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HEALTH RISK ASSESSMENT OF PAH IN BREAD

BY

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

To investigate the health risk assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in bread, Monte Carlo simulation was used to estimate the probabilistic cancer risk. A dietary survey at Kwadaso sub-metro Kumasi was conducted to determine bread consumption and as input variables in cancer risk assessment. The concentration of 12 PAHs in 47 bread samples was performed by HPLC/FLD. Six of the PAHs were detected and benzo [a] pyrene was not detected in any of the bread samples. The concentrations of the PAHs ranged from 0.54 to 1212.5 µg/kg whiles benzo [a] pyrene equivalent (BaP_{eq}) concentration ranged from 0.0021 to 3.99 µg/kg on wet weight (w.w). Naphthalene and it derivatives 1-methylnaphthalene and 2-methylnaphthalene were the dominant PAHs in the bread samples. The mean daily exposure to PAH in bread was $2.55E^{-10}$ mg/kg, $bwday^{-1}$. The 95% percentile risk ($2.06E^{-7}$) is lower than 10^{-6} , the cancer risk 'acceptable' range (less than 10^{-6}) and it could be concluded that PAH in bread is likely not a health risk. Further studies are however needed on the content of PAHs in bread and other cereal products to effectively control dietary exposure to PAH since it is a major source of PAHs in diet.

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LIST OF ABBREVIATIONS

1-MNAP	1-Methyl Naphthalene
2-MNAP	2-Methyl Naphthalene
Ace	Acenaphthene
Ant	Anthracene
AhR	Aryl hydrocarbon Receptor
ATSDR	Agency for Toxic Substances and Disease Registry
BbF	Benz [b] fluoranthene
BkF	Benz [k] fluoranthene
BaP	Benzo [a] pyrene
CDI	Chronic Daily Intake
CSF	Cancer Slope Factor
CYP	Cytochrome P450
DBA	Dibenz [a,h] anthracene
DNA	Deoxyribonuclic Acid
EC	European Commission
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization
FLD	Fluorescent Detection
Flt	Fluoranthene
Flu	Fluorene
GC-MS	Gas Chromatography with Mass Spectrometry

HPLC	High Pressure Liquid Chromatography
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
JECFA	Joint Expert Committee on Food Additives
LD_{50}	Lethal Dose 50%
LOAEL	Low Observable Adverse Effect Level
LOD	Limit of Detection
Nap	Naphthalene
NOAEL	No Observable Adverse Effect Level
PAHs	Polycyclic Aromatic Hydrocarbons
PEC	Potency Equivalent Concentration
PYR	Pyrene
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, Safe
SPE	Solid Phase Extraction
TEF	Toxic Equivalency Factors
US	United State (of America)
UV	Ultraviolet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

It has to be emphasized that, diet is one of the main route of exposure to chemical contaminants into the body, resulting in multitude of health related concerns. Thus, the risk of exposure to chemical contaminants through food is assessed for chemicals that present a public health concern (Zelinkova and Wenzl, 2015 and Xia *et al.*, 2010). Polycyclic aromatic hydrocarbons (PAHs) represent substantial number of organic compounds with two or more fused aromatic rings. Incomplete pyrolysis or combusting of organic matter generate over hundred different PAHs occurring in complex mixtures in a number of activities. (Zelinkova and Wenzl, 2015; Kuo *et al.*, 2006 and Agerstad and Skog, 2005). These activities include the use of petrochemical products such as oil by industries and other uses or daily activities involving fires such as tobacco smoking and cooking.

Bread is widely consumed by Ghanaians daily and it is a major staple food taking the highest cash expenditure within the food sub-group (Bediako, 2008). Depending on local customs and convenience, bread may be served in various forms during meals and may be eaten as a snack or used as an ingredient in other food preparations. It is mostly baked using traditional clay wood oven even though the use of gas oven is increasingly becoming popular. A number of researchers have detected the occurrence of PAH in bread in various countries (Ahmed et al., 2000; Rey-Salgueiro *et al.*, 2008; Orecchio and Papuzza, 2009; Al-Rashdan *et al*., 2010 and Ciecierska and Obiedzinski, 2013). Although the levels of PAHs in these studies were low, some of the samples showed high PAH values and thus the need for further research.

The level of PAH generated is significantly influenced by temperature and ventilation and this can be seen in the difference between traditional smokehouse and recent methods where temperature and ventilation are controlled as well as generating smoke externally (EC, 2002). Cereal and cereal based foods including bread has been shown to be among foods with high PAH content in a number of study (EC, 2008).

Polycyclic aromatic hydrocarbons are ubiquitous environmental contaminants and its generation and contamination of food especially during food preparation present a human health risk concern (IPCS, 1998). The carcinogenic effects of a number of PAHs further heighten this concern. PAH identification and levels in air, food, water and the environment at large create the need to determine its effects on human health. The source, formation and occurrence of PAHs have been widely studied the world over (Maliszweska- Kordybach, 1999). PAH usually occur as mixtures in food and other media with varying health effects including genotoxic and carcinogenic effects whiles others are not (EC, 2008). The health effects of PAHs has been evaluated by both national and international agencies including the World Health Organisation (WHO, 2006), Food and Agriculture Organization (FAO) and the International Agency for Research on cancer (IARC, 2008).

Food is one of the major routes of human exposure to PAHs and these compounds can reach the food chain through air, water, soil and sediment, modes of cooking, food processing and food additives. PAHs may be formed during processing and domestic food preparation at high temperatures such as smoking, drying, roasting, baking, frying or grilling are recognized as the major source of contamination by PAHs (Moret *et al.*, 2005; Phillips, 1999 and Guillen *et al.*, 1997). The formation of PAH is directly influenced by the length of cooking time which increase the level of pyrolysis and the

exposure of food to smoke and heat for long periods of time (Kazerouni *et al*. 2001). It has been found in a number of foods including smoked meat, fish, cereals, vegetables, dairy products and thus raising concern for PAHs analysis in food (Plaza-Bolanos *et al.*, 2010 and Kazerouni *et al*., 2001).

Risk assessment is the scientific evaluation of potential or known health effects from human exposure to hazardous substances. It is a very structured process involving qualitative and quantitative information characterizing the nature and likelihood of adverse health effects. Risk assessment provides an effective framework for determining the relative urgency of problems and the allocation of resources to reduce the risk. It is however associated with some degree of scientific uncertainties (IPCS, 1998).

1.2 Statement of the problem

Bread is a convenience food in Africa for both urban and rural communities with increased consumption in Ghana (Bediako, 2008). Most homes consume bread as part of their breakfast and also in relation to other beverages during the day. The bread is baked with imported and locally made stoves most of which are poorly ventilated. Baking of bread or cooking with intense heat yield flames containing a number of PAHs which may adhere to the surface of the food. Genotoxic substances and other toxicant of various kinds including PAH are shown to be generated from food processing and cooking at high temperatures (Agerstad and Skog, 2005). Food cooked over open flames has been found to contain some of the highest concentrations of PAHs (Phillips, 1999 and Guillen *et al*., 1997). It has been reported that PAHs are mutagenic and carcinogenic agents in animals and human (Agerstad and Skog, 2005).

According to Kazerouni *et al*., (2001), bread, cereal and grain constitute the highest Benzo[a] pyrene total daily percentage intake to the general population. Bread can be contaminated with PAHs through the raw ingredients, particularly flour and through the baking process. The risk of exposure to PAHs depends on diet, cooking and processing practices, eating habits and other human activities of a particular region. Foods contributing to high exposure to contaminant have been targeted for analysis as recommended by WHO (WHO, 1995). Appropriate dietary staples should be continuously monitored since it likely constitutes significant intake sources. Bread exposed to PAHs as a result of baking method may present health risk since it is consumed some few hours to days after baking and also it consumption by a large percentage of the general population.

Although a lot of work including reviews and books on PAHs has been published, accumulation of PAHs in diet and health risk and exposure assessment strategies to these PAHs has gotten less attention (Ramesh *et al.*, 2004). No or very little systematic clinical studies or health risk assessment of the level these contaminant in bread in the country has been investigated. However, significant contribution of the nutritional requirements of a number of Ghanaians is provided by the baking industry (bread).

1.3 Justification

The presence of PAHs in food present concerns that requires continuous monitoring since over 70% of exposure to PAHs arises from diet. Dietary factors have been attributed in part to be the cause of cancers in a number of epidemiological studies in recent years (Doll, 1996). There is the need to evaluate activities such as cooking and processing at high temperatures to assess the risk to contaminants presenting both carcinogenic and genotoxic properties.

Determination of the types and levels of PAHs in bread would be helpful in appreciating the exposure of these hazards in bread consumption and in designing strategies to reduce them. The suitability of bread for human consumption should be assessed by investigating the levels of PAHs in bread due to the significant health effect PAH present. This would also inform risk managers about the possible magnitude of health concerns at different levels of bread intake in humans since exposure to genotoxic carcinogen such as PAHs should be as low as reasonably practicable.

1.4 Objectives;

Main objective;

To determine the dietary risk due to PAH in the consumption of bread.

Specific objectives

The specific objectives were to;

1. Determine the concentrations of PAH in the different types of bread consumed.
2. Assess the dietary risk due to PAH in the consumption of bread.

CHAPTER 2

LITERATURE REVIEW

2.1 Bread

Bread is an important staple food for people of different geographical origin and the most widely consumed bakery product. It been processed and consumed by man for a number of centuries (Hui, 2006). Depending on local customs and convenience, bread may be served in various forms at any meal of the day and may be eaten as a snack or used as an ingredient in other food preparations. It is a fermented confectionary product produced mainly from white wheat flour, water, yeast and salt by a series of processes involving mixing, kneading, proofing shaping and baking. It is processed into different shapes and sizes and baked under different baking conditions for colours, textures and flavours (Datta and Mondal, 2008).

Bread is often generally identified as leavened and unleavened. Leavened agents such as baker's yeast and baking powder are added to the dough in making leavened bread. Unleavened bread on the other hand does not contain gas producing agents or undergo fermentation. Bread can also be identified as either quick bread or yeast bread (Datta and Mondal, 2008).

Baking is the step in the bread making process where heat in a baking oven transforms raw dough into bread. The oven conditions such as the amount of heat applied and humidity as well as bread residence time significantly affect the final quality of bread (Hui, 2006). The baking oven is usually powered by firewood, electricity or gas and all these energy supplies have safety concerns. These energy sources greatly influence the amount of PAH produced and consumed in bread (Ahmed *et al.*, 2000).

2.2 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) represent a group of organic compounds with two or more fused aromatic rings. Incomplete pyrolysis or combusting of organic matter generate over hundred different PAHs occurring in complex mixtures in a number of activities (Kuo *et al.*, 2006 and Agerstad and Skog, 2005). The production of PAHs is highly favored when there is oxygen-deficient flame, temperatures in the range of 650-900^{o}C and fuel which are not highly oxidized (Byeong-Kyu, 2010; Skupinska *et al.*, 2004 and Maliszewska-Kordybach, 1999). The highest levels of PAH in the environment are mostly due to human activities even though it occur naturally.

PAHs are ubiquitous environmental contaminants belonging to the group of persistent organic pollutants (POPs) which are resistant to degradation, can remain in the environment for long periods and have the potential to cause adverse health and environmental effects. Ingestion of food and inhalation are the main route of exposure including other minor route such as drinking water, dust, soil and the use of PAH-contaminated products. The source, formation and occurrence of PAHs have been widely studied the world over (Maliszweska- Kordybach, 1999).

2.2.1 Physical and chemical properties

The physical and chemical characteristics of PAHs generally vary with molecular weight. Aqueous solubility decreases with increasing molecular weight whiles melting point, boiling point and the log kw (octanol/ water partition coefficient) increases, suggesting increased solubility in fats, a decrease in resistance to oxidation and reduction. The high lipophilicity of PAHs is measured by water-octanol partition coefficients (k_{ow}). The behavour and distribution of PAHs in the environment and their

biological effects vary substantially for PAHs of different molecular weight (Maliszewska-Kordybach, 1999 and Eisler, 1987).

