollars) annually (Martin,1988).

2.7 Nutritional importance of bush meat

In sub-Saharan Africa, the proportion of wild animal meat in total protein supplies is exceptionally high. For example, communities living near a forest in Nigeria obtained 85% of their animal protein from bush meat and in Ghana; approximately 75% of the population consume wild animal products regularly (FAO, 1989). Many researchers have emphasised the importance of bush meat as a source of animal protein (Asibey, 1987, Ajayi, 1979) noted that wild animals are good sources of carbohydrates (ranging from 1% in red river hog to 6% in forest) whereas the range for domestic animals in similar environment is 0.1% in pork and beef to 1.3% in mutton. Ajayi (1979) added that the protein content of bush-meat ranges from 16-55% compared to 11-20% for domestic animals. In addition bush-meat is often a source of minerals and vitamins.

2.8 Bush-meat preservation

A delay in selling bush-meat, particularly when the meat is still fresh can cause severe health problems. This is more or less solved by smoking or even drying the bush-meat. This practice is widely applied when the bush-meat is to be transported over long distances. Throughout West Africa, the most common method used to preserve bush-meat is by smoking. This may be by soft/wet or dry smoking. The smoking is usually done in a mud-built smoking chamber or drum. Meat is smoked and then sold in major towns where it is much sought after. In Kumasi, Falconer (1992) reported that smoked meat comes from the Savanna regions north of Kumasi from as far as Bolgatanga, 450 km away.

2.9 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) also known as polyaranes or polynuclear aromatic hydrocarbons, are ubiquitous contaminants in the environment. PAHs constitute a very large group of organic compounds that contain two or more fused benzene rings (Harvey, 1997). Theoretically, PAHs could have infinite benzene rings, and the arrangement of those benzene rings could produce a copious number of isomers. However, PAHs most studied contain eight or less rings, with the most environmentally significant ones having seven or less (Harvey, 1997; Boehm *et al*., 2002). PAHs may also be alkylated (substituted) meaning straight-chained hydrocarbons are attached to the rings at one or more points (Boehm *et al*., 2002). PAHs form homologous series, containing a non-alkylated PAH known as the parent compound and alkylated PAHs that have the parent compound as the base.

PAHs attached by one alkyl chain are classified as C-1 members, those with two alkyl chains are classified as C-2 members, and those with three alkyl chains are C-3 members and so on. Commonly associated with PAHs are heterocyclic, compounds in which an atom of nitrogen, sulphur or oxygen has replaced one carbon atom in a ring (Boehm *et al*., 2002).

These compounds result from pyrolytic processes and the incomplete combustion of organic matter at high temperatures, and anthropogenic activities are the main source of PAHs in the environment. Because of their adverse effects on human health, their persistence in the environmental matrices, and their reactivity and ability to transform into more active species, PAHs have been classified as priority pollutants by the European Environment Agency (1999).

2.9.1 Occurrence of PAH in foods

Raw foods should usually not contain high levels of PAH. In areas remote from urban or industrial activities, the levels of PAH found in unprocessed foods reflect the background contamination, which originates from long distance airborne transportation of contaminated particles and natural emissions from volcanoes and forest fires. In the neighbourhood of industrial areas or along highways, the contamination of vegetation can be ten-fold higher than in rural areas (Hancock *et al.*, 1970, Larsson and Sahlberg, 1982).

Processing of food (such as drying and smoking) and cooking of foods at high temperatures (grilling, roasting, frying) are major sources generating PAH (Guillen *et al.*, 1997; Phillips, 1999). Levels as high as 200 µg/kg food have been found for individual PAH in smoked fish and meat. In barbecued meat, 130 µg/kg has been reported whereas the average background values are usually in the range of 0.01-1 µg/kg in uncooked foods. Contamination of vegetable oils (including olive residue oils) with PAH usually occurs during technological processes like direct fire drying, where combustion products may come into contact with the oil seeds or oil (Speer *et al.*, 1990; Standing Committee on Foodstuffs, 2001).

2.9.2 Sources of environmental PAH contamination

Foods can be contaminated by PAH that are present in air (by deposition), soil (by transfer) or water (deposition and transfer). The sources, natural and mostly anthropogenic, of PAH in the environment are numerous and include (IPCS, 1998):

- Stubble burning (Ramdahl and Moller, 1983) and spreading of contaminated sewage sludge on agricultural fields (Hembrock-Heger and Konig, 1990; cited by IPCS, 1998), exhausts from automobile sources (motor vehicles and

aircrafts). Close to an emission source such as a motorway, very high concentrations of PAH were detected in the surface layer, but soil at a depth of 4-8 cm was two times less contaminated (Butler *et al.*, 1984; cited by IPCS, 1998). Close to highways, concentrations of PAH in the soil in the range of 2-5 mg/kg can be found whereas in unpolluted areas, the levels are in the range of 5-100 µg/kg. The distribution and concentration of PAH in soil, leaf litter, and soil fauna depend broadly on the distance from the roadside.

- Industrial plants (e.g. aluminium foundries, incinerators).
- Wood preservation, use of tar coated wood. Oysters and mussels grown in beds with tar or creosote coated wood posts may be contaminated with PAH.
- Domestic heating with open fireplaces. Levels of PAH in the atmosphere appear to be higher in the winter than in the summer period.
- Burning of coal for thermal and electric energy. The quantity emitted varies widely depending on the quality of coal and on the combustion process.
- Burning of automobile tires or of creosote treated wood releases considerable amounts of PAH.
- PAH present in tobacco smoke contaminate both the atmosphere of the kitchen and the foods during preparation and cooking.
- Oil pollution of surface waters and soils.
- Forest fires and volcanic eruptions (Hites *et al.*, 1980; cited in IPCS, 1998).

2.9.3 Physical and chemical properties of PAH influencing their occurrence in foods

The occurrence of PAH in foods is influenced by the same physicochemical characteristics that determine their absorption and distribution in man. These are their relative solubility in water and organic solvents, which determine their capacity for

transport and distribution between different environmental compartments and their uptake and accumulation by living organisms. The transportation of PAH in the atmosphere is influenced by their volatility. The chemical reactivity of PAH influences adsorption to organic material or degradation in the environment. All these factors determine the persistence and capacity of PAH to bioaccumulate in the food chain. PAH are lipophilic and generally have a very poor aqueous solubility. PAH accumulate in lipid tissue of plants and animals. PAH will not tend to accumulate in plant tissues with a high water content and limited transfer from the soil to root vegetables will occur. The rate of transfer varies widely and is also influenced by soil characteristics, the plant and the presence of co-pollutants. PAH adsorb strongly to the organic fraction of soils and do not penetrate deeply into most soils, therefore limiting both leaching to groundwater and availability for uptake by plants.

Some PAH are semi volatile but most of them tend to adsorb on organic particulate matter. Heavier PAH preferentially associate with particulate matter so atmospheric fall out is a principal route of contamination (Edwards, 1983; Nielsen *et al.*, 1996). PAH with 5 or more aromatic rings are found predominantly on particulates, (usually on small (< 2.5 μm) particles such as fly ash and soot). Consequently, vegetables with large leafs, grazing cattle and poultry which may ingest particulate matter from soil are susceptible to contamination by PAH adsorbed to particles. PAH with 2 or 3 rings are almost entirely in the vapour phase, those with 4 rings being in an intermediate position.

The waxy surface of vegetables and fruits can concentrate low molecular mass PAH mainly through surface adsorption. PAH concentrations are generally greater on plant surface (peel, outer leaves) than on internal tissue. Careful washing may remove up to 50% of the total PAH. Particle bound PAH are easily washed off the surface whereas

those in the waxy layer are less efficiently removed, washing may alter the apparent high to low molecular mass PAH profile.

When particulates fall out into surface water, they are transported in suspension and surface adsorbed PAH finally end up in fresh water or marine sediments. PAH are strongly bound to these sediments which constitute a potential pollution reservoir for PAH release under specific conditions. Sediment-dwelling and filtering organisms are most susceptible to contamination. Most organisms have a high bio-transformation potential for PAH resulting in no significant bio-magnification in the aquatic food chain. However filter-feeding bivalves (e.g. mussels and oysters) filter large volumes of water and have a low metabolic capacity for PAH, they may accumulate PAH. The water-soluble low molecular mass PAH are rapidly degraded in water but sustained release of PAH in waste can result in elevated concentrations in bivalves grown close to industrialized areas. The accumulation of sediment-adsorbed PAH depends on the contaminant (Baumard *et al.*, 1998, personal communication from Lambré, C., 2002).

2.9.4 Degradation

PAH are chemically stable and very poorly degraded by hydrolysis (Howard *et al.*, 1991; cited in IPCS, 1998) but are susceptible to oxidation and photo-degradation in light. PAH half lives in air range from a few hours to days and estimated PAH half lives in soils vary from several months to several years. Abiotic degradation may remove 2-20% of two and three ring PAH in contaminated soils (Park *et al.*, 1990; cited in IPCS, 1998). PAH with 4 or more aromatic rings persist in the environment but they are often strongly adsorbed to organic matter. Following degradation, oxidized reaction products may be formed which tend to react with biological components. Reaction with nitrogen oxides and nitric acid in the atmosphere can form

nitro derivatives, which could contaminate foods. Thus although parent compounds cannot always be detected in PAH contaminated foods, degradation products or derivatives, some of which have significant toxicity, may be present. The half lives in soil and air depends on various parameters (e.g. type of adsorption onto particles, molecular weight) and range from hours to days for air and months to years for soil.

2.9.5 Biodegradation

The most significant information on biodegradation is summarized below (IPCS, 1998): The biotransformation potential of aquatic organisms depends on the activity of their cytochrome P450-dependent mixed-function oxidases (James, 1989; cited in IPCS, 1998). Biotransformation mainly takes place in liver, lung, kidney, placenta, intestinal tract, and skin (Cerniglia, 1984; cited in IPCS, 1998). The initial transformation step in invertebrates is usually slower than in vertebrates. Invertebrates excrete PAH metabolites inefficiently. There are marked differences in biotransformation for different PAH within each species of crustaceans. Limited information prevents conclusions on biotransformation by algae, plants and fungi.

2.9.6 Contamination of food with PAHs during processing and smoking

Processing procedures, such as smoking and drying, and cooking of food is commonly thought to be the major source of contamination by PAH. Depending on a number of parameters: time, fuel used, distance from the heat source and drainage of fat, type (grilling, frying, roasting), cooking results in the production in the food of a number of compounds including PAH. Although not precisely known, it is likely that there are several mechanisms of formation of PAH such as melted fat that undergoes pyrolysis when dripping onto the heat and pyrolysis of the meat due to the high temperature (Lijinsky and Shubik, 1965 a, b). A comparison of PAH levels in duck

breast steaks, undergoing various processing and cooking treatments for 0.5 hour to 1.5 hours, showed that charcoal grilled samples without skin contained the highest amount of total PAH (320 μg/kg), followed by charcoal grilling with skin (300 μg/kg), smoking (210 μg/kg), roasting (130 μg/kg), steaming (8.6 μg/kg) and liquid smoke flavouring (0.3 μg/kg). For PAH that are classified as carcinogenic (IARC class 1 or 2 A and B), the trend was the same with the exception that smoked samples contained the highest amount (35 μg/kg). In addition, the highest amounts of total and carcinogenic PAH were observed after smoking of duck breast samples for 3 hours (53 μg/kg) (Chen and Lin, 1997). Contamination of water may lead to intake of PAH through drinking water and cooked foods. The levels are usually below 1 ng/L in drinking water but can be higher when asphalt or coal tar coating of storage tanks and water distribution pipes are used.

2.10 Analytical Methods

PAH are extracted using different techniques prior to clean up and purification. PAH are most often identified and quantified using either gas chromatography (GC) with flame ionization detection (FID) or coupled to mass spectrometry (MS) or high performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection.

In addition to potential losses of PAH during homogenization, extraction and clean-up, there are a number of other factors that may lead to erroneous results. During sample collection and storage it is important that the sample is not exposed to light and high temperatures (leading to volatilization and/or chemical conversion). Also storage for a prolonged time before analysis may result in the reaction of some PAH with components of the food matrix.

Attention should also be paid to the possible co-elution of some PAH. For example, under the gas chromatographic conditions generally used, chrysene + triphenylene, the benzo [*b+j+k*] fluoranthenes, and the dibenzo [*a,h+a,c*] anthracenes may co-elute and give rise to only a single peak.

2.11 Barbecue

PAH formation during charcoal grilling was shown to be dependent upon the fat content of the meat, the time of cooking and the temperature (Mottier *et al*., 2000). For example a heavily barbecued lamb sausage contained 14 µg/kg of carcinogenic PAH (Mottier *et al.*, 2000). The presence of PAH was studied in several samples of meat and fish that were grilled on two geometrically different gas barbecues. In contrast to a horizontal barbecue, the vertical prevented fat from dripping onto the heat source, and the PAH level were very low and 10-30 times lower than with the horizontal system (Saint-Aubert *et al*., 1992).

