

ASSESSMENT OF PAEDIATRIC HEALTH RISK ASSOCIATED WITH TRACE
METALS AND POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)
CONTAMINATION IN CEREAL-BASED BABY FOODS FROM THE GREATER
ACCRA REGION

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BY

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DECLARATION

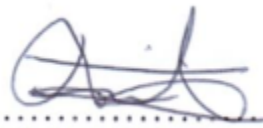
I, Domfeh Augustine, do hereby declare that, with the exception of well acknowledged references of other researchers` work, this thesis is in entirety mine under the supervision of Dr. Anita Asamoah and Dr. Alfred Kwablah Anim. This work has not been presented to any University elsewhere for a degree either in part or in whole.


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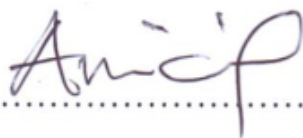
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DEDICATION

This thesis is dedicated to my dear sister, Lydia Fosua and my lovely mother, Madam Faustina Addai, for their immense support and words of encouragement. With a grateful heart, I say “Merci Beaucoup.”

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
BAPEQ	Benzo(a)Pyrene Equivalent
BDH	British Drug House
BDL	Below Detection Limit
BW	Body Weight
CDI	Chronic Daily Intake
EC	European Commission
ED	Exposure Duration
EF	Exposure Frequency
EFSA	European Food Safety Authority
EI	Electron Impact
EPA	Environmental Protection Agency
EU	European Union
FAAS	Flame Atomic Absorption Spectrometer
FAO	Food and Agriculture Organization
GAEC	Ghana Atomic Energy Commission
GC-FID	Gas Chromatography – Flame Ionization Detector
GC-HRMS	Gas Chromatography – High Resolution Mass Spectrometry
GC-MS	Gas Chromatography – Mass Spectrometry
GC-MS/MS	Gas Chromatography – Mass Spectrometry / Mass Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometer

GS	Ghana Standards
HDPE	High-Density Polyethylene
HI	Hazard Index
HMW	High Molecular Weights
HQ	Hazard Quotient
IARC	International Agency for Research On Cancer
ICP	Inductively Coupled Plasma
IR	Intake Rate
JECFA	Joint Expert Committee on Food Additives
KBTH	Korle Bu Teaching Hospital
LMW	Low Molecular Weights
LOD	Limit of Detection
LOQ	Limit of Quantification
MAE	Microwave Assisted Extraction
MEF	Mutagenic Equivalent Factors
MEQ _{BAP}	Benzo(a)Pyrene Mutagenic Equivalent
MMW	Medium Molecular Weight
NCERC	Nuclear Chemistry and Environmental Research Center
PAHs	Polycyclic Aromatic Hydrocarbons
PET	Polyethylene Terephthalate
PLE	Pressurized Liquid Extraction
PSA	Primary Secondary Amines
PTFE	Polytetrafluoroethylene

QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RfD	Oral Reference Dose
SCF	Scientific Committee of Food
SF	Slope Factor
SFE	Supercritical Fluid Extraction
SPE	Solid – Phase Extraction
TEF	Toxicity Equivalent Factors
TEQ _{BAP}	Benzo(a)Pyrene Carcinogenic Equivalent
TDS	Total Diet Study
UNICEF	United Nations International Children`s Emergency Fund
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organization
XRF	X – Ray Fluorescence

ABSTRACT

Dietary intake is a major route of human exposure to contaminants. Cereal-based baby foods, which are often used to complement breastfeeding can be contaminated with toxic compounds. Consequently, the health of babies can be compromised upon exposure to such contaminants depending on the concentrations and/or as a result of contaminant bioaccumulation even at trace levels. It should be expected that cereal-based baby food must be manufactured using high-quality raw materials and the best processing technology. However, this is not often the case as contaminants such as polycyclic aromatic hydrocarbons (PAHs) and trace metals (eg. cadmium and lead among others) can be introduced into baby foods due to poor processing protocols.

This work, therefore, sought to evaluate the presence of polycyclic aromatic hydrocarbons (PAHs) and trace metals in locally produced cereal-based baby foods and estimate their toxicological effect on babies. To achieve the objectives, 50 samples comprising of varying cereal compositions (maize, rice, millet, wheat) were sampled from sales outlets including major markets and hospitals in the Greater Accra region of Ghana. Samples were analyzed for metal content using atomic absorption spectrometry (AAS) following acid digestion (HNO_3 - H_2O_2). PAHs were extracted following the QuEChERS method and analysed using Gas Chromatography-Mass spectrometry (GC/MS).

The results showed contamination of both metals and PAHs in the samples. The concentrations of the metals across all the 50 samples were in a range: 0.87–34.74 mg/kg (Fe), <0.001–1.00 mg/kg (Zn), <0.003–0.92 mg/kg (Cu), <0.002–0.82 mg/kg (Cd), <0.001–0.26 mg/kg (Cr), <0.005–0.23 mg/kg (Co), <0.001–0.58 mg/kg (Pb), and <0.001–

0.20 mg/kg (Ni). The mean concentrations (mg/kg) of metals that were predominantly measured were: 8.60 (Fe), 0.06 (Pb), and 0.20 (Cd). The risk indices (HI) were in a range of 0.10–59.84, 0.13–84.53, and 0.20–123.69 for the ages 6–8 months, 9–11 months and 12–23 months respectively with cadmium as the main contributing factor to the risk index. This observation is indicative of potential child health risks.

Mean concentrations of Σ PAHs (15 PAHs), Σ PAH4 [benzo(a)anthracene, chrysene, benzo(a)pyrene, and benzo(b)fluoranthene)], and benzo(a)pyrene [BaP] were $276.7 \pm 256.2 \mu\text{g/kg}$, $16.0 \pm 32.2 \mu\text{g/kg}$, and $5.0 \pm 9.0 \mu\text{g/kg}$ respectively. Approximately 33.3 % of the samples analysed had concentrations of carcinogenic PAHs exceeding the FAO/WHO recommended limit of $1.0 \mu\text{g/kg}$. The calculated carcinogenic risks (R_{car}) for babies were in the ranges: 2.47×10^{-6} – 1.40×10^{-5} (6–8 months) , 3.32×10^{-6} – 1.88×10^{-5} (9–11months), and 5.10×10^{-6} – 2.88×10^{-5} (12–23 months) , while mutagenic risk (R_{mut}) were in the ranges: 2.47×10^{-6} – 2.05×10^{-5} , 3.32×10^{-6} – 2.75×10^{-5} and 5.10×10^{-6} – 4.23×10^{-5} for the ages 6–8 months, 9–11 months, and 12–23 months respectively.

The health risk assessment showed that the presence of Pb, Cd, and BaP in the samples could potentially cause both carcinogenic and non-carcinogenic risks to babies in the Greater Accra region if they should be exposed via consumption of cereal-based baby foods.

The outcome of such studies is necessary towards safeguarding public health, particularly babies.

CHAPTER ONE

INTRODUCTION

1.1 Background

Human populations can get exposed to contaminants depending on the quality of the food they eat. Adults, particularly breastfeeding mothers, can also transfer such contaminants to their babies. Babies, therefore, stand a higher risk of exposure to feeding-related contaminants since they take in more food and water than adults relative to their body size (Rintala et al., 2014; Suomi et al., 2017). To complement breastfeeding, babies depend mostly on cereal-based foods (Bonsi et al., 2014). However, cereal-based baby foods can be contaminated with potentially toxic compounds. The processing of cereal-based foods involves two main stages: roasting of the cereals at high temperatures and milling to obtain smaller or finer particles. These routes of processing can introduce contaminants such as trace metals and polycyclic aromatic hydrocarbons (PAHs) in cereal-based baby foods.

Aside the processing methods, the type and source of cereals used can also be a cause of concern as they can greatly contribute to the contamination of cereal-based baby foods. A variety of cereals including rice, wheat, maize, millet, oat, sorghum, and barley can be used in the manufacture of baby foods. These cereal grains are dried through various technologies. The cereal grains, either one variety or a combination of two or more are roasted at high temperatures in the range of 120 to 150 °C till they brown (Ocheme et al., 2014). The formulation of cereal-based baby foods usually has in addition plant protein such as soyabeans and groundnut. The only animal protein source which may be added to the formulation is milk.

It should be expected that cereal-based baby food must be manufactured using fresh and high-quality raw materials and with the best technology in a hygienic environment (Ciecierska and Obiedzin'ski, 2010). However, this is not often the case as contaminants can be introduced into baby foods due to poor processing protocols in addition to any contaminants in the raw materials.

In the commercial production of baby foods, most manufacturers use commercial milling machines. These milling machines, unfortunately, are potential sources of trace metals and other contaminants in baby foods. For example, it has been found by Adeti (2015) that the milling metal plates commonly used in Ghana in all the commercial milling machines have similar trace metals content but differs in terms of their concentrations. The metal contamination due to wearing and tearing of the milling plates was reported to decrease with duration of use, and notably, milling metal plates manufactured in Ghana were the highest source of metal contamination in the milled foods (Adeti, 2015). It is also worth noting that cereal grains can also be contaminated with trace metal at the point of cultivation (Kumar et al., 2019; Pirsahab et al., 2016). This contamination may arise from proximity to a mining site or polluted soil, urbanization, or unsafe irrigation water (Fageria et al., 2011). These trace metals are known to have serious health impacts when their concentrations exceed the regulated levels established by the Ghana FDA and FAO/WHO (Codex Alimentarius Commission, 2011; GS Codex Stan 193, 2019).

The degree and severity of the health impact of trace metal contaminations, of which babies are the most vulnerable, can be influenced by the type of heavy metal exposure, the concentration, and the level of exposure. Trace metals such as iron, zinc, copper, cobalt,

chromium, and nickel are essential elements in the human body that perform various biological functions when in their right proportion. (Codex Alimentarius Commission, 2011; Winters, 2011). The limited supply of these essential metals results in various deficiency-related diseases and conditions such as anemia, fatigue, poor growth, poor enzyme and metabolic performance, decrease immunity, etc. (Fowler, 2015; Owens, 2001; Reilly, 2002). Aside the great benefits, metals can pose serious harm to the body when the permissible limits are exceeded.

Health impacts of excess metal concentrations in the body include: high risk of atherosclerosis, arrhythmia, and increases the risk of liver, breast, gastrointestinal and hematologic cancers, heart disease, arthritis, osteoporosis, hair growth complications, and even death (Brizio et al., 2016; Codex Alimentarius Commission, 2011; Jurowski et al., 2019; Reilly, 2002). Some metals like cadmium (Cd) and lead (Pb) have no biological function in the human body and even at very low concentrations are known to be carcinogenic, which is associated with the decline in child intelligence and development, gastrointestinal toxicity, pulmonary toxicity, nephrotoxicity, and neurotoxicity (Alihttps et al., 2019; Codex Alimentarius Commission, 2011; Gardener et al., 2019; Khan et al., 2015; Kumar et al., 2019; Nasreddine et al., 2007; Suomi et al., 2018; Toxicological Factsheet Series, 2009).

Apart from metal contamination of baby cereal foods, polycyclic aromatic hydrocarbons (PAHs) have been identified with food processing methods such as smoking, frying, drying, roasting, and grilling (Essumang et al., 2012; Friedrich et al., 2017; Ledesma et al., 2016; Rose et al., 2015; Toxicological Factsheet Series, 2015). PAHs are known to be

environmentally persistent with various structures and varied toxicity through various actions (Aldrighi et al., 2018; Essumang et al., 2006; Singh et al., 2016). While smokers may be exposed directly to PAHs, the major route of exposure for non-smokers such as babies is through feeding. The contamination of food can be done via environmental sources (like soil, air, and water), industrial processing of food, and domestic cooking practices (Ciecierska and Obiedzin'ski, 2010). PAHs have been found in different foods such as vegetable oils, margarine, mayonnaise, dairy products, fruits, vegetables, smoked products, tea, yerba mate, coffee, sugar, cereal, water, seafood, grilled food, beverages, etc. (Ciecierska and Obiedzin'ski, 2010; Rose et al., 2015). Processed cereal-based foods can be a significant source of exposure to PAHs and more specifically to babies (Rozentale et al., 2017).

PAHs have been evaluated by international bodies such as the Scientific Committee on Food (SCF), the International Programme on Chemical Safety (IPCS), and the Joint Expert Committee on Food Additives (JECFA) of the FAO/WHO (JECFA, 2005; SCF 2002). Following such experimental observations, SCF classified 15 PAHs compounds as potentially genotoxic and carcinogenic to humans (Toxicological Factsheet Series, 2015). PAHs, therefore, represent a priority group in the assessment of the risk of long-term adverse health effects following dietary intake of PAHs. The IARC (2012) classification for carcinogenicity has grouped benzo[a]pyrene in food matrix as Group 1 (definite carcinogenic Group) whiles dibenzo(a, l)pyrene, dibenzo(a, h)anthracene, and cyclopenta(c, d)pyrene were grouped as Group 2A (probably carcinogenic Group) to human.

In this work, cereal-based baby food samples were sourced from major metropolitan and municipal trading outlets in Accra, the capital of Ghana to assess the levels of metals and PAHs contamination. The study area represents more than 50% of the entire population in the Greater Accra region (Ghana Statistical Services, 2019). The market systems operated in the Greater Accra region and the country as a whole, are the open-market system and regulated-market system with the majority of the population depending on the open-market system for most of their products. Hence, different types of baby foods including those of poor quality can easily find their way into the market without adequate checks. To achieve quality results for this work, robust analytical procedures were followed taking cognizance of quality assurance and quality control measures. The metal analysis was carried out using atomic absorption spectrometry following acid digestion of the powdered samples (Brizio et al., 2016; Das, 2016; Jurowski et al., 2019; Winters, 2011). Samples were analysed for PAHs using triple quadruple Gas Chromatography-Mass Spectrometry (GC-MS) after extraction following the QuEChERS method (Aldrighi et al., 2018; Escarrone et al., 2014; Urkude & Dhurvey, 2015).

Although PAHs and trace metals contamination of food can pose health implications, most of the baby foods found on the Ghanaian market, for instance, do not have the Food and Drugs Authority (FDA) approved certification and other essential product information on their labels. The lack of such vital label information makes it difficult for nursing mothers and the general public who patronize these products to ascertain the quality of cereal-based baby foods purchased in the market to feed their innocent and vulnerable babies. It is therefore very prudent to evaluate the quality of locally prepared cereal-based baby foods on the Ghanaian markets and particularly assess any potential health risks associated with

the consumption of these products. Such studies are necessary to assure the public of food safety and also provide vital inputs towards the revision or stipulating of food safety guidelines.

1.2 Problem Statement

There is little monitoring of cereal-based baby foods manufactured locally on the Ghanaian markets, with most of the products without Food and Drugs Authority approved certification. Hence producers with little or no knowledge of best practices for preparing baby food can still get their products on the open market.

PAHs, a potential carcinogen is associated with some food processing methods and cooking practices, of which cereal-based baby foods are inclusive (Ciecierska and Obiedzin'ski, 2010; Rozentale et al., 2017). Some of the food processing methods that could introduce PAHs into baby's foods are smoking, roasting, grilling, baking, frying, and drying (Essumang et al., 2014; Friedrich et al., 2017; Ledesma et al., 2016; Rozentale et al., 2017). There is, therefore, the potential of exposing babies to dietary PAHs.

Apart from PAHs, trace metals could also be introduced into food, particularly during the milling stage of food processing (Adeti, 2015). In the commercial production of cereal-based baby foods, producers mostly use the commercial milling machines because of the stress in using the blender for large productions. This practice is a likely source of trace metals contamination of the food through wear and tear of the milling plates (Adeti, 2015). Apart from the milling, there is also a natural source of trace metal concentration of the

cereals through cultivation (Doamekpor et al., 2018; Kumar et al., 2019; Reilly, 2002). Trace metals may pose a health threat with several health implications, especially to babies when their concentrations exceed permissible thresholds (Codex Alimentarius Commission, 2011; Reilly, 2002; Suomi et al., 2018; Suomi et al., 2017).

Since PAHs and some trace metals can be potentially toxic and cause health implications, of which babies are the most vulnerable. Hence it is worth assessing the levels of these contaminants in baby foods on the Ghanaian market.

1.3 Objectives of Study

1.3.1 Overall Objective

To evaluate the presence of polycyclic aromatic hydrocarbons (PAHs) and trace metals in locally produced cereal-based baby foods; and estimate their toxicological effect on babies.

1.3.2 Specific Objectives

- (i)** To assess the levels of Polycyclic Aromatic Hydrocarbons (PAHs) in baby foods,
- (ii)** To evaluate the concentrations of trace metals present in baby foods,
- (iii)** To ascertain if the levels of the PAHs and trace metals in cereal-based baby food pose health risks to babies.

1.4 Relevance and Justification

Food processing could be a major route of PAHs and trace metals contamination in cereal-based baby foods. For example, food processing procedures using the conventional methods of smoking, roasting, grilling, baking, frying, and drying have been recognized as major sources of PAHs contamination in food (Essumang et al., 2014; Friedrich et al., 2017; Ledesma et al., 2016; Rozentale et al., 2017). Several PAHs are genotoxic and carcinogenic even at low concentration (Aldrighi et al., 2018; Essumang et al., 2010; Ledesma et al., 2016; Palm et al., 2011; Sen & Field, 2013; Toxicological Factsheet Series, 2015).

Similarly, trace metals can also be introduced into cereal-based baby foods through processing methods including milling (Adeti, 2015; Fageria et al., 2011; Kumar et al., 2019; Pirsahab et al., 2016). Apart from processing methods, cereals can also be contaminated with trace metals during the cultivation stages, particularly when farm soils have already been contaminated (eg. near mining areas) or through the use of contaminated irrigation water. Metal contamination in foods can pose health problems even at trace concentrations due to bioaccumulation. For instance, while Pb and Cd have been found to have high-risk factors ranging from mild to severe toxicological effect including mental retardation, carcinogenicity (Islam et al., 2018; Rintala et al., 2014; Stroheker et al., 2019; Suomi et al., 2018), some essential metals (eg. iron, zinc, and copper) can also pose health implications when present in foods at concentrations exceeding their permissible limits (Chao et al., 2014; Fowler, 2015).

In Ghana, babies are be introduced to cereal-based foods in their early stages of life to complement breastfeeding (Bonsi et al., 2014). Since babies take in more food than adults relative to their body size, even trace amounts of PAHs and/or metals contamination in their diets can result in potential health implications (Pelto et al., 2011; Stroheker et al., 2019).

Due to the open market system operated in Accra (the capital city of Ghana), it is likely to have unwholesome cereal-based baby foods infiltrating the markets. It is therefore imperative to assess concentrations of PAHs and trace metals in cereal-based baby foods and also evaluate any associated health implications.

1.5 Organization of Thesis

This thesis consists of five chapters. A summary of each chapter is provided as follows:

- I. Chapter 1 provides background information and highlights the motivation for this work. It also outlines the aims and objectives.
- II. Chapter 2 presents a review of the current literature regarding PAHs and metal contamination in baby food. The chapter further describes the toxicological effects of these trace metals and PAHs on humans when exposed to which babies are the most vulnerable.
- III. Chapter 3 presents a detailed description of the analytical procedures carried out in this work, taken cognizance of quality assurance and quality control measures. A brief description of the demography of the research area with a detailed mapping of the sampling area is also provided.

- IV. Chapter 4 presents a discussion of the experimental results. Metals and PAHs contamination in the variety of cereal-based baby foods were thoroughly discussed with reference to the objectives of this study. Health risks assessment due to exposure to metals and PAHs is also detailed.
- V. Chapter 5 gives a summary of the thesis as well as provides direction for future works. The appendix contains a full description of all the benchmark methodologies, calculations, and raw data.

CHAPTER TWO

LITERATURE REVIEW

2.1 Baby Foods

Babies obtain most of their nutrition from the food they eat. A well-balanced diet provides babies with nutrition such as proteins, carbohydrates, fats and oils, vitamins, and mineral salts which help in building them up in their growth and development, and resistance to diseases (Nicklas et al., 2020; WHO, 2019). Exclusive breastfeeding (first six months) for babies as recommended by the United Nations International Children's Emergency Fund (UNICEF) and the World Health Organization (WHO) has been established to provide all the nutritional needs to babies (Davis et al., 2003; Paiva et al., 2019). Subsequently, the growth of the infant is considerably increased in size and weight demands for additional appropriate food to complement the increasing energy requirements for further growth. Baby foods serve as the source of all their nutritional needs, provide high immunity to diseases, and maintain adequate growth in babies (Jurowski et al., 2019). There is therefore the need to introduce them to other forms of foods including cereal-based foods to complement breastfeeding. Some babies are introduced to these cereal-based foods before they are six months old. This is because most mothers are in the working class and may not be available to breastfeed their babies. Also, diseases, allergies, and/or dietary problems are contributing factors (Jurowski et al., 2019).

Baby foods come in different forms including infant formulae and cereal-based baby foods. The cereal-based foods are prepared from the different varieties of cereals including maize, rice, wheat, millet, sorghum, oats, and barley (Sopade, 2017). They are prepared with one

variety of cereals or a mixture of two or more cereals. They are mostly nourished with plant proteins such as soyabeans and groundnut. There are a few occasions when they are nourished with skimmed milk (Bahwere et al., 2016). They are mostly served with or without milk after cooking. Their nutritional value mostly depends on the types of raw materials used. There are no preservatives, flavoring, and coloring additives added. Because of the roasting, the food then assumes a light brown to deep brown coloring depending on the starting materials, the temperature, and the duration of roasting.

Baby foods are expected to be prepared from fresh and high-quality raw materials devoid of contaminations, prepared under hygienic conditions, and best cooking practices are to be employed to limit contaminations (Iwegbue et al., 2010; Ciecierska and Obiedzinski, 2010). Unfortunately, that is not the case, baby foods contamination may result from the raw materials used, through to the processing and final state (Adeti, 2015; Loutfy et al., 2012). There are various stages in which contamination may be introduced to food. They include: cultivation, preparation, and storage (Palm et al., 2011; Rose et al., 2015; Soceanu et al., 2016; Wang et al., 2017). Several contaminants such as heavy metals, trace metals overload, polycyclic aromatic hydrocarbons, pesticide residues, aflatoxins, and microbial infections can be associated with baby foods (Ghuniem et al., 2020; Kortei et al., 2019; Loutfy et al., 2012).

2.2 Polycyclic Aromatic Hydrocarbons

2.2.1 Characteristics of Polycyclic Aromatic Hydrocarbons

Polycyclic Aromatic Hydrocarbons (PAHs) are a large complex group of organic compounds characterized by the presence of two or more aromatic rings fused in a linear, cluster, or angular arrangement (Rozentale et al., 2017; White et al., 2004). PAHs are ubiquitous environmental contaminants that are of special importance due to their potential toxicity, carcinogenicity, and mutagenicity to humans (Toxicological Factsheet Series, 2015; White et al., 2004).

PAHs in general are not chemically synthesized for industrial purposes. However, few ones are produced for research purposes and as intermediaries in the pharmaceutical, agricultural, and thermosetting plastics chemical industrials (Abdel-shafy & Mansour, 2016). PAHs such as acenaphthene, anthracene, fluoranthene, fluorene, and pyrene were used for wood preservation, dyes, and pigments, pesticides, plastics, and resins (Abdel-shafy & Mansour, 2016).

Polycyclic aromatic hydrocarbons are mostly colorless and can be white, or pale yellow solids (Abdel-shafy & Mansour, 2016). PAHs are generally characterized by high melting and boiling points (Toxicological Factsheet Series, 2015). The low vapor pressure and very low aqueous solubility of PAHs decrease greatly for each additional ring or increase in molecular weight (Abdel-shafy & Mansour, 2016; Satyaprakash & Padal, 2017). However, their resistance to oxidation and reduction rather increases when molecular weight increases. Although, PAHs are non-polar and generally insoluble in water, some few ones with lower molecular weight have been found to dissolve in water. PAHs are very soluble

in organic solvents (non-polar) because of their high lipophilicity and hence have a high potential for biomagnification through trophic transfers (Satyaprakash & Padal, 2017). PAHs are also described by their semi volatility, persistence, and bioaccumulation effects in the environment (Palm et al., 2011).

PAHs also have characteristics including conductivity, light sensitivity, emit ability, heat resistance, corrosion resistance, and physiological action (Abdel-shafy & Mansour, 2016). PAHs possess unique ultraviolet (UV) absorbance spectra and fluorescence properties for each PAHs ring structure which are very useful for the identification of individual PAHs. (Abdel-shafy & Mansour, 2016).

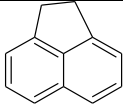
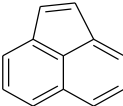
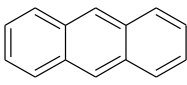
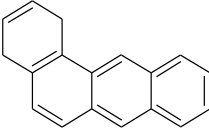
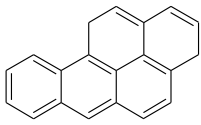
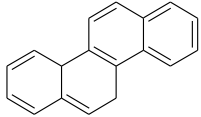
2.2.2 Types and Categories of PAHs

PAHs have been grouped into three major categories based on their molecular weights. Members of each category have a similar fate in the environment. The categories are Low Molecular Weights (LMW) PAHs, Medium Molecular Weight (MMW) PAHs, and High Molecular Weights (HMW) PAHs (Palm et al., 2011). The LMW PAHs are those PAHs containing two or three rings with a molecular weight ranging from 128 to 178 g/mol. The MMW PAHs have four rings and are those with a molecular weight of around 202 g/mol. The HMW PAHs are those that usually contain from 5 – 7 rings or more and have weights ranging from 228 to 278 g/mol (Palm et al., 2011).

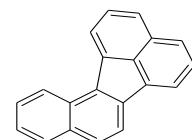
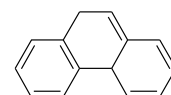
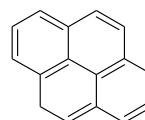
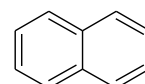
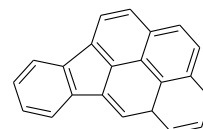
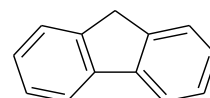
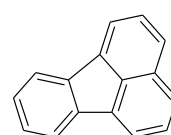
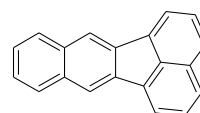
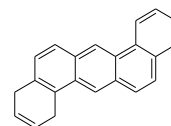
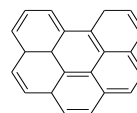
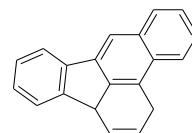
PAHs are a large group of environmental contaminants which include several hundred individual compounds found and characterized, yet few are of interest for scientific studies (Tsibart & Gennadiev, 2014). Out of these numerous PAHs, 16 of them were classified as

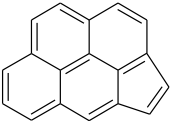
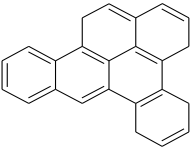
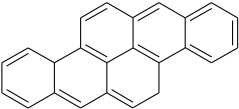
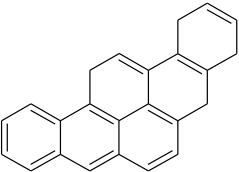
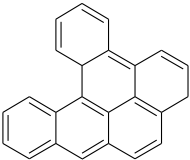
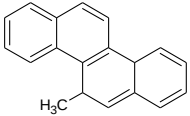
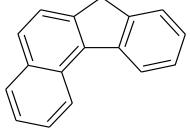
pollutants by the United States Environmental Protection Agency (USEPA) as priority pollutants or contaminants due to their level of toxicity (Satyaprakash & Padal, 2017). The European Scientific Committee of Food (SCF) assessed the risk posed by 33 PAHs and concluded that 15 PAHs have clear genotoxicity and are carcinogenic to animals (Toxicological Factsheet Series, 2015). The common PAHs of concern by the EPA, SCF, and EU together with their toxicity classification by IARC (Table 2.1).

Table 2.1 Names of common PAHs with their IARC toxicity classification

Common PAHs	Abb.	Recommen dation Body	Molar mass	*IARC Toxicity classification	Structure
Acenaphthene	ACP	EPA	154	3	
Acenaphthylene	ACY	EPA	152	NA	
Anthracene	ANT	EPA	178	3	
Benz[a]anthracene	BAA	EPA, SCF, EU	228	2B	
Benzo[a]pyrene	BAP	EPA, SCF, EU	252	1	
Chrysene	CHR	EPA, SCF, EU	228	2B	

Benzo[b]fluoranthene	BBF	EPA, SCF, EU	252	2B
Benzo[g,h,i]perylene	B(ghi)P	EPA, SCF, EU	276	3
Dibenzo[a,h]anthracene	DB(ah)A	EPA, SCF, EU	278	2A
Benzo[k]fluoranthene	BKF	EPA, SCF, EU	252	2B
Fluoranthene	FA	EPA	202	3
Fluorene	FLR	EPA	166	3
Indeno[1,2,3-c,d]pyrene	IP	EPA, SCF, EU	276	2B
Naphthalene	NPH	EPA	128	2B
Pyrene	PYR	EPA	202	3
Phenanthrene	PHE	EPA	178	3
Benzo[j]fluoranthene	BJF	SCF, EU	252	2B



Cyclopenta[cd]pyrene	CPcdP	SCF, EU	226	3	
Dibenzo[a,e]pyrene	DBaeP	EU+ SCF	302	3	
Dibenzo[a,h]pyrene	DBahP	EU+ SCF	302	2B	
Dibenzo[a,i]pyrene	DBaiP	EU+ SCF	302	2B	
Dibenzo[a,l]pyrene	DBalP	EU+ SCF	302	2A	
5-methyl chrysene	MCH	EU+ SCF	242	2B	
Benzo[c]fluorine		EU	216	3	

*IARC (International Agency for Research on Cancer): Group 1 = carcinogenic to humans, Group 2A = probably carcinogenic to humans, Group 2B = possibly carcinogenic to humans, Group 3 = Not Classifiable as to carcinogenicity to humans.

2.2.3 Formation of PAHs in food

Incomplete combustion or pyrolysis of the organic substance in food substance or the fuel, together with other factors contribute to the formation of PAHs (Ledesma et al., 2016). Combustion or Pyrolysis of organic substances produces a large variety of compounds aside PAHs. These significant contaminants are formed during thermal food processes such as roasting, drying, frying, smoking, and grilling (Aldrighi et al., 2017; Bansal et al., 2017; Ciecierska and Obiedzin'ski, 2010; Ledesma et al., 2016; Palm et al., 2011; Rose et al., 2015).

The PAHs formation is known to occur at temperatures above 200 °C and is highly stimulated at higher temperatures over 700 °C (Esumang et al., 2012). However, Friedrich et al. (2017) recorded values of PAHs in corn grains dried at 60 and 80 °C and reported that probably the high values could be due to the longer time of drying. Aldrighi et al. (2018) also studied rice subjected to drying by different fuels at different temperatures of 40, 60, 80, and 100 °C. He reported that the lowest contamination of PAHs at 40 °C is due to the dilution of the combustion air with ambient air to reach the desired temperature and the time of exposition of the samples (Aldrighi et al., 2018). These studies imply that temperature alone is not the determining factor in the formation of PAHs, and hence other factors such as the type of fuel and duration of heat exposure, can cause PAHs to be formed at lower temperatures.

The concentration of PAH also depends on a complex interaction of factors including the type of fuel, the flame temperature, the oxygen concentration, the type of burner, cooking method, the time of grain exposure to the heated air, the physical state of the fuel (solid, liquid, or gas), combustion efficiency, and type of grain. This was affirmed by Aldrighi et al. (2018) when they reported that the total PAHs content varied with the

heating source: wood (highest), liquefied petroleum gas (LPG), and electric heating. Studies on PAHs have been associated with some cooking practices such as grilling, steaming, smoking, roasting, and liquid smoke flavoring (Ciecierska and Obiedzin'ski, 2010; Rose et al., 2015). An increase in roasting time is relative to PAH formation and roasting induced more PAH formation (Rose et al., 2015). USEPA (2008) also reported that these factors: burner design, operating parameters, and insufficient ventilation, can influence the production of PAHs.

PAHs are produced in the environment as a result of various natural and anthropogenic processes (Rozentale et al., 2016; Ledesma et al., 2015). Naturally, PAHs are formed by processes such as carbonization. Examples of natural sources of PAHs are forest fires, volcanic eruptions, petroleum seeps, post-depositional changes of biogenic precursors, and also biosynthesis origin of some plants and fungi. Anthropogenic activities are, however, considered as the major sources of PAHs in the environment. The petrogenic and pyrolytic sources are considered to be the two main anthropogenic sources that introduce PAHs in the environment (Abdel-shafy & Mansour, 2016; Rozentale et al., 2017). Pyrogenic is the incomplete combustion of coal, wood, oil, garbage, or other organic substances from food and mostly formed at high temperatures above 350 °C (Abdel-shafy & Mansour, 2016; Manzetti, 2013). Examples include exhaust from automotive sources, industrial plants, domestic heating with open fire, cooking, etc. (Satyaprakash & Padal, 2017). The petrogenic process is associated with the contamination from petroleum products or organic substances including food at low temperatures (Manzetti, 2013). Examples of anthropogenic sources that may be pointed out include the combustion of fossil fuels, leaking of petroleum and derivatives,

incineration of waste, production of coke, pitch, asphalt, cracking of petroleum, and industrial effluents (Satyaprakash & Padal, 2017).

PAHs are found in every part of the environment such as air, soil, water, and vegetative structures (Aldrighi et al., 2018). They are found in soil in varying depths including farming soils adsorbed to the soil particles (Tsibart & Gennadiev, 2014). PAHs have been found in water sediments and aquatic organisms including the fishes and Mollusca (Alexander et al., 2008; Bansal & Kim, 2015; Essumang et al., 2012). PAHs have been found in the air, especially due to high urbanization and industrialization growth areas (Essumang et al., 2006). PAHs have been associated with plants' vegetative structures: stems, roots, and leaves (Aldrighi et al., 2018).

2.2.4 Exposure of PAHs to Babies

For humans, the two major modes of exposure are smoking and diet (Rose et al., 2015; Santonicola et al., 2017). While for non-smokers, such as babies, the major route of exposure is in food consumption. Smokers may receive a level of exposure resulting from inhalation of smoke similar to average dietary intake (Alexander et al., 2008; Rose et al., 2015; Santonicola et al., 2017). Although air and drinking water may be responsible for some human exposure, babies can also be exposed to PAHs through breastfeeding. There are reports on PAHs contamination in human breast milk (Asamoah et al., 2019). The highest PAHs intake is associated with occurrence in diet (Palms et al., 2011; Ciecierska and Obiedzin'ski, 2010). This makes PAHs analysis in food a major course for concern especially for babies since it may be the only main route of their exposure.

The presence of PAHs in food, whether industrialized or not, is mainly due to their formation during certain types of heat processing and direct drying with wood, air, and water pollution and its presence in terrestrial and marine soils (Froehner et al., 2018; Ledesma et al., 2016). Substantial quantities of PAHs in food are due to food processing and cooking techniques, preservation, and storage including barbecuing, roasting, drying, refining, smoking, frying, and grilling (Ciecierska and Obiedzin'ski, 2010; Janska et al., 2006; Ledesma et al., 2016; Palm et al., 2011; Rose et al., 2015). These food processing steps are known to generate and increase the level of PAHs in the food. Thus, PAHs are found in a wide range of food matrices: vegetable oils, margarine, mayonnaise, dairy products, fruits, vegetables, smoked products, tea, coffee, sugar, cereal, water, seafood, grilled food, beverages, etc. (Aldrighi et al., 2018; Ledesma et al., 2016; Palm et al., 2011).

Considering consumers' exposure to PAHs, oils, and fats, cereals, some vegetables, and fruits were identified as major sources of PAHs in diets (White et al., 2004). A study by Rozentale et al. (2017) shows that processed cereal-based foods constitute one of the major contributing sources of PAHs exposure. Generally, smoked foods contain a large amount of PAHs in them while processed cereal-based foods are usually characterized by low levels of PAHs contamination (Mohammadi et al., 2013). Nevertheless, the high consumption rate of these processed cereal-based foods makes them a very significant dietary source of PAHs.

Babies are introduced to cereal-based foods in the early stages of their life to complement breastfeeding (Davis et al., 2003). They consume a lot of the different varieties of cereals based foods. These cereal-based foods are mainly cooked in the form of porridge and they come in the form of commercial instant cereals, home-

processed cereals, enriched cereals/ weanimix purchased from health centers or market areas (Davis et al., 2003). Therefore babies stand a greater chance of exposure from cereal-based foods when there is small contamination in terms of heavy metals, trace metals overload, or polycyclic aromatic hydrocarbons.

2.2.5 Previous Studies on PAHs contamination in baby foods

Babies' nutrition has been of major concern due to the severity of the effect of baby food contamination on babies (<https://www.who.int/health-topics/infant-nutrition>). Currently, there is an increasing number of research works on PAHs in baby foods. Several PAHs have been identified and quantified in baby foods in other countries and devastating effects of PAHs in baby's foods and even in very low concentration calls for more concern. Though cereal-based foods form the major food component introduced to babies after their 6 months of exclusive breastfeeding, yet there are limited research works done on cereal-based baby foods in Ghana. Table 2.2 shows a summary of PAHs range recorded in baby food.

Table 2.2 Data on PAHs in Cereal-Based Baby Foods

Matrix	Country	PAHs range $\mu\text{g/kg}$	Reference
Cereals	Latvia	0.22–1.62	Rozentale et al. (2017)
Baby foods	Poland	0.28–7.45	Ciecierska and Obiedzin'ski (2010)
Rice grains	Brazil	1.0–5.0	Escarrone et al. (2014)
Corn grains	Brazil	1.0–6.8	Friedrich et al. (2017)
Baby food (meat / fish-based)	Italy	11.82	Santonicola et al. (2017)
Baby foods	Romania	2.52–6.7	Soceanu et al. (2016)
Infant formulae and follow-up formulae	Nigeria	0.102–1.98 (0-6months) 0.054–1.98 (6-12 months) 0.081–2.54 (1-3 years) 0.51–0.70 (0-12 months)	Iwegbue et al. (2014)

2.2.6 Toxicity of PAHs to babies.

Regarding toxicity, PAHs have been identified and quantified in virtually all segments of the environment. Studies on individual PAHs in animals have shown various toxicological effects such as carcinogenicity, genotoxicity, mutagenicity, cytotoxicity, haematotoxicity, immunotoxicity, reproductive and developmental toxicity even at very low concentrations (Ciecierska and Obiedzin'ski, 2010; Essumang et al., 2010; Friedrich et al., 2017; Palm et al., 2011; Sen & Field, 2013). The PAHs may also induce growth inhibition, degradation of the lymphatic system, and interference in hematopoietic functions (Masood et al., 2017).

PAHs carcinogenicity, mutagenicity, and bioaccumulative effects have been reported by the FAO/WHO, IARC, USEPA, the European SCF, and the European Food Safety Authority (EFSA) and (EFSA 2009; IARC 2012; Lerda, 2011; SCF 2002; Toxicological Factsheet Series, 2015). Accordance to the current classification on the carcinogenicity of PAHs by the IARC monograph, it has been established that benzo[a]pyrene is in Group 1 (a definite carcinogenic group) and dibenz[a,h]anthracene in Group 2A (probably carcinogenic group), whereas a number of them are in Group 2B (possible human carcinogens group) as shown in Table 2.1 (Essumang et al., 2012; Lerda, 2011; Sen & Field, 2013).

2.2.7 Regulations on PAHs Contamination in Foods

PAHs have been of major scientific concern due to their toxicity since the 1970s. There have been several regulations by various international bodies in charge of food safety on PAHs in food and the environment as a whole. These regulations have undergone various changes as scientific studies on PAHs increases with increasing knowledge on the dangers of PAHs toxicity. These regulatory bodies include the USEPA, FAO/WHO, ESFA, JECFA, and FDA in Ghana (EFSA 2009; IARC 2012; Lerda, 2011; SCF 2002; Toxicological Factsheet Series, 2015).

The European Commission amended regulation (EC) 466 (2001) to regulation (EC) No 1881 (2006) to set maximum limits for PAHs in key foodstuffs, including baby's dietary exposure. For example, the maximum limit of 1.0 µg/kg was stipulated for benzo(a)pyrene in for foodstuffs such as dietary foods for infants with specific medical needs, processed cereal-based foods and baby foods for infants and young children,

infant formulae and follow-on formulae (European Commission Regulation No 1881, 2006).

Based on the EFSA opinion, the benzo[a]pyrene alone was not good enough as a marker for PAHs. In 2011 the European Commission extended the scope of the regulation to include other types of foods as well as limits for PAH4 (Codex Alimentarius Commission, 2011; Mohammadi et al., 2013). Accordingly, the maximum limits set for benzo[a]pyrene (BaP) and the sum of benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, and chrysene (PAH4) were both 1.0 µg/kg for processed cereal-based foods, baby foods, and foods for infants, young children, and pregnant women (Aldrighi et al., 2017; European Commission Regulation No 1881, 2006; Rozentale et al., 2017). The latest European legislation on PAHs in foods was introduced in 2015 and the same maximum limit for processed cereal-based foods and baby foods for infants and young children was maintained at 1.0 µg/Kg for benzo[a]pyrene and sum of PAH4 (Rozentale et al., 2017).

In Ghana, even though PAHs are potentially harmful to the human health of which babies are the most vulnerable, there is no specific legislation by Food and Drug Authority on the subject and hence there are no upper boundary limits set for foods including baby's foods. However, the recommended maximum limit set by the European Commission for PAHs in foods and baby foods is considered applicable. A document available to Ghana Standard Authority (GSA) has recommended for the establishment of a monitoring system for PAHs and recommended protocols to reduce PAH levels in food to acceptable limits (GS 1131, 2017). In Ghana, PAHs concentration in cereal-based baby food, infant formulae, and follow-on formulae are not available, though there are several studies of PAHS on breast milk, smoked fish,

smoked meat, traditional kitchen smoke, ambient air, vehicle exhaust, generator, industrial chimneys and many more (Asamoah et al., 2019; Essumang et al., 2012; Essumang et al., 2014; Essumang et al., 2010; Essumang et al., 2006; Palm et al., 2011). It is therefore prudent for this study to be carried out to identify and quantify the level of PAHs concentration in cereal-based baby food in the Ghanaian market.

2.3 Trace Metals

2.3.1 Trace Metals Contamination in Baby Foods

Assessment of trace metals in baby foods has been of major concern. Babies grow and develop very rapidly in their early years of life, their energy requirements and food consumption rate are much higher relative to their body weight than adults and children (Pandelova et al., 2012; Saracoglu et al., 2007). Hence metal contamination in their meals has a very devastating effect on their development. The common metals found in baby foods include iron, zinc, copper, magnesium, calcium, selenium, chromium, lead, and cadmium (Pandelova et al., 2012). This work is targeted to work on six (6) trace essential metals and two (2) hazardous trace metals as discussed in detail below. Table 2.3 shows research articles on baby foods.

Table 2.3. Recent Research Articles on Baby Foods

Matrix	Metals	Country	Reference
Imported infant cereals	Al, Br, Ca, Cl, K, Mg, Na	Ghana	Ayivor et al. (2011)
Baby foods .	Pb, Cd	USA	Gardener et al. (2019)
Baby foods	Reviewed paper	Brazil	Paiva et al. (2019)
Baby foods	Cd, Pb, Hg, Mn, Zn, Cu, Fe, Ca, Ni, Se	Germany (European market)	Pandelova et al. (2012)
Cereals	Al, Cd, Cu, Zn, Ni, Pb	Italy	Brizio et al. (2016)
Baby foods: special medical purpose and modified milk products	Cu, Zn, Mn	Poland	Jurowski et al. (2019)
Baby foods and meat	Pb, Cd, Zn, Co, Cu, Fe, Cr	Turkey	Das (2016)
Baby foods	Cu, Mn, Fe, Zn, Se, Ni, Cr, Al, Co	Turkey	Saracoglu et al. (2007)
Children and Baby foods	As, Cd, Hg, Pb	Finland	Suomi et al. (2017)
Baby foods	Environmental impact on babies	UK	Sieti et al. (2019)
Cereals	Pb, Cd, Cr, Ni, Cu, Zn	Iran	Pirsaheb et al. (2016)

2.3.2 Toxicity of Lead

Lead is a naturally occurring element found in small amounts in the earth's crust (WHO, 2015). Through human activities such as mining, burning of fossil fuel, and industries,

the level of lead has been enhanced in every part of the environment including water, air, soil, and foods (Reilly, 2002). The non-biodegradable nature of lead is the reason for its prolonged persistence in the environment.

Lead has a wide range of applications including processes and materials such as metal products, leaded fuel, batteries, paints, toys, etc. (Codex Alimentarius Commission., 2011; WHO, 2015). The use of lead has resulted in the prevalence of lead as an environmental pollutant and human toxin. The use of lead has come under scrutiny in the past decades leading to interventions that prevent and minimize its uses due to its high toxicity.

The level of lead has been elevated and hence lead contamination in foodstuffs has increased. This contamination has been attributed to the cultivation of a polluted environment resulting in rapid and unorganized urban and industrial development (Kumar et al., 2019; Pirsahab et al., 2016). Milling of cereal grains also introduces lead into foods and its deposition is through the wearing and tearing of the milling plates, and the use of lead shims to repair a particular type of axle assembly for the millstone. (Adeti, 2015; Loutfy et al., 2012).

Lead has been found in the diet, water, air, soil, dust, electronics, and paints (Fowler, 2015). Food is the predominant source of lead exposure to the general public and children in particular (Codex Alimentarius Commission, 2011; Fowler, 2015). Inhalation of lead may be significant when lead in the air is high especially in an industrially polluted environment for workers (Toxicological Factsheet Series, 2009). For children, ingestion has been the major source of exposure as confirmed by Laurence et al. (1997). According to their report, over 75 % of the daily intake of lead in children occurs through the food they consume (Loutfy et al., 2012). Mothers can also pass on

lead to fetuses and breastfed infants through the mobilization of Lead accumulated in the skeleton during pregnancy and lactation respectively (Winters et al., 2011).

Lead does not occur naturally in the human body and therefore serves no purpose in the body. Its presence is usually as a result of pollution-related exposure or diet exposure (Fowler, 2015). Lead is detrimental to human health, affecting virtually every organ in the human body (Saracoglu et al., 2007). The maximum limit for lead in cereals foods and processed cereal foods is 0.20 mg/kg (European Commission Regulation No 1881, 2006; Codex Alimentarius Commission, 2011; Toxicological Factsheet Series, 2009). The human digestive system is not able to metabolize Lead and this leads to the accumulation of Lead in the body, more especially in the skeletal system (Winters, 2011). Lead has been classified as potentially hazardous to most forms of life by USEPA (Doamekpor et al., 2018). IARC has also classified lead as possibly carcinogenic to humans (group 2B) while inorganic lead compounds are probably carcinogenic to humans (Group 2A) (Loutfy et al., 2012).

Lead affects the peripheral and the central nervous system, the renal function, blood cells, and the metabolism of vitamin D. Chronic toxicity has always been a major concern, especially in children compared to acute toxicity which is mostly related to rare industrial exposure in adults (Winters, 2011). Lead can affect calcium-dependent or related processes by mimicking or inhibiting calcium (Lanre-Iyanda, 2012). Lead has also be reported to cause an adverse effect on the cardiovascular system by increasing blood pressure and the risk of cardiac problems in adults (Suomi et al., 2018).

Lead is a well-known neurotoxin (Kumar et al., 2019; Winters, 2011) and its chronic neurological effect notably on fetus and babies or children is well reported (Doamekpor et al., 2018). Infants and children are more sensitive and more vulnerable to lead

poisoning than adults. Lead poisoning can adversely affect the development of the brain and the nervous system (Loutfy et al., 2012; Saracoglu et al., 2007). Because the infant's brain develops rapidly both before birth and after birth, and hence lead exposures during this critical period are particularly detrimental to the future intellectual potential of children (Koka & Koka, 2011). Lead affects the peripheral and central nervous system leading to diminished Intelligent Quotient, encephalopathy, destruction of the neurons, stunted cognitive development, learning and memory problems, and attention span in children (Codex Alimentarius Commission, 2011; Kumar et al., 2019; Lanre-Iyanda, 2012; Winters, 2011). Excessive lead exposure and accumulation in an organism can lead to death (Koka & Koka, 2011).

2.3.3 Toxicity of Cadmium

Cadmium is one of the trace metals that occur naturally in relatively small quantities (Winters, 2011). Industrial applications of cadmium, metal mining as well as burning of fossil fuels end up introducing cadmium in food. (Paiva et al., 2019; Winters, 2011). Cadmium is regarded as an indicator of carcinogenic processes (Koka & Koka, 2011). The USEPA has placed cadmium as a probable human carcinogen (Group 2B) while cadmium and cadmium compounds are recognized as carcinogenic to humans (Group 1) by the IARC (Loutfy et al., 2012).

Cadmium is ubiquitous in the environment: found in food, water, air, and soil. Cadmium has been found in soils under certain environmental conditions, thus, the future risk of exposure through food during cultivation. Cadmium has also been reported to contaminate cereals during the milling stage of the cereal processing through wearing and tearing of the milling plates (Adeti, 2015). Food is the main source

of cadmium exposure to the general population (Stroheker et al., 2019). Inhalation of cadmium may be very significant for smokers and industrial workers. Cadmium has a half-life of 30 years in humans, hence exposing babies or children to it has serious implications on their health (Koka & Koka, 2011).

Cadmium is known to have no biological function in the human body and therefore its presence in the body is extremely toxic even in low concentrations (Lanre-Iyanda, 2012). The maximum limit for cadmium in cereals foods and processed cereal foods is 0.20 mg/kg (European Commission Regulation No 1881, 2006; Codex Alimentarius Commission, 2011; Toxicological Factsheet Series, 2009). Cadmium bio-accumulates in organisms and ecosystems. The human body absorbs and holds cadmium because the body's metabolic processes cannot efficiently get rid of it. Cadmium ends up accumulating in its critical target organs namely: the kidney and the bones (Toxicological Factsheet Series, 2009). Cadmium has also been found to accumulate in babies during the fetal stage (Koka & Koka, 2011). Cadmium exposure to women is more hazardous than men, due to increased Cadmium absorption is related to lowered body iron storage (Loutfy et al., 2012).

The presence of cadmium in food in the body interferes with the functions of some essential metals. Cadmium inhibits zinc enzyme reactions and nutrient utilization. It causes metabolic disturbances of iron and calcium in the body (Koka & Koka, 2011).

Acute toxicity of cadmium poisoning by ingestion may lead to stomach lining irritation which induces vomiting and diarrhea (Fowler, 2015; Prasad, 2008). This is easily treated medically through chelation therapy. Increased lung cancer has been reported due to inhalation exposure in occupational settings and smoking (Anim et al. 2011;

Chao et al., 2014; Escarrone et al., 2014). Acute toxicity has not been a major concern but rather its chronic toxicity which exhibits the most severe effects of exposure since it generally occurs in the long-term. Chronic toxicity may be seen in various body systems (Winters, 2011).

The main target organ of cadmium poisoning is the kidney leading to kidney diseases and kidney failure (Codex Alimentarius Commission, 2011; Prasad, 2008). Cadmium damages the proximal tubular cells a condition known as cadmium renal tubular damage which is irreversible (Koka & Koka, 2011). This condition decreases the filtration ability of the nephron and weakens the renal tubular function which turns to increase excretion of low-molecular-weight proteins and other essential ions into the urine (Stroheker et al., 2019; Suomi et al., 2018; Winters, 2011). Cadmium toxicity is also associated with endocrine disruption, estrogen-mimicking activities, osteomalacia, osteoporosis, and damage of olfactory senses which lead to teeth discoloration and anemia (Codex Alimentarius Commission, 2011; Stroheker et al., 2019; Winters, 2011).

2.3.4. Benefits and Negative Effects of Iron

Iron is one of the most abundant elements exceeded only by aluminum, silicon, and oxygen. Most dietary iron comes from meat and human can absorb iron from animal sources than from plant sources. Babies and young children obtain iron from plant sources such as cereals, vegetables, and green beans since their digestive system is not well developed to obtain from meat. These plant foods are often fortified with iron or zinc to obtain the recommended daily allowance for babies and young children (Saracoglu et al., 2007; Winters, 2011).

Iron is an essential trace metal in the human body that plays important role in the biological systems. Iron, as an essential part of many enzymes and proteins, interacts with other metals viz. copper, zinc, manganese, and chromium. Iron-binding proteins bind with these metals to transport them (Prasad, 2008). Iron is involved in oxygen uptake and transport, regulation of normal cell growth, and differentiation (Das, 2016; Winters, 2011).

Iron deficiency is more common than iron overload, especially in babies. Iron deficiency will limit the oxygen delivery to cells resulting in fatigue, poor work performance, decreased immunity, and even death (Codex Alimentarius Commission, 2011; Das, 2016; Winters, 2011). Iron deficiency in babies can be more problematic because normal growth and development could be disrupted. Excess iron intake in the body can result in iron overload and iron toxicity which can lead to health implications such as arrhythmia, heart failure, increased risk of atherosclerosis, and increased risk of liver, breast, gastrointestinal and hematologic cancers (Codex Alimentarius Commission, 2011).

2.3.5. Benefits and Toxicity of Zinc

Zinc is one of the several essential trace metals in the human body. Zinc occurs naturally in the environment and is released into the environment by activities such as mining, burning of fossil fuels, refining metal ores, and steel production (Winters, 2011). Zinc is found in a wide range of foods including cereals, meat, fruits, vegetables but a large amount is found in protein-rich foods like clams, beef, chicken, lamb, pork, crabmeat, turkey, lobster, and salmon (Jurowski et al., 2019; Tongesayi et al., 2013). Zinc gets into the body mainly through the ingestion of food. Zinc can be introduced into cereal-

based food during milling. This occurs through wear and tear of the milling plates (Adeti, 2015).

Zinc is one of the most important trace metals for newborns babies and infants due to its vital role in the body. The zinc present in an adequate amount in the body reduces or prevents absorption and accumulation of cadmium and its adverse effect (Loutfy et al., 2012). Zinc stimulates and forms an essential part of more than 200 enzymes, which promote major biochemical reactions such as digestion, metabolism, and reproduction (Das, 2016; Prasad, 2008). In the digestion system, Zinc plays a central role in the synthesis and decomposition of proteins, lipids, carbohydrates, and nucleic acids and their metabolism (Winters, 2011). Zinc supports normal growth and development especially for babies (Loutfy et al., 2012). Zinc is an immune system booster, and it is involved in the nucleotide transcription processes, DNA synthesis, wound-healing, and maintains body sense of taste and smell (Loutfy et al., 2012; Winters, 2011).

Zinc can be very detrimental to the human body when the right amount of it is not met. Inadequate dose of zinc in the body causes zinc deficiency: a condition that leads to cognitive defects, impaired immune system, skin sore, slow wound healing, birth defects, loss of appetite, taste, and smell (Codex Alimentarius Commission, 2011; Das, 2016; Tongesayi et al., 2013). The overdose of zinc in the body causes zinc toxicity: gastrointestinal toxicity, pulmonary toxicity, nephrotoxicity, and neurotoxicity, and may inhibit copper intake by competition for transporters (Prasad, 2008). Zinc toxicity is reported to cause stomach cramps, nausea, and anemia. A high level of zinc can damage the pancreas, disrupt protein metabolism, and can even cause arteriosclerosis (Codex Alimentarius Commission, 2011; Prasad, 2008; Tongesayi et al., 2013).

2.3.6 Benefits and Toxicity of Copper

Copper is an essential trace metal with a wide range of importance in both the physical and mental development of the human body. Copper is found in many foods particularly vegetarian foods such as cereal grains, nuts, and seeds. Copper aids in iron assimilation and hemoglobin synthