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HEALTH RISK ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN PROCESSED COCOA FOODS

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi or any other educational institution, except where due acknowledgment is made in the thesis.

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DEDICATION

I dedicate this work to God and Family.

ABSTRACT

Poly Aromatic Hydrocarbons (PAH) have carcinogenic, mutagenic and toxic properties, making their presence in food and environment dangerous for human health. This study accessed the health risk due to PAH associated with the consumption of processed cocoa foods in Kumasi. A total of 60 food samples (matrices) were sampled from 5 locations in Kumasi metropolis - About 300 respondents in a survey helped determine the body weights, amounts consumed and their contact rate as input variables for the risk assessment. A probabilistic risk assessment framework based on incremental lifetime cancer risk (ILCR) approaches was used to quantitatively estimate their risk of exposure. The oral-ILCR for the 95^{th} percentile which represents the highest consumable group

of the population was $2.59x10^{-6}$. This level of risk exposure was above the acceptable 10^{-6} range of the de minimis. This indicates that there is a cancer risk on the consumers used for the study, given that cocoa products are the only source of exposure. The sensitivity analysis indicated that, the contact rate has a strong positive correlation and regression to the risk exposure. It is therefore important to reduce the rate of consumption as to reduce the incidence of PAH accumulation in the body.

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ABBREVIATIONS

PAH – Poly Aromatic Hydrocarbon

BaP – benzo[a]pyrene

HPLC- High Performance Liquid Chromatography

GC-MS – Gas Chromatography mass spectrometry

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Cocoa seed is much desired, appreciated by a great passion of people all over the world because of some characteristics that cannot be found in any other product (Rusconi and Conti, 2010). Some major cocoa producing countries include Cote d'Ivoire, Ghana, Cameroon, Brazil, Malaysia and Indonesia. Post-harvest procedures of cocoa influence the final quality of cocoa beans largely. Whatever reduces the quality of the beans may certainly affect the quality of the finished products intended for direct consumption such as cocoa powder, chocolate bar and chocolate confectionery (Lowor, 2012). Many contaminants are likely to be introduced into cocoa beans during post harvesting and processing stages.

Polycyclic aromatic hydrocarbons (PAHs) have recently been identified as a serious contaminant of cocoa. It is an important class of environmental contaminants with prevalent and demonstrated adverse health effects (IARC, 1987). 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 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, 2006). In 2001, PAHs ranked 9th on the list of the most threatening compounds to human health (King *et al.*, 2002). They have a relatively low solubility in water, but are highly lipophilic. For most of the PAHs, their carcinogenic and genotoxic potential constitute the critical factor for the hazard and risk characterizations (King *et al.*, 2002).

Occurrence and toxicity of PAHs have been evaluated by numerous organizations such as the United States Environmental Protection Agency, the International Agency for Research on Cancer, the Scientific Committee on Food. There exists about 660 PAHs in the environment but there has been reselection to about 16 PAHs (Sander and Wise, 1997).

Simal-Gandara *et al*., (2005), reported presence of polycyclic aromatic hydrocarbons in instant coffee using minimal clean up and rapid determination. Lodovici *et al*. (1995) analysed as the mixed sample of three different types of chocolate and found a Benzo[a]Pyrene (BaP) content of 0.33mg/kg. The BaP contents in six chocolate samples determined by Dennis *et al.* (1991) ranged between 0.13 and 0.32 µg/kg. In one chocolate candy, 0.18µg BaP/kg was detected (Kazerouni *et al.*, 2001).

The most critical step through which PAH can gain entrance into cocoa beans is the drying stage when cocoa beans are dried on asphalt, bitumen or by using direct firing model (Misnawi, 2012). Furthermore, cocoa beans can be contaminated with PAH during storage and transport in jute or sisal bags that had been treated with batching oil (Grob *et al*, 1993). However, since 1998, International Standards recommend that ingredients of batching oils must be non-toxic and approved for use in packaging materials (Ziegenhals *et al.*, 2009). Among 660 compounds belonging to the PAH group benzo[a]pyrene has been found to be the most toxic and carcinogenic and therefore, used as a marker (Sander *et al.*, 1997). Garcia-Falcon et al. detected Benzo [a] pyrene in alcoholic drink, in 2005.

Cocoa contains a lot of polyphenols (catechins, anthocyanidins) and oxidation of these compounds produced reactive compounds which in turn are hypnotized to produce numbers of derivate and

possibly contribute to the rise in PAH concentration in dried cocoa beans (Wollgast and Anklam, 2000).

1.2 PROBLEM STATEMENT AND JUSTIFICATION

Flavour development in cocoa beans continues during roasting and drying steps in the processing of cocoa beans with also the development of characteristic brown colour. This makes the drying step, important in cocoa processing. These processes involve critical parameters such as temperature, time, humidity, types of control and smoke used, design and the type of smoke house (Jahnckeadn and Herman, 2001). In the drying process by use of asphalt, bitumen or usage of direct firing method, cocoa beans are burnt at high temperatures, which lead to the likely formation of PAHs. During de-shelling of the dried cocoa beans, pieces of the shells remain on the cocoa nib and are milled together at the manufacturing stage. This results in the deposition of PAH in the milled cocoa mass for production.

The consumption of cocoa products (cocoa powder, chocolates, chocolate spreads) have continuously grown amongst all age groups and this has led to the introduction of more new cocoa products on the market. However, the PAH levels of these products are not known. Exposure levels of the consuming population are not known.

This research will inform producers about the safety of the cocoa processed products. Producers will use this information to improve the safety quality of cocoa food products. Consumers will be informed on the choices made when consuming cocoa processed products in Ghana.

1.3 OBJECTIVES

1. To evaluate the PAH levels of processed cocoa foods.

2. To determine the health risk associated with the consumption of processed cocoa foods.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTORY OF COCOA PRODUCTION

Historically, it is believed that cocoa was first discovered in between 1400 BC and 1100 BC in Honduras as a fermented alcoholic drink (Maugh II, 2007). Cocoa got to Mexico where the people of Aztec integrated it into their culture, thinking that the "god of air" brought it to them.

Cocoa was used for trading purposes and a form of currency to the king where the Native Americans prepared chocolate drink or chocolate (Delbourgo, 2011). Around the 17th century, cocoa had spread to Central America, and then moved to Brazil during the 18th century. It spread from Brazil through to Sao Tome in 1854 and around 1882 it got to the island of Fernando Po in West Africa (Sundiata, 1974). The growth of cocoa then spread to other parts of West Africa that is Ghana (then Gold Coast), Nigeria and the Ivory Coast (Afoakwa, 2010).

Tetteh Quarshie, a Ghanaian from Osu, travelled to Fernando Po where he worked as a blacksmith. He returned in 1879 with a cocoa pod by name Amelonado and made a cocoa farm in the Eastern Region at Akwapim Mampong. Tetteh Quarshie was successful in his cocoa nursery (Hill, 1997). In 1883, Tetteh began selling cocoa pods to farmers. Three bags of cocoa were the first to be exported from Ghana (then Gold Coast) to Germany in 1885. 1,000 tonnes of cocoa were produced in Gold Coast for export and between 1910 and 1911, Ghana became the world leading producer of Cocoa until the year 1977 (Darkwah and Verter, 2014).

The Ghana government recognized the Ghana Cocoa Board (COCOBOD) in the year 1947 to regulate the development of the cocoa sector. The establishment was done due to the importance of cocoa in the economic development of Ghana (Essegbey and Ofori-Gyamfi, 2012).

Currently, of the ten administrative regions in Ghana, six areas that grow cocoa in Ghana are; Ashanti, Volta, Brong Ahafo, Central, Western and Eastern Region.

2.1.1 Medicinal History of Cocoa

Colonial era documents such as the Florentine Codex of 1590 (León-Portilla, 2012) presented a treatment of cocoa beans, maize and the herb *Calliandra anomala*. This is to lessen the severity of fever, and in treatment of heart diseases. Three reliable medicinal importance of cocoa have been established. The intake of cocoa helps sick and lean patients to gain weight. This also improves digestion by countering effects of weak stomachs and stimulated kidneys. It also helps improve the nervous system of apathetic and sick patients by stimulating their nervous ends(Dillinger *et al.,* 2000).

Cocoa paste was used as a medium of drug administration as well as to mask the bitter sense of taste of other pharmacological additives. In addition cocoa barks and leaves were used to treat burns, wounds, cuts and skin irritations (Katz *et al.,* 2011, Dillinger *et al.,* 2000).

2.1.2 World Cocoa Production

Every year, about 3,000,000 tonnes of cocoa are produced for the past 30 years. Production of cocoa occurs mainly in Belize, Mexico, Ecuador, Peru, Costa Rica and Brazil in Latin America.

In West Africa, production of cocoa occurs mainly in Cote d'Ivoire, Ghana, Cameroon, Nigeria, and Sao Tome. In Indonesia, cocoa production occur mainly Sulawesi and Central Sumatra (Wessel and Quist-Wessel, 2015).

Worldwide, Cote D'Ivoire and Ghana found in West Africa are among the three largest cocoa producers and countries that have land used for cocoa production. An estimated land of 2.4 million hectares in cocoa is cultivated in Cote D'Ivoire. The country produced a total of 1.4 million metric tons in the year 2000, and Ghana had an estimated 1.5 million hectares in cocoa production and yielded 436,600 metric tons. Other countries in West Africa that produce cocoa are Nigeria with a

966,000 hectares in production and Cameroon with a 370,000 hectares in production (Clay, 2004).

2.2 COCOA PRODUCTION IN GHANA

In Ghana, cocoa is produced in the forested areas of the country. These areas are found in six out of the ten regions in Ghana. The regions that grow cocoa are Ashanti, Brong-Ahafo, Central, Eastern, Western and Volta Regions as shown in Figure 1. The rainfall level required for the growing of cocoa ranges from 1,000 – 1,500 mm per year. The cocoa growing year in Ghana begins in October that is during the times consumptions of the main cocoa crop begin, whereas the light crop starts during July. Sale of cocoa beans in Ghana is traded at a fixed price to Ghana Cocoa Board. It is shown from researches that about a quarter of all cocoa farmers obtain just over half of the total cocoa income (Porto *et al.*, 2011).

Source: Cadbury, 2010

FIGURE 2.1: Cocoa-growing regions in Ghana

The government started to reform the cocoa sector, focusing on controlling the industry through the Cocoa Marketing Board in 1979. The board was dissolved and re-formed as the Ghana Cocoa Board (COCOBOD). Alterations such as the removal of subsidies for inputs used in production were made in the sector. These include fertilizers, insecticides, fungicides, and equipment.