2.12 Smoked foods

On a quantitative basis, the data reported in the literature are highly variable. Such variations can be attributed in part to the different procedures used to evaluate the presence of PAH, but the main reason for such discrepancies is the difference in procedures used for smoking. Such variables include: the type and composition of wood, type of generator, oxygen accessibility, temperature of smoke generation, and smoking time. PAH content in smoked fishery products from modern smoking kilns with external smoke generation and products from traditional smoking kilns have been compared. The average benzo [*a*] pyrene concentration determined for the traditional kilns was 1.2 µg/kg with a sum of carcinogenic compounds of 9 µg/kg, and 0.1 µg/kg and 4.5 µg/kg respectively, for the modern kilns (Karl and Leinemann,

1996). In Annex II of Directive 88/388/EEC (EEC, 1988), a maximum level of 0.03 µg benzo[*a*]pyrene per kg as a result of the use of (smoke) flavourings has been set for foodstuffs or beverages as consumed.

Regarding the generation of liquid smoke flavorings, it has been showed that poplar wood generated the highest number and concentration of both total and carcinogenic PAH, while oak, cherry tree, beech samples were similarly less effective. Hardwoods instead of softwoods have also been recommended, indeed, dry woods generate more PAH because of their higher smoke generation temperature (Guillen *et al.*, 2000).

2.13 Measures to reduce PAH contamination of foods

The amount of PAH formed during cooking or processing of food depends markedly on the conditions used. Simple practices are known to result in a significantly reduced contamination of foods by PAH (Lijinsky and Ross, 1967; Lijinsky, 1991; Knize *et al.*, 1999) as well as by other undesirable contaminants. This may include selecting preferentially lean meat and fishes, avoiding contact of foods with flames for barbecuing, using less fat for grilling, and, in general, cooking at lower temperature for a longer time.

Broiling (heat source above) instead of grilling can significantly reduce the levels of PAH. Actually the fat should not drip down onto an open flame sending up a column of smoke that coats the food with PAH. The use of medium to low heat, and placement of the meat further from the heat source, can greatly reduce formation of PAH. The intensity of flavour is not necessarily associated with the depth of the brown colour of grilled foods. It is therefore needless to overcook the food to get the flavour. However, cooking must always remains effective as regards inactivation of any possible contaminating bacteria or endogenous toxins.

In some countries (e.g. Norway) the food authorities have banned the sale of shellfish and mussels caught in areas contaminated with PAH. The public is advised not to catch and consume PAH-contaminated shellfish and mussels. Depuration of contaminated mussels in clean water will not reduce their level of PAH significantly. The waxy surface of vegetables and fruits can concentrate low molecular mass PAH mainly through surface adsorption. The concentrations of PAH are generally higher on plant surface (peel, outer leaves) than in internal tissue. Consequently, careful washing may remove, on average, up to 50% of the total PAH. Particle bound high molecular mass PAH which remain on the surface are easily washed off whereas low molecular mass compounds which are in the vapour phase can penetrate the waxy layer of fruits and vegetables and, therefore, are less efficiently removed by washing.

2.14 Absorption

The two major determinants of gastrointestinal absorption are aqueous solubility and lipophilicity, since absorption requires compounds to go into solution in the lumen of the intestine, pass through the cell walls of intestinal cells and be removed to the circulation. PAH are lipophilic compounds with low aqueous solubility. Those considered in this opinion have log K_{ow} values ranging from 3.4 to 7.3 and aqueous solubilities from 0.17 to 31740 μg/L at 25 °C. Although aqueous solubility generally decreases as the log K_{ow} value increases, there is considerable variability amongst compounds with similar log K_{ow} values reflecting the influence of molecular structure on aqueous solubility. There are three main routes of absorption in humans, lung and respiratory tract following inhalation of aerosols or particulates containing PAH, dermal following skin contact and gastrointestinal tract following ingestion in water or food. For the purposes of this evaluation only the last route will be considered in detail.

Rees and colleagues in 1972 showed rapid absorption of benzo [*a*] pyrene following intragastric administration in rats with highest levels seen in the thoracic lymph nodes after 3-4 hours. Based on results in whole animals and intestinal sacs, these workers suggested that absorption of benzo [*a*] pyrene involved two phases, uptake by the mucosa followed by diffusion through the intestinal wall (Rees *et al.*, 1972 as cited in IPCS, 1998). Laurent and colleagues (2001) described studies on the absorption of two PAH (benzo[*a*]pyrene (log K_{ow} 6.5, aqueous solubility 3.8 µg/L) and phenanthrene (log K_{ow} 4.6, aqueous solubility 1290 µg/L)) following oral administration to pigs in a lipophilic milieu. Two castrated Large White pigs were catheterised in the portal vein and brachiocephalic artery. Fourteen days post-surgery they were fed 1 litre of milk containing 50 µCi of [7, 10-14C]-benzo[*a*]pyrene or 15 µCi of [9-14C]-phenanthrene and 10 ml arterial and portal blood samples were collected before administration and then hourly for 6 hours at 9 and 24 hours. Radioactivity was detectable within 1 hour and peaked at 5-6 hours and reached background by 24 hours. The peak radioactivity was higher for phenanthrene despite the 3- fold lower dose. However the amounts of the PAHs in the portal vein were slightly higher than those in the brachiocephalic artery.

The peak time corresponds with that observed for milk fat and is much longer than glucose (45 minutes) or protein (30 minutes). No areas under the curves (AUCs) or other pharmacokinetic parameters are reported. Rahman and colleagues (1986) showed the presence of bile increased the intestinal absorption of PAH in Sprague-Dawley rats, absorption of benzo [*a*] pyrene (log Kow 6.50, solubility 3.8 µg/L) (and 7,12-dimethylbenz[*a*]anthracene) being affected more than that of anthracene (log Kow 4.5, solubility 78 µg/L) or pyrene (log Kow 5.18, solubility 135 µg/L) (Rahman *et al.*,1986 as cited in IPCS, 1998). Kawamura and co-workers (1988) demonstrated

that the composition of the diet influenced the absorption of co-administered 14C-benzo [*a*] pyrene in Wistar rats. The radio-labelled benzo [*a*] pyrene was orally administered in a solution, emulsion or suspension of 200 mg of food or food component and blood samples collected over the first six hours and at 24 hours post-administration. The foods and components studied were triolein, soya bean oil, cellulose, bread, rice flake, lignin, water, starch, katsuobushi (dried bonito), ovalbumin, potato flake and spinach. The AUCs for administration in lipophilic foods (triolein and soya bean oil) were 50% and 42% of that following intravenous administration in saline. The AUCs for the other foods tested were 20-25% of that following intravenous administration in saline except for cellulose (around 30%); all were significantly lower than the lipophilic foods. These data suggest that the bioavailability of PAH from food will be in the range of 20-50% and that it increases with increasing content of lipophilic components in the food.

2.15 Distribution

Distribution of PAH has been studied in rodents and levels in tissues are influenced by several factors; the PAH, route of administration, vehicle, time of tissue sampling after treatment and presence or absence of inducers or inhibitors of hydrocarbon metabolism. However three common traits are observed; there are detectable levels of PAH (probably more accurately PAH-derived material) in almost all organs, those organs rich in adipose tissue act as depots from which material is slowly released and high levels are found in the gastrointestinal tract irrespective of the route of administration.

Following intravenous administration benzo [*a*] pyrene was rapidly removed from the bloodstream with a distribution half-life of less than 1 minute. The rate of clearance of

radioactivity after administration of radio-labelled benzo [*a*] pyrene was increased after pre-treatment with inducers of metabolism either benzo [*a*] pyrene or phenobarbital. Whole body autoradiography was used to study distribution in mice and their foetuses following intravenous administration of 14C-labelled 3-methylcholanthrene to pregnant hams. Radioactivity was widely distributed in maternal tissues and was detected in foetuses showing that it crossed the placenta (Takahashi and Yasuhira 1973, Takahashi 1978 as cited in IPCS, 1998). Similar results have been obtained following inhalation, intragastric or intravenous administration of benzo[*a*]pyrene and 7, 12-dimethylbenz[*a*]anthracene to rats and mice (Shendrikova *et al.*, 1973; 1974; Shendrikova and Aleksandrov 1974; Neubert and Tapken 1988; Withey *et al.*, 1992, as cited in IPCS, 1998). In a small study in humans, samples of milk, placenta, maternal and umbilical cord blood were taken from 24 women and analysed for selected PAH. The highest levels of benzo [*a*] pyrene, dibenz [*a,c*] anthracene and chrysene were observed in milk and umbilical cord blood but levels were only above the detection limit in half of the samples. Nevertheless the authors concluded that both foetuses and infants were exposed to PAH which were presumed to be from the maternal diet (Madhavan and Naidu, 1995 as cited in IPCS, 1998).

2.16 Harmful effects of Polycyclic Aromatic Hydrocarbons

PAH health effects have been widely studied primarily because of their potential carcinogenic and mutagenic properties. Several toxicological studies in animals [World Health Organization-International Programme on Chemical Safety (WHO-IPCS) 1998] and occupational studies in humans (Armstrong et al., 2004) demonstrate an excess risk of lung cancer associated with PAH inhalation. The potential influence of PAH exposure on the development of bladder and urinary system cancer also has

been studied (Bosetti *et al*., 2007). In general, the carcinogenic properties of PAHs depend on the number of aromatic rings. Benzo [a] pyrene (BaP) has been the most extensively studied PAH and is the usual marker for carcinogenic levels of PAHs in environmental studies. However uncertainties about the suitability of BaP as a cancer risk indicator have also been discussed (Bostrom et al., 2002). The International Agency for Research on Cancer (IARC) classified BaP as carcinogenic to humans (group 1); other PAHs such as dibenzo [a,h] anthracene (DahA), as probably carcinogenic to humans (group2A); and other PAHs, such as naphthalene (NaP), Benzo[a]anthracene (BaA), Chrysene (Chr), benzo[b]Fluoranthene (BbF), Benzo[j]Fluoranthene (BjF) and indeno[1,2,3-cd]pyrene (Ind) as possibly carcinogenic to humans (group2B).

2.17 Toxic levels and bioaccumulation of PAHs

PAHs can also metabolize and become reactive electrophilic intermediates that can form adducts, which may induce mutations and ultimately tumors (IARC 2010). Some PAHs, such as Fluoranthene (FLT), which IARC classified as weak carcinogens (group3), have mutagenic characteristics and may therefore play an important role in carcinogenesis (Bostrom *et al* 2002). Studies of other outcomes have suggested effects of PAHs on the development of arteriosclerosis (WHO 2000), reproductive outcomes such as intrauterine growth retardation (Dejmek *et al*.,2000; Sram *et al*.,2005) and children's neurological development (Perera *et al*.,2009). Furthermore, because PAHs are highly hydrophobic, they have an affinity for environmental matrices such as sediments, soils, and biota and can bioaccumulate in adipose tissues and become magnified through the food chain (WHO-IPCS 1998). Because of their lipophilic characteristics and limited biodegradation, PAHs are classified as persistent organic pollutants.

Ingestion is quantitatively the main route for PAH human exposure. However, inhalation is also significant route because of the ubiquitous presence of these compounds in the atmosphere [Agency for Toxic Substances and Disease Registry (ATSDR) 1995; Li *et al.,* 2010]. PAHs can be associated with the atmospheric gas phase and particulate phase (Ravindra *et al.,* 2001; Gerde et al., 1993). Risk estimation for PAH exposures is complex for several reasons. There are few reported human epidemiological studies of individual PAHs, and individual PAHs are likely to induce cancer through different mechanisms. As mentioned above, BaP is the most studied PAH, and other PAHs have been ranked according to cancer potency relative to BaP using toxic equivalency factors (TEFs). The application of TEFs combined with the WHO quantitative risk assessment (QRA) methodology (WHO 2000) can be used to estimate the excess life time risk of lung cancer due to PAH exposures.

2.18 Methods of analysis of PAHs

The determination of PAHs in environmental matrices has been subject of great scientific attention and during the latest years, as the accuracy and sensitivity of analytical methods need to be improved in order to be able to detect the compounds of interest in a complex matrix such as meat. Therefore, significant research is being devoted to the optimization of analytical methodologies. The major problems associated with analysis of PAHs in foods are as follows:

- Most PAHs are present in trace amounts (ppb or ppm) in foods, which can make extraction difficult;
- Many PAH-like impurities can be co-extracted with PAHs from foods, which can interfere with the subsequent separation and identification of PAHs;

- Most PAHs are structurally similar and present in isomeric forms, which make their separation and identification difficult.