PAHs are generally considered to be semi-volatile organic compounds (SVOCs) and their volatility is strongly related to the number of aromatic rings as well as the molecular structure. The low volatility of most PAH increases it tendency to adsorb on organic particulate matter. PAHs belong to a group of compounds known as persistent organic pollutants (POP) due to it lipophilic characteristics and limited biodegradation and this persistence increase with ring number and condensation degree. PAHs have low vapour pressure (Joa *et al.*, 2009). These properties clearly demonstrate the ubiquitous nature of PAHs which present in trace contaminant in air, soil and water.

The aromatic rings of PAHs consist of 2 to13 rings with PAHs containing up to 4 rings known as light molecular weight (LMW) PAHs and more than 4 rings being heavy molecular weight (HMW) PAHs. The later have low aqueous solubility and vapour pressure and they are more stable and toxic than the light ones. PAHs in the environment is transported and distributed mainly due to its high log k_{ow}, low vapour pressure and very low water solubility. Many PAHs contain the same amount of rings but the differences in the configuration of rings may lead to differences in the compounds properties whiles substituted PAHs with functional groups such as –OH, -NO_2, =O and CH_3 are also found in the environment.

PAHs are very poorly degraded by hydrolysis and chemically stable. PAHs are susceptible to photodegradation and oxidation in the presence of light. When they react, they undergo two types of reactions; i.e. electrophilic substitution and addition. As the latter destroys the aromatic character of the benzene ring that is affected, PAHs tend to

form derivatives by the former reaction; addition is often followed by elimination, resulting in net substitution.

Photodecomposition in the presence of air, strong ultraviolet light and sunlight forms a number of oxidative products, including quinines and endoperoxides. Nitro derivatives of PAHs are formed by reaction with nitric acid and nitrogen oxides; they also react with sulfur oxides and sulfuric acid (in solution) to form sulfinic and sulfonic acids. The information on the fate and reactions of PAHs in food is limited and thus are assumed to be stable in different food matrices due to its chemical stability. Pure PAHs are usually pale yellow-green solids, colourless, or white. PAHs are used in plastics, dyes, pesticides or medicines (Skupinska *et al.*, 2004).

2.2.2 Sources of PAHs

Anthropogenic activities and natural sources are the main sources from which PAH are derived. Volcanic eruption, accidental burning of woodland and forest including decaying organic matter are natural sources contributing to atmospheric levels. Temperature, humidity and wind are the meteorological conditions as fuel characteristics and type determines the degree of PAHs production.

The highest levels and continuous increase of PAHs in the environment are mostly due to human (anthropogenic) activities even though it occurs naturally. This includes agricultural activities, mobile emission and stationary sources (including industrial and domestic sources). Stationary sources including domestic heating and cooking as well as industrial processes mainly with coal, garbage, wood, oil and other organic substances. More than 100 PAHs have been identified in atmospheric particulate matter and in emissions from coal-fired residential furnaces and about 200 compounds have

been found in tobacco smoke (IPCS, 1998). The emission of PAHs in the environment from domestic sources is high and thus is one of the main routes of contamination to food. The prevalence of these sources in indoor environment makes it a major health concern. About 32.8% of total indoor PAHs emission is attributed to cooking, contributing significantly to the contamination of different food varieties and beverages (Zhu *et al.*, 2009).

Raw food has been found not to contain of high level of PAHs, however, the presence of PAHs in uncooked food has been found in areas where activities relating to PAHs occur and it usually accumulate on the surface. Substantial quantities of PAHs are found in some foods depending on the mode of cooking, preservation and storage. Cooking and heating process such as drying, smoking, grilling, roasting, and baking have been shown to generate considerable concentrations of PAHs (Guillen *et al.*, 1997; Philips, 1999 and Moret *et al.*, 2005). Fatty meat and meat products charcoal grilled/ barbecued under prolonged and severe conditions have been reported to contain high PAH concentrations (EC, 2008 and El-Badry, 2010). Food component such as fat facilitate the generation of PAHs through either thermal degeneration or polymerization and affect the PAH generated quantitatively (Philips, 1999). The closeness of the heat source, the duration of exposure of the food to the flames and increased fat content increases the amount of PAH produced. PAHs levels in processed foods are strongly influenced by the type of heat source and cooking method (EC, 2008). PAH in food does not occur as single individual compounds but rather as mixtures. However, international bodies have not recommended specific list of PAHs to be analysed in food. In this study, 12 PAHs were analysed some of which are classified as Group 1 and 2A by IARC.

Industrial sources are mostly as a result of raw materials processing involving burning of fuels such as oil, gas and coal. Industrial activities known for substantial PAHs production include asphalt industries, rubber tire and cement manufacturing bitumen, coke and primary aluminum production, petrochemical industries, waste incineration, commercial power and heat generation and wood preservation. A number of studies on emission of PAHs from these industries have been reported in recent years (Byeong-Kyu, 2010).

PAH emission in most urban areas is mainly from mobile sources which are made up of vehicles exhaust fumes of automobiles, aircraft, ships, railways and other vehicular motors. It usually involves the use of gasoline, coal, oils, diesel and oil lubricant. Agricultural sources of PAH is mostly through burning (open burning) of straw, brushwood and stubble. Open burning of biomass is noted to produce significant amount of PAHs.

2.3 Human exposure

2.3.1 Dietary intake

PAH in food is ubiquitous in nature and this makes dietary intake of PAH very difficult to estimate, however, the assessment of consumption and PAH concentrations from occurrence data in corresponding food groups has been helpful in this regard. Food can become contaminated by PAHs in the soil in which food grows or deposition of particles from the air, from water used in growing or cooking or via certain processing and cooking methods, such as smoking, drying, grilling or barbecuing. Concentrations in some animal foods (e.g. meat, eggs, milk and poultry) are low due to the metabolism of PAHs in these organisms, however, some marine animals, such as lobsters, oysters

and mussels can absorb and accumulate PAHs from contaminated water (EC, 2002). PAHs are lipophilic and therefore tend to accumulate in lipid tissues of plant and animals (IPCS, 1998 and EC, 2002).

There are no international limits for PAHs in food set by the Codex Alimentarius Commission. International, bodies such as European Union have set limits for smoked foods. Contamination as a result of environmental pollution, food packaging and processing has made food to be a significant source of PAHs for humans. However variation in exposures to PAHs depends on area of residence (higher in industrial areas), level of soil contamination, types of food consumed, food preparation practices and the water source used for drinking and preparing foods. A wide range of foods are included in intake assessment in order to obtain a good estimate, because it has been demonstrated by numerous sources that PAHs occur in a very broad range of foods and beverages. Comprehensive assessments on PAHs have been conducted by the Scientific Committee on Food of European Commission (EC, 2002; 2008) and WHO (IPCS 1998).

Food is a significant source of PAHs for humans and benzo [a] pyrene has often been regarded as an indicator of PAHs in food. The review by the Scientific Committee on food (EC, 2002) referred to benzo [a] pyrene as a good indicator only for higher-molecular-mass PAHs from benzofluorenes upwards. Intake assessments of individual PAHs tend to focus more on PAHs that are classified carcinogenic or probable human carcinogens by US-EPA and EFSA. The most abundant PAHs in food tend to be smaller compounds that are less harmful than those classified by these bodies (Philips, 1999). The two general approach used to measure PAH levels in diet is the measurement of benzo [a] pyrene (Kazerouni *et al*., 2001) or a large number of PAHs

as a surrogate marker for all PAHs (Philips, 1999). Although individual PAHs may vary extensively in any particular food, a more realistic background to the overall burden of PAH from diet is provided by the second approach.

PAHs can contaminate foods by generation through pyrolysis during cooking and certain methods of preservation as well as other environmental sources. PAHs content of foods exposed to smoke especially grilled foods are likely to be high. Food is exposed to PAHs and other potentially carcinogenic compounds through smoking and other methods of preservation. Atmospheric PAH and PAH uptake from water, soil and sediment including deposition can contaminate unprocessed foods such as dairy products, fruits, vegetables, vegetable oils and sea foods. Vegetables grown on contaminated soils or close to highways can be highly contaminated. Considerable levels of PAHs are contained in maize and vegetable oils from various sources such as olive oil, sunflower, rapeseed and soya bean (Ramesh *et. al*., 2004) rather probably contaminated during processing than airborne.

Contaminated feed consumption leads to PAHs accumulation in foods of animal origin, particularly livestock in addition to smoking and grilling. Considerable amount of PAHs in diet may be contributed by seafood consumption particularly bottom-feeding fishes (Ramesh *et al*., 2004). As a result of high capability to metabolize PAHs, human and other higher trophic-level consumers do not bioaccumulate..

The major contributing foods for the majority of intake assessments tend to be cereals and vegetable fats and oils, followed by vegetables. Cereals are a contributor because they make up a major part of the diet and vegetable fats and oils are major contributors due to their relatively higher concentration of PAHs. Generally, despite their usually higher concentrations of PAHs, smoked fish and meats and barbecued foods do not

contribute significantly, particularly where they are a small component of the diet. They do, however, make larger contributions leading to higher PAH intakes where these foods make up a larger part of the diet.

Methods of extraction, identification and quantification as well as selection of food items are the required issues considered in the estimation and comparison of PAHs daily intake across studies. Countries such as USA, UK, Germany, Australia etc. have conducted PAH daily dietary intake estimate as summarized in Table 2.1. The European Food Safety Authority (EFSA) concluded based on available data the overall average dietary exposure to be 235 ng/day for benzo [a] pyrene across European countries (3.9 ng/kg b.w. per day assuming a body weight of 60 kg) (EC, 2008). From the evaluation of PAHs, JECFA selected 4 ng/kg b.w. and 10 ng/kg b.w. per day benzo [a] pyrene as a representative mean intake and high-level intake respectively (JECFA, 2005). Population groups who habitually or regularly consume foods cooked over open flames or barbecues and also foods from higher contaminated areas were noted by the committee.

Table 2.1: Dietary intakes of PAHs in various countries

Country	Intake (µg/person/day)	Reference
USA	0.16-1.6 0.04-0.06[a] 0.12-2.8[a]	Santodonato *et al*., (1981) Kazerouni *et al*., (2001) Hattemer-Frey & Travis (1991)
United Kingdom	3.7	Dennis et al., (1991)
Germany	0.02-0.04	State Committee for Air Pollution Control (1992)
Australia	3.4 (0.7-15.6)[b]	Pfannhauser (1991)
Italy	3.0	Lodovici *et al*., (1995)
Spain	6.3-8.4	Falco *et al*., (2003)
Greece	1.6-4.5	Voutsa & Samara (1998)
Netherlands	5-17	de Vos *et al*., (1990)
Sweden	0.08	Beckman Sundh *et al*., (1998)

From Ramesh *et al*., (2004) as cited in IARC,2010
[a] Values reported were for benzo [a] pyrene concentrations only
[b] Median and range for the sum of 16 PAHs

The main route of exposure to PAHs is clearly dietary intake for majority of non-occupationally and nonsmoking exposed population notwithstanding the observed differences in international studies (Ramesh *et al*., 2004). Philips (1999) estimated in a study that dietary sources accounted for about 70% of PAHs exposure for the non-occupationally exposed who are nonsmokers.

2.4 Hazard identification and characterization

2.4.1 Toxicokinetics

Toxicokinetics is the study of how substances get into the body what happens to it in the body. It four processes (Absorption, Distribution, Metabolism and Excretion) are all essential for understanding the systematic toxicity of a chemical substance.

2.4.2 Absorption

The size and lipophilicity of a molecule mainly define PAHs absorption from diet since it has to go into solution passing through the cell walls of intestinal cells into circulation. It is also affected by conditions in the digestive tract such as the presence of bile, the lipid content of the diet and the dose ingested. Benzo [a] pyrene gastrointestinal absorption in experimental animals is well documented.

Aqueous solubilities ranging from 0.17 to 31740 µg/L at 25^{o}C and log k_{ow} values from 3.4 to 7.3 have been the PAHs considered in this regard. However, compounds with similar log k_{ow} values showed considerable variability as molecular structure influence on aqueous solubility reflected from increase in log k_{ow} values as aqueous solubility generally decreased.