Additionally, in 1982, there was an introduction of new system for paying for dried beans during purchasing. In previous times, produce buying clerks paid cocoa farmers with false checks and

abused funds. Under the new payment system, a farmer was made to cash a check from a bank of his choice that had the signature of the produce buying clerk (Bell *et al.,* 1994).

In the early 1990s, there was the continuation of the liberalization and privatization of the cocoa marketing. Prices were raised and a new system was introduced to enable private traders get some incentives. Cocobod agreed to pay traders a minimum producer price as well as an additional fee to cover the buyers' operating and transportation costs and to provide some profit. Cocobod was in still control of shipping of cocoa to overseas and export of cocoa to ensure quality control (Akiyama *et al.,* 2001).

The government of Ghana made an effort to reform cocoa production system. Farmers with cocoa farms that have trees more than thirty years old (about a quarter of the total number of trees in 1984) were provided with seedlings to replace these trees in 1983. Farms that were lost to the drought were also replaced with cocoa seedlings. Till the early 1990s, there was an expansion of cocoa farms in Ghana with a projected 40 hectares added to the total area of 800,000 hectares under cocoa production each year (Obeng-Ofori *et al*. 2014). In addition, there was a launch to construct 3000 kilometres new feeder roads and upgrade existing ones to provide easy transportation and sale of cocoa from some very fertile cocoa growing areas on the border with Cote D'Voire. Likewise, major importance was placed on extension services, drought and disease research and the use of fertilizers and insecticides to help boost the production of cocoa from 300 kilograms per hectare to compete with Southeast Asian productivity of almost 1,000 kilograms per hectare. The results of these measures were to be seen in rising cocoa production in the early 1990s (Ajayi and Place, 2012).

Cocoa bean is rich in polyphenols. Food products made from cocoa (cocoa powder, cocoa liquor and chocolates) contain varied polyphenol concentration. About 12-18% of the dry weight of

whole bean is made of polyphenols. Some polyphenols found in cocoa are simple phenols, benzoquinones, phenolic acids, acetophenones, phenylacetic acids, hydroxycinnamic acids, phenylpropenes, coumarines, chromones, naphtoquinones, xanthones, stilbenes, anthraquinones, flavonoids, lignans and lignins. The different groups of polyphenols identified in the beans of cocoa are catechins or flavan-3-ols ca. 37%, anthocyanins ca. 4% and proanthocyanidins ca. 58% (Kim and Keeney, 1984, Hii *et al.,* 2009).

Oxidation of these compounds produce reactive compounds which in turn are hypnotized to produce numbers of derivate and possibly contribute to the rise in PAH concentration in dried cocoa beans (Wollgast and Anklam, 2000). Enzymatic oxidation of cocoa bean polyphenol by polyphenol oxidase in the presence of oxygen produces quinone which is very reactive agents and can react further with amino acids and proteins into dark coloured covalent bond complex (Kattenberg and Kemmink, 1993). The occurrence of defective smoke smell in over fermented and or/ abnormal fermented cocoa bean due to the excessive content of mono-phenolic compounds supported the suggestion that PAH compounds could be produced during drying and fermentation (Minifie, 2012, Minifie, 1989).

2.3 COCOA PROCESSING

Cocoa processing starts when beans taken out of the cocoa pod and undergoes fermentation for about a week. Development of flavour precursors that contribute to the taste of the cocoa product is also produced during fermentation. After fermentation follows drying where beans are transported to industrial plants to be manufactured into finished products for commercial purposes(Kamphuis, 2009). The studies of microbes evolving during fermentation of beans have

been done (Lima *et al.*, 2011). From different studies, it has been shown that microbial activities of yeasts, lactic acid bacteria are present.

Dried and fermented beans are then roasted. Roasting of the cocoa beans helps to develop the taste of the chocolate. The fermentation step results in flavour precursors developing into actual flavour. Amino acids and sugars react to produce tasty chemicals in a Maillard reaction caused because of heating the beans. The roasting process results in the loss of more volatile acids that occur naturally in the beans. This process also kills bacteria that are present because of beans exposure to dry or open air during either fermentation or drying. The process of roasting and drying are important critical control points in the production. They are important because they are responsible for the development of the distinctive cocoa flavour, and also results in the destruction of *Salmonella*. Drying of cocoa on asphalt, on bitumen in the sun or by sun drying are also ways that can contaminate cocoa beans (Gutiérrez and Pérez, 2015).

Roasting directly influences the flavour of cocoa beans which goes on to affect the final product. After roasting, winnowing of the beans is done whereby the valuable product, the nib, is separated from the by-product (shell). The cocoa beans undergo alkalization by adding potassium carbonate to develop more flavour and colour. Milling of the nibs is done to create the cocoa liquor which has cocoa particles suspended in cocoa butter (Dimick & Hoskin, 1981). Production of chocolate is done by using cocoa liquor through the addition of cocoa butter and other useful ingredients. These ingredients include sugar, milk and emulsifying. The ingredients are added in different proportions and this depends on the chocolate being made. Conching is the process of refining the chocolate by kneading and smoothening which further helps to develop flavour and taste. The mixture undergoes tempering where it undergoes a heating, cooling and another heating process.

Formation of crystalline that results in discolouration is prevented by the tempering process. The mixture is put in moulds and cooled which is later packaged for usage (Gutiérrez and Pérez, 2015).

2.4 POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

This is a complex group of organic compounds that are formed by the fusion of two or more aromatic rings and has carbon and hydrogen atoms through industrial processing activities like the pyrolysis of organic matter, waste burning, fuel combustion and some other human activities. Combustion is a natural process e.g. burning of wood through which PAHs are formed and in this process there is a conversion of organic substances to a residue which contains carbon through pyrolysis and destructive distillation. In this process, there is high boiling and melting point, low vapour pressure, low solubility of water which increases when molecular mass decreases. Polycyclic aromatic hydrocarbons are lipophilic, that is they are fat soluble (which means they mix readily with oil than water). Larger PAHs easily evaporates and not soluble in water. In our environment, PAHs are found in the soil sediments (Kislov *et al.*, 2013).

There is a quantity of PAH in the natural crude oil, coal deposits because of the chemical conversion of steroids to aromatic hydrocarbons. Processed oil, tar and fossil fuels contains appreciable amount of PAHs which is an organic pollutant. PAHs are formed because of incomplete combustion of fuels which contain carbon like fat, wood, tobacco, coal, incense and diesel (Kislov *et al.*, 2013).

The kind of PAH formed depends on the process of combustion, that is coal burning produces different mixture than forest fire, or even the combustion of motor fuel which makes the compound useful as a marker. The chemical and physical properties determine the contamination level of food with environmental PAH and the properties are chemical reactivity, biotic and abiotic degradability relative solubility in water and organic solvents. Plant tissues with high water content

cannot accumulate PAH therefore limited transfer of PAH from contaminated soil to roots. PAHs do not penetrate deeply in soils because of their strong adsorption to organic fraction of soil, therefore leaching to groundwater and uptake by plants is low (Kislov *et al.*, 2013).

Polycyclic aromatic hydrocarbons are poorly broken down by hydrolysis and are therefore very stable chemically. They undergo photo-degradation and oxidation in the presence of light. PAHs are broken down by microbial activity. Half-life of PAH in the air can range from few hours to days depending on some parameters like molecular mass, type of adsorption onto particles etc. The half-life in soil may vary for every specific PAH from months to years. Breakdown of PAH may lead to formation of oxidized reactive compounds (Howard, 1991).

2.4.1 Sources of Polycyclic Aromatic Hydrocarbons

The pathway for exposure to PAH is through inhaled air and food ingestion. They are released into the environment via the atmosphere from pyrolysis and combustion process. Carbonization can also lead to the introduction of polycyclic aromatic hydrocarbons into the atmosphere. Non Smokers are exposed to PAH through ingestion of food and inhalation of air. Ingestion of drinking water, use of products like coal-tar containing preparations contaminated with PAH, ingestion of dust and soil are also other minor routes through which non-smokers are exposed to PAH. Polycyclic aromatic hydrocarbons have been found in foods like vegetables, fishes and muscles in contaminated water. Some approaches used in food preparation such as roasting, grilling and smoking causes the formation of poly aromatic hydrocarbons. Benzo[a]pyrene and other PAH compounds have been identified as carcinogenic with possible genotoxic properties (IARC, 1987).

2.4.1.1 Combustion Process

Emission of the 16 environmental protection agency's (EPA) PAHs into the atmosphere worldwide was 530,000 tons in 2004 alone. China was leading followed by India and the USA, and they emitted 114,000 90,000 and 32,000 tons respectively (Zhang and Tao, 2009)

2.4.1.2 Diffusion of PAH into the environment

Sewage treatment and other diffuse sources are various ways through which PAHs enter into water. The inputs of PAHs were studied by the federal environmental agency where emissions into the atmosphere are of the greatest significance (Fuchs and Toshovski, 2016). Aside depositing directly onto water surfaces, there is deposition of substances onto urban grounds which are then washed into the water bodies through surface run-off and erosion. Atmospheric deposition influences as high as 80% of the PAHs into water bodies. Large quantities of PAH are introduced into the environment through leakages, petroleum refinery and transport systems. The environmental load remains local when oil is spilled into the soil. If there is a high amount of oil spillage into the lakes and rivers, the entire ecosystem can collapse because the load from PAHs makes a significant contribution to the collapse. Accidental oil spills into the seas also destroy ecosystems in vast areas (Alloway and Ayres, 1997, Rena, 2008).

2.4.2 Harmful Effects of Polycyclic Aromatic Hydrocarbons

The effects of PAH exposure on health on humans and animals have been widely studied mainly because of their potential cancer risks. Several studies showed the toxic effects in animals and occupational studies in humans show that there is a risk of lung cancer associated with PAH exposure through inhalation (Armstrong *et al.*, 2004).

Studies have reported a correlation between the development of bladder and urinary system cancer as a result of PAH exposure (Bosetti *et al.,* 2006, Rota *et al.,* 2014). A major characteristic of

cancer causing PAH is the number of aromatic rings present in it. Benzo [a] pyrene (BaP) serves as an indicator for carcinogenic levels of PAH as many studies was done on it. However, uncertainties surrounding the use of BaP as a maker for cancer risk have been discussed. The International Agency for Research on Cancer (IARC), has categorized BaP as carcinogenic to humans (group 1); other PAHs such as dibenzo [a,h] anthracene (DahA), as probably carcinogenic to humans (group 2A); and other PAHs, such as naphthalene (NaP), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[j]Fluoranthene (BjF) and indeno[1,2,3cd]pyrene (Ind) as possibly carcinogenic to humans (group 2B) (Boström *et al.,* 2002).