The most common method for extraction of PAHs from foods usually involves saponification of lipids by methanolic KOH, followed by liquid-liquid partition and liquid-solid chromatography. The separation of PAHs has been previously achieved by thin-layer chromatography (TLC) .However; the method proved to be lengthy and failed to resolve various PAHs in foods. Gas chromatography in combination with mass spectrometry (GC-MS) has been widely used to determine PAHs in foods (Kolarovic et al., 1982; Afolabi et al., 1983; Lawrence et al., 1984a; Karlesky et al., 1986; Hopia *et al*., 1986; Castello *et al*., 1993). PAHs may be degraded if exposed to high temperatures during separation. Also , a number of isomeric PAHs such as benzo[b]Fluoranthene and benzo[k]Fluoranthene are difficult to separate. In view of these problems, many high performance liquid chromatographic (HPLC) methods have been developed to PAHs from foods (Schimdt *et al*., 1971; Takatsuki et al., 1985; Joe et al., 1982; Yabiku *et al*., 1993; Gomma *et al*., 1993; Chen *et al*., 1996; Chiu *et al*., 1996).

An ideal extraction technique theoretically should allow complete removal of impurities from sample so that the subsequent separation and identification of PAHs by GC-MS or HPLC would not be disturbed. Saponification is often employed to remove unwanted lipid, free fatty acid and water-soluble impurities. However it has been reported that benzo [a] pyrene is decomposed under alkaline conditions (Takatsuki *et al*., 1985). In view of this problem a more sophisticated extraction method is required for meat products. Theoretically, an ideal separation technique should allow complete resolution of all sixteen PAHs with low detection limit, high reproducibility, high sensitivity and short retention time. However, it is often

impossible to achieve these goals simultaneously. Therefore one must choose a particular technique for a specific type of sample so that the best results can be obtained. HPLC is often used in combination with UV, Fluorescence or Diode-array detection. GC is often used in combination with flame ionization detection (FID), photo ionization detection (PID), quadrupole analyzer mass spectrometry (QUAD), and Ion-trap mass detection. The newly developed Ion-trap mass detector permits the entire mass spectra to be obtained with a sensitivity greater than that of QUAD (SIM) and the identification of compounds by means of automatic library search, and the simultaneous use of ITD and FID allows quantitation to be carried out without the need for standard samples of all of the detected PAHs (Castello et al., 1993). In addition to GC-MS, the combination of HPLC with mass spectrometry(HPLC-MS) is also a powerful tool for identification and quantitation of PAHs. The resolution power of a chromatographic system depends primarily upon column efficiency and separation selectivity. GC is often used for separating complex PAHs because of its superior column efficiency.

2.19 Studies conducted on PAHs in environmental samples

In a study conducted by Yurchenko et al, the levels of six PAHs (benzo [a] pyrene, benz[a]anthracene, benzo [k] Fluoranthene, benzo [b] Fluoranthene, benzo [ghi] perylene and indeno [123-cd] pyrene) were determined in 97 various samples of smoked fish, 11 samples of fresh fish, and18 olive- and14 rape-oil samples. For cleaning of the sample, a gel chromatography was used. PAHs were separated by gas chromatography and detected by positive-ion chemical ionization using ammonia as reagent gas. The HP 6890 plus GC/HP 5973 MSD with positive-ion chemical ionization option was used in the selected ion monitoring mode. The limit of detection of PAHs using this method was approximately 0.3 ppb with about 75% recovery. The

results were confirmed by high-pressure liquid chromatography. The samples of domestic (Estonian) smoked fish was analysed during one year period, the sum of the average of six PAHs content was found to be 12.37ug/kg , and in samples of fresh fish it was not detected.

Kazerouni etal., created a Benzo[a]pyrene, database of selected food products that could be linked to Food Frequency Questionaires (FFQs) to estimate BaP intake. BaP levels were measured for each food line-item which consisted of a variety of foods in a FFQ. Composite sample parts were derived from the second National Health and Nutrition Examination Survey (NHANES II) which represents the most common food items consumed by the general population. Meat samples were cooked by different techniques in controlled conditions, and by various restaurants and fast-food chains. Non-meat products were purchased from the major national supermarket chains. The quantities of BaP were measured using a thin-layer chromatography (TLC)/ spectrofluorometer technique and were highly correlated with both BaP (r=0.99) and sum of carcinogenic PAH (r=0.98) measured by HPLC technique. They linked their database to the results from a FFQ and estimated the daily BaP intake of various food items in 228 subjects in the Washington, DC metropolitan area. The highest levels of BaP (up to about 4ng BaP/g of cooked meat) were found in grilled/barbecued very well done steaks and hamburgers and in grilled/barbecued well done chicken with skin. BaP concentrations were lower in meats that were grilled/barbecued to medium done and in all broiled or pan-fried meat samples regardless of doneness level. The BaP levels in non-meat items were generally low. However, certain cereals and greens (e.g. kale, collard greens) had levels up to 0.5ng/g. In their population, the bread/cereal/grain, and grilled/barbecued meat, respectively, contributed 29 and 21 percent to the mean daily intake of BaP.

Akpambang *et al.,* (2009) investigated PAH levels in some smoked/grilled fish and meat products commonly consumed in Nigeria and to estimate the potential risk associated with the consumption of such traditionally processed foodstuffs. Unprocessed samples were also analysed too. A quick and efficient microwave assisted (MAE) method, involving simultaneous saponification and solvent extraction, was used for sample preparation before high performance liquid chromatography (HPLC) determination and spectrofluorometric detection of 15 Environmental Protection Agency (EPA)-priority PAHs. Attention was focused on the EPA priority PAHs as they include the eight PAHs indicated (together with a subgroup of four PAHs) as the most suitable indicators of the presence of carcinogenic and genotoxic PAHs in foodstuffs (EFSA 2008).Traditionally smoked and/or grilled fish and meat from the Nigerian market were found to be heavily contaminated with benz (a) pyrene (BaP) in amounts often exceeding by far the limit of 5 µg/kg settled in 2005 by the European Commission. Margin of exposure (MOE) values lower than 10,000, which according to EFSA (2008) indicate a potential concern for consumer health, were generally found in commercially smoked samples for both BaP and eight polycyclic aromatic hydrocarbons (PAHs). This demonstrates the need for legal limits in traditionally smoked foodstuffs in Nigeria and possible risk management action. In with previous works, it has been demonstrated that the use of charcoal smoke instead of smoke from burning wood can help in lowering the final PAH load at levels around 2 ug/kg.

In the work of Wretling *et al.,* (2009), thirty-eight samples of smoked meat and meat products and thirty-nine samples of smoked fish were analysed for BaP and other polycyclic aromatic hydrocarbons (PAHs) using the high resolution gas chromatography-mass spectrometry (HRGC-MS) method complying with the criteria

for official control according to commission Regulation (EC) No333/2007. Nine samples of smoked meat showed high BaP levels ranging from 6.6 to 36.9ug/kg, exceeding the 5.0ug/kg maximum level for smoked meat and fish established by the European commission (Regulation (EC) No 208/2005). The samples analysed were produced by the traditional "sauna" smoking, where the food is directly exposed to hot smoke from a burning log fire. Six samples of smoked fish had BaP levels exceeding 5.0 µg/kg, the concentrations ranging from 8.4 to 14.4µg/kg. Samples of meat and fish smoked by indirect technique, using smoke from an external smoke generator, all had BaP levels below the limit of quantification, i. e. 0.3µg/kg.

Santos *et al.*, (2011), investigated the contamination levels in traditional dry fermented sausages manufactured in Alentejo (South Portugal), a total of 66 samples of smoked meat sausages and blood sausages picked up from seven producers were taken for analysis.

The amounts of sixteen PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, Fluoranthene, pyrene, benzo (a) anthracene, chrysene, benzo (b) Fluoranthene, benzo (k) Fluoranthene, benzo (a) pyrene, dibenzo (a,h) anthracene, benzo (g,h,i) perylene, indeno (1,2,3-cd) pyrene were quantified.

Smoked meat products are still produced in traditional way in Zlatibor region, Serbia. Beef and pork ham were smoked by beech wood smoke, both in traditional (TS) and industrial smokehouses (IS). Smoke samples were collected from both smokehouses using different types of tubes (PUF and XAD-2) during meat smoking. The sum of 16 EU priority PAHs in final smoked beef and pork ham was (µg/kg): beef ham - 3.9/TS, 1.9/IS; pork ham – 4.9/TS, 4.2/IS. The total emission of the analysed PAHs in smoke samples was (mg/m3): in PUF 1.1/TS, 3.8/IS and in XAD-2 0.9/TS, 11.0/IS. PAH

fingerprints in smoke and smoked beef and pork ham were compared. Chrysene was found to be the most predominant PAH compound in smoke, both in PUF and XAD-2 tubes from TS, while benzo[c]fluorene (BcL) was the most predominant PAH in smoke from IS. For PAHs with lower MW (BcL to BaP) similar fingerprints between smoke-beef and smoke-pork ham were observed, while the fingerprints for dibenzopyrenes (MW=302) were different, both in TS and IS. BaP equivalent concentrations (BaPeq) were calculated, both in smoke and smoked meat products.

In a study in Istanbul-Turkey, (Serden et al., 2010) 24 samples of smoked fish products were analysed in order to investigate the levels of the potently carcinogenic PAHs. Salmon and rainbow trout samples were tested with a liquid choromatographic (HPLC) method. In none of the smoked fish products benzo (a) pyrene, one of the potential carcinogenic PAH compounds limited in food codex alimentarious was detected. However, benzo (a) antracene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (g,h,i) perylene, compounds which could be carcinogenic for humans, were detected in the smoked samples. There is a significant correlation between the fish fat and the total PAHs level. Consequently the average fat contents of the smoked salmon were significantly higher than that of the smoked rainbow trout. It was also detected that the total PAH amount variations of the smoked fish samples were due to the non-homogenous smoke dispersion in the traditional ovens and it was difficult to obtain homogenous smoked fish products from traditional ovens.

The contents of the 15+1 EU priority PAH were analysed from 113 representative commercial smoked German meat products collected in the year 2006 with a Fast-GC/HRMS method. The median of benzo [a] pyrene content was 0.03 g/kg and therefore greater than a factor of 100 below the maximum level of 5 g/kg. The highest content of benzo [a] pyrene was detected in a Frankfurter-type sausage (0.43 g/kg).

The sum content of benzo [a] pyrene, benzo [a] anthracene, chrysene and benzo [b] Xuoranthene ("PAH4"), as proposed by the European Food Safety Authority to be a good marker for PAH in food, was 0.28 g/kg in median, and the sum content of the 15+1 EU priority PAH was 0.64 _g/kg in median. The analysed smoked meat products showed an increasing presence of PAH in the following order: cooked ham (n = 17) < raw sausages (n = 25) < liver sausages (n = 25) < raw ham (n = 23) < Frankfurter-type sausages (n = 23). The correlation coefficient (R) between BaP and the sum of the 15+1 EU priority PAH was 0.90. To increase the safety of the consumer, a lowering of the BaP maximum level to 1 g/kg is proposed and critical aspects using "PAH4" as a marker for PAH in food surveillance are discussed.

A study was conducted to determine the levels of Polycyclic Aromatic Hydrocarbons (PAH) in smoked *Scomba japonicus* sampled from some Ghanaian markets. By way of preparation, smoked fish comes into contact with smoke or extremely high temperature which are potential sources of PAH generation. Levels of 20 individual PAHs including acenaphthene, acenaphtyelene, anthanthrene, anthracene, benz (a) anthracene, benzo (a) pyrene, benzo (b) fluoranthene, benzo (e) pyrene, benzo (ghi) perylene, benzo (j) fluoranthene, benzo (k) fluoranthene, chrysene, cyclopenta(cd)pyrene, dibenzo (ah) anthracene, fluoranthene, fluorene, indeno (1, 2, 3-cd) pyrene, naphthalene, phenanthrene and pyrene were determined in 34 smoked fish samples using gas chromatographic techniques with flame ionization detector. Benzo (a) pyrene, which is one of the very few PAHs for which a legal limit exists in different types of food matrices in addition to other high molecular weight PAHs suspected to be carcinogens, were detected in most samples.

The concentrations of benzo [a] pyrene and 11 other polycyclic aromatic hydrocarbons (PAHs) were analysed from 322 commercial, cured meat products and

14 home-grilled meat samples as part of the Estonian food safety monitoring programme during 2001–2005. The maximum acceptable concentration of 5 $mgkg^{-1}$ for benzo [a] pyrene was exceeded in 3.4% of samples. The highest PAH concentrations were detected in home-grilled pork samples. Using of disposable grilling unit resulted in 1.6 times higher PAH concentrations compared to the traditional wood-burning grill. The average intake of Benzo [a] pyrene and sum of 12 PAHs from meat products were estimated for children (age 1–16 years) on the basis of an individual food consumption questionnaire and, for the general population, based on national food consumption data.

The highest total PAH concentrations detected were 16 $mgkg^{-1}$ in smoked meat and ham, 19 $mg\ kg^{-1}$ in smoked sausage and 6.5 $mgkg^{-1}$ in smoked chicken samples. Since smoking and grilling are prevalent meat-cooking methods in Estonia, the impact of meat products is assessed to be significant in overall PAH intake.