Food and water ingestion through the gastrointestinal tract, skin through dermal and inhalation of particle containing PAHs through the respiratory tract are the main routes of absorption of PAHs in humans. The gastrointestinal absorption is considered in detail here.

In a study with Spraque-Dawley rats, the presence of bile was found to increase intestinal absorption of PAHs such as benzo [a] pyrene and 7, 12 – dimethylbenzanthracene to a greater degree than that of anthracene and pyrene (Rahman *et al.*, 1986).

Radiolabelled benzo [a] pyrene absorption in wistar rat was investigated following oral administration in solution, emulsion or various food components. The increase in lipophilic content of food component increased bioavailability which was in the range of 20-50%. Absorption from triolein and soya bean oil resulted in a greater area under the curve, compared with absorption from less lipophilic foods (kawamura *et al.*, 1988). The metabolite 1-dydroxyprene was detected in urine by Buckley and Lioy (1992) after very low levels benzo [a] pyrene diet ingestion as indirect evidence of gastrointestinal absorption of PAH in humans.

2.4.3 Distribution

Radiolabelled and unlabeled PAHs administered orally or by intravenous in rodents have been mainly used to investigate tissue distribution of PAHs. The PAH, vehicle and route of administration, hydrocarbon metabolism inhibitors and inducers presence or absence and tissue sampling time after treatment are all factors that influence tissues levels. All lipids-rich tissues and the gastrointestinal tract were found to have the highest levels of PAHs and/or metabolites although it was detected in almost all organs (IPCS, 1998).

Fatting tissues can serve as storage absorbed and from which they are then released. In a study by Kang *et al.*, (2007) rats treated by oral gavage once a day for 30 days with benzo [a] pyrene, pyrene and phenanthrene with concentrations of 0.15, 2.7 and 4.3 mg/kg b.w. respectively. Day 20 of the treatment recorded the highest concentration (34.5 ng/g) benzo [a] pyrene in the muscle of treated animals. The muscle (47.1 ng/g) and in fat (118.8 ng/g) showed the highest concentrations of phenanthrene while muscle (29.7 ng/g) and in fat (219.9 ng/g) was for pyrene in treated rats.

Benzo [a] pyrene (12.5,25,50 and 100 mg/kg b.w.) administered in a single dose by gavage to rats showed benzo [a] pyrene and it metabolites (benzo [a] pyrene – trans-7,8- dihydrodiol (+), benzo [a] phyrene – trans-9,10- dihydrodiol (+), benzo [a] pyrene – trans-4,5-dihydrodiol (+), 3-hydroxybenzo [a] pyrene and benzo [a] pyrene – 3,6-dione,) in a significant amount in a study by Saunders *et al.*, (2002). The diol metabolites (4,5;7,8,9,10 –diols) were predominant in cerebellum and cortex relative to plasma across various time points. Considering the time – course distribution of the various metabolites, the diol metabolites dominated in the earlier time points (up to 12hours post exposure) and the hydroxyl metabolites in the later (from 24-96 hour post exposures time points. These result are in accordance with previous data (Lipnaik and Brandys, as cited in EC 2008 and Modica *et al.*, 1983) publish on other PAHs (flouranthene, benzo [a] anthracene, chrysene, pyrene and benz [a] anthracene) and indicates the ability of these compounds or their metabolites to cross the blood - brain barrier.

In a study of 24 women in south India samples of umbilical cord, maternal and placenta blood and milk were examined for the presence of selected PAHs. The highest levels were detected in umbilical cord blood and milk (0.013-0.6, 0.002-2.8 and 0.005-041 ppm for chrysene, dibenz [a,c] anthracene and benzo [a] pyrene respectively). The authors concluded that maternal diet probably accounted for the developing fetus and newborn infant being exposed to these PAHs (Madhavan and Naidu, 1995 as cited in IPCS, 1998). Benzo [a] pyrene, 3-methylcholanthrene and 7,12 – dimenthyl benz [a] anthracene in studies in pregnant mice and rats showed wide distribution in maternal tissue and indicated crossing the placenta through their detection in fetuses (EC, 2002).

2.4.4 Metabolism

The metabolism of PHs occurs in all tissue and it makes them more excretable by increasing their water solubility. This is after PAHs have penetrated cellular membranes due to their lipophilicity. Enzymatic actions of varying degrees in several pathways make up the metabolic process. These actions and enzyme affinities determines the metabolic pathway in a particular tissue (ATSDR, 1990).

Metabolic studies of PAHs *in vivo* and *in vitro* has been conducted extensively with the most commonly used system being rat liver incrosomat fraction, although other species are also used. Cells and cultured tissues from human and other animals have also significantly contributed to the elucidation of the PAH metabolic scheme.

The metabolism of benz [a] anthracene, anthracene, chrysene, benzo [a] pyrene, pyrene, dibenz [a,h] chrysene, phenanthrene, naphthalene and 3 – methycholanthrene involving whole animal studies has been conducted. The structural similarity of PAHs contributes to the similarities that exist in their biotransformation. PAHs are first oxidized to form phase 1 primary (eg. dihydrodiols, phenols and Epoxides) and secondary (epoxides and diol) metabolites. The metabolites of phase 1 are then conjugated with glutathione, sulfate or glucuronic acide to form phase II metabolites, which are more polar and water – soluble than the parent hydrocarbons and the therefore more readily excreted. Benzo [a] pyrene metabolism is used as PAH metabolism model as it is the most extensively studied and reviewed PAH as shown in Figure 2.1 below.

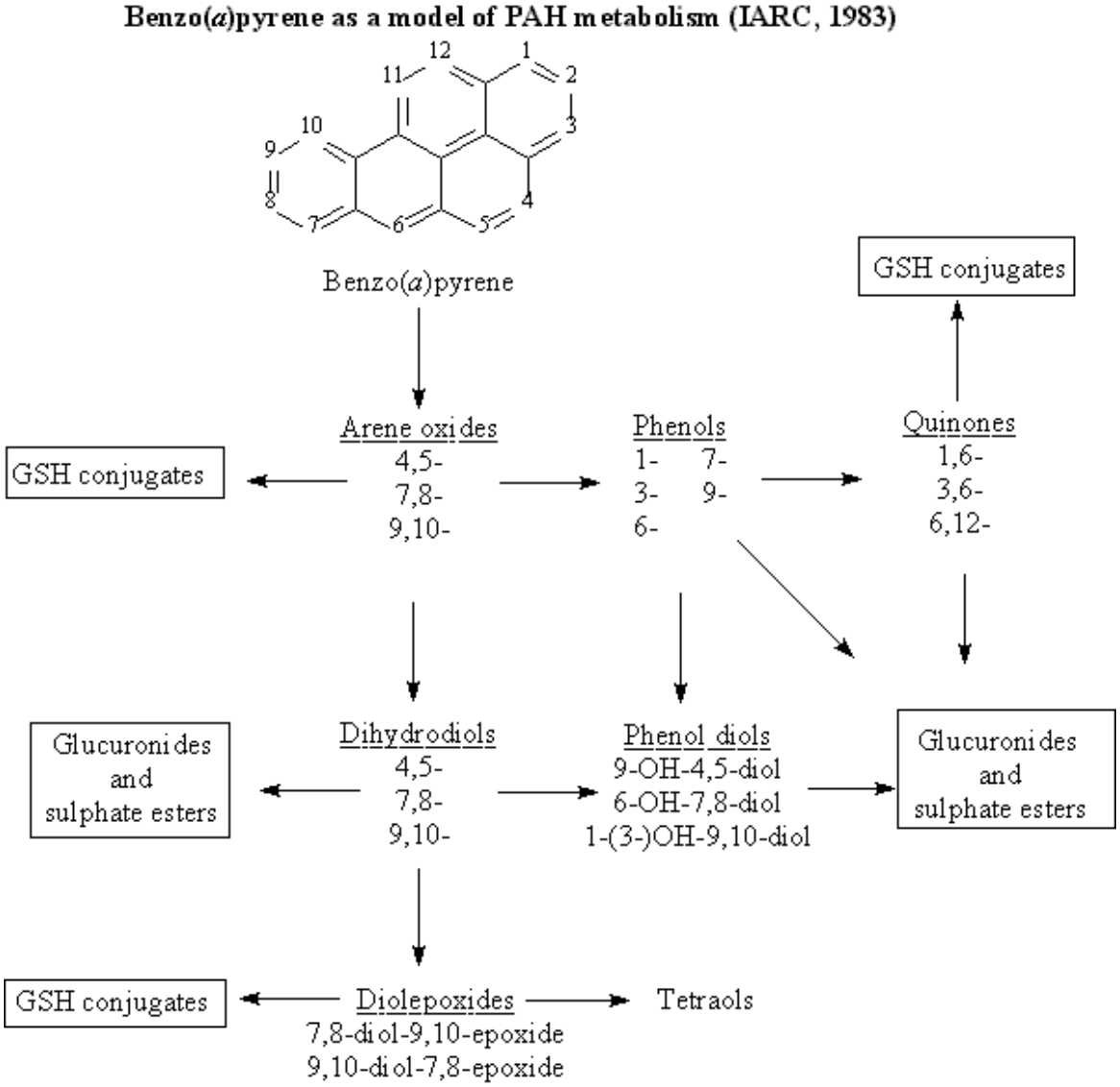

Figure 2.1: Metabolism of PAHs (Adapted from IARC, 1983)

The metabolic transformations of PAH involves a number of sequential and simultaneous activities and this makes particular metabolites biological effects difficult to ascertain after treatment. Number human tissue preparations of benzo [a] pyrene metabolism have been studied. Human and animal tissues metabolic profiles are quantitatively similar including the identity of metabolites identified. Quantitative differences of benzo [a] pyrene metabolism and activation by different human or animal tissues account for the difference in susceptibility to it carcinogenic properties. Species dependent bioactivation has however, been indicated in studies involving PAH such as benzo [a] anthracene (EC, 2002). Benzo [a] pyrene metabolism is initiated by cytochrome P450 (CYP) dependent formation of several expoxides which may undergo rearrangement spontaneously to phenols by hydrolysis. It is then conjugated with glutathione non – enzymatically or catalysed by glatathione – S – transferase or hydrolysed to dihydrodiols. The primary metabolites may similarly undergo further oxidation, hydrolysis and /or formation of glutathione; sulfate or glucuronicle conjugates (IARC, 1983).

Dihydrodiols can also further undergo oxidative metabolism. The cytochrome P- 450 system metabolizes benzo [a] pyrene – 4;5 –dhydrodiol to a number of uncharacterized metabolites, while the 9,10 – diol – 7, 8-epoxide being formed.

Benzo [a] pyrene-4,5-dihydrodiol is metabolized by cytochrome P-450 system to various uncharacterized metabolites including the formation of 9, 10-diol-7,8-epoxide. There is specifically the formation of 7,8-diol-9,10-epoxide (with less or no triol) from 7,8-hydrodiol. Pentols and triol epoxide may be formed further from the metabolism of triols and diol epoxides. Glutathione-5-transeferase catalyse diol epoxides or chemically through glutathione conjugation and some are hydrolysed spontaneously to

tetraols. The k- region 4,5-dihydrodiol is formed from the oxidation of 9-hydroxybenzo [a] pyrene. Tetraols and diol epoxides are also formed from the oxidative metabolism of 7,8-diol by various enzymes such as lipoxygenases, amyeloperoxidases system and prostaglandin H synthase. There is further spontaneous or metabolic oxidation of 3-or 6-hydroxybenz [a] pyrene by prostaglandin H synthase to yield 1,6-3,6-and 6,12 quinone. Inflammation, chronic irritation or uninduced cells leads to low CYP levels which these enzymes significant (EC, 2008).

Recent studies indicate that the doil metabolites of a number of PAH many undergo oxidation catelysed by aldo – keto reductases to redox-active o-quinones. Redox cycling of the quinines species, which many contribute to the toxicity of PAHs (Penning, 2004 and Palackal, *et al.,* 2001, 2002).