2.4.3 Toxic levels and bioaccumulation of PAH

Mutations and untimely tumors can occur when polycyclic aromatic hydrocarbons metabolize and become reactive electrophilic intermediates (IARC, 1987). Fluoranthene (FLT) has been classified as weak carcer causing PAH but has mautagenic characteristics and so it plays a role of causing cancer (Boström *et al.,* 2002). Poly aromatic hydrocarbons have affinity for soils and bio-data and so therefore can accumulate in adipose tissues and form in larger quantities through the food chain. Because of their lipophilic characteristics and limited biodegradation, PAHs are classified as persistent organic pollutants(Edwards, 1983; Phillips, 1999).

Consumption of food is the major route through which PAH is exposed to humans. However, inhalation is also an important route of human exposure to PAH because of the universal presence

of these compounds in the air (Li *et al.*, 2010). Poly Aromatic Hydrocarbons can be associated with the atmospheric gas phase and particulate phase (Ravindra *et al.*, 2006, Grob *et al.*, 1993)

The complexity of risk estimations happens for several factors. The cancer causing characteristics of individual PAH can happen through different mechanisms. Risk estimation for PAH exposures is complex for several reasons. The use of toxic equivalency factors (TEFs), other PAHs have been listed and ranked according to cancer potency relative to BaP. The combination of TEFs with the quantitative risk assessment (QRA) method can be used to determine the estimation of the excess life time risk of lung cancer due to PAH exposures (Ramírez *et al.*, 2011).

2.5 METHODS OF ANALYSIS OF PAH

Analysis of PAH in various matrices in the environment is very important these last years. The analytical methods used in PAH analysis needs its accuracy and sensitivity to be improved. Some major problems associated with analysis of PAH in food include the fact that most PAHs are present in foods in very small amounts usually in ppm making their extraction difficult. The most used method for PAH analysis in foods usually involved the use of saponification of lipids by methanolic KOH, followed by liquid-liquid partition and liquid-solid chromatography. Previously, PAH separation was achieved by thin-layer chromatography (TLC) but this technique was long and failed to resolve PAH in foods. There has been a widely use of Gas chromatography in together with mass spectrometry (GC-MS) to identify PAHs in foods(Lawrence and Weber, 1984, Karlesky *et al.*, 1986, Afolabi *et al.*, 1983, Kolarovič and Traitler, 1982, Hopia *et al.*, 1986, Castello and Gerbino, 1993)

Upon exposure to high temperatures during separation, Polycyclic Aromatic Hydrocarbons may be degraded. There is a difficulty in separating PAHs that are isomers such as benzo[b]fluoranthene and benzo[k]fluoranthene. These problems have resulted in the development of high performance liquid chromatographic (HPLC) methods to extract PAH from foods (Takatsuki *et al.,* 1984, Ishizaki *et al.,* 2010, Yabiku *et al.,* 1993).

There has to be complete removal of impurities from matrices so as not disrupt the subsequent separation and identification of PAHs by GC-MS or HPLC analysis. However, it has been reported that benzo [a] pyrene is decomposed under alkaline conditions. The occurrence of polycyclic aromatic hydrocarbon is as a result of the incomplete combustion of organic material (Takatsuki *et al.,* 1985).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. MATERIALS

3.1.1 Study Sites

Using simple random sampling, five locations within Kumasi were selected, and these were; Ahodwo, Ayigya, Gyinyase, Bantama and Santasi which are labelled in red triangles in Figure 3.1.

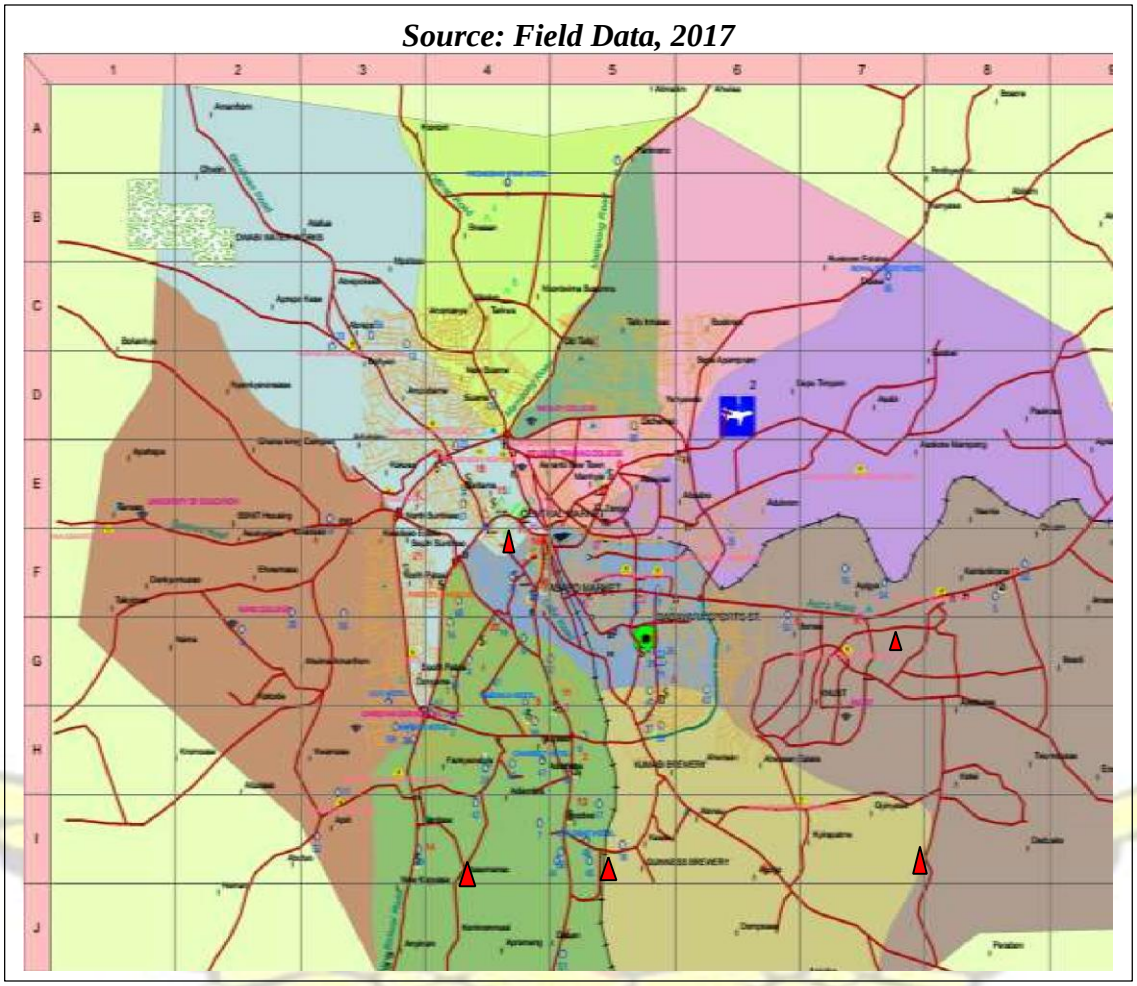
3.1.2 Matrices

The matrices for the study were purposively selected because of their high content of PAH precursors (cocoa products) and therefore susceptibility to PAH formation (Bansal and Kim, 2015, Lowor *et al.,* 2012, Raters and Matissek, 2014).Matrices are Cocoa powders: "Richoco", "Chocolim", "Milo", "This way Cocoa Powder" and "Royale", chocolate bars: "Golden Tree Chocolate" Chocolate spreads: "Choco Delight"; Toffees: "Pebbles", "Chocomilo"; Icecream: "Fanchoco". These matrices used were of different batch numbers. In all, 60 matrices were obtained from five locations in Kumasi metropolis.

3.1.2 Sampling

Simple Random sampling was used to select 5 locations using a list of towns from the Kumasi metropolitan assembly as a sampling frame and available and three hundred willing respondents were interviewed using a semi-structured questionnaire (Appendix 1).

Source: Field Data, 2017



Source: Kumasi Metropolitan Assembly

FIGURE 3. 1: Map of Kumasi metropolis and its sub-metros.

Five enumerators assisted with the data collection. The enumerators were equipped with a oneday training on the nature of the study, the structure of the questionnaire, survey techniques and questionnaire administration. Together with the researcher, they moved to sampled communities to gather data. The matrix sampling was done after the town sampling by going through streets and picking samples from every third vendor. Each sample was weighed to ascertain the weight in grams consumed by the respondent.

3.2 ANALYTICAL METHOD

3.2.1 Preparation of standards

A standard mix of PAH was purchased from Sigma Aldrich. Standard mix contained 100 ug/ml each of anthracene, benz[a]anthracene,benzo[b]fluoranthen, benzo[k]fluoranthene, fluoranthene, pyrene; 200 ug/ml each of benzo[ghi]perylene, fluorene and 1000 ug/ml each of 1methylnaphthalene, 2-methylnaphthalene, naphthalene, acenaphthene. Calibration standards of 5,

10, 15, 20, 25…, 125 ng/g. A 100 ul were prepared from the standard mix. HPLC Set-up was done based on protocol by Shimadzu Application Note (LC-022) Demuro (Date) with some modifications. A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 254 nm, Em: 390 nm) with PhenomenexHyperClone BDS C18 Column (150 x 4.60 mm, 5 um). Mobile phase composition was Pump A (Acetonitrile) and Pump B (Deionized Water) at 0.8 ml/mim. Gradient elution was used with the following combination, 0 min – 5min = 60% A,

40% B; 5 min; 15 min = 90% A, 10% B; 20 min 100% A, 0% B; 28 – 30 min = 60%A, 40% B. PAH in samples were identified using the retentions times against the standards and quantified using the calibration curve obtained.