Analysis for the presence of sixteen priority polynuclear aromatic hydrocarbons (PAHs) (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz [a] anthracene, chrysene, benzo [b] fluoranthene, benzo [k]fluoranthene, benzo [a] pyrene, benzo [ghi] perylene, dibenz [a, h] anthracene and indeno [1,2,3-cd] pyrene) was carried out on four different species of fish found in the Niger Delta region of Nigeria. The fish species included *Parachanna obscura*, *Pseudolithus elongatus, Liza dumerillii* and *Clarais gariepinnus*. Individual PAHs were identified through both retention time match with authentic standards and simultaneous maximization of several major ions from gas chromatography/mass spectrometry (GC/MS) data. Four isotopically-labeled internal standards namely D10-acenaphtalene, D12-chrysene, D10-phenanthrene and D12-perylene, were used for quantitation. All four species of fish were found to contain

high levels of PAHs ranging from 0.41 to 39.64 ug/kg. The high molecular weight PAHs such as benzo [ghi] perylene, dibenzo [a,h] anthracene and indeno [1,2,3-cd] pyrene were consistently present in much higher amount than other PAHs in all four species of fish studied, suggesting higher resistance of these compounds to degradation.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Reagents and chemicals

All reagents were of analytical grade and included; 96.5% hexane, 99.5% acetone, 20% diethylether, 100% methanol, anhydrous sodiumsulphate, silicagel (5% water content).Millipore-Q water was used.

3.2. Equipments and Instruments

Centrifuge, rotary evaporator, nitrogen gas evaporator isolute SPE PAH HC 1g columns, reservoir 6 mL, glasswool, Gas-chromatography/mass spectrometer.

3.3. Sampling

Commercially smoked bushmeat (about 2.5 kg) of twelve different species commonly consumed in Ghana, namely antelope (cephalophus maxwellii), Grass cutter (Thryonomys swinderianus), Bushcow (Syncerus caffer nanus), bushbuck (Tragelaphus sciptus), Rat (cricetomys gambianus), squirrel (protoxerus strangeri), bushpig (potamochoerus porcus), bushrabbit (lepus spp), cockbird, bush guineafowl, bushcat (felis aurata), monitorlizard (varanus niloticus), were purchased from three different market centres from local vendors in Kumasi, Ghana.

Each of the twelve different bush-meat samples were purchased from three different market centres in Kumasi including Asafo, Central market, Atwemonom (Kejetia) from commercial local meat vendors. Samples from different vendors were pooled together to obtain representative samples (for A, B, C, D, E, F, G, H,I, J, K, L) for each of twelve types of meat products analysed. Representative smoked bushmeat

were deboned, cut into smaller pieces. A small portion of each of the representative sample so obtained (about 100g) was milled, packed in aluminium foil wraps and stored in the freezer at -20ºC before analysis.

3.4. Extraction

PAH extraction was carried out by applying the method by (Wretling *et al.,* 2010), with some modifications. About 1 g of milled sample was weighed into a test tube and 10 mLof distilled water was added. The mixture was taken through the centrifuge at 3000 rpm (revolutions per minute) for 10 minutes. The supernatant was transferred into a new tube. To the residue was added 10 mL of 1:2 acetone: hexane and shaken for 10 minutes. The mixture was taken through the centrifuge at 3000 rpm for 10 minutes. The supernatant obtained was passed through anhydrous sodium sulphate. About 30 mL of hexane was added to the residue, mixture shaken and put in ultrasonic for 10 minutes and the supernatant obtained was passed through anhydrous sodium sulphate. The sample obtained after passing through anhydrous sodium sulphate was concentrated by rotary evaporator, transferred into a test tube, the flask was washed three times with hexane and added to the test tube and shaken well. The mixture was taken through the centrifuge at 2000 rpm for 5 minutes. The supernatant (hexane layer) was transferred into a new tube, the process was repeated and the supernatants are added together and made up to10 ml with hexane.

3.5 Clean-up:

The 10 ml supernatant (hexane layer) was concentrated to approximately 1 ml, 300 uL of methanol was added and SPE column chromatography was performed. Elution of sample carried out with10 ml of (1:4) i.e. 20 % diethylether: 80 % hexane. Eluate was

concentrated to 2 ml and transferred into a sample vial and stored in a refrigerator for analysis.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

Target analytes included sixteen non-alkylated PAHs. Isolation, identification, and quantification of the 16 Priority Pollutants via Stout *et al*., (2001b) which follows a standard procedure of organic extraction, sample clean-up, and analysis using GC-MS capabilities.

Method of analysis for Non-alkylated PAHs

Calibration standards included 8270 LCS Mix 1 (Supelco, Inc., St. Louis, MO; lot number LB21442). This mix of semivolatile compounds included the following target analytes: acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene, dibenzofuran, fluoranthene, fluorene, indeno(1,2,3-c,d)pyrene, naphthalene, phenanthrene, pyrene, 1-methylnaphthalene, and 2-methylnaphthalene. This prepared solution contained a concentration of 100 µg/mL of all compounds mixed in a solution of 90:10 methylene chloride:acetone. Neat standards of the remaining non-alkylated target analytes, benzo(e)pyrene (Supelco, Inc., St. Louis, MO; lot number LB21553), biphenyl (Supelco, Inc., St. Louis, MO; lot number 24 LB15806), dibenzothiophene (Supelco, Inc., St. Louis, MO; lot number LB20805), and perylene (Supelco, Inc., St. Louis, MO; lot number LB20731) were prepared to a 10 ppm solution by adding 1.00 mg of each to methylene chloride to fill a 100 mL flask.

Each standard (surrogate, LCS 8270 Mix, and prepared neat) was analyzed using a Shimadzu GCMS-QP2010 Gas Chromatograph coupled to a Hewlett Packard 5972 Mass Selective Detector (Hewlett Packard L.P., Palo Alto, CA) in full-scan mode.

The GC instrument specifications followed those published by Stout *et al.,* (2001b) with some modification:

Instrument: SHIMADZU GCMS-QP2010

Column: HP5MS 30m x 0.25µm x 0.25mm id

Carrier Gas: Helium

Column Flow: 1.2ml/Min

Injection temp.:250^{o}c

Injection Mode: Splitless

MS CONDITIONS

Acquisition mode: selected ion monitoring mode (SIM)

Ion source temp.: 250^{o}c

Interface temp.: 280^{o}c

Solvent cut time: 4 min

Table 3: Oven Temperature Program

RATE	TEMPERATURE (^{O}C)	HOLD TIME (min)
-	80	1
10	200	5
5	280	8
10	300	2

Total Runtime: 46min

Table 4: M/Z Parameters Used for the MS

S/N	Compound	Quant ion	Qual ions
1	Naphthalene	128	129, 127
2	2-Methylnaphthalene	142	141,115
3	Acenaphthylene	152	151, 153
4	Acenaphthene	153	154, 152
5	Fluorene	166	165, 167
6	Phenanthrene	178	176, 179
7	Anthracene	178	176, 179
8	Fluoranthene	202	200, 203
9	Pyrene	202	200, 203
10	Benzo(a)anthracene	228	114, 226
11	Chrysene	228	114, 226
12	Benzo(k)Fluoranthene	252	253, 250
13	Benzo(b)Fluoranthene	252	253, 250
14	Benzo(a)pyrene	252	253, 250
15	Benzo(g,h,i)perylene	276	277, 138
16	Dibenzo(a,h)anthracene	278	279, 139
17	Indeno(1,2,3-cd)pyrene	276	138, 227

Elution times for all non-alkylated target and surrogate compounds were confirmed through replicate analyses in which the elution time for each individual component remained consistent (±0.1 min). Once the elution times were identified, the PAHs were confirmed with comparison of mass-to-charge (m/z) ratios to library database values (NBS-75K, 1995).

Quantification of compounds was not performed using this method. The sensitivity of full-scan mode is markedly decreased due to the full range of m/z ratios being scanned. Instead, full-scan was used as a qualitative assessment of the whole sample and as a way to evaluate elution times of non-alkylated compounds.

All samples were analyzed by GC/MS under the previously described instrument parameters. Each sample was analyzed using four separate programs utilizing the selected ion monitoring (SIM) mode. Tables 3 and 4 list each program and the mass-to-charge ratio(s) used to identify and quantify the compound(s). Analytes targeted within a certain time range were distinguished from each other by elution time within that time period and m/z.

Quality control measures were taken to validate data integrity, as follows. Matrix blanks were run between each sample and batch standards were run to confirm elution time and response factors. Detection limits were compound specific, and were set at the lowest concentration of the calibration curve where each individual component was identified, with adequate signal to noise ratio for confidence. Due to the use of selected ion monitoring mode, peaks were not limited to a 3:1 signal to noise ratio for identification, but were identified based on a consistent elution time from sample to sample. All extracts were analyzed by GC/MS in triplicate. All data in subsequent tables and figures are represented as means.

CHAPETER FOUR

4.0 RESULTS AND DISCUSSIONS

In this study, 12 samples of different smoked bush meats were analyzed and the concentrations of PAHs were determined. The data collected after the analysis of the bush meat were processed and presented in graphs to explain the results of the experiments. The PAHs levels of the bush meat samples from the 12 bush meat species from Kumasi in the Ashanti Region of Ghana were far below the European Commission standard maximum level of 5µg/kg in smoked meat.

According to Figure 1 samples of smoked bush meat contained Naphthalene in variable concentrations. On the basis of the results it was found that smoked Antelope contained Naphthalene in concentrations substantially higher (0.353µg/kg) in comparison with the other 11 smoked bush meat samples. Smoked rat had the least (0.011µg/kg) Naphthalene concentration.

4.1 Concentration of Naphthalene in various bushmeat

Clearly analyzed samples contain Naphthalene concentrations below the EU permitted maximum limit of 5 µg/kg. The highest content of Naphthalene (Fig. 1) was detected in smoked Antelope (0.353 µg/kg), the lowest, in smoked Rat (0.011µg/kg). This result clearly indicates that the production of smoked bush meat with Naphthalene levels less than 1µg/kg is possible in non-intensely smoked bush meat. Taking into account properties of Naphthalene, the EU recommended that the Naphthalene content in smoked bush meat should be as low as reasonably achievable (ALARA).

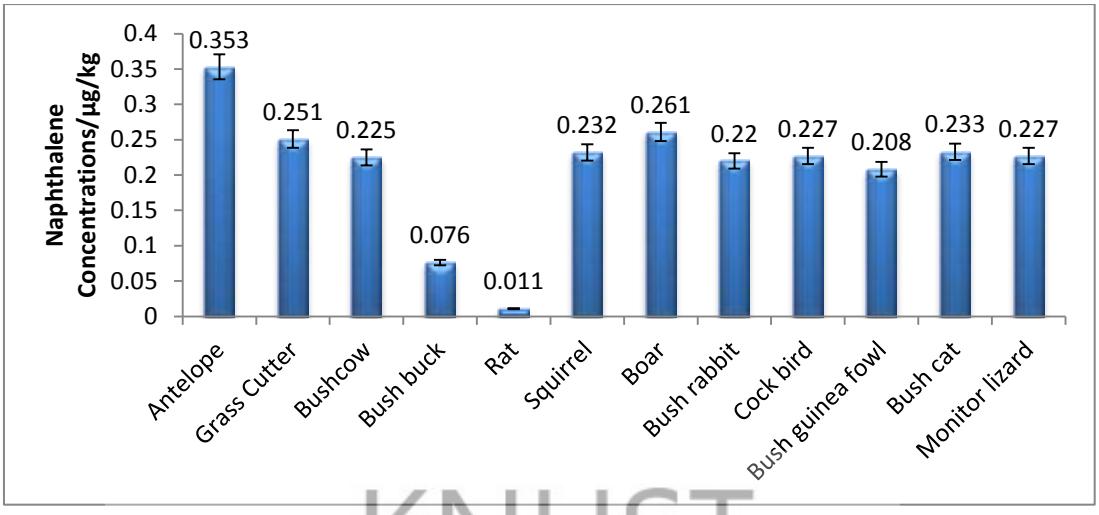


Figure 1: Various bush meat sample

4.2 Concentration of 2-Methylnaphthalene in various bushmeat

According to Figure 2 the various concentrations of 2-Methylnaphthalene in the bush meat samples range between 0.527 µg/kg to 0.067 µg/kg. On the basis of obtained results it was found that smoked Antelope contained 2-Methylnaphthalene in concentrations substantially higher (0.527µg/kg) in comparison with the other 11 smoked bush meat samples. The mean concentrations for smoked Boar, Bush cat, Cock bird, Bush cow, Squirrel, Monitor lizard, Bush guinea fowl, Bush rabbit, smoked Grass cutter and Bush buck were (0.519 µg/kg), (0.478 µg/kg), (0.47 µg/kg), (0.428 µg/kg, (0.422 µg/kg), (0.421 µg/kg), (0.411 µg/kg) (0.404 µg/kg), (0.399 µg/kg), (0.067 µg/kg) respectively.

According to Figure 2, analyzed samples contain 2-Methylnaphthalene concentrations that were below the EU permitted maximum limit. The highest content of 2-Methylnaphthalene (Fig. 2) was detected in smoked Antelope (0.527 µg/kg), the lowest; in smoked bush buck (0.067 µg/kg). This result clearly indicates that the production of smoked bush meat with 2-Methylnaphthalene levels less than 1µg/kg is possible in non-intensely smoked bush meat. Taking into account properties of 2-

Methylnaphthalene, the EU recommended that the 2-Methylnaphthalene content in smoked bush meat should be as low as reasonably achievable (ALARA).