The enzymes involved in activation and detoxification of PAHs are present in the tissues of the gastrointestinal tract, although generally at lower levels than in the liver. The small intestine contributes to first – past metabolism of ingested and absorbed PAHs. Ingestion of PAHs has been shown to cause a rapid and marked induction of CYP1A1. Colon tissue expresses cox, which is inducible by PAHs. Human COX – 1 and COX -2 have been reported to activate benzo [a] pyrene - 7,8-diol and may be relevant for a possible role of dietary PAHs in colorectal carcinogenesis (Ramesh *et al.*, 2004)

Electrophilic metabolites of PAH may bind to macromolecules such as protein or DNA, forming adducts. Particular attention is focused on DNA adducts because of its potential to result in mutations leading to carcinogenicity. A number of metabolites of PAH shown to react with DNA are mostly diol epoxide including vicinal diol epoxide in the “bay region” formed between three adjourning aromatic rings. However, adducts

may also form with some non – bay region epoxides, such as that formed from benzo [j] fluoranthene or other electrophilic ion formed from the sulfate ester of methyl – substituted PAHs such as 9 – hydroxymethyl anthracene (Flesher *et al.*, 1998).

Damage including strnd nicking can result from interaction with DNA which can significantly be involved in carcinogenensis. Godschalk *et al.*, (2000) in a study following administration to rats measured the formation of DNA adduct in lung, skin, stomach and white blood cells. This was through 10 mg/kg b.w. benzo [a] pyrene by intratracheal, dermal and oral gavage routes. It was shown from the result that formation of adduct occurs both systematically and at the site of contact with no benzo [a] pyrene total systemic or peak exposure correlation.

2.4.5 Excretion

Many of the metabolites of PAH are excreted in bile, faces and urine. Rats were treated with pyrene (2.7 mg/kg b.w.), phenanthrene (4.3 mg/kg b.w.) and/or benzo [a] pyrene alone for 30 days by oral gavage once a day. These compounds together with their metabolites were monitored in urine (Kang et al., 2007). Benzo [a] pyrene and it major metabolites 3-hydroxybenzo [a] pyrene was not detected during treatment in both (benzo [a] pyrene + pyrene + phenanthrene) or benzo [a] pyrene alone exposed groups. Unchanged phenanthrene (41-69 ng/mL), pyrene (9-17 ng/mL), 3-hydroxyphenanthrene (114-161 ng/mL) and 1- hydroxyphenanthrene (201-263 ng/mL) were the major compounds found during treatment. There was rapid decrease in the levels of phenanthrene, pyrene and their metabolites in urine after treatment withdrawal.

The excretory process of PAH such as pyrene, benzo [a] pyrene, anthracene and 7, 12-diemthylbenzo [a] anthracene has been demonstrated to go through bile into the intestine, reabsorbed and returned via portal circulation to the liver (enterohepatic recirculation) (Ramesh *et al.*, 2004). Enterohepatic recirculation may lead to longer reactive PAH metabolites halve lifes as a result of the extension of residence time of PAH in the body. Although there has been studies involving animal models for enterohepatic recirculation, it confirmation in human studies is not known (de-Kok and van Maanen, 2000).

A study involving the oral administration of a single dose of 14 C-labeled (100 µg) pyrene, phenanthrene and benzo [a] pyrene in lactating goats and their 22 elimination kinetics (Grova *et al*., 2002). During a five day period of administration, a cumulated excretion of radioactivity in milk was 1.9, 1.5 and 0.2% of dose respectively. Goat milk excretion of pyrene and phenanthrene occure mainly as 1-hydroxypyrene and 3-hydroxyphenanthrene respectively (Lapole *et al.*, 2007).

2.4.6 Receptor mediated biochemical and toxicological effects

Polycyclic aromatic hydrocarbons (PAH) elicit many adverse biological effects, including immunosuppression, teratogenicity, tummour promotion and hormonal effects. These biological effects are believed to be mediated by the sustained activation of the arylhydrocabon receptor (AhR) and the subsequent disturbance of cellular homeostage. The ALR is a ligand – dependent transcriptional regulator of several genes including enzymes involved in the metabolism of xenobiotics eg. Cytochrome P4501A family as well as genes encoding factors involved in cell growth and differentiation. 2,3, 7,8-tefrachlorodibenzo – p – dioxin (TCDD) is the most efficient ligand known for

the ALR, that has subsequently been termed the dioxin receptor (Wilson and Safe, 1998).

The ALR is located in the cytoplasm and following binding of the ligand (eg. PAH) ligand – AhR complex enters the nucleus where it binds to specific DNA sequences flanking the genes regulated by the AhR. The result of AhR binding is up – regulation of transcription followed by increase in messenger RNA (in RNA) and protein levels of a large number of sense referred to as the aryl hydrocarbon (AhR) gene battery, Including genes coding for various CYPs, glutathione transferases and glucuronosyltranferases, oncogenes, growth factors and signal transduction (Lai *et al.*, 1996). Carcinogenic agents including PAH present in diet can induce this expression and the resulting carcinogenic process.

The AhR has been detected in most cells and tissues, including the gastrointestinal tract. Large difference in interstrain and interspecies AhR acentration has been reported. The receptor has been shown in lung colon, liver and placenta of human with the lung having the highest levels. Studies in AhR – null mice have shown the existence of AhR independent mechanisms for benzo [a] pyrene metabolic activation. Benzo [a] pyrene single dose (100 µmol/kg b.w) intraperitonial treatment resulted in marked induction of CYP1A1 and CYP1A2 in wild – type mice, but not in AhR – null mice. A number of DNA-adducts of benzo [a] pyrene was present in the livers of both wild type and AhR- null mice. The total numbers of adducts did not differ significantly between the two strains. However, level of five adducts were significantly decreased and the level of one adduct was significantly increased in AhR – null mice, compared with wild-type mice. Although the adducts were not identified, the major adduct was reported to exhibit chromatographic properties similar to those of the adduct derived

from benzo[a] pyrene 7,8-dihydrodiol -9,10-epoxide and the levels did not differ significantly between the wild – type and ALR – null mice (Kondraganti *et al.*, 2003)

2.4.7 Toxicological studies

2.4.8 Acute and short – term toxicity of PAH

There are relatively few studies of PAH acute toxicity. Oral median lethal dose (LD_{50}) values in mice and rats for fluoranthene, anthracene, phenanthrene and benzo [a] pyrene varying from 700 to 18000 mg/kg bw have been listed. Oral Lethality data for naphthalene are more numerous, with LD_{50} values in rats ranging from 490 to 9430 mg/kg bw and in mice from 354 to 710 mg/kg bw. The acute toxicity of PAH is indicated to be low to moderate from LD_{50} values presented (IPCS, 1998).

Acute oral toxicity study with benzo [a] pyrene was performed by administering single doses of 0.100, 600 or 100 mg/kg bw by garage as a solution in peanut oil to F- 344 rats. The animals were labeled after 14 days and the toxicological end-points examined included haematology blood biochemistry, body weights, tissue weights and tissue histopathology on selected tissues. The effects observed were limited to increased liver weights (at all doses levels, males only). No histological effect were found (knuckles *et al.*, 2001).

In a subchronic study in Wistar rats treated with 0, 3, 10 or 30 mg/kg b.w. of benzo [a] pyrene 5 days for 3 months, NOEL was 3 mg/kg b.w. per day on the basis of toxicity in the liver (Kroese *et al*., 2001).

2.4.9 Long-term studies of toxicities and carcinogenicity

The carcinogenicity of PAHs has been assessed in a number of studies relating to hazard and risk characterizations of adverse effects. With the exception of oral toxicity

of benzo [a] pyrene which is prominent, there is limited number of PAH toxicity. Oral administration of benzo [a] pyrene produced tumours of liver, gastrointestinal tract, lungs and mammary glands of mice and rats. Benz [a] anthracene and dibenz [a,h] anthracene also produced tumours of the liver, lungs and gastrointestinal tract in mice of the carcinogenicity test for other few PAH.

The oral administration of naphthalene, fluorine, benzo [a] anthracene and phenanthrene, however, did not produce increase in tumour incidence in rats seen. Oral administration test for the carcinogenicity of other PAH has not been found (ATSDR, 1994; IPCS, 1998 and kroese *et al.*, 2001).

2.4.10 Genotoxicity

The genotoxic action of 15 individual PAHs is shown in standard assays in vitro and in vivo and concluded to be genotoxic based on available information by JECFA (WHO, 2006). They are benzo [g,h,i] perylene, benzo [j] fluoranthene, [cd] pyrene, dibenz [a] anthracene, benzo [a] pyrene, dibenzo [a,h] pyrene dibenzo [a,i] pyrene, benzo [a] anthracene, dibenzo [a,l] pyrene, indeno [1,2,3-cd] pyrene, benzo [b] fluoranthene, 5-methyl chrysene and dibenzo [a,e] pyrene., Chrysene, benzo [a] pyrene and benz [a] anthrancene gave evidence of genotoxicity in germ cells. The carcinogenicity of naphthalene, pyrene, acenaplthylene, benzo [a] fluorine, coronene, acenaphthene, benzo [e] pyrene, phenanthrene and benzo [b] fluorine was considered questionable. However, pyrene, naphthalene, benzo [a] fluorine and anthracene produced negative, or generally negative results and thus were considered probably not or not genotoxic. Naphthalene, anthracene, benzo [a] fluorine, pyrene were the four PAHs that seemed to lack genotoxic activity (IPCS, 1998). A summary of the genotoxicity of some PAHs is presented in table 2.2 below.

Table 2.2: Genotoxicity of some PAHs

PAH	Genotoxicity	IARC Classification
Acenaphthene	Questionable	3
Anthracene	Negative	3
Benz [a] anthracene	Positive	2B
Benz [b] fluoranthene	Positive	2B
Benz [k] fluoranthene	Positive	2B
Benzo [a] pyrene	Positive	1
Chrysene	Positive	2B
Dibenz [a,h] anthracene	Positive	2A
Fluoranthene	Positive	3
Fluorene	Negative	3
Naphthalene	Questionable	2B
Phenanthrene	Questionable	3
Pyrene	Questionable	3

*IARC Classification (Adapted from IPCS, 1998)
Group 1: Carcinogenic to humans
Group 2A: Probably carcinogenic to humans
Group 2B: Possibly carcinogenic to humans
Group 3: Not classified as to its carcinogenicity to humans

The interaction of PAHs with DNA has been examined extensively with the important observation being PAHs active metabolites binding to DNA mainly to adenine and guanine amino groups. The N_2 position of desoxyguanosine is where the main adduct is formed, leading to stable adducts. The DNA adduct is the precursor lesion for mutations which occur at DNA adducts sites or close by them through replication errors during DNA synthesis. The correlation between the levels of adducts formation and actual tumour formation is an important tissue for cancer risk assessment of DNA adducts formation by electrophilic metabolites is mostly regarded as one of the foremost steps in PAH carcinogenesis.

In an experiment concerning DNA adduct formation in rat chronic carcinogenicity assay, 32p-postlabelling method was used to detect DNA adducts which were found in all tissue with remarkable high levels in organs devoid of any tumour development (i.e. kidneys, Lungs). The authors concluded that DNA adducts formation in addition to other factors are critical for the development of tumour by benzo [a] pyrene, and then postulated that the critical additional factor might be local cell proliferation (Kroese *et al.*, 2001).

2.4.11 Reproductive toxicity

The lipohilicity of PAHs has led to the assumption that most PAHs probabily pass into the fetus and embryo as some PAHs (and/or their metabolites) have been shown to cross the placenta. Benzo [a] pyrene, chrysene, dibenz [a,h] anthrancene, fluoranthene, pyrelene and phanathrene have been shown to induce CYP enzymes in rat placenta, and it is anticipated that induction would increase toxicity in the fetus. Fetal tissue and human placenta have been found with DNA-adducts of PAH indicating the transfer and activation of PAH by the human fetus (Benford *et al.*, 2010).

Benzo [a] pyrene has been shown in studies involving intraperitoneal injection to impair female fertility by destruction of oocytes. This effect is AhR-mediated, involving increase expression of the Bax gene in oocytes. In some studies however, the consequence of strain differences in inducibility of the AhR does not correlate with oocyte destruction, leading to the conclusion that the sum of activation, detoxification and repair seems to be decisive (IPCS, 1998).