3.2.2 Hazard content determination

A 5.0 g of homogenized sample was placed into a 50 ml centrifuge tube. A 10 ml of HPLC grade acetonitrile was added and vortex for 1 min. Agilent Bond ElutQuEChERS AOAC extraction salt packet containing 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous Sodium Acetate were added to the tube. The sample tubes were vortexed again for 1min and then centrifuged at 4000 rpm for 5min. A 6.0 ml of aliquot of the upper acetonitrile layer was transferred into a Bond

ElutQuEChERS AOAC Dispersive SPE 15 ml tube which contains 400 mg of Primary secondary amine (PSA), 40 mg of C18 EC and 1200 mg of anhydrous $MgSO_4$. The tube was vortex for 1 min and then centrifuged at 4000 rpm for 5 min. A 4ml aliquot of the extract was filtered through a

0.45 um PVDF syringe filter, then 100 ul of the extract was inject into the HPLC system.

3.3 DATA ANALYSIS

The data from the questionnaire was entered in excel spreadsheet columns as consumption frequency, mass of medium consumed, exposure frequency, exposure duration and body weight. Also total hazard concentration derived from the PAH analysis of matrices used in the study was input into an excel spreadsheet column. The columns of the data were fitted to a best fit distribution using the @Risk "distribution fitting" function and specifying whether it is discrete or continuous and writing the aggregate output to a designated cell. The values that were generated were added to an excel cell using the "add output" tool and simulated at first order Monte Carlo and ten thousand iterations. The data from the iterations were presented in a distribution information table showing the mean, 5^{th} percentile, 50^{th} percentile and 95^{th} percentile values.

3.3.1 Health Risk assessment

The health risk of the consumers using the equation 3.1.

$$Risk\ of carcinogenicity = \left[\frac{HC \times CR \times EF\ X\ ED}{BW} \times \frac{1}{AT}\right] \times PF \cdots\cdots\cdots\cdots (3.1)$$

Table 3. 1 Model parameters and data sources used for estimating risk.

Risk parameters	Data source
Hazard concentration (HC)	Hazard content analysis
Consumption rate (CR)	Food dietary recall survey
Exposure frequency (EF)	365 days
Exposure duration (ED)	1 year
Body weight (BW)	Food dietary recall survey
Averaging time (AT) for carcinogenicity	70 years (Gerba, 1999)
Potency Factor (PF)	

This model assume that, and for that matter limited to the extent that, the oral Reference Dose (RfD) and Potency Factor of Water (PF) of water is the same as that of food and that there is total absorption of ingested poly aromatic hydrocarbons (Gerba, 2000). The RfD and PF are determined essentially on the basis of route and hazard. The assumptions are justified on the basis that because both water consumption and food consumption use the oral route, their RfD and PF should be identical in either case for the same hazard, poly aromatic hydrocarbon.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter is estimates the empirical model and discusses the findings. There are also comparisons with previous studies by other authors in same subject of work. The results of the diagnostic test as discussed in the Chapter 3 as well as the descriptive analysis of the various variables used in the study are also presented. The result of the risk estimation is also interpreted.

4.1 DEMOGRAPHIC ANALYSIS

The demography of this study describes the general overview of respondents that were selected using the simple random sampling during the survey. In this study, the demographic topics include gender, age, education level and occupations of these respondents. The population used for is study was a total of 300 respondents. Out of the 300 people used in the study, 60% represented females and 33.3% represented males. A 6.7% of the respondents had their gender not available. The population of Ghana as at 2016 was 28,875,509 of which 49.1% represent females and 50.9% represent males (Countrymeters, 2016). Even though the percentage of females in the country is lower than males, the location of some respondents resulted in the gender distribution being different in this study. Markets and busy commercial areas have lots of women involving in trade and therefore resulted in this high number of female respondents.

The education level of the respondents was also taken into consideration. A 26.7% of the respondents were illiterates and 15% of them had basic education. Respondents with Senior high

school and tertiary education were 10 and 41.7% respectively. About 6.7% of the respondents had their education level not available. The level of literacy was 71.5% from the 2010 census conducted.

The highest percentage of age category that represented in the survey was the 15-24 year group at a percentage of 65.

Table 4. 1 A Cross Section Showing the Various Characteristics of the Respondents (n=300)**.**

Parameter	**Frequency, %**
Gender	
Male	33.3
Female	60.0
n/a	6.7
Education	
No Formal	26.7
JHS	15.0
SHS	10.0
Tertiary	41.7
n/a	6.7
Work	
Civil servant	16.7
Trader	40.0
Farmer	0.0
Student	26.7
None	10.0

n/a	6.7
Age (years)	
0-14	7.0
15-24	65.0
25-54	19.0
55-64	2.,0
>65	1.0
n/a	7.0

This is followed by the 25-54 years at a 19% representation. Only 7% of the respondents used in this survey represent the 0-14 years age group and 2% for the group of 55-64 years. In 2016, the age structure in Ghana had 38.9% of its population in the 0-14years category. The 15-24 years age group represented 18.9% and 25-54 years age group represented 33.5% of Ghana's population. The older age group of 55-64 years are 4.0% of the country's population (Population Census, 2010). The 65 years and above were 4.19% of the population. The variation in the ages that represented in the study can be seen in respondents with the 15-24 years age category where 65% where the percentages of the age in the country of them were present in the study.

4.2 DESCRIPTIVE ANALYSIS

Results obtained from the survey and laboratory work was put through various analysis techniques and the output was put in Table 4.2. In order to find the risk associated with consumption of processed cocoa foods in Ghana, a risk analysis was done as well as regression and correlation analysis among the variables under study. The results generated from the sensitivity analysis indicate that there is a relationship between lifetime risk levels and contact rate, number of times

of consumption per week and body weight of consumers of processed cocoa products**.** The statistics presented in this section include mean, mode, standard deviation, maximum and minimum values, 5% percentile, 50th percentile and 95th percentile of all the variables used in the study. Thedataset used in this study contains few missing values, though no observation was dropped due to data unavailability. The statistics presented in this section represents the summary of the values of the variables that were gathered during the survey.

Table 4. 2 Overall Parameter Estimates of PAH Related Matrices in the Study.

Variables	Central tendencies			Percentiles		
	Mean	**StD**	**Mode**	**5th**	**50th**	**95th**
PAH content (µg/kg)	18.83	18.83	18.83	18.83	18.83	18.83
Contact Rate(g/day)	55.32	274.31	11.38	5.92	25.63	166.84
Exposure Frequency(day)	173.73	50.25	208	82.26	180	247.59
Body weight(kg)	65.01	17.04	59.27	44.06	62.28	94.64
Risk × 10^{-6}	0.82	4.38	0.13	0.07	0.04	2.60

StD= standard deviation

Source: Field Data, 2017

4.2.1 Aggregate PAH Concentration

From Table 4.2, the combined PAH concentration of all the food samples used: processed cocoa powders: “Richoco”, ‘Chocolim”, “Milo”, “This way cocoa powder”, ‘Royale”, “Golden Tree

chocolate bars"; "Choco delight chocolate spreads"; "Pebbles", "Chocomilo"; "Fanchoco" was found to be 18.83 µg/kg. This indicated the presence of PAH in the processed cocoa foods as seen in other works done on PAH and other cocoa products. For total PAH, the maximum acceptable limit for cocoa beans and its derived products is 30 µg/kg set by Regulation (EC) 1881/2006. This shows that, the total PAH (18.83 µg/kg) obtained from the research was lower than the maximum acceptable level of 30 µg/kg. This is the safety level intended for human consumption. The level of PAH obtained from 143 cocoa butter samples was tested for a minimum of 6 PAHs and a maximum of 16 PAHs. Benzo a pyrene, the indicator substance for the PAHs had a mean of 1.93µg/kg with 95.8% of the samples detectable concentrations of the substance (Petersen, 2015). Also, a work carried on 4065 food products of different categories, BaP was consistently found in barbequed meat, dried tea, cocoa butter, chocolate and bivalve molluscs. High concentrations that were found in spice will not reflect on the exposure assessment since spices are consumed in low amounts. However, high concentrations found in cocoa butter are equivalent to the levels found in chocolate if the amount of cocoa butter in chocolate is considered since chocolate must contain at least 18% cocoa butter (Iwegbue *et al.*, 2015, Owusu, 2013).

There is a variety of processes that expose food for consumption to PAH contamination. These processes are due to commercial or home techniques. In the area of the sample matrixes used in the study, the contamination of the food products would be as a result of the roasting and drying processes cocoa goes through from the various stages after harvest to develop the desirable taste and flavour.

It is known that flavour and taste of the cocoa beans used in producing these various products was derived from critical processes that not controlled can expose the food to considerable contamination of PAH. These processes are roasting and drying of the cocoa beans which involve

critical parameters such as temperature, time, humidity, types of control and smoke used, design and type the smoke house. Modern kilns have monitored and regulated temperature and other critical parameters involved in PAH formation (Jahnckeadn and Herman, 2001). Over the past years, the traditional smoking process in commercial food production has been increasingly replaced by the use of liquid smoke flavourings. These smoke flavourings are produced from smoke that have been subjected to purification and so their health concern are lower compared to the traditional type since they result in low PAH contamination. This method also results in uniform distribution of flavour throughout the product and in this case, the cocoa beans *(*Guillén *et al.,* 2000).

However, the influence of different smoking techniques used for drying and roasting in the food commodities have been investigated by many authors. The BaP levels of 27 smoked fish samples from traditional kilns ranged from 0.2µg/kg to 4.1µg/kg, with a mean of 1.2µg/kg (Karl and Leinemann, 1996). Research programs in Latvia inter alia showed that there is a difference up to 6 times in PAH concentrations in smoked fish which have been processed by using different wood chips for smoking. Chen and Lin (1997)studied the effect of processing methods, smoking, steaming, charcoal grilling, and Liquid Smoke Flavouring (LSF) on the formation of PAH in duck breast. These results showed that charcoal grilling, smoking and roasting showed high levels of carcinogenic PAH. Steaming and LSF showed no presence of carcinogenic PAH after the experiment. It is therefore observed that the roasting of cocoa beans during the postharvest stages results in the formation of PAH when not controlled.

4.2.2 Overall Contact Rate of PAH

The contact rate is defined as the amount of contaminated medium contacted per unit time (Gerba, 2000). All the matrices were found to be 55.32 g/day, 11.38 g/day, and 25.63 g/day by mean, mode

and median respectively. The mean contact rate of 55.32 g/day indicates the average mass of the cocoa processed food consumed by the respondents in a day is 55.32g. The contact rate for the 5^{th} percentile is 5.92g/day and this shows the population that consumes the least grams in a day. The 95^{th} percentile of the study population found to be 166.84g/day and this shows the highest consumable mass of the cocoa foods. The contact rate of the 50^{th} percentile group of the population is 25.63g/day.

4.2.3 Overall PAH probable carcinogenicity risk by oral route

Health risk estimation can be calculated using PAH exposure through one of the following exposure pathways: ingestion, inhalation or dermal exposure(Chiang *et al.*, 2009, Ramesh *et al.*, 2004). In this study, ingestion of foods contaminated with PAHs was considered. Health risk assessment of carcinogenic PAHs cannot be related to the sole total concentration, because each PAH has a different carcinogenic potential. For this purpose, toxicity equivalency factors (TEFs) were used to quantify the carcinogenic potential of other PAHs, relative to BaP (Nadal *et al.*, 2004).

The cumulative frequency, fig 4.1 describes the lifetime risks of the groups in the population used in the study. The maximum acceptable limit (de minimis) of the incremental lifetime cancer risk is $10^{-06.}$ It can be seen that, the 5% of the population had a risk level of $6.9657x10^{-08}$ whiles the 95% of the population also recorded a risk of $2.59x10^{-06}$. The 5^{th} percentile represents the lower 5% consumable group in the population used in the study. An estimated risk exposure of $6.965x10^{08}$ is below the de minimis and therefore, consumers at this level are safe and not exposed to any risk of cancer. The highest consumable group (95^{th} percentile) recorded an estimated risk of $2.59x10^{-06}$ which is above the de minimis. For every million people in a population, 3 people are at risk of cancer as a result of consuming these processed cocoa foods at 166.84 g in a day.

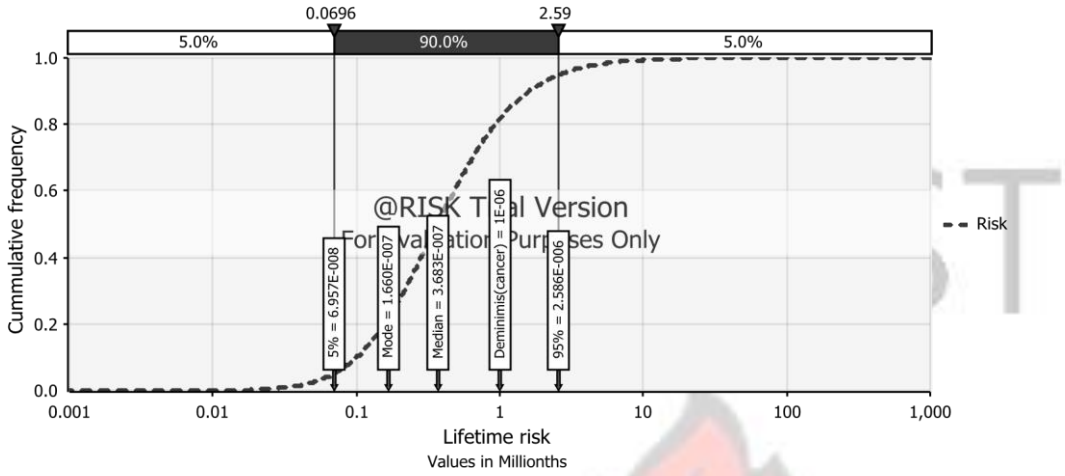


FIGURE 4. 1: The incremental lifetime cancer risk (ILCR) for the 50th percentile

4.2.4 Correlation Analysis

From the Fig 4.2, it can be noted that contact rate has a strong correlation (0.91) with the risk of consumption. This means, the higher the contact rate with processed cocoa products, the higher the risk of consumption. It is therefore necessary to reduce the contact rate so as to reduce the risk upon consumption.

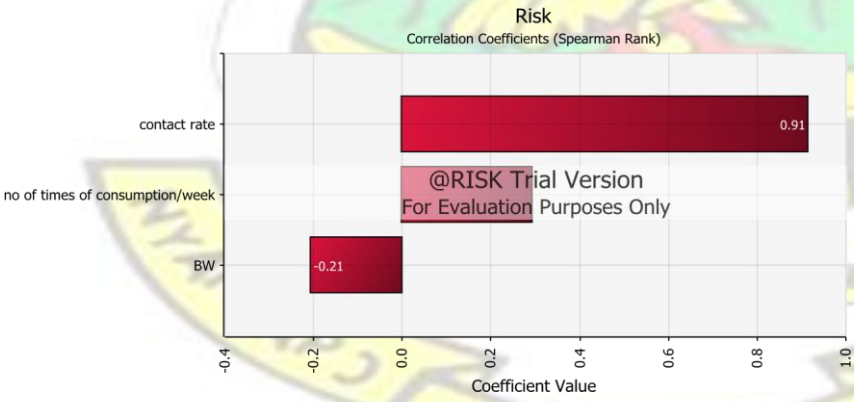


FIGURE 4. 2 Correlation Coefficients

4.2.5 Regression Analysis

From Fig 4.3 it can be noted that contact rate has a strong regression (0.92) with the risk of consumption. This means, the higher the contact rate with processed cocoa products, the higher the risk of consumption. It is therefore necessary to reduce the contact rate so as to reduce the risk upon consumption.

FIGURE 4. 3: Regression coefficients

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSIONS

Based on the results of the study, it is concluded that cocoa foods are exposed to PAH contamination through stages it goes through in production. Drying and roasting during processing to achieve desired flavours and smells exposes the cocoa beans to PAH formation.

The mean total PAH concentration of the 60 samples obtained from Kumasi metropolis was 18.83 µg/kg which was below the maximum acceptable level for cocoa beans and its derived products (30 µg/kg) meaning these foods are safe for human consumption.

The population of the people in the 95% percentile are at risk because their estimated cancer risk is above the de minimis. It is therefore important to reduce the intake of these foods as the regression and correlation curves both show that there is a relationship between the contact rate and the estimated risk.

5.2 RECOMMENDATIONS

Even though the total PAH in processed cocoa foods sampled were within acceptable limits, Farmers can be educated on the use of modern roasting and drying methods that regulate the temperature exposure to cocoa beans in order to reduce PAH formation. Manufacturers can be advised to adopt techniques in production of these processed cocoa foods that causes little or no PAH contamination. Also, this study took into consideration of only the cocoa food products and therefore an aggregate analysis that involves other food items can be done to determine the contribution of other foods when it comes to PAH contamination. Consumers should be educated

on how to reduce their exposure to PAH through all possible routes (dermal and inhalation) and not only oral to reduce cancer effects.

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APPENDIX

1. QUESTIONNAIRE ADMINISTERED TO RESPONDENDTS

DEPARTMENT OF SCIENCE AND TECHNOLOGY
COLLEGE OF SCIENCE, KNUST

Please kindly answer the following. Estimate if you are not sure.

1. DEMOGRAPHY

EDUCATION	No Formal	JHS	SHS	Tertiary
WORK	non- skilled	Farmer	Trader	civil servant
GENDER	Male	Female		

2. ANTHROPOMETRY

AGE/YEARS	
WEIGHT/kg	

3. CONSUMPTION RATE: I usually buy

OPTIONS	Small	Medium	Large	Extra Large
MILO	1 Table sp	2 Table sp	3 Table sp	> Table sp
FAN CHOC	1 pack	2 packs	3 packs	> 3 packs
THIS WAY	1/2 sachet	1 sachet	2 sachets	3 sachets
CHOCO MILO	1 Table sp	2 Table sp	3 Table sp	> Table sp
CHOCOLATE	1 bar	2 bars	3 bars	>3 bars
RICHOCO	1 Table sp	2 Table sp	3 Table sp	> Table sp
PEBBLES	3 pebbles	5 pebbles	10 pebbles	>10 pebbles
ALL TIME	1 Table sp	2 Table sp	3 Table sp	> Table sp
ROYALE	1 Table sp	2 Table sp	3 Table sp	> Table sp
CHOCOLIM	1 Table sp	2 Table sp	3 Table sp	> Table sp

4. EXPOSURE FREQUENCY/week

Over the week, when you ate the cocoa processed cocoa foods, how often did you eat?

OPTIONS	never	1 time	2 times	3 times	4 times	5 times	6 times
MILO							
FAN CHOC							
THIS WAY							
CHOCO MILO							
CHOCOLATE							
RICHOCO							
PEBBLES							
ALL TIME							
ROYALE							
CHOCOLIM							
OTHERS							

5. EXPOSURE FREQUENCY/day

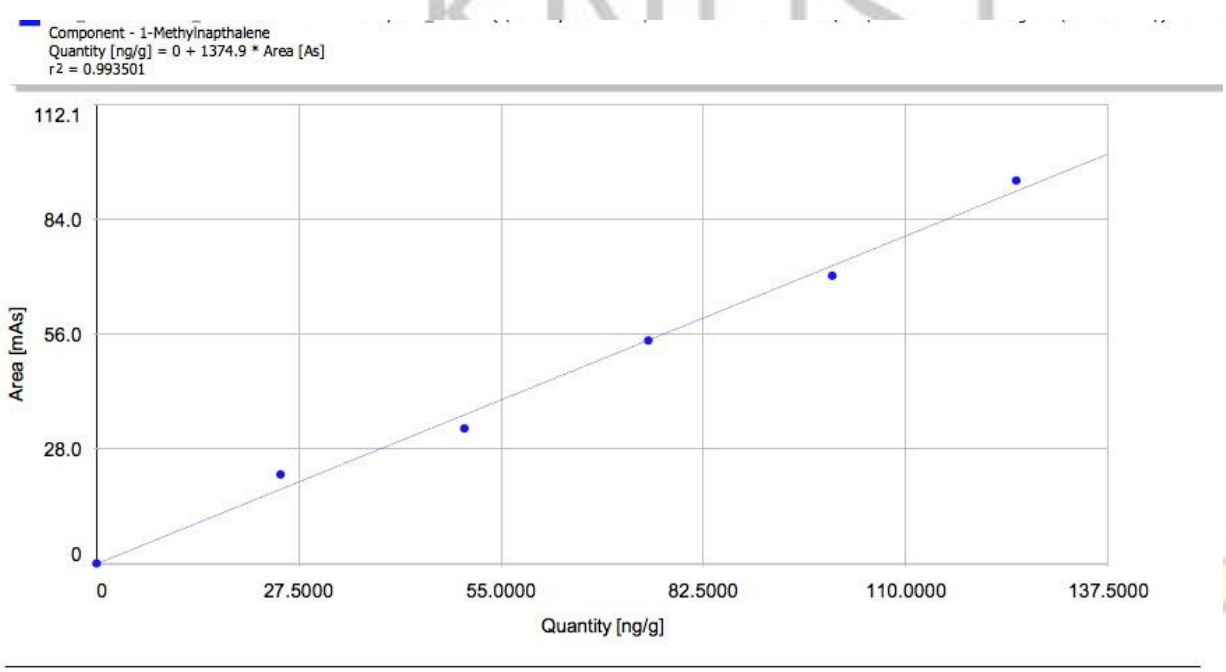
In a day, when you ate the cocoa processed cocoa foods, how often did you eat?

OPTIONS	never	1 time	2 times	3 times	4 times	5 times	6 times
MILO							
FAN CHOC							
THIS WAY							
CHOCO MILO							
CHOCOLATE							
RICHOCO							
PEBBLES							
ALL TIME							
ROYALE							
CHOCOLIM							
OTHERS							

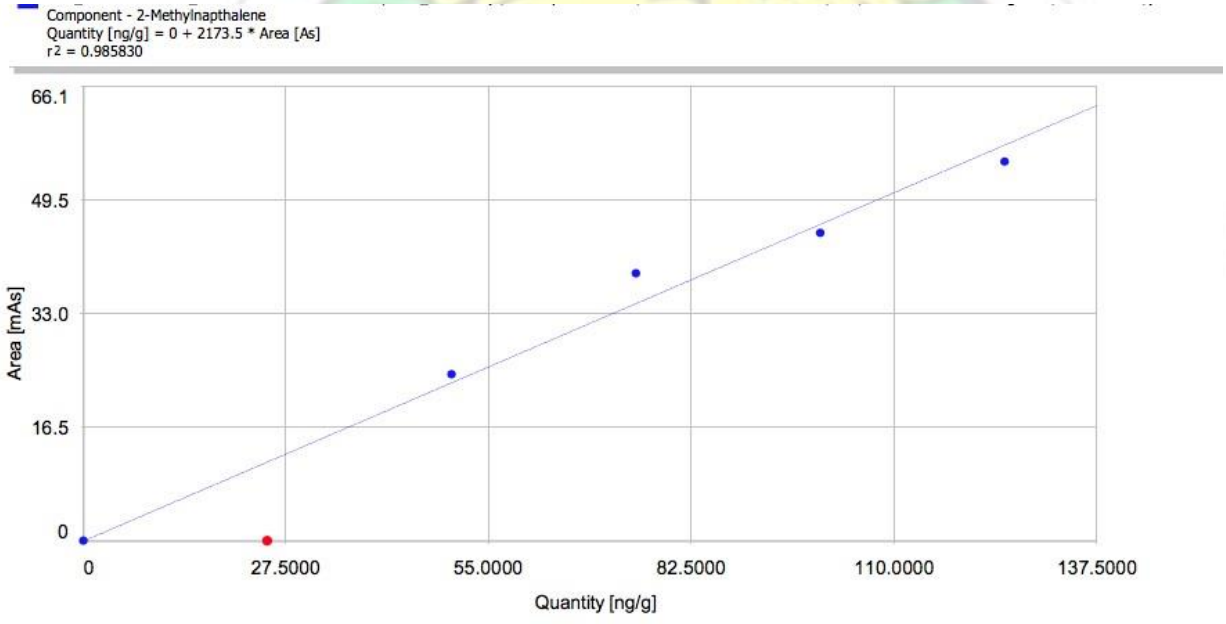
UNIT	WEIGHT/g
1 table sp	
1 bar	
1sachet	
1 pack	

2. CALIBRATION CURVES OF PAH STANDARDS

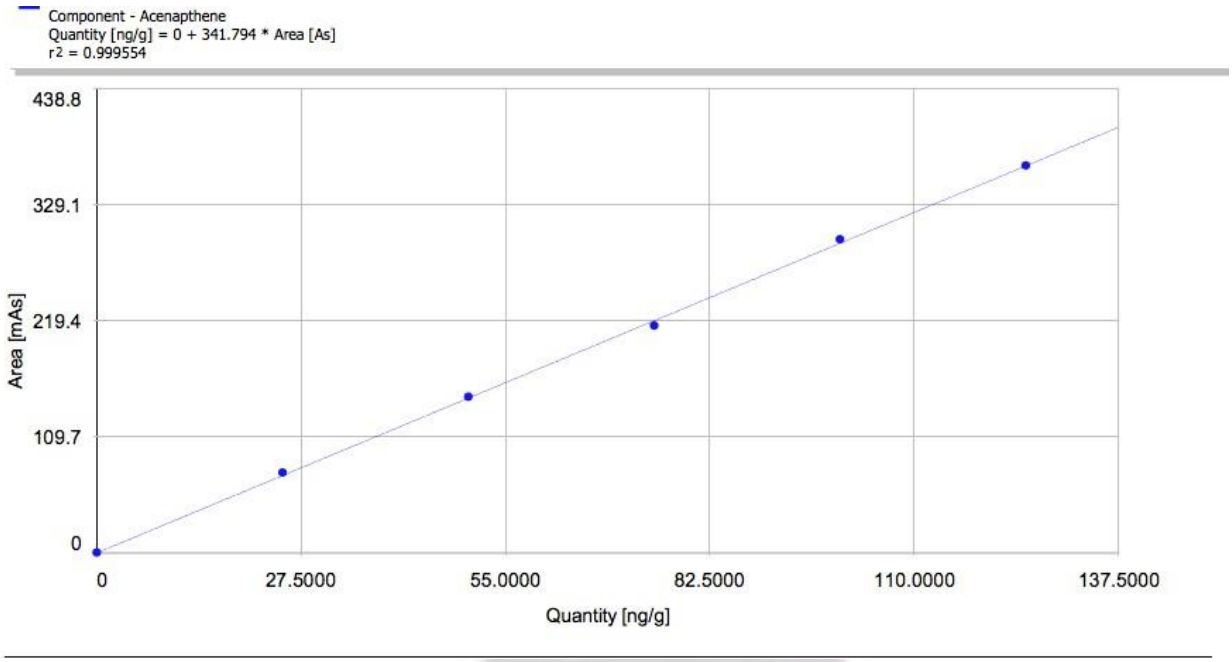
A. 1-METHYLNAPTHALENE



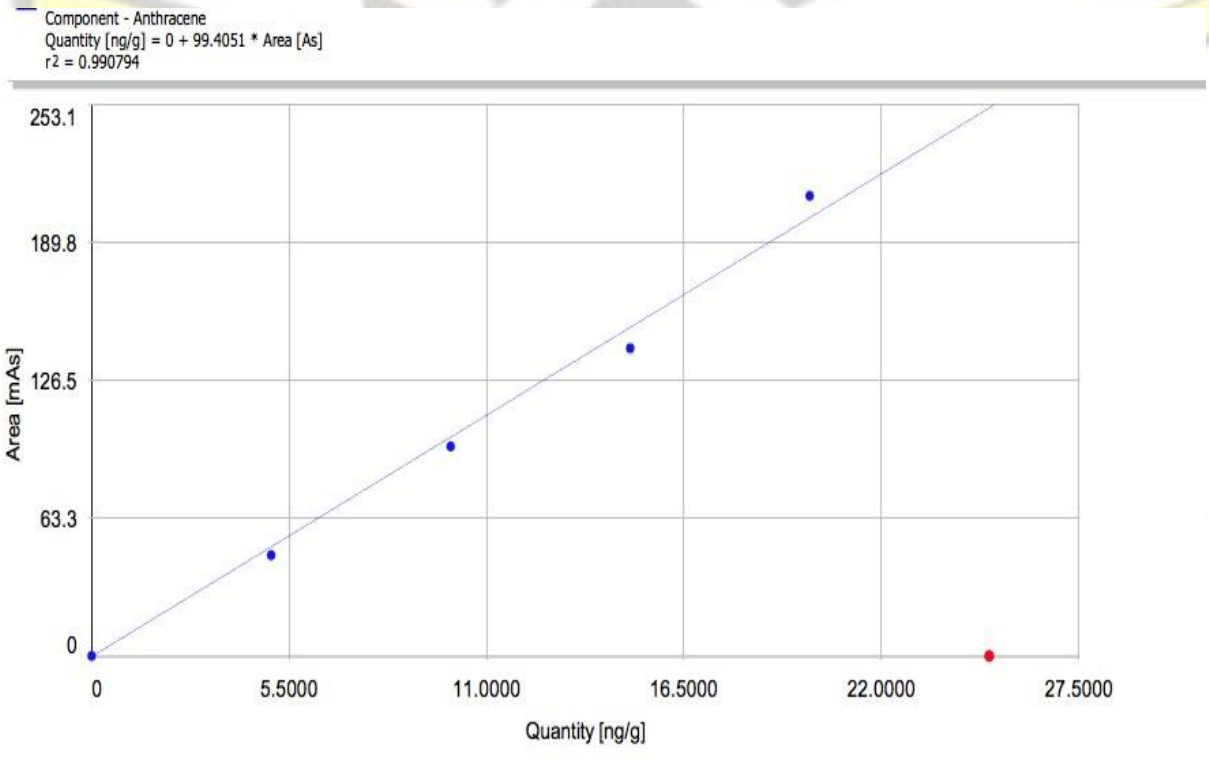
B. 2-METHYLNAPTHALENE



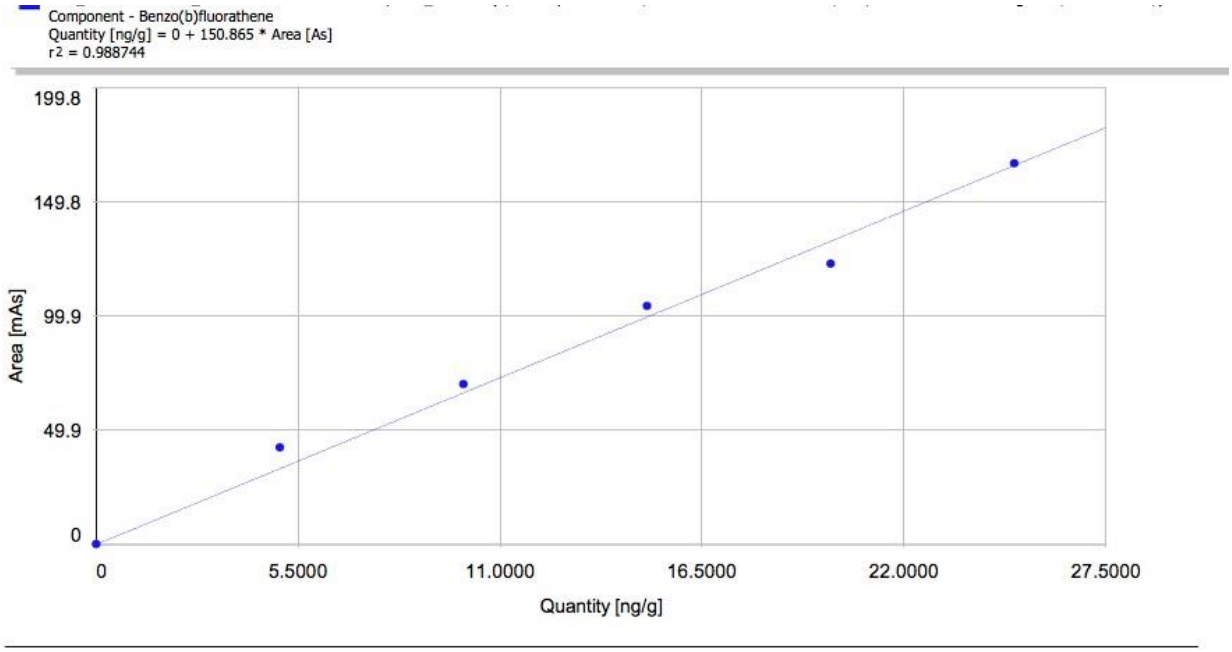
C. ACENAPHTHENE



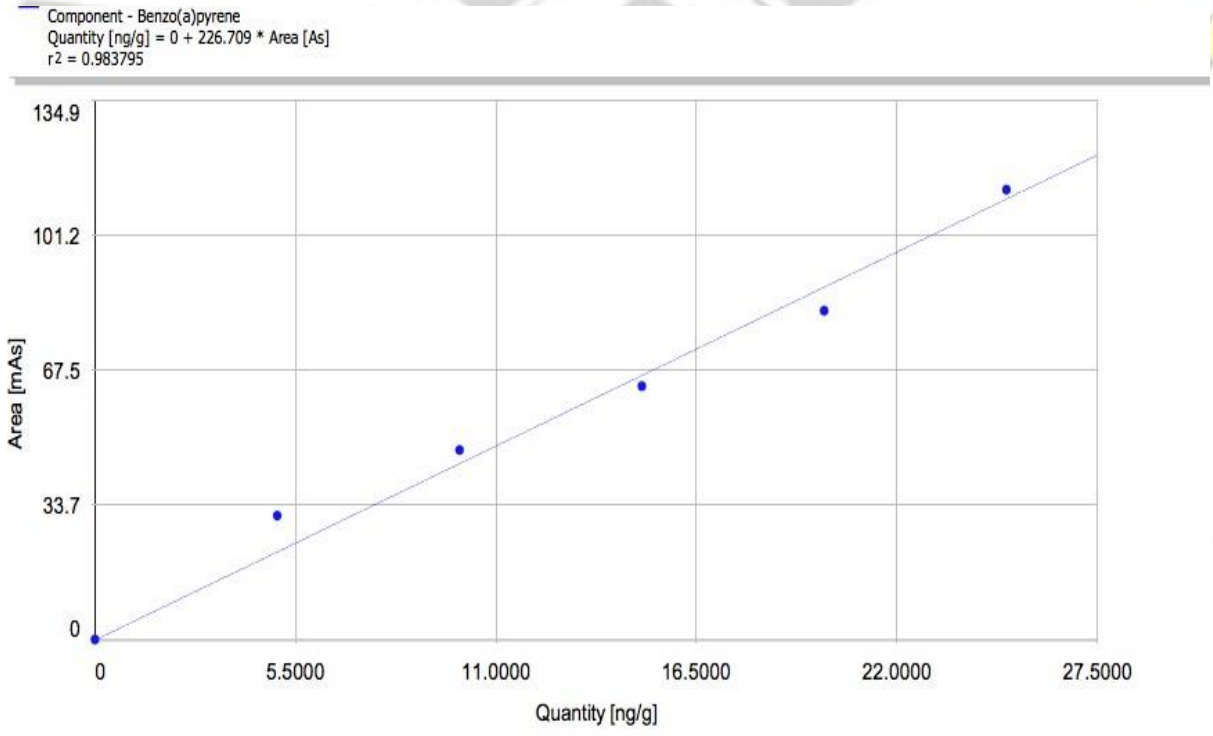
D. ANTHRACENE



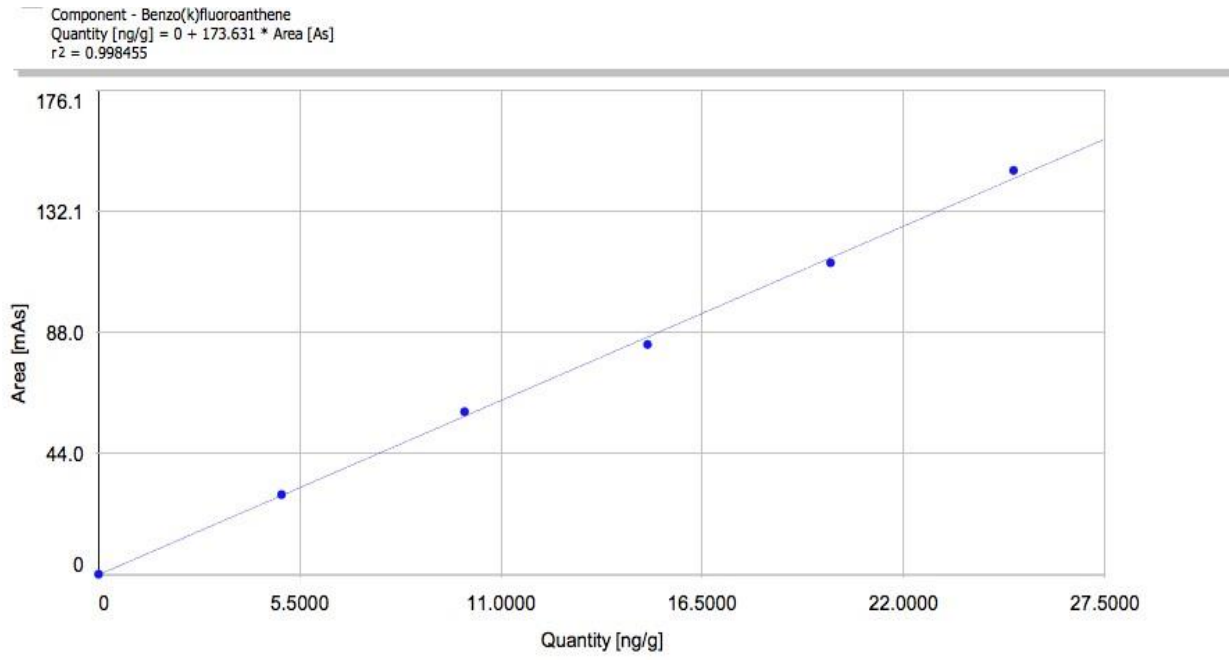
E. BENZ(B)FLUORANTHENE



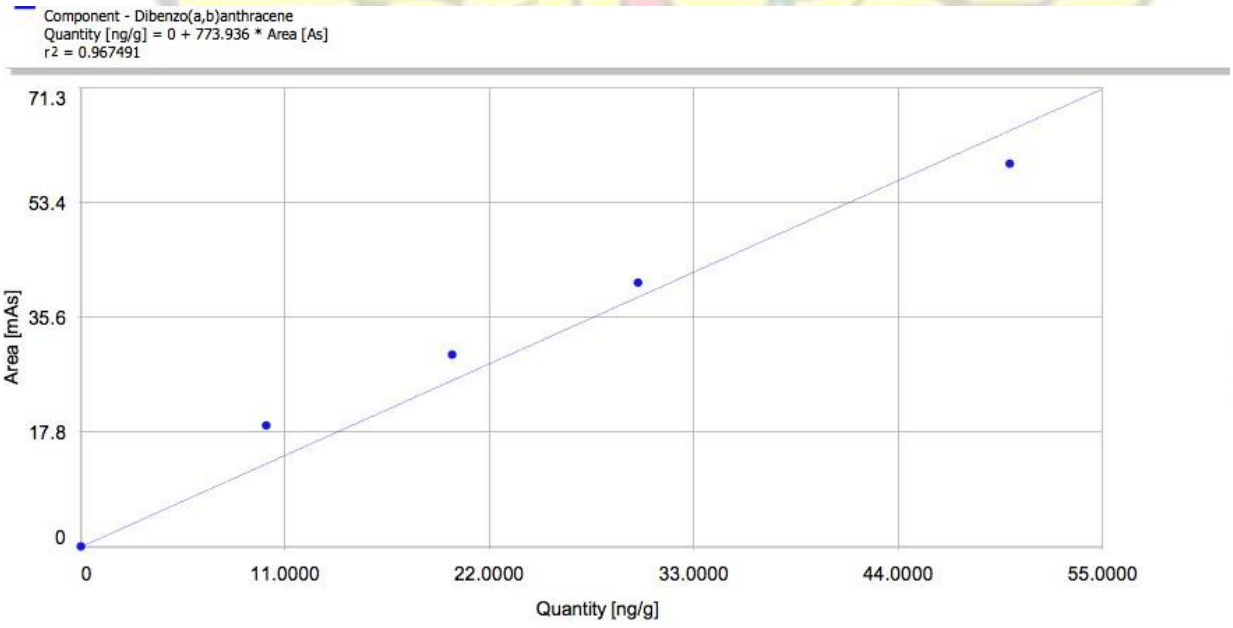
F. BENZ(A)PYRENE



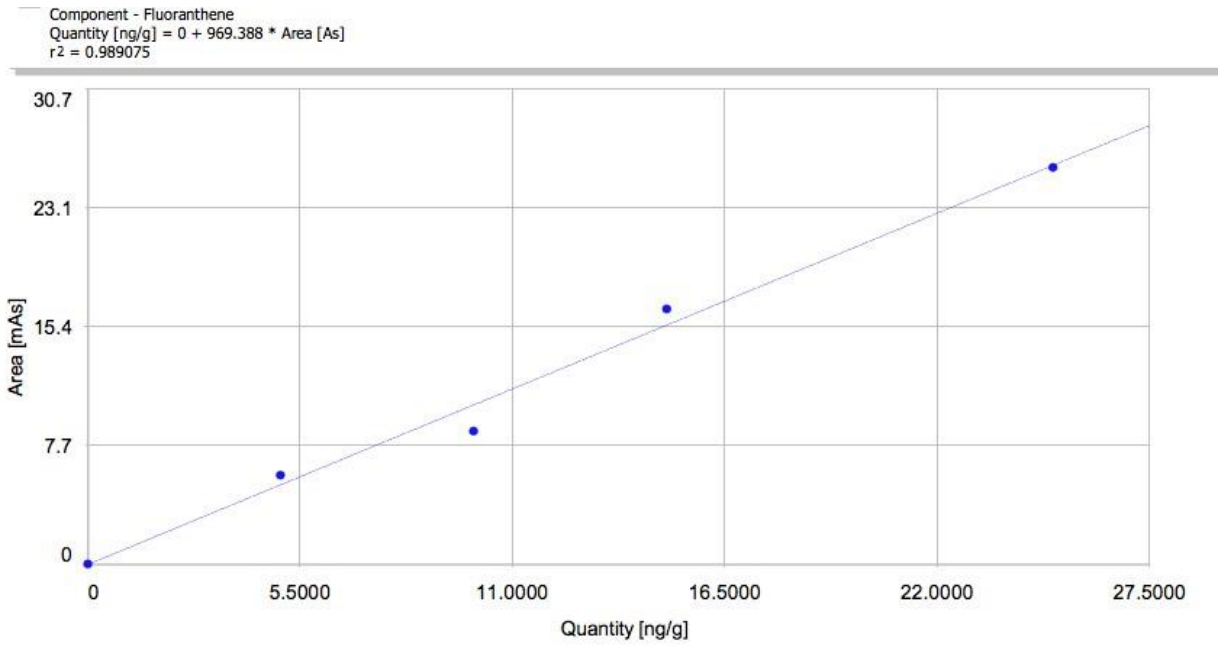
G. BENZ(K)FLUROANTHENE



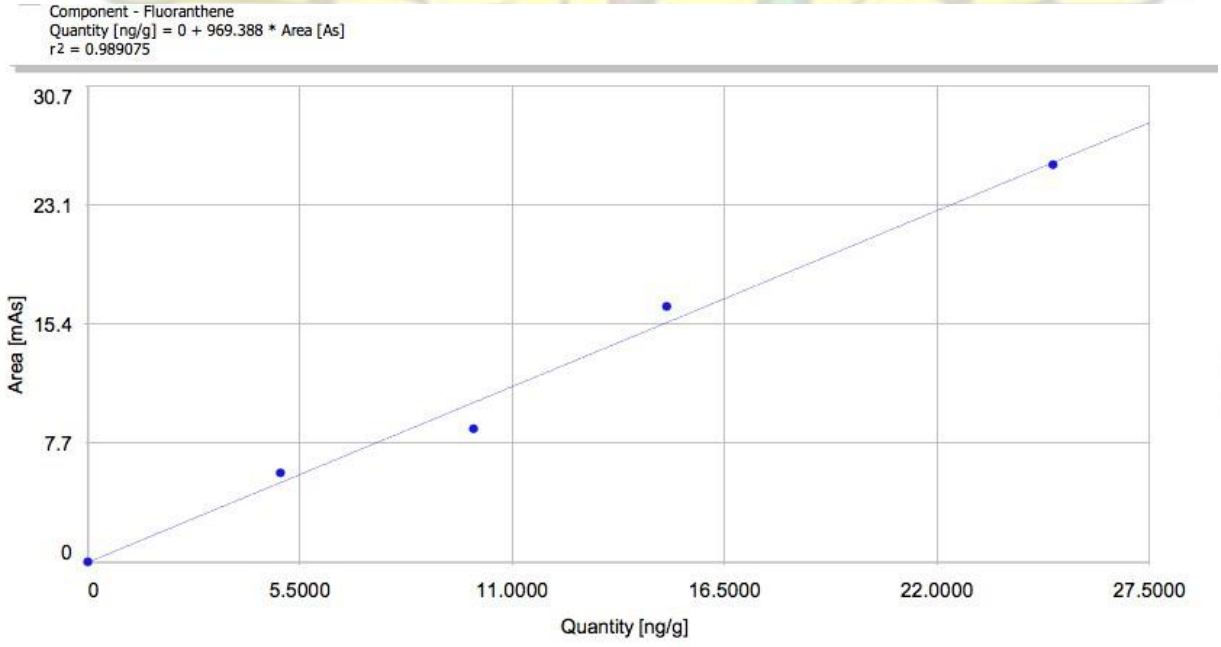