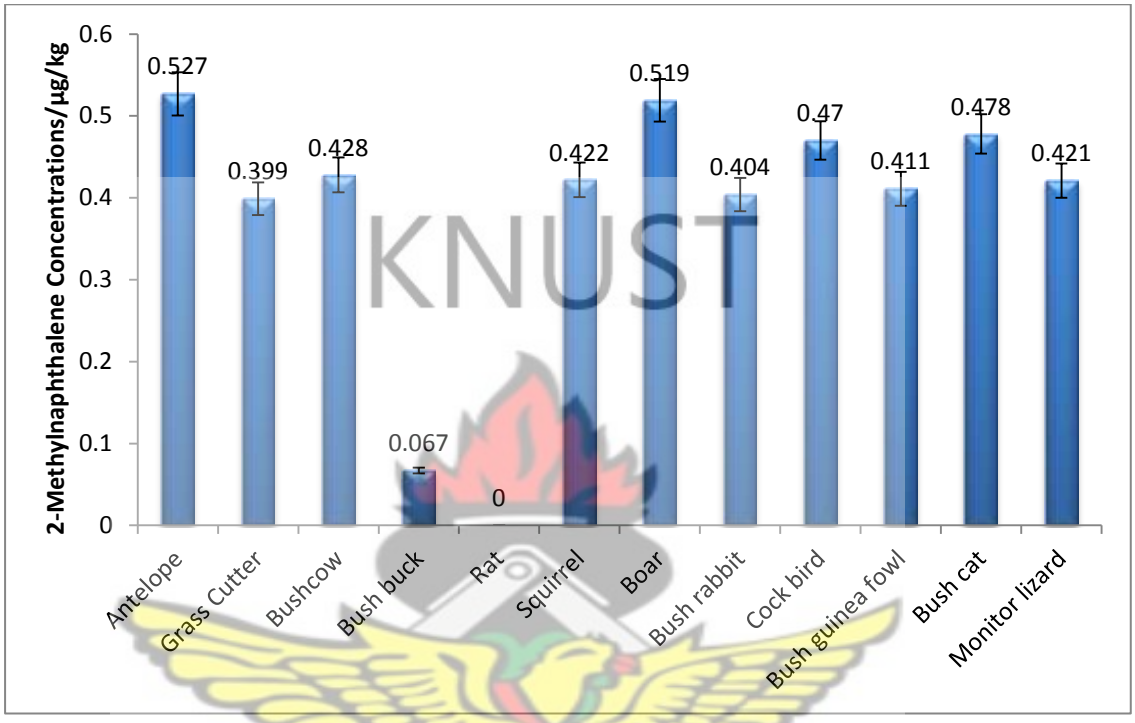


Figure 2: Various bush meat samples

4.3 Concentration of Acenaphthylene in various bushmeat

According to Figure 3, concentrations of Acenaphthylene in the bush meat samples ranged between 0.114µg/kg to 0.087µg/kg. On the basis of obtained results it was found that smoked Antelope contained Acenaphthylene in concentrations substantially higher (0.114µg/kg) in comparison with the other 11 smoked bush meat samples. Followed by smoked Boar (0.104 µg/kg), Bush cat (0.1µg/kg), Cock bird (0.1µg/kg), Bush rabbit (0.093 µg/kg), Bush guinea fowl (0.093 µg/kg), Bush cow (0.092 µg/kg), Squirrel (0.088 µg/kg), Monitor lizard (0.087 µg/kg), smoked Grass cutter (0.085 µg/kg) and Bush buck (0.054 µg/kg), respectively. Comparing the levels

of Acenaphthylene to the naphthalene and 2-Methylnaphthalene, the Acenaphthylene had relatively lower concentrations in the smoked meat samples.

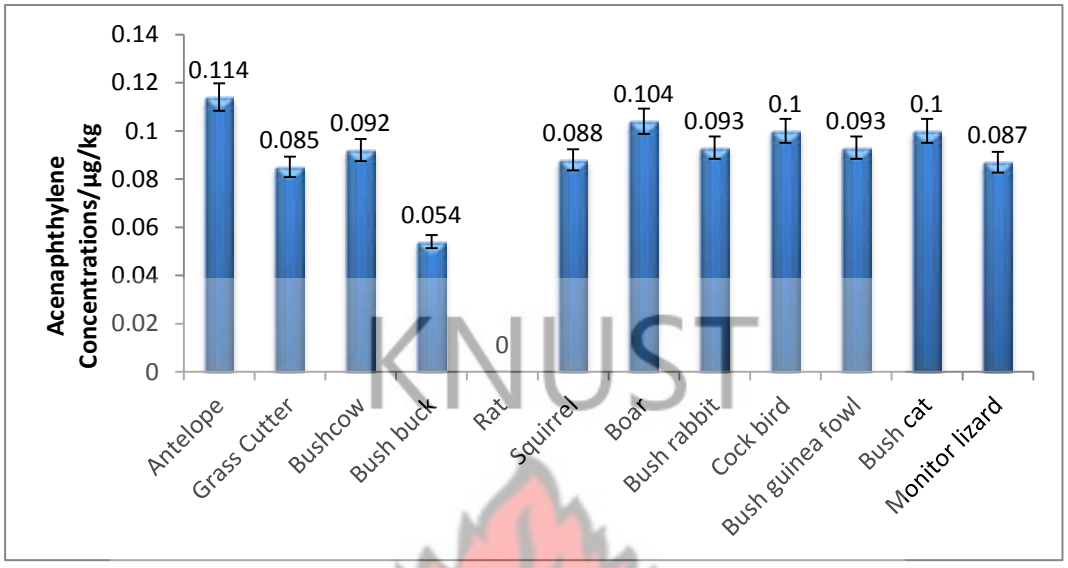


Figure 3: Various bush meat samples

4.4 Concentration of Fluorene in various bushmeat

Fluorene was slightly higher in bush cat as compared to the bush meat species (Figure 4) and as a result it was significantly different ($P \leq 0.05$) from the other species but not bush cock bird and monitor lizard (Figure 4). The Fluorene content ranged from the least of 0.049 µg/kg in squirrel and a maximum of 0.132 µg/kg in bush cat. However, fluorene was absent in bush cow and rat. Analysis based on comparative examination with the EU standards showed that all the recorded values of fluorene in the various bush meat samples were within the safety limits (Figure 4).

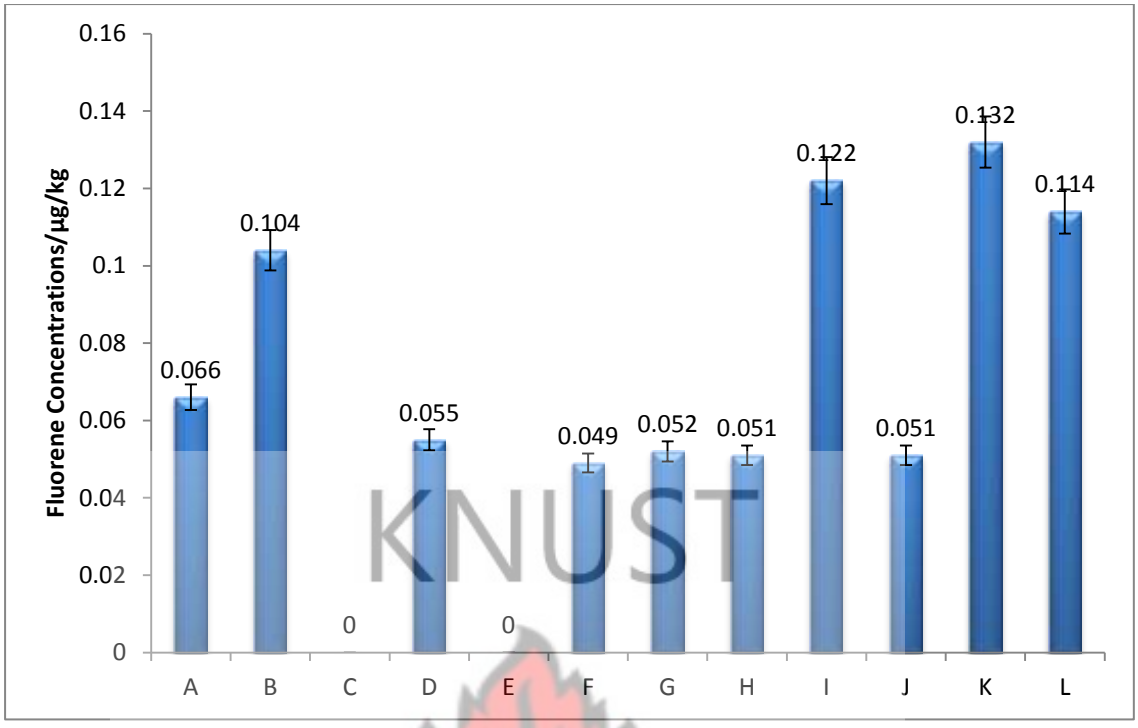


Figure 4: Various bush meat samples

4.5 Concentration of Phenanthrene in various bushmeat

The phenanthrene content presented in Figure 5 varied significantly among bush meat samples across the various species. The phenanthrene content ranged from a minimum of 0.053 µg/kg in rat and a maximum value of 0.92 µg/kg antelope. The phenanthrene content was statistically different ($p \leq 0.05$) across the bush meat species. Boar and Bush cat recorded the next highest phenanthrene levels in sequence. The EU values for the various bush meat species (Figure 5) compares favorably with our findings. Thus all measured values, in all cases less than 1 µg/kg, for the various bush meat samples in this research are within the safety limits.

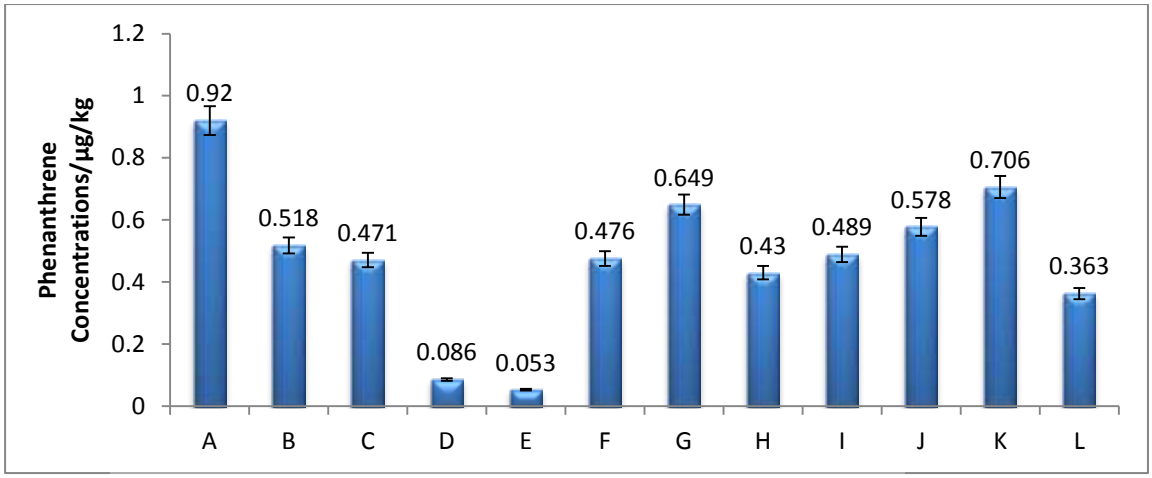


Figure 5: Various bush meat samples showing Phenanthrene levels

4.6 Concentration of Anthracene in various bushmeat

The analysis of Anthracene content in bush meat of different bush meat species was not different ($P \geq 0.05$) in the various bush meat samples as indicated in Figure 6. The minimum recorded amount for Anthracene was 0.043 µg/kg in rat which rose to a maximum value of 0.096 µg/kg in antelope. The results for Anthracene analysis indicated that the Anthracene content of the bush meat samples was very uniform (SE = 0.004) across the twelve bush meat samples as expressed in Table 3.