Developmental toxicity (malformations, embryo lethality and reduced fetal weight) has been reported in response to benzo [a] pyrene, dibenz[a,h] anthracene, benz [a] anthracene and in the presence of maternal toxicity, naphthalene (IPCS, 1998).

2.4.12 Immunotoxicity

PAHs have been reported to be immunotoxic, mostly causing immunosupression, which could be associated with an increase susceptibility to development of cancer or infectious diseases. Immunotoxicity oocuring from PAH contaminated food ingestion is considered by assessing experimental studies involving the evaluation of immunotoxicity after oral intake of PAH contaminated diet. Dietary intake of PAH may lead to the induction of DNA adducts of PAH in the lungs, translocations from one organ to another may result in "at distance" effects. It should be noticed that reactive epoxides metabolites rather than parent compound are mostly responsible for the immunotoxic effects reported for PAH. These effects are mediated via the AhR or based on the results of in vitro studies, via modulation of intracellular calcium concentration signaling (Pessah *et al.,* 2001).

2.4.13 Neurotoxicity

Acute oral neurotoxity studies with benzo [a] pyrene and fluoranthene respectively on groups of 8-week-old. F -344 rats by giving single garage dose levels of the test compound. There were evaluations for motor activity and behavior for 5 days or submitted to a functional observational test battery (FOB) on eight occasions throughout the 5-day post dosing period. Bath compounds induced marked decreases in locomotor activity and clear dysfunction in the FOB. The NOAELS for these effects

were 12.5 mg/kg b.w. for benzo [a] pyrene (LOAEL, 25 mg/kg b.w.) and 100 mg/kg b.w. for fluoranthene (LOAEL 200 mg/kg b.w.) (Saunders *et al.*, 2001, 2003).

2.4.14 Cardiovascular effects

Benzo [a] pyrene effects have been investigated in apolipoprotein E knockout mice, a transgenic model in which diet – dependent atherosclerotic lesion develop that resemble human atherosclerosis. A gavage administration of Benzo [a] pyrene at 5 mg/kg b.w. once a week for 12 (n=31) and 24(n – 19) weeks. Benzo [a] pyrene – DNA binding products were detected at high levels in the aorta. There was no influence of benzo [a] pyrene on the location on number of atherosclerotic lesions, but plaques were larger, were more prone to lipid – core development and plaque layering and contained more T- lymphocytes and macrophages in animals treated with benzo [a] pyrene than in controls. It was suggested by the authors that benzo [a] pyrene did not initiate atherosclerosis in apolipoprotein E knockout mice, but accelerated the progression of atherosclerotic plaques via a local inflammatory response (Curfs *et al.*, 2004).

2.5 Human health risk assessment process

Risk assessment is part of the components of risk analysis which is made up of risk assessment, risk management and risk communication as described by WHO and FAO. The risk manager gives direction to how the whole process is undertaken and assumes the responsibility for health risk management on the population's behalf. The risk manager uses the hazard identification as the basis for deciding whether to undertake a full assessment in the light of other risk priorities and available resources, based on preliminary information. Risk managers with respect to food safety ensure that

measures undertaken to assess human health risk in various countries conform to relevant international organizations risk assessment techniques (WHO and FAO).

The examination of potential health risk of PAH in food has been undertaken in many countries through their food safety authorities. This process involves the characterization of the hazard and assessing it exposure in order to characterize the risk for their population. The evaluation of PAH international bodies such as Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Scientific Committee on Food (SCF) and by the International Program on Chemical Safety (IPCS) spans two decades. About 15 PAHs was concluded by SCF to have shown clear evidence of mutagenicity/ genotoxicity in experimental animals *in vivo*. This group of PAHs are therefore prioritized in health risk assessment of dietary PAH intake as reasoned by the SCF (EC, 2008).

Risk assessment is a multid**imensional process of evaluating and estimating both the probability of occurrence and** magnitude of health effects to a population. It provides the platform for decision making based on scientific information using both quantitative and qualitative information. It also involves the integration of activities of stakeholders with fundamental knowledge in some relevant disciplines. The information provided clarifies potential health effects such as cancer and serves as guidance for risk management. The toxicity and exposure to a substance are quantified to determine the probability of adverse health effects or the risk in general.

Risk assessment provides the backbone to risk management by providing information including clarifying the nature of the problem and risk reduction resources to be allocated. These helps in initiating strategies to prevent, remediate and direct effort towards sources and populations with the greatest risk. International and national

agencies have broadly adopted risk assessment. Risk assessment is made up of four stages as defined by WHO and FAO (FAO/ WHO, 1995). These stages are;

1) Hazard identification
2) Hazard characterization
3) Exposure assessment
4) Risk characterization.

2.5.1 Hazard identification

Hazard identification involves the process of determining the hazard and the nature of harm or adverse effects. These effects can be from chemical or biological agent and relevant review on it toxicological information including animal and epidemiological studies is also examined.

2.5.2 Hazard characterization

Hazard characterization is the quantification of adverse effects from exposure to a hazard depending on the degree of exposure. Studies are conducted in animals or humans and adverse health effects are observed (dose-response assessment). Mathematical models are used to extrapolate the relationship between exposure and effect including review of relevant data and uncertainty factors (IPCS, 1998).

2.5.3 Exposure assessment

Exposure assessment is the determination of the concentration of an agent and it intake rate in a targeted organism. It takes into account the duration, extent, magnitude, frequency and the route of exposure.

2.5.4 Risk characterization

Risk characterization integrates hazard identification, hazard characterization and exposure assessment to estimate the potential effect of the hazard. The estimate is based on available information from toxicity profile, relevance to humans, mechanism of action, potential human exposure and dose response. It also includes presenting limitations and uncertainties of the process. This is the final step of risk assessment process and risk managers used the information to manage the risk (FAO/WHO, 1995).

2.6 Analytical methods

The identification and quantification of PAHs in foods are mostly done with either High-performance liquid chromatography (HPLC) with fluorescence or ultra-violet detection and gas chromatography (GC) with mass spectrometer (MS). Flame ionization detection (FID) with GC or an ultraviolet (UV) or a photo-diode array detector (PDA) with HPLC were used by researchers earlier than now even though each of these methods has its relative advantages and disadvantages (poorer selectivity and sensitivity). Critical reviews on the analytical methods for PAHs determination in different foods such as fats and oils, marine, smoked foods have been published (Benford *et al.*, 2010).

The extraction of PAHs in food samples by an extraction method is mostly dependent on the nature of food component or matrix. Solid food samples (fish, meat, bread etc.) are mostly extracted with organic solvents whiles liquid samples (vegetable oils etc.) often by liquid-liquid extraction. There is less frequent use of automated extraction techniques such as supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) for solid food matrices are also applied, but less frequently. The isolation of

PAHs from interfering matrix substances is mainly achieved by solid phase extraction (SPE), gel permeation chromatography (GPC) and column chromatography.

Food matrix, method and compound are the factors that affect recoveries and LODs. The range for LODs for PAHs is usually from 0.1 to 1.0 µg/kg. Some PAHs however have lower and higher LODs (even up to 20 µg/kg) (EFSA, 2007). LODs for both HPLC and GC-MS methods are adequate to detect the concentrations normally encountered in foods. There is usually over 70% recoveries for PAH by GC and HPLC methods for different food samples but there is lower recoveries for some PAH (FAO/WHO, 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Reagents and Chemicals

All reagents were of analytical or HPLC grade and included; magnesium sulfate, sodium acetate, acetonitrile (CH_3CN) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A PAH custom standard (PAH Mix 3) of product code, 861291 and lot number LC00389 containing the analytes; acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo [b] flouranthene, benzo [g,h,I] perylene, benzo [k] flouranthene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorine, indeno [1,2,3-cd] pyrene, naphthalene, phenanthrene, pyrene, 1-methylnaphthalene and 2-methylnaphthalene were purchased from Supelco, Bellefonte, PA, USA. Milli-Q system (Milford, Mass, USA) water was used to prepare all solutions. The mobile phase was filtered through a Whatman membrane filter (47 mm diameter and 2 μm pore size).

3.1.2 Preparation of Standards

A standard mix of PAH contained 100 ug/mL each of anthracene, benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, fluoranthene, pyrene; 200 ug/mL each of dibenz [a, h] anthracene, fluorene; 1000 ug/mL each of 1-methylnaphthalene, 2-methylnaphthalene, acenaphthene, naphthalene. Calibration standards of 5 – 125 ng/g per 100 uL were prepared from the standard mix.

3.1.3 Equipment and Material

The analysis was performed on Cecil-Adept HPLC (Cecil Instruments Ltd, Cambridge CB24 6AZ, England) equipped with binary pump and a fluorescence detector (Shimadzu 10AxL) set at excitation and emission wavelengths. The selection of the excitation and emission wavelengths for detection was based on the optimum responses for the various PAHs. Separation of the compounds was achieved on Phenomenex HyperClone BDS CI8 150 x 4.6 mm, 5 µm column.

Extraction and cleanup were achieved with Agilent Bond Elut Buffered QuEChERS AOAC Extraction kit, p/n 5982-5755 and Bond Elut QuEChERS AOAC Dispersive SPE kit, p/n 5982-5158, (Agilent Technologies).

3.2 Methods

3.2.1 Survey

The survey area was Kwadaso Sub-metro in the western part Kumasi metropolis in the Ashanti region of Ghana. There were four (4) centre's purposively selected based on the social and economic diversity of the people, namely Kwadaso market, Ohwimase, Edwinase and Agric Nzema (Figure 3.1). The survey forms consisted of questions which were administered by face to face interview, where respondents were asked on the frequency of bread intake either per day, per week. Respondents were also requested to respond on the number of servings consumed each time they ate bread, the duration of storage and the type of bread consumed. Questionnaires also consisted of socio-demographic variables which included gender, body weight, age group and family size.

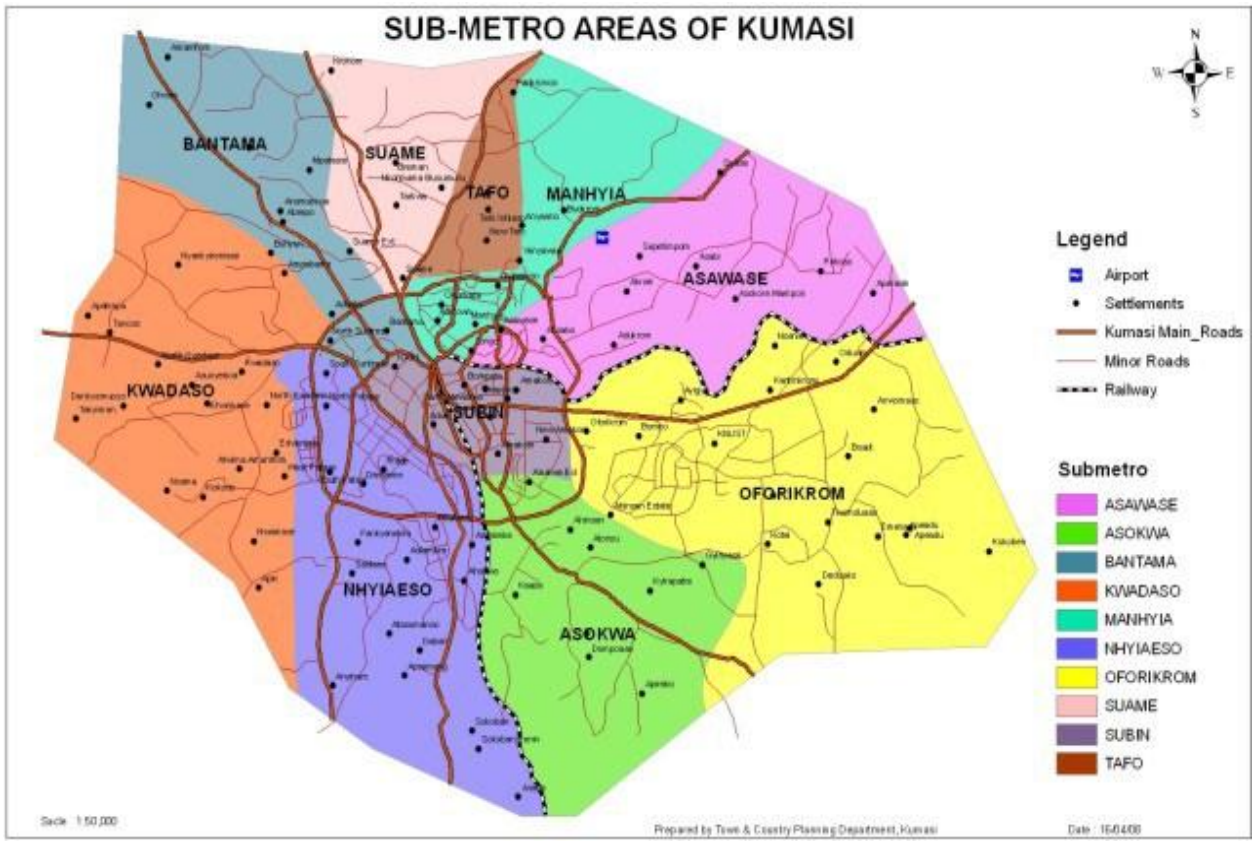

Figure 3.1: Sub-Metro Areas of Kumasi

Source: Google images

3.2.2 Sampling

Three types of commercial bread were selected as target sample based on the frequency of consumption in the short survey. Tea bread, sugar bread and butter bread of similar sizes (about 500 g) were purchased from local vendors and bakeries using either gas or firewood as source of fuel. Samples were bought in the morning from vendors and immediately after baking from bakers and sent to the Department of Food Science and Technology laboratory, Kwame Nkrumah University of Science and Technology for analysis. The bread samples were homogenized in whole and minced into smaller fillets. Fifty grams (50 g) of the sample was collected into paper bags and stored in the freezer at -20 ^{o}C until analysis.