H. DIBENZ(A,B)ANTHRACENE



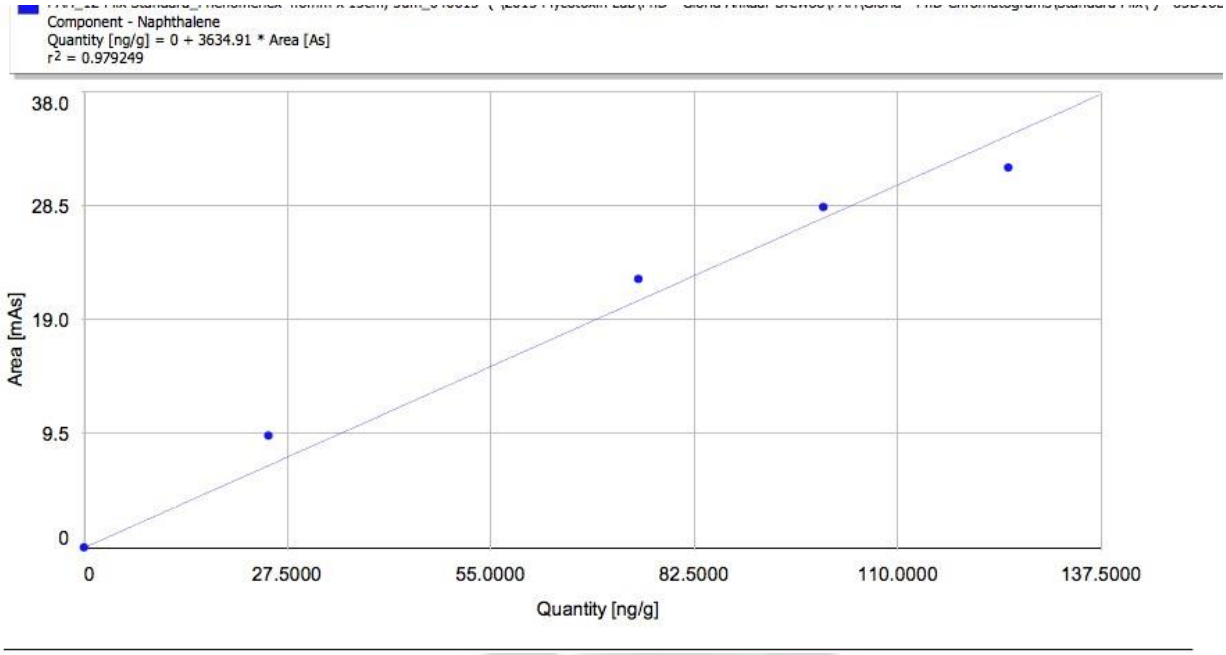
I. FLUORANTHENE



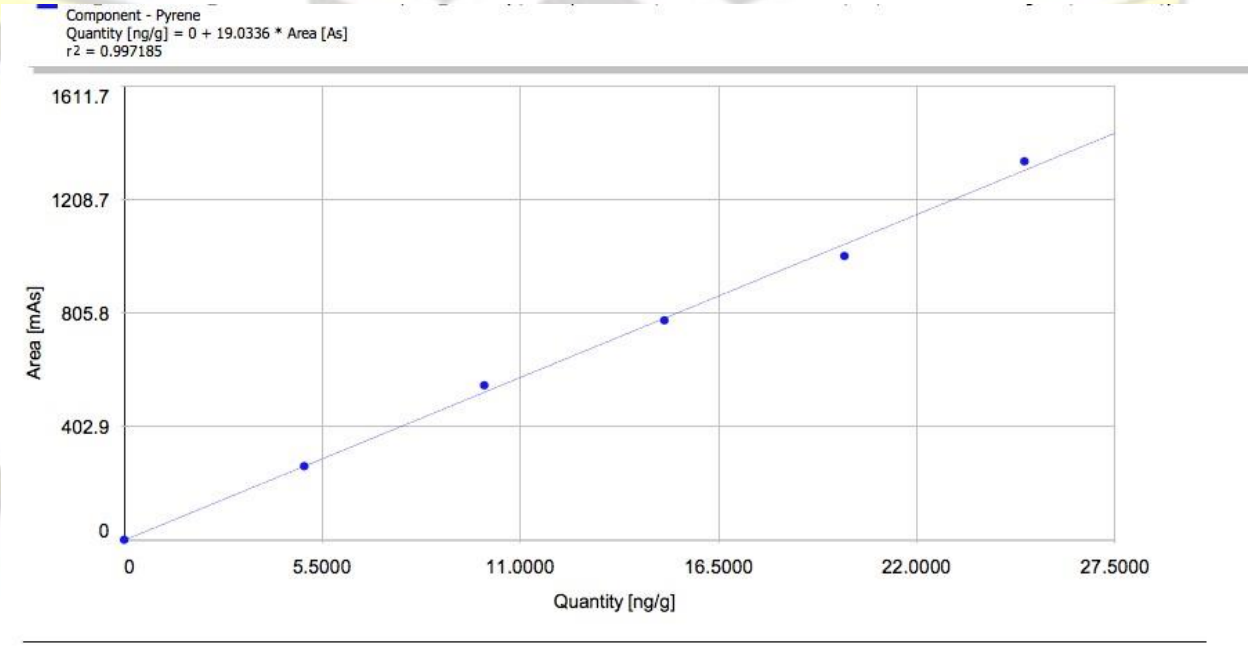
J. FLUORANTHENE



K. NAPTHALENE


Component - Naphthalene
Quantity [ng/g] = 0 + 3634.91 * Area [As]
r2 = 0.979249
38.0
28.5
19.0
9.5
0
Area [mAs]
0
27.5000
55.0000
82.5000
110.0000
137.5000
Quantity [ng/g]

L. PYRENE


Component - Pyrene
Quantity [ng/g] = 0 + 19.0336 * Area [As]
r2 = 0.997185
1611.7
1208.7
805.8
402.9
0
Area [mAs]
0
5.5000
11.0000
16.5000
22.0000
27.5000
Quantity [ng/g]