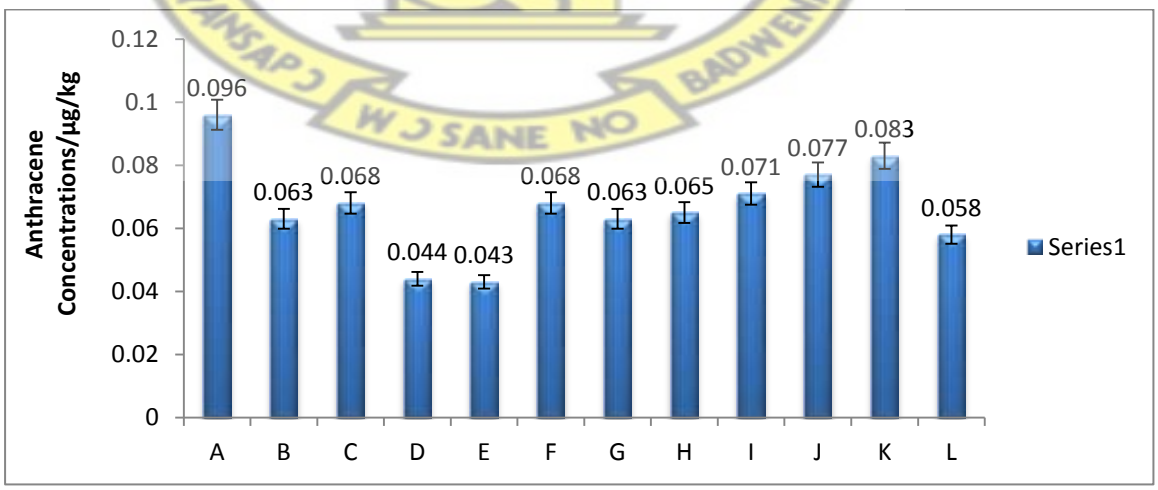


Figure 6: Various bush meat samples showing Anthracene levels

4.7 Concentration of Fluoranthene in various bush meat

The statistical analysis of the Fluoranthene content indicated that there was significant difference ($P \leq 0.05$) across the various bush meat species. The Fluoranthene content ranged from 0.00 µg/kg in rat, squirrel, boar, bush rabbit, bush guinea fowl and monitor lizard to 0.521 µg/kg in antelope. Fluoranthene content was also lower in bush buck (0.079 µg/kg) as seen in Figure 7. Fluoranthene from the results presented in Figure 7 compares favourably with EU standards.

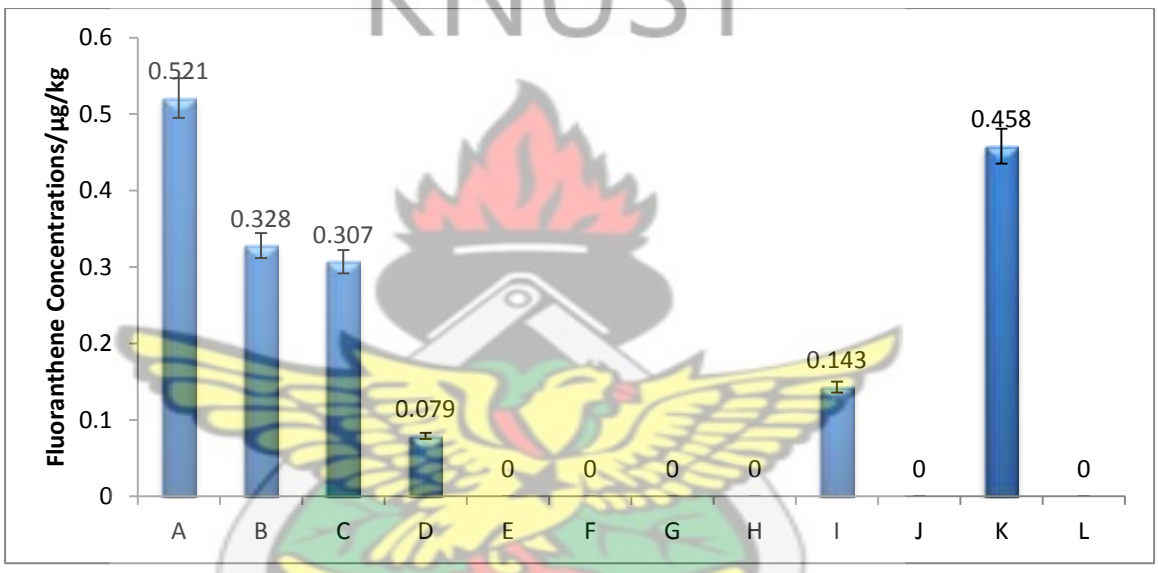


Figure 7: Various bush meat samples showing Fluoranthene levels

4.8 Concentration of Pyrene in various bushmeat

Pyrene content of the various bush meat samples was not significantly different ($P \geq 0.05$) from one another as was presented in Figure 8. The Pyrene content for the twelve bush meat species were less than 0.5 µg/kg and lower than the EU standards as was shown in Figure 8. Pyrene content ranged insignificantly from 0.00 µg/kg to 0.44 µg/kg across the meat species. The cock bird in general produced higher Pyrene content as compared to the others (Figure 8). Pyrene content was also higher in the

bush guinea fowl than the bush rabbit and bush buck (Figure 8). These values of pyrene compares well with those presented by the EU as found in Figure 8.

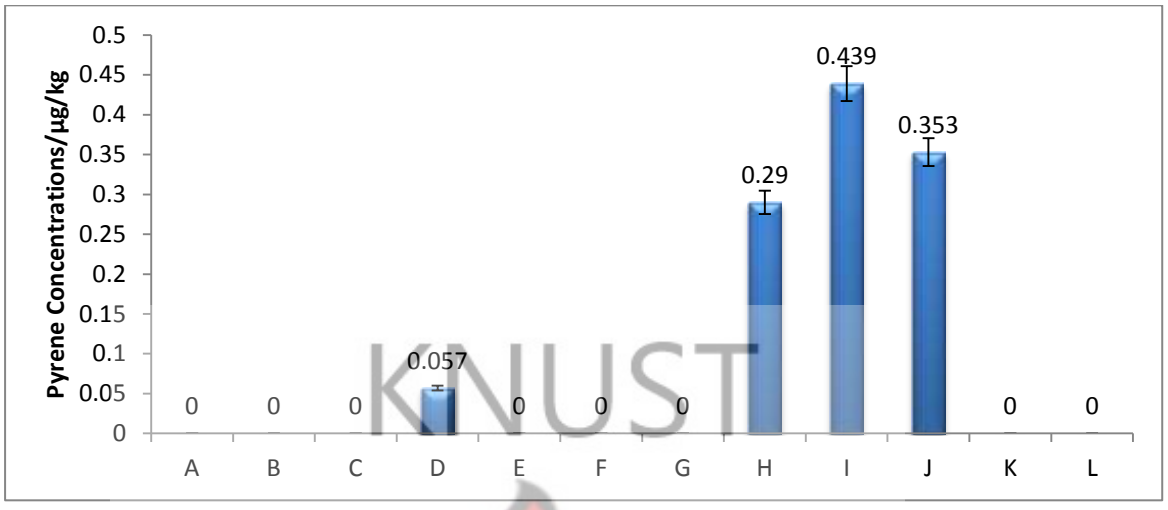


Figure 8: Various bush meat samples showing Pyrene levels

Table 5: PAHs mean contents (µg/kg) detected in different types of bush meat products

					95% Confidence Interval for Mean			
Groups	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Naphthalene	12	0.21033	0.087494	0.025257	0.15474	0.26592	0.011	0.353
2-Methylnaphthalene	12	0.37883	0.167504	0.048354	0.27241	0.48526	0.000	0.527
Acenaphthylene	12	0.08417	0.030181	0.008712	0.06499	0.10334	0.000	0.114
Acenaphthene	12	0.32900	0.132663	0.038296	0.24471	0.41329	0.063	0.515
Fluorene	12	0.06633	0.043700	0.012615	0.03857	0.09410	0.000	0.132
Phenanthrene	12	0.47825	0.240866	0.069532	0.32521	0.63129	0.053	0.920
Anthracene	12	0.06658	0.014878	0.004295	0.05713	0.07604	0.043	0.096
Fluoranthene	12	0.15300	0.197443	0.056997	0.02755	0.27845	0.000	0.521
Pyrene	12	0.09492	0.164197	0.047400	0.00941	0.19924	0.000	0.439
Total	108	0.20682	0.199070	0.019156	0.16885	0.24480	0.000	0.920

Table 6: Analysis of Variance for bush meat samples

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.257	8	.282	14.081	.000
Within Groups	1.983	99	.020		
Total	4.240	107			

Table 7: Correlations among the first four PAHs identified with bush meat

Variables	Naphthalene	2-Methylnaphthalene	Acenaphthylene	Acenaphthene
Naphthalene	1			
2-Methylnaphthalene	.948**	1		
Acenaphthylene	.932**	.948**	1	
Acenaphthene	.962**	.952**	.894**	1

**. Correlation is significant at the 0.01 level (2-tailed).

Table 8: Correlations among the last five PAHs identified with bush meat

Variables	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene
Fluorene	1				
Phenanthrene	.357	1			
Anthracene	.334	.939**	1		
Fluoranthene	.305	.604*	.648*	1	
Pyrene	.177	.008	.144	-.300	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

The PAHs content of the bush meat samples in Ghana are heartwarming. Naphthalene, 2-Methylnaphthalene, Acenaphthylene, Acenaphthene, Fluorene, Anthracene, Fluoranthene and Pyrene etc were all, on the average, lower than 0.4 µg/kg (Table 5) and they were followed by Phenanthrene (0.5 µg/kg). These are all precarious chemicals for the destruction of human life: they are carcinogenic. In addition to the lower PAHs levels across the various bush meat species, the levels were also below the safety limits. This study revealed 0.00 µg/kg minimum levels of 2-Methylnaphthalene, Acenaphthalene, Fluorene, Fluoranthene, and Pyrene and a maximum value of 0.920 µg/kg in Phenanthrene (Table 3).

Most PAHs get into the human system through either direct or indirect ingestion. These PAHs at certain significant concentrations can be very risky by the consumption of man. For instance Phenanthrene which was present in the bush meat

samples inhibits and affects the fluid balance of the body and promotes the abnormal functioning of the body nerves and muscles.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

All the polycyclic aromatic hydrocarbons (PAHs) found in this study were among the prioritized PAHs considered as carcinogenic. Most PAHs get into the human system through either direct or indirect ingestion. PAHs at certain significant concentrations can be very dangerous to the health of humans. Thus, Phenanthrene which was present in the bush meat samples inhibits and affects the fluid balance of the body and promotes the abnormal functioning of the body nerves and muscles. The levels of PAHs across the bush meat species were below the safety limits considering that this study revealed minimum levels of 2-Methylnaphthalene, Acenaphthalene, Fluorene, Fluoranthene and Pyrene as below detection. Benzo[a]pyrene was not detected at all. A maximum value of 0.920 µg/kg for Phenanthrene was found in antelope but was still below the safety limit.

5.2 Recommendations

- Bushmeat from the Ghanaian market is safe for consumption
- However continuous consumption of bush meat could pose potential health hazard due to the possibility of bioaccumulation of the PAHs and their secondary metabolites
- Actors in policy formulation, Government and NGOs should collaborate to establish a policy framework for preparation of bush meat in Ghana.

REFERENCES

Afolabi, A.O., Adesulu, E.A., Oke, O.L. (1983). Polynuclear Aromatic Hydrocarbons in Some Nigerian Preserved Freshwater Fish Species. Comparative Ratio of Benzo(a)pyrene (%) in Fish and Oil Fractions. *Journal of Agricultural and Food Chemistry*, 31:1083-1090.

Agency for Toxic Substances and Disease Registry (ATSDR) (1995). Toxicology Profile for Polycyclic Aromatic Hydrocarbons, US Department of Health and Human Services, Public Health Service, Atlanta, GA.

Ajayi S. S., (1979). Utilisation of Forest Wildlife in West Africa. Miscellaneous/ 79/26. *Food and Agriculture Organisation*. Rome. 79.

Akpambang V. O. E, Purcao, G., Lajide, L., Amoo, I. A., Conte, L. S. and Moret, S. (2009). Determination of PAHs in Commonly Consumed Nigerian Grilled Fish and Meat. *Food Additives and Contaminants*. Part A Chemical Analysis Control Exposure and Risk Assessment. 26(7): 1096 -10103.

Alonge, D.O. (1987). Factors Affecting the Quality of Smoke-dried Meats in Nigeria. *Acta Aliment*. 16: 263-270.

Alonge, D.O. (1988). Carcinogenic Polycyclic Aromatic Hydrocarbons (PAH) Determined in Nigerian Kundi(Smoke-dried Meat). *Journal of the Science of Food and Agriculture*. 43: 167-172.

Andrée S., Jira W., Schwind K. H., Wagner F. and Schwagele F. (2010).Chemical Safety of Meat and Meat Products.*Meat Science*. 86: 38–48.

Anyakora, C., and Coker, H., (2006). Determination of Polynuclear Aromatic Hydrocarbons (PAHs) in Selected Water Bodies in the Niger Delta.African Journal of Biotechnology. 5: 2024-2031.

Anyakora, C., and Coker, H., (2007). Assessment of Polynuclear Aromatic Hydrocarbon Content in Four Species of Fish in the Niger Delta by Gas Chromatography/Mass Spectrometry. *African Journal of Biotechnology*. 6: 737-743.

Armstrong, B., Hutchinson E., Unwin J. and Fletcher T. (2004). Lung Cancer Risk after Exposure to Polycyclic Aromatic Hydrocarbons: A Review and Meta-analysis. Environmental Health Perspectives.