3.2.3 Extraction

PAH extraction was carried out by applying the method by (Pule *et al*, 2012, Agilent Technologies, Inc) with some modification. A 5.0 g sample of bread homogenate was weighed into a 50 mL centrifuge tube. The extraction kit Bond Elut QuEChERS AOAC was added and the tube was centrifuged at 4000 rpm for 20 sec. Next, 20 mL of HPLC grade acetonitrile (CN_3CN), then an Agilent Bond Elut QuEChERS AOAC extraction salt packet (p/n 5982-5158) containing 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous NaOAc were added to the tubes. The sample tubes were vortex for 1 min, and then further centrifuged at 4000 rpm for 5 min. The supernatant (acetonitrile layer) was transferred into a new tube.

3.2.4 Clean-up

A 6.0 ml aliquot of the supernatant (CH_3CN layer) was transferred into a Bond Elut QuEChERS AOAC Dispersive SPE 15 mL tube. The SPE tube contained 400 mg of

PSA, 400 mg of C18EC and 1200 mg of anhydrous $MgSO_4$. The SPE tube was vortex for one minute after which it was centrifuged at 4000 rpm for 5 minutes. A 4 mL supernatant of the extract was filtered through a 0.45 µm PVDF syringe filter; then 1000 µL of the extract was injected into the HPLC – FLD system for analysis.

3.2.5 Instrumental analysis

The polycyclic aromatic hydrocarbon analysis was carried out by means of Cecil-Adept Binary Pump high performance liquid chromatographic system, equipped with Shimadzu 10AxL Florescence detector (HPLC-FLD).

The calibrated standards included PAH Mix 3 and the prepared solution contained a concentration of100 µg/mL of all compounds mixed in a solution of 50:50 methylene chloride: methanol. The HPLC instrument specifications followed the protocol by Shimadzu Application Note (LC-022) Demuro (2001) with some modifications.

The column used was Phenomenex HyperClone BDS CI8 150 x 4.6 mm, 5 µm with column temperature set at 30 ^{o}C. A mobile phase of Acetonitrile (Pump A) and Deionised water (Pump B) was used for the analysis based on the following gradient program: 0 min, 60 % A; 5 min, 90 % A; 20 min, 100 % A; 28 min, 60 % A and 30 min, 60 %. Fluorescence detection (Ex: 254 nm, Em: 390 nm) was used.

Identification of PAHs in the samples on the comparison of the retention times with those in a standard solution and quantified using the calibration curve obtained. Duplicate analyses were performed for all samples. Results obtained from the HPLC analysis was multiplied a factor of 20 to obtain the PAH content per gram of sample.

3.2.6 Human health risk assessments

The risk assessment guidelines advocated by US EPA as described by Cheung *et al.*, 2007 was used to assess the carcinogenic risk from the exposure to PAHs in bread. The carcinogenic health risk of a PAHs mixture is estimated by conversion of the carcinogenic potency of each individual PAH relative to that of BaP which is usad as a marker. The potency of the mixtures of PAHs (BaP_{eq}) can be calculated as the sum of the product of the concentrations of individual PAHs and BaP toxic equivalency factors (TEF) as seen in equation 1. The toxic equivalency factors of each individual PAH are shown in Table 3.1 (Nisbet and Lagoy, 1992).

PEC =$\sum$ (TEF X C) Equation (1)

Where C concentration of PAH; TEF the toxicity equivalency factor of PAH (Table 3.1).

Table 3.1: Toxicity equivalency factors (TEFs) for individual PAHs relative to BaP

Compound	TEF	Compound	TEF
Naphthalene (Nap)	0.001	Benzo [a] Pyrene (BaP)	1
Acenaphthene (Ace)	0.001	Benzo [b] Fluoranthene (B[b]F)	0.1
Anthracene (Ant)	0.01	Benzo [k] Fluoranthene (BkF)	0.1
Fluoranthene (Flt)	0.001	Fluorene (Flu)	0.001
Pyrene (Pyr)	0.001	Dibenz[a,h] Anthracene (DBA)	1

The chronic daily intake (CDI) was calculated to determine the exposure rate of PAH in bread from equation 2 (Cheung *et al.*, 2007).

$$CDI = \frac{C(PEC)*CR*EFD}{BW*AT} \qquad \text{Equation 2.}$$

Where C (PEC) is the concentration of PAH in bread (mg/kg), CR is the consumption rate (kg/day), EF is the exposure frequency (365 day/year), ED is the exposure duration, which is 70 years to assess lifetime cancer risk, BW is body weight (kg) from survey data and AT is duration over which the dose is averaged (365 day/ year*70=25550 days). The bread consumption rate used to calculate the dose were determind in the bread consumption survey conducted at Kwadaso sub-metro.

The cancer risk was calculated by multiplying the estimated dosage (CDI) by the Cancer Slope Factor (CSF) of 7.3 $mgkg^{-1}day^{-1}$ for BaP ingestion, which was adapted from USEPA-IRIS (2014) in equation 3.

$$Risk = CDI*CSF \qquad \text{Equation 3.}$$

3.2.7 Statistical Analysis

All experiments were done in duplicate and the data recorded as mean were analyzed using Microsoft Excel (2007). Descriptive statistical analysis were used to describe the demographic data obtain and the concentration of PAHs in bread.

Monte Carlo simulation was employed to determine the probabilistic risk assessment to minimize uncertainties as used by Qu *et al.*, 2014 with some modification. Uncertainties exist in risk assessment as a result of data scarcity, model limitation and variability of individual human characteristics (Mari *et al.*, 2009). The Monte Carlo simulation was performed using @ Risk Software (version 7.0; Palisade Cooperation; Ithaca, NY, USA). Simulations were run at 10,000 iterations with each parameter sampled independently to ensure stability of results.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Socio-demographic characteristics of respondents

The mean age of respondents was 32 (10.5) years old while the mean weight was 67.4 kg with a standard deviation (8.12). The respondents were made of slightly overrepresented males (51.7%) and 48.3% females. They comprised of students, bakers, vendors, shop owners and adults in the street and houses of Kwadaso. The minimum and maximum age was 18 and 74 years respectively. The distribution of respondents as to sex and age group are shown in Table 4.1

Among both sexes, respondents aged between 24 to 40 years were overrepresented (57.4%) with respondents over 40 years representing 19.3%. With the exception of one respondent who did not consume bread, all respondent consumed at least one type of bread analysed.

Table 4.1: Total Respondents (%) (n = 300)

Parameters	**Respondents % (n)**
Gender	
Female	48.3 (144)
Male	51.7 (156)
Age group	
Less < 23	23.3 (70)
24-27	20 (60)
28- 32	18.7 (56)
33- 4	18 .7 (56)
>40	19.3 (58)

4.2 The consumption pattern of bread

The three types of bread frequently consumed by the respondents were Butter, Sugar and Tea. Butter bread had the highest consumption rate (91.2%) followed by Sugar (56.7%), Tea (26.3%), Wheat (7.6%) and Brown (2.3%). The names of these bread types are based on flour composition and ingredients used (Ellis *et al.*, 1997). Wheat and Brown were less consumed and thus was not included in the PAH analysis. Among the respondents, 27.3% consumed Butter only whiles 5.7% consumed only Sugar with no respondent consuming Tea, Wheat and Brown only. Also just (2.3%) did not consume any of the three bread types analysed as in Table 4.2

Only one respondent did not consume bread as the rest consumed at least one type of bread. Approximately 33% of the respondents were consumers of Butter and Sugar only, with almost halve of the respondent (49.6%) consuming two types of bread. Respondent who consumed three types of bread was 17.3% with just 2.3% consuming Brown and Wheat bread which were not used in the analysis of PAH in bread. The total percentage of respondents who consumed each type of bread is shown in Figure 4.1.

Bread consumption has been increasing over the years and has become an integral part of many Ghanaian daily meals (Ellis *et al.*, 1997). This increase can however be related to bread being relatively cheap and not needing any preparation as it is mostly commercially baked. Bread and cereals make the highest share (20%) of food budget in all localities in Ghana (Bediako, 2008). It is usually consumed during breakfast and also with other foods for the other part of the day. It is a good source of energy and contains significant amount of vitamins and minerals essential for human nutrition (Ciecierska and Obiedzinski, 2013). Bread is sold in malls, shops, restaurant, market and even on the street of towns and cities.

Table 4.2: The types of bread and the number and percentage of respondents who consumed them.

Types of Bread	Frequency (%)
Butter	82 (27.3)
Sugar	17 (5.7)
Butter and sugar	99 (33.0)
Butter and Tea	31 (10.3)
Butter and Wheat	10 (3.3)
Sugar and Tea	2 (0.7)
Brown and Wheat	7 (2.3)
Butter, Tea and Sugar	46 (15.5)
Butter, Sugar and Wheat	6 (2.0)

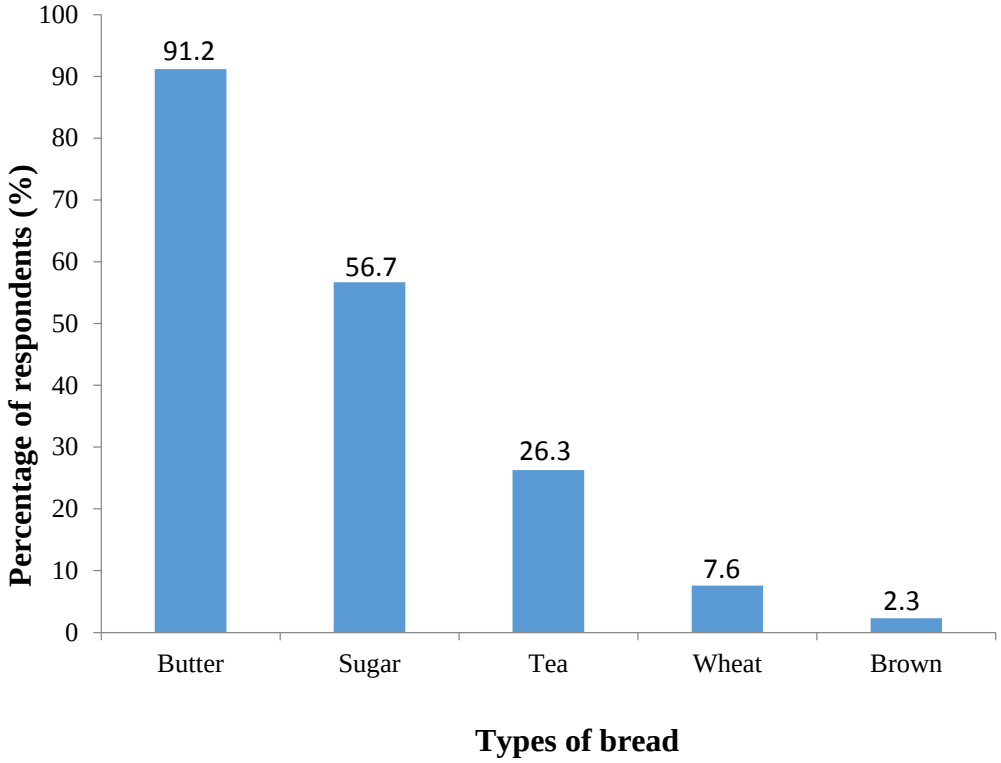

Figure 4.1: The total percentage (%) of respondents who consumed these types of bread

4.3 PAH content in bread

In this study, 47 samples of commercial bread were analyzed and the concentrations of PAHs were determined. The PAH concentration of bread samples is presented in Table 4.3, 4.4 and 4.5. The PAH concentration observed for naphthalene, 1-methyl naphthalene and 2-methyl naphthalene in the bread samples were higher than the other PAH. Most of the samples showed values below the limit of detection (LOD) and thus were recorded as not detected. Low molecular weight PAH which constitute two and three benzene rings have been shown through survey data to dominate PAHs in food stuffs (Zelinkova and Wenzl, 2015). In all, six of the PAH were detected (naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, anthracene, fluoranthene and dibenzo[a,h] anthracene) while the others were not detected. The concentration of the 6 PAH varied from 0.54 to 1212.5 µg/kg (Table 4.3-4.5).

Table 4.3: PAH concentrations, ∑PAH and BaP equivalents in Butter bread (µg/kg wet wt.)

PAH	Nap	1MNap	2MNap	Ace	Ant	B[b]F	BkF	B[a]P	DBA	Flt	Flu	Pyr	∑PAH	BaP Eq
B1	1212.50	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1212.5	1.21250
B2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.00	0.00000
B3	3.24	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.24	0.00324
B4	7.77	201.86	4.75	nd	nd	nd	nd	nd	nd	nd	nd	nd	214.38	0.21438
B5	3.57	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.57	0.00357
B6	8.00	3.21	2.80	nd	nd	nd	nd	nd	3.00	nd	nd	nd	17.01	3.01401
B7	7.10	1.50	3.50	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.10	0.01210
B8	7.30	2.94	5.40	nd	nd	nd	nd	nd	2.76	nd	nd	nd	18.40	2.77564
B9	5.30	2.70	2.80	nd	nd	nd	nd	nd	3.41	nd	nd	nd	14.21	3.42080
B10	5.98	2.90	7.20	nd	nd	nd	nd	nd	3.97	nd	nd	nd	20.05	3.98608
B11	3.49	4.00	4.50	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.99	0.01199
B12	5.97	2.60	nd	nd	nd	nd	nd	nd	2.78	nd	nd	nd	11.35	2.78857
B13	8.60	2.50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.10	0.01110
B14	10.03	4.54	1.50	nd	nd	nd	nd	nd	nd	nd	nd	nd	16.07	0.01607
B15	8.65	2.62	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.27	0.01127
B16	12.40	3.86	1.90	nd	nd	nd	nd	nd	nd	nd	nd	nd	18.16	0.01816
B17	721.50	2.19	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	723.69	0.72369
B18	2.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.01	0.00201
B19	8.89	nd	3.67	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.56	0.01256
B20	10.74	2.94	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	13.68	0.01368
T0tal	**2053.04**	**240.36**	**38.02**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**15.92**	**0.00**	**0.00**	**0.00**	**2347.34**	**18.25142**

nd= not detected

Table 4.4: PAH concentrations, ∑PAH and BaP equivalents in Sugar bread (µg/kg wet wt.)

PAH	Nap	1MNap	2MNap	Ace	Ant	B[b]F	BkF	B[a]P	DBA	Flt	Flu	Pyr	∑PAH	BaP Eq
S1	7.90	2.20	2.77	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.67	0.01067
S2	6.20	2.70	3.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.90	0.01190
S3	10.5	4.80	3.04	nd	nd	nd	nd	nd	nd	1.0	nd	nd	19.34	0.01934
S4	102.50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	102.5	0.10250
S5	3.85	nd	2.77	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.62	0.00662
B6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.00	0.00000
S7	6.05	nd	2.10	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.15	0.00815
S8	6.62	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.62	0.00620
S9	12.60	nd	6.10	nd	nd	nd	nd	nd	nd	1.47	nd	nd	20.17	0.02017
S10	5.10	nd	2.29	nd	nd	nd	nd	nd	1.35	nd	nd	nd	8.74	1.35739
S11	6.60	nd	3.09	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.69	0.00969
S12	5.40	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.40	0.00540
S13	8.40	3.1	1.38	nd	0.54	nd	nd	nd	nd	nd	nd	nd	13.42	0.01828
S14	4.83	nd	2.77	nd	nd	nd	nd	nd	nd	nd	nd	nd	7.60	0.00760
S15	7.17	nd	5.17	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.34	0.01234
S16	2.46	2.05	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.51	0.00451
S17	6.21	3.56	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.77	0.00977
S18	nd	nd	3.84	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.84	0.00837
S19	4.86	nd	3.51	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.37	0.00837
T0tal	**207.25**	**18.41**	**41.83**	**0.00**	**0.54**	**0.00**	**0.00**	**0.00**	**1.35**	**2.47**	**0.00**	**0.00**	**269.65**	**1.62274**

nd= not detected

Table 4.5: PAH concentrations, ∑PAH and BaP equivalents in Tea bread (µg/kg wet wt.)

PAH	Nap	1MNap	2MNap	Ace	Ant	B[b]F	BkF	B[a]P	DBA	Flt	Flu	Pyr	∑PAH	BaP Eq
T1	10.60	2.20	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.80	0.01280
T2	6.95	nd	4.10	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.05	0.01105
T3	11.44	nd	3.87	nd	nd	nd	nd	nd	nd	nd	nd	nd	15.31	0.01531
T4	6.49	1.90	6.50	nd	nd	nd	nd	nd	2.39	nd	nd	nd	17.28	2.40489
T5	2.73	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.73	0.00273
T6	10.60	3.00	nd	nd	nd	nd	nd	nd	1.57	nd	nd	nd	15.17	1.58360
T7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.00	0.00000
T8	2.06	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.06	0.00206
Total	**50.87**	**7.10**	**14.47**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**3.96**	**0.00**	**0.00**	**0.00**	**76.4**	**4.03244**

nd= not detected

Naphthalene and it derivatives (1-methylnaphthalene and 2-methylnaphthalene) were found in most of the bread samples analysed as compared with the other PAH. Naphthalene represented the highest concentration of PAHs in bread samples with 2.3 mg/kg followed by 1-methylnaphthalene and 2-methylnaphthalene with 0.27 mg/kg and 0.94 mg/kg respectively. The total concentration of each PAH is shown in Fig. 4.2

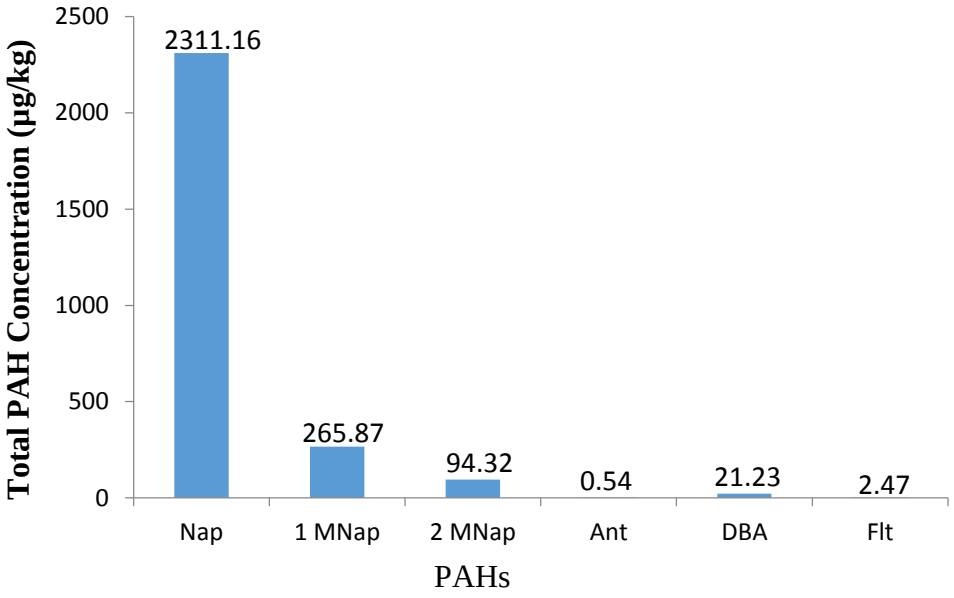

Figure 4.2: Total concentration of PAH

Although the number of the bread types analysed was not the same, Butter bread had the highest concentration of naphthalene (2.1 mg/kg) followed by Sugar bread (0.21 mg/kg) and Tea bread (0.05 mg/kg). The same trend was seen with 1-methylnaphthalene with Butter (0.24 mg/kg), Sugar (0.018 mg/kg) and Tea (0.007 mg/kg). The trend was however different with 2-mehtylnaphthalene where the highest concentration was found in Sugar (41.8 µg/kg) followed by Butter (38.0 µg/kg) and Tea (14.5 µg/kg). Naphthalene has been shown in a number of studies to be one of the most abundant PAHs in bread (Al-Rashdan *et al.*, 2010 and Marti-Cid *et al.*, 2008). The levels of naphthalene is reported to be 1.9 µg/kg and 5.15 µg/kg in Marti-Cid *et al.*, 2008 whiles Al-Rashdan *et al.*, reported 1.0 to 177 µg/kg. It must be noted that naphthalene is one of the most abundant PAH in bread. However, it was not included in most studies due to high uncertainties in its source fingerprints and it mostly evaporates during chemical analyses. Also it derivatives, 1-methylnaphthalene and 2-mehtylnaphthalene was not analysed in most studies. The level of naphthalene in this study was higher than all studies considered and the reason stated could be the course.

Anthracene was detected (0.54 µg/kg) in only one sample. It was not detected in any of the samples considered by Al-Rashdan *et al.*, 2010 whiles in a number of studies, few samples with values below 0.7 µg/kg was detected. Fluoranthene was detected in two Suagr bread with 1.0 µg/kg and 1.4 µg/kg whiles Dibenz[a,h]anthracene was detected in eight samples with a range of 1.35 µg/kg to 3.97 µg/kg in all the three types of bread. Al-Rashdan *et al*, 2010, reported Fluoranthene to range from 0.5 µg/kg to 28.3 µg/kg and as one of the three most abundant PAHs in bread including Naphthalene and Phenanthrene. Similar values were also reported by Orecchio and Papuzza, 2009 and Marti-Cid *et al.*, 2008. The levels

of Dibenz[a,h]anthracene is similar to that reported by Orecchio and Papuzza, 2009 with no detection in some studies such as Ciecierska and Obiedzinski, 2013.

These six other PAHs (Acenaphthene, Benz[a]fluoranthene, Benz[k]fluoranthene, Benz[a]pyrene, Fluorene and Pyrene) were not detected in any of the bread samples. Low levels of Benz[a]fluoranthene and Benz[k]fluoranthene were found in some studies including Ciecierska and Obiedzinski, 2013 and Zelinkova and Wenzl, 2015. In the studies, these PAH were not detected in most of the samples analysed. Benz[a]pyrene was also not detected in most bread samples in other studies but in studies where it was detected in some part of the bread and also bread baked at higher temperatures. The crust of bread was reported to constitute the highest BaP and total PAH content (Zelinkova and Wenzl, 2015 and Ciecierska and Obiedzinski, 2013). Al-Rashdan *et al*., 2010 and Rey-Salgueiro *et al.*, 2008 detected low levels of BaP in some of the few samples they analysed.