Asibey E. O. A.(1974b).Some Ecological and Economic Aspects of The GrasscutterThryonomysSwinderianus(Temminck) (Mammalia, Rodentia, Hystricimorpha) in Ghana. Unpublished PhD Thesis, University of Aberdeen, UK, 305.

Asibey E. O. A., (1966). Why not Bush meat too? *The Ghana Farmer*, 10: 165-170.

Asibey E. O. A., (1974 a). Wildlife as a Source of Protein in Africa South of the Sahara. *Biological Conservation*, 6 (l): 32-39.

Asibey E. O. A., (1977). Expected Effects of Land-use Patterns on Future Supplies of Bush meat in Africa South of the Sahara. *Environmental Conservation*. 4: 43-50.

Asibey E. O. A., (1978). Wildlife Production as a Means of Protein Supply in West Africa with Particular Reference to Ghana. Proceedings of the 8th Forestry Congress. 3: 869-881.

Asibey E. O. A., (1986). Wildlife and Food Security. Pages prepared for FAO Forestry Department, Rome.

Asibey E. O. A., (1987). The Grasscutter. Paper prepared for the Ghana Livestock Show 87, Accra. 4.

Asibey E. O. A., (1989). Bush meat Production in West Africa. Paper Presented at the Symposium on Extractive Economies in Tropical Forests: A Course of Action, Nov 30- Dec 1 1989, Washington Dc, USA. 34.

Bartle, K. D. (1991). Analysis and Occurrence of PAHs in Food. *Food contaminants*: Sources and Surveillance, Cambridge: Royal Society of Chemistry: 41-60.

Baumard P., Budzinski H., Garrigues P., Dizer H. and Hansen P.D.(1999). Polycyclic Aromatic Hydrocarbons in Recent Sediments and Mussels (Mytilusedulis) from the Western Baltic Sea: Occurrence, Bioavailability and Seasonal Variations. *Marine Environmental Research.* 47: 17–47.

Boehm P.D., Loreti C.P., Rosenstein A.B. and Rury P.M. (2002). A Guide to Polycyclic Aromatic Hydrocarbons for the Non-Specialist. *American Petroleum Institute,* Washington D.C. Special Publication Number 4714: 54.

Bostrom C. E., Gerde P., Hanberg A., Jernstrom B., Johansson C., Kyrklund T., (2002). Cancer Risk Assessment, Indicators, and Guidelines for Polycyclic

Aromatic Hydrocarbons in the Ambient Air. *Environmental Health Perspectives*. 110(3): 451–488.

Butler J.D. and Crossley P. (1981). Reactivity Of Polycyclic Aromatic Hydrocarbons Adsorbed on Soot Particles. *Atmosphere and Environment*. 15: 91-94.

Chen S-C. and Liao C. M. (2006). Health Risk Assessment on Human Exposed to Environmental Polycyclic Aromatic Hydrocarbons Pollution Sources. *Science of the Total Environment*. 366: 112–123.

Chen, B.H. (1997). Analysis, Formation and Inhibition of Polycyclic Aromatic Hydrocarbons in Foods. An Overview. *Journal of Food and Drug Analysis*. 5: 25 – 42.

Chen B.H. and Lin Y.S. (1997). Formation of Polycyclic Aromatic Hydrocarbons during Processing of Duck Meat. *Journal of Agricultural and Food Chemistry*. 45: 1394-1403.

Chiu W-L, Niwa Y., Zeng W, Hirano T. Kobayashi H. and Sheen J. (1996). Engineered GFP as a Vital Reporter in Plants.*Current Biology*. 6: 325–330.

Dejmek J., Solansky I., Benes I., Lenicek J., and Sram R. J. (2000).The Impact of Polycyclic Aromatic Hydrocarbons and Fine Particles on Pregnancy Outcome. *Environmental Health Perspectives*. 108: 1159-1164.

DouAbul A. A., Heba H. M. A. and Fareed K. H. (1997). Polynuclear Aromatic hydrocarbons (PAHs) in Fish from the Red Sea Coast of Yemem. *Hydrobiologia*. 352: 251 - 262.

Edwards, N.T. (1983). Polycyclic Aromatic Hydrocarbons (PAHs) in the Terrestrial Environment - A Review. *Journal of Environmental Quality*. 12:427-441.

European Commission (EC), (2005). Commission Recommendation of 4 February 2005 (2005/108/EC) on the Further Investigation into the Levels of Polycyclic Aromatic Hydrocarbons in Certain Foods. Official Journal of the European Union 34: 43–45.

European Environment Agency (EEA) (1999). Criteria for Euroairnet: The EEA Air Quality Monitoring and Information Network. Technical Report No. 12. Available: *http://www.eea.europa.eu/publications/TEC12* [accessed 1 December 2010].

Falconer J. (1992). Non-timber Forest Products in Southern Ghana. Kent: Natural Resources Institute.

Garcia-Falcón, M.S., Simal-Gándara, J., 2005. Polycyclic Aromatic Hydrocarbons in Smoke from Different Woods and their Transfer during Traditional Smoking into Chorizo Sausages with Collagen and Tripe Casings. *Food Additives and Contaminants.* 22: 1–8.

Gerde P., and Scott B. R. (2001). A model for absorption of low-volatile toxicants by the airway mucosa. *Inhalation Toxicology*. 13: 903–929.

Gerde P., Ewing P., Låstbom L., Waher J., Lidén G., and Ryrfeldt Å. (2004). A Novel Method to Aerosolize Powder for Short Inhalation Exposures at High Concentrations. Isolated Rat Lungs Exposed to Respirable Diesel Soot. *Inhalation Toxicology.* 16:45-52.

Gerde P., Medinsky M. A., and Bond, J. A. (1991).The Retention of Polycyclic Aromatic Hydrocarbons in the Bronchial Airways and in the Alveolar Region.A Theoretical Comparison. Toxicology and Applied Pharmacology. 107, 239-252.

Gerde P., Muggenburg B. A., and Henderson R. F. (1993a). Disposition of Polycyclic Aromatic Hydrocarbons in the Respiratory Tract of the Beagle Dog: III. Mechanisms of the Dosimetry. Toxicology and Applied Pharmacology. 121: 328-334.

Gerde P., Muggenburg B. A., Hoover M. D. and Henderson, R. F. (1993). Disposition of Polycyclic Aromatic Hydrocarbons in the Respiratory Tract of the Beagle Dog in the Alveolar Region. *Toxicology and Applied Pharmacology*. 121:313–318.

Gerde P., Muggenburg B. A., Lundblad M., and Dahl A. R. (2001). The Rapid Alveolar Absorptionn of Diesel-soot Adsorbed Benzo(a)pyrene. Bioavailability, Metabolism, and Dosimetry of an Inhaled Carcinogen. *Carcinogenesis* 22: 741–749.

Gerde P., Muggenburg B. A., Sabourin P. J., Harkema J. R., Hotchkiss J. A., Hoover M. D., and Henderson R. F. (1993c). Disposition of Polycyclic Aromatic Hydrocarbons in the Respiratory Tract of the Beagle Dog: II. The Conducting Airways.Toxicology and Applied Pharmacology. 121: 319.327.

Gerde P., Muggenburg B. A., Stephens T., Lewis J. L., Pyon K. H., and Dahl A. R. (1998a). A Relevant dose of 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone is Extensively Metabolized and Rapidly Absorbed in the Canine Tracheal Mucosa. *Cancer Research*. 58: 1417–1422.

Gerde P., Muggenburg B. A., Thornton-Manning J. R., Lewis J. L., Pyon K. H., and Dahl A. R. (1997). Benzo(a)pyrene at an Environmentally Relevant Dose is Slowly Absorbed by, and Extensively Metabolized in, Tracheal Epithelium. *Carcinogenesis*. 18:1825-1832.

Gerde P., Muggenburg B.A., and Henderson R. F. (1993a). Disposition of Polycyclic Aromatic Hydrocarbons in the Respiratory Tract of the Beagle Dog. III. Mechanisms of the Dosimetry. Toxicology and Applied Pharmacology. 121: 328–334.

Gerde P., Stephens T., and Dahl A. R. (1998b). A Rapid Combustion Method for the Accurate Determination of Low Levels of Tritium in Biological Samples. *Toxicology Methods*. 8: 37-44.

Gilbert E., Dodoo D. K., Okai-Sam F., Essuman K. and Quagraine E. K. (2006). Characterization and Source Assessment of Heavy Metals and Polycyclic Aromatic Hydrocarbon (PAHs) in Sediments of the Fosu Lagoon, Ghana. *Journal of Environmental Science* and *Health.* 41: 2747–2775.

Gomaa E.A., Gray J.I., Rabie S., Lopez-5.Bote C. and Booren A.M. (1993). Polycyclic Aromatic Hydrocarbons in Smoked Food Products and Commercial Liquid Smoke Flavourings. *Food Additives and Contaminants*. 10: 503-521.

Grimmer G., Brume H., Dettbarn G.,Naujack K. W., Mohr.U. and Hartung, R. W. (1988). Urinary and Faecal Excretion of Chrysene and ChryseneMetabolites by Rats after Oral, Intraperitoneal, Intratrachealor Intrapulmonary Application. *Archives of Toxicology*. 62: 401-405.

Grova N., Feidt C. Laurent C. and Rychen G. (2002). Milk, Urine and Faeces Excretion Kinetics in Lactating Goats after an Oral Administration of polycyclic aromatic hydrocarbons. *International Dairy* Journal. 12: 1025-1031.

Grova N., Feidt C., Crepineau C., Laurent C., Lafargue Hachimi A, Rychen G. (2002). Detection of Polycyclic Aromatic Hydrocarbon level in Milk Collected Near Potential Contamination Sources. *Journal of Agricultural and Food Chemistry*. 50: 4640 - 4642.

Guillen M.D. and Sopelana P. (2004). Occurrence of Polycyclic Aromatic Hydrocarbons in Smoked Cheese. *Journal of Dairy Science*. 87: 556-564.

Bartle, K. D. (1991). Food Contaminants: Sources and Surveillance. *The Royal Society of Chemistry*, Cambridge, U. K. 154-218.

Guillen M. D., Sopelana P. and Partearroyo M. A. (2000). Polycyclic Aromatic Hydrocarbons in Liquid Smoke Flavorings Obtained from Different Types of Wood. Effect of Storage in Polyethylene Flasks on their concentrations. *Journal of Agricultural and Food Chemistry*. 48: 5083 - 5087.

Guillen M. D., Sopelana P. and Cid C. (1996). Presence of Polycyclic Aromatic Hydrocarbons in the Foods that form part of the diet of Different European Countries. *Alimentaria*. 34(278): 41–47.

Guillen, M. D., Sopelana, P. and Partearroyo, M.A. (1997).Food as a Source of Polycyclic Aromatic Carcinogens Reviews on Environmental Health. 12, 133-146.

Guillen M.D., Sopelana P. andPartearroyo M.A. (1997). Food as a Source of Polycyclic Aromatic Carcinogens. *Reviews on Environmental Health*. 12: 133-146.

Harvey, R.G. (1997). Polycyclic Aromatic Hydrocarbons. New York. *Wiley Publishers*. 667.

Haugen A., Becher G., Benestad C., Vahakangas K., Trivers G. E., Newman M. J. (1986). Determination of Polycyclic Aromatic Hydrocarbons in the Urine, benzo[a]pyrenediol epoxide-DNA Adducts in Lymphocyte DNA, and Antibodies to the Adducts in Sera from Coke Oven Workers Exposed to Measured Amounts of Polycyclic Aromatic Hydrocarbons in the Work Atmosphere. *Cancer Research*.46:4178–83.

Hladik, C. (1987). Feeding in Rainforest: Differential Food Anthropology Populations Humid Forest Regions. Africa. Research Team Report No 263. Paris, CARS.

Hopia A. Pyysalo H. and Wickstrom K. (1986). Margerines, Butter and Vegetable Oils as Sources of Polycyclic Aromatic Hydrocarbons. *Journal of the American Oil Chemists' Society*. 63: 889-893.

Hopia A., Huang S., Schwartz K., German B. and Frankel E. (1996). Effect of Different Lipid Systems on Antioxidant Activity of Rosemary Constituents Carnosoland Carnosic Acid with and without a-Tocopherol. *Journal of Agriculture and Food Chemistry*. 44: 2030-2036.

Howard P.H., Boethling R.S., Jarvis W.F., Meylan W.M., and Michalenko E.M. (1991). Handbook of Environmental Degradation Rates. Printup, *H. T. Lewis Publishers.* Chelsea, Michigan.

IARC (2010). Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 92: 1–853.

IPCS, (1998). Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons. Environmental Health Criteria. International Programme on Chemical Safety, World Health Organization, Geneva.

James M.O., (1989). Biotransformation and Disposition of PAH in Aquatic Invertebrates. In: Varanasi, U. (Edition.), Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press Incoperation. 69–91.

JECFA (2005).Joint FAO/WHO Expert Committee on Food Additives.(2005). Sixty-fourth meeting. Rome, 8-17 February 2005..

Joe F. L., Jr., Salemme J. and Fazio T. (1984). Liquid Chromatography Determination of Trace Residues of Polynuclear Aromatic Hydrocarbons in Smoked Foods. *Journal of Association of Official Analytical Chemists*.67: 1076-1082.

Karl H. and Leinemann M. (1996). Determination of Polycyclic Aromatic Hydrocarbons in Smoked Fishery Products from Different Smoking Kilns. *ZLebensmUntersForsch*. 202: 458-464.

Kawamura Y., Kamata, E. and Ogawa Y. (1988).The Effect of Various Foods on the Intestinal Absorption Of Benzo(a)Pyrene in Rats. *Journal of the Food Hygienic Society of Japan.* 29(1): 21-5.

Kazerouni N., Sinha R., Hsu C-H., Greenberg A. and Rothman N. (2001). Analysis of 200 Food Items for Benzo(a)pyrene and Estimation of its Intake in an Epidemiologic Study. *Food and Chemical Toxicology*. 39: 423-436.

King G., Murray C. J. L., Salomon J. A. and Tandon A. (2002). Enhancing the Validity of Cross-cultural Comparability of Survey Research.Submitted for Publication. Downloadable from www.gking.harvard.edu/files/vign.pdf.

King S., Meyer J. S., Andrews A. R. J. (2002). Screening Method for Polycyclic Aromatic Hydrocarbons in Soil Using Hollow Fibre Membrane Solvent Microextraction. *Journal of Chromatography*. 982:201-208.

Knize M.G., Salmon C. P., Pais P. and Felton J. S.(1999). Impact of Processing on Food Safety. Advances in Experimental Medicine and Biology. 459: 179–193.

Lijinsky W. (1991). The Formation and Occurrence of Polynuclear Aromatic Hydrocarbons Associated with Food. *Mutation Research*. 259(3– 4): 251–261.

Koyano M., Mineki S., Tsunoda Y., Endo O.,Goto S. and Tadahiro T. (2001). Effect of Fish (Mackerel Pike) Broiling on Polycyclic Aromatic Hydrocarbon Contamination of Suspended Particulate Matter in Indoor Air. *Journal of Health Sciences*.47:452 - 459.

Larsson B.K., Sahlberg G.P., Eriksson A.T. and Busk L.A. (1983). Polycyclic Aromatic Hydrocarbons in Grilled Food. *Journal of Agriculture and Food Chemistry*. 31: 867-873.

Lawrence J. F. and Weber D. F. (1984). Determination of Polycyclic Aromatic Hydrocarbons in Some Canadian Commercial Fish, Shellfish, and Meat Products by Liquid Chromatography with Confirmation b Capillary Gas Chromatography-mass Spectrometry. *Journal of Agricultural and Food Chemistry*. 32:7942-797.

Li Z., Mulholland J. A., Romanoff L. C., Pittman E.N., Trinidad D. A., Lewin, M. D. (2010). Assessment of Non-occupational Exposure to Polycyclic Aromatic Hydrocarbons through Personal Air Sampling and Urinary Biomonitoring. *Environmental Monitoring and Assessment*. (Formally Journal of Environmental Monitoring).

Lijinsky W., and Shubik P.(1965a). Benzo(a)pyrene and other Polynuclear Hydrocarbonsin Charcoal-broiled Meat. *Science*. 145: 53-55.

Lijinsky W. and Shubik P. (1965b). Polynuclear Hydrocarbon Carcinogens in Cooked Meat and Smoked Foods. *Industrial Medicine and Surgery*. 34: 152-154.

Liu, X., Korenga, T. (2001).Dynamic Analysis for the Distribution of Polycyclic Aromatic Hydrocarbons in Rice. *Journal of Health Sciences*. 47:446-451.

Martin G. H. G., (1983). Bushmeat in Nigeria as a Natural Resource with Environmental Implications. *Environmental Conservation*. 10: 125- 132.

Martson C. P, Pareira C. and Ferguson J. (2001). Effect of Complex Environmental Mixture from Coal Tar Containing Polycyclic Aromatic Hydrocarbons (PAH) on TumorInitiation, PAH DNA Binding and Metabolic

Activation of Carcinogenic PAH in Mouse Epidermis. *Carcinogenesis.* 22(7): 1077–1086.

Mottier P., Parisod V., and Turesky R. J. (2000). Quantitative Determination of Polycyclic Aromatic Hydrocarbons in Barbecued Meat Sausages by Gas Chromatography Coupled to Mass Spectrometry. *Journal of Agricultural and Food Chemistry*. 48: 1160-1166.

Neilson A. H. (1998). The Handbook of Environmental Chemistry Journal: PAHs and Related Compounds. Biology.Springer-Verlag, Berlin, 312-350.

Neubert D. and Tapken S. (1988). Transfer of Benzo(a)pyrene into Mouse Embryos and Fetuses. *Archives of Toxicology*. 62: 236-239.

Nielsen T., Jorgensen H. E., Larsen J. C. and Poulsen M. (1996). City Air Pollution of Polycyclic Aromatic Hydrocarbons and other Mutagens: Occurrence, Sources and Health Effects. Science of the Total Environment. 189-190, 41-49.

Nieva-Cano M. J., Rubio-Barroso S., Santos-Delgado M. J. (2001). Determination of PAH in Food Samples by HPLC with Flourimetric Detection Following Sonication Extraction Without Clean-up. *The Analyst*.126:1326-1331.

Ntiamoa-Baidu Y. (1998). Sustainable Harvesting, Production and Use of Bushmeat. Wildlife Department, Accra: Ghana.

Park K. S.,Sims R. C. and DuPont R. (1990). Fate of PAH Compounds in Two Soil Types. Influence of Volatilization, Abiotic Loss and Biological Activity. *Environmental Toxicology and Chemistry*. 9:187-195.

Perera B., Reece, M., Monahan P., Billingham R., and Finn P. (2009). Childhood Characteristics and Personal Dispositions to Sexually Compulsive Behavior Among Young Adults. *Sexual Addiction and Compulsivity*. 16(2): 131-145.

Perera F., Li T. Y., Zhou Z. J., Yuan T., Chen Y. H, Qu L. (2008). Benefits of Reducing Prenatal Exposure to Coal-burning Pollutants to Children's Neurodevelopment in China. *Environmental Health Perspectives*.116:1396-1400.

Perera F. P., Li Z., Whyatt R., Hoepner L., Wang S. Camann D. (2009). Prenatal Polycyclic Aromatic Hydrocarbon Exposure and Child Intelligence at Age Five. *Pediatrics*.124(2): 195–202.

Phillips D. H. (1999). Polycyclic Aromatic Hydrocarbons in the Diet. *Mutation Research* - Genetic *Toxicology* and *Environmental Mutagenesis*. 443: 139–147.

Ravindra K., Bencs L., Wauters, E., de Hoog J, Deutsch F., Roekens E. (2006). Seasonal and Site-specific Variation in Vapour and Aerosol Phase PAHs over Flanders (Belgium) and their Relation with Anthropogenic Activities. *Atmospheric Environment*. 40: 771–785.

Ravindra K., Bencs L., Wauters E., de Hoog J., Deutsch F., Roekens E., Bleux N., Bergmans P. and Van Grieken R. (2006a). Seasonal and Site Specific Variation in Vapour and Aerosol Phase PAHs over Flanders (Belgium) and their Relation with Anthropogenic Activities. *Atmospheric Environment*. 40: 771-785.

Ravindra K., Mittal A.K., Van Grieken R., (2001). Health Risk Assessment of Urban Suspended Particulate Matter with Special Reference to Polycyclic Aromatic Hydrocarbons. A Review. *Reviews on Environmental Health*. 16: 169-189.

Ravindra K., Sokhi R., Van Grieken R. (2008). Atmospheric Polycyclic Aromatic Hydrocarbons: Source Attribution, Emission Factors and Regulation. Atmospheric Environment.4242(13):2895–2921.

Roseiro L., Gomes A. and Santos C. (2011). Influence of Processing in the Prevalence of Polycyclic Aromatic Hydrocarbons in a Portuguese Traditional Meat Product. *Food and Chemical Toxicology*. 49: 1340–1345.

Schmit J. A. Henry R. A. Williams R. C. and Dieckman J. F. and Chromagr J. (1971). Applications of High Speed Reversed-phase Liquid Chromatography. *The Journal of Chromatographic Science*. 9: 645-651.

Scientific Committee on Food (SCF) (2002). Opinion of the Scientific Committee on Food on the Risks to Human Health of Polycyclic Aromatic Hydrocarbons in Food Source: http://www.europa.eu.int/comm/food/fs/sc /scf/out153_en.pdf

Sikorski Z. E. (2004). Traditional Smoking. In Jensen W. K. (editions), Encyclopedia of Meat Sciences, *Elsevier Ltd*. 1265–1277.

Simko P. (2002). Determination of Polycyclic Aromatic Hydrocarbons in Smoked Meat Products and Smoke Flavouring Food Additives. *Journal of Chromatography*. 770: 3-18.

Simko P. (2005). Factors Affecting Elimination of Polycyclic Aromatic Hydrocarbons from Smoked Meat Foods and Liquid Smoke Flavorings. *Molecular Nutrition and Food Research*. 49: 637–647.

Simko P. (2005). Factors Affecting Elimination of Polycyclic Aromatic Hydrocarbons from Smoked Meat Foods and Liquid Smoke Flavourings. *Molecular Nutrition Food Research*. 49: 637–647.

Srám R. J., Binková B., Dejmek J. andBobak M. (2005). Ambient Air Pollution and Pregnancy Outcomes: A Review of the Literature. *Environmental Health Perspectives.* 113:375-382.

Sram R.J., and Binkova B. (2000). Molecular Epidemiology Studies on Occupational and Environmental Exposure to Mutagens and Carcinogens. *Environmental Health Perspectives*. 108 (1): 451-60.

Takatsuki K. Suzuki S. Salo N. and Ushizawi (1985). Liquid Chromatography Determination of PAHs in Fish and Shell Fish. *Journal of the* Association *of Official Analytical Chemists.* 68: 945 – 949.

Toth L. and Blass W. (1972). The Effects of Smoking Technology on the Content of Carcinogenic Hydrocarbons in Smoked Meat Products. Effect of Smoldering Temperature of Wood and of Cooling, Washing and Filtering of Smoked. *Fleischwirtsch*. 52: 14-19.

U.S. EPA (U.S. Environmental Protection Agency). 1994. Amendments to 1990 Clean Air Act-List of 189 Hazardous Air Pollutants. Washington, DC:U.S. EPA.

US EPA (1994). Soxhlet Extraction - Method 354.

US EPA (1999).Method TO-13A, Compendium of Methods for Toxic Air Pollutants.

Van der Oost R., Heida H., Opperhuizen A. and Vermeulen P. E. (1991). Interrelationships between Bioaccumulation of Organic Trace Pollutants (PCBs,

Organochlorine Pesticides and PAHs, and MFO Induction in Fish. *Comparative Biochemistry* and *Physiology*.100: 43-47.

Viksna I.S., Bartkevics V., Kukare A. and Morozovs A. (2008). Polycyclic Aromatic Hydrocarbons in Meat Smoked with Different Types of Wood. *Food Chemistry*. 110: 794–797.

Wenzl T., Simon R., Anklam E. and Kleiner J. (2006). Analytical Methods for Polycyclic Aromatic Hydrocarbons (PAHs) in Food and the Environment Needed for New Food Legislation in the European Union. Trends in Analytical Chemistry. 25, 716–725.

WHO (1998). Polynuclear Aromatic Hydrocarbons. In: Guidelines for drinking-water quality, 2nd ed. Vol. 2. Health Criteria and Other Supporting Information. Geneva, World Health Organization. 123-152.

WHO–IPCS (World Health Organization–International Programme on Chemical Safety) (1998). Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons. Environmental Health Criteria Available: http://www.inchem.org/documents/ehc/ehc/ehc202.htm

Withey J. R., Law F. C. and Endrenyi L. (1991). Pharmacokinetics and Bioavailability of Pyrenein the Rat. *Journal of Toxicology and Environmental Health*. 32: 429-447.

Withey J. R., Shedden J., Law F. P. C, and Abedini S. (1992). Distribution to the Fetus and Major Organs of the Rat Following Inhalation Exposure to pyrene. *Journal of Applied Toxicology*. 12: 223-231.

World Health Organisation (WHO) (1998). International Programme on Chemical Safety. Environmental Health Criteria Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons.

Wretling S., Eriksson A., Eskhult G. A. and Larsson B. (2010).Polycyclic Aromatic Hydrocarbons (PAHs) in Swedish Smoked Meat and Fish.Journal of Food Composition and Analysis. 23: 264–272.

Yabiku H. Y., Martins M. S. and Takahashi M. Y. (1993). Levels of Benzo[a]pyrene and other Polycyclic Aromatic Hydrocarbons in Liquid Smoke Flavor and Some Smoked Foods. *Food Additives & Contaminants*. 10: 399-405.

Yun T., Tainling Z. and Xinhong W. (2003). PAH Contamination and PAH Degrading Bacteria in Xiamen Western Sea Chemical Speciation and Bioavailability. 14:25-34.