The total PAH found in the different types of bread showed similar trend with naphthalene and it derivatives dominating though with different sample size. The most consumed bread (Butter) showed a total PAH of 2311.1 µg/kg with 15.9 µg/kg being dibenz[a,h]anthracene and the rest being naphthalene and it derivatives. It was followed by Sugar and Tea with 269.6 µg/kg and 76.4 µg/kg respectively. Figure 4.3 shows the concentration of PAH in the different bread types.

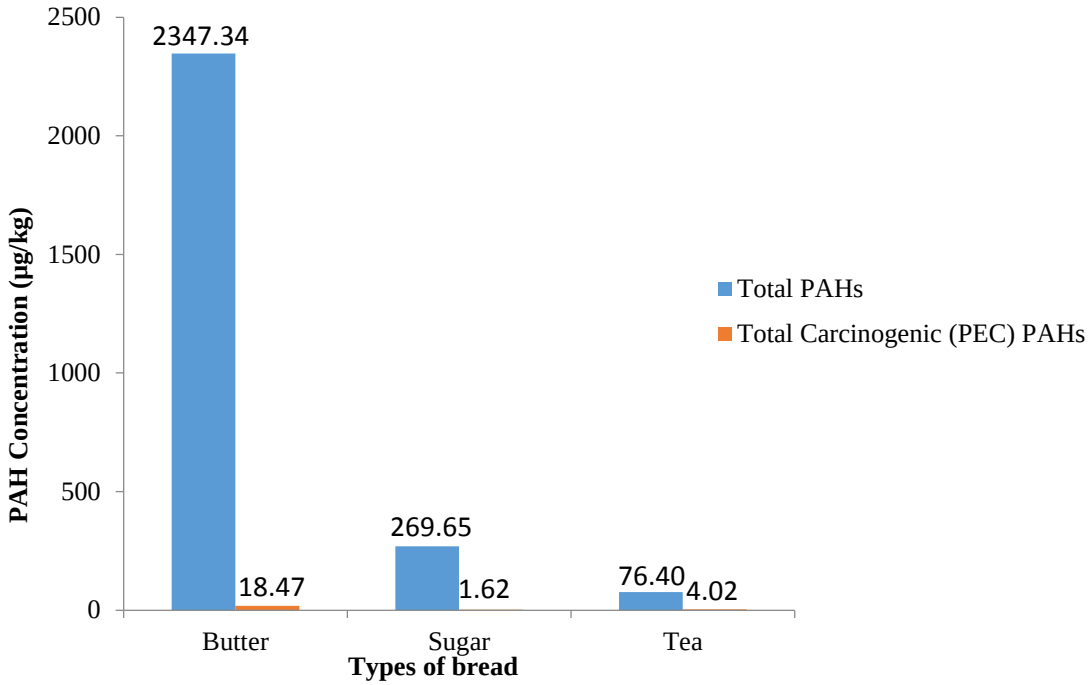

Figure 4.3: Total PAH in different bread types

Table 4.6 Risk parameters used for the Monte Carlo simulation

Parameter	value	unit	Source
C(PEC)	-	$mg/kgday^{-1}$	Survey
CR	-	mg/day	Survey
EF	-	days/year	Survey
ED	1	years	Cheung *et al*., 2007
BW	-	kg	Survey
AT	25550	days	Cheung *et al*., 2007
PF	7.3	$mg/kgday^{-1}$	USAEPA-IRIS (2014)

4.4 Risk Assessment

The chronic daily intake (CDI) from the exposure assessment estimated using equation (2) and Monte Carlo simulation was used for the carcinogenic risk assessment. The parameters used for the estimation of the CDI mg/kg of body weight (BW) per day and carcinogenic risk are shown in Table 4.6 The cumulative probability distribution of health risk of PAH through bread consumption is shown in Figure 4.4. The simulation result showed that health risk caused by PAH in bread were $2.52E^{-10}$ at 5%, $1.87E^{-9}$ at 50% and $2.06E^{-7}$ at 95% respectively. The 95% percentile risk ($2.06E^{-7}$) is lower than 10^{-6}, the cancer risk is at acceptable range. This indicates that PAH in bread would not cause significant health risk through bread consumption in the USEPA acceptable range (less than 10^{-6}). Risk may be acceptable if just a section rather than the entire population is exposed at the higher end of the range (10^{-4} rather than 10^{-6}) (Gerba, 2010). That notwithstanding, the risk indicate the probability of getting cancer (not the probability of dying of cancer) and the corresponding chronic daily intake (CDI) over an assumed 70 year human lifetime (USEPA, 2005).

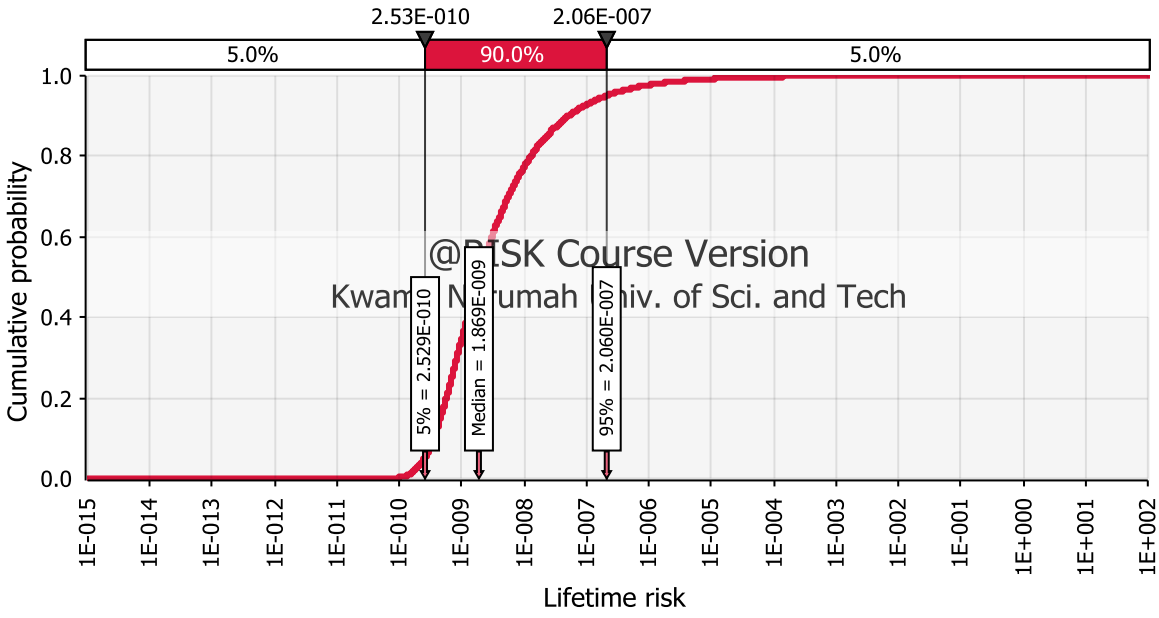

Figure 4.4 Cumulative Probability distribution of excess lifetime risk

Long term exposure to PAHs may cause health effects including kidney problem, liver damage, cataracts and jaundice (Omodara *et al*, 2014). The genotoxic effects of a number of PAH have been shown in both in vitro in mammalian cell (including human) and prokaryotes as well as in vivo in rodents (IPCS, 1998). PAH such as benzo[a]pyrene, benzo[a]anthracene and dibenz[a,h]anthracene are classified as Group 2A (probable carcinogenic to humans) and thus cause greater health concern (EC, 2002). Some PAHs do not show genotoxic effect but also present significant health concern especially for high level consumers. Naphthalene which was the dominant PAH in this study is one of them. However, naphthalene if ingested or inhaled in large amount can cause the breakdown of red cells (EC, 2002). Hence effort should be made to minimize PAHs exposure as far as practicable.

4.5 Methods to Control PAH in Bread

A number of approaches can be considered to overcome the PAH content in bread. These methods are proposed and adopted to control or reduce PAH production during baking. The determining factors for the contamination of PAH in bread are raw materials, fuel types, baking method and variation in oven temperature.

The PAHs in baked bread were reported to be 2-6 times higher than the flour used for production (Ciecierska and Obiedzinski, 2013). This means that the baking process when controlled will reduce the contamination of PAH in bread drastically.

The foremost approach for the reduction of PAH is the use of alternative cooking method and temperature control. The use of a well-ventilated oven coupled with baking at lower temperatures reduces the production and contamination of bread. Cooking methods

involving the use of gas and electricity which can be well controlled as compared to wood is shown to reduce PAH contamination in a number of studies (Ciecierska and Obiedzinski, 2013; Ciemniak and Witczak, 2010 and Ahmed *et al.*, 2000). The use of low temperature and keeping food further from the heat source lower PAH production and contamination (Bansal and Kim, 2015).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The present study analyzed 12 PAHs in bread samples and presented the probabilistic risk assessment due to PAH intake through the consumption of bread in Kwadaso submetro of Kumasi. There was a general decrease in the PAH concentration observed except for naphthalene and it derivatives 1-methylnaphthalene and 2-methylnaphthalene. The study proved the increasing bread consumption and bread and cereal making the highest share of food budget (20%) as stated by the Ghana Statistical Service. The PAHs in most of the samples were below the limit of detection (LOD) and six of the twelve PAHs were detected. The concentration of the PAH in bread ranged from 0.54 to 1212.5 µg/kg and naphthalene and it derivatives (1-methylnaphthalene and 2-methylnaphthalene) were the dominant PAH. None of the bread samples showed detection levels for benzo [a] pyrene but the benzo[a]pyrene equivalent (BaP_{eq}) concentration ranged from 0.0021 to 3.99 µg/kg. The mean daily exposure to PAH in bread was $2.55E^{-10}$ mg/kg, $bwday^{-1}$. The results show that the contamination of PAHs detected in the bread samples and Monte Carlo simulation is likely not a health risk to human consumers.

5.2 Recommendations

This study could provide useful information on human exposure to PAHs in bread and it risk management and reduction strategies. However, further studies on PAHs content in bread and other cereal products are still needed to effectively control dietary exposure to

PAH since it is a major source of PAHs in diet. Additionally, further research on staple foods especially foods cooked over open flame should be conducted since the total dietary exposure to PAH might be high.

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APPENDIX I

Sample Questionnaire Administered to Respondent

The researcher is a student of the Kwame Nkrumah University of Science and Technology conducting a research aimed at assessing the health risk of PAH in bread.

The information you provide will be treated as confidential and be used only for academic purposes. Thank you for your time and cooperation.

Demographic information

1. Gender of respondent

(i) Male [ ] (ii) Female [ ]

2. Age [ ] years

3. Weight [ ] kg

4. Height [ ] cm

Bread Consumption

5. Do you eat bread? Yes [ ] No [ ]

6. How many times in a week [ ]?

7. What type(s) of bread do you eat? [ ]

8. How much (serving size) do you eat?

(i) ½ slice [ ] (ii) 1 slice [ ] (iii) more than 1 slice [ ]

9. Do you eat only the bread or with other food?

(i) only bread [ ] (ii) with other food [ ]

10. What is your family size?

(i) 2 [ ] (ii) 3 [ ] (iii) 4 [ ] (iv) more than 5 [ ]

11. Do they eat the bread? (i) Yes [ ] (ii) No [ ]

12. How long do you store the bread before you eat?

(i) a day [ ] (ii) 2 days [ ] (iii) 3 days [ ] (iv) more than 3 days [ ]

APPENDIX II

Limit of detection and quantification of PAHs

PAH	LOD	LOQ
Naphthalene	0.45	1.51
1-Methylnaphthalene	0.61	2.03
2-Methylnaphthalene	0.10	0.33
Acenaphthene	2.35	7.84
Fluorene	4.15	13.83
Anthracene	3.79	12.65
Fluoranthene	2.17	7.24
Pyrene	4.68	15.59
Benzo[b]fluoranthene	0.05	0.18
Benzo[k]fluoranthene	6.46	21.54
Benzo[a]pyrene	2.28	7.59
Dibenz[a,h]anthracene	3.18	9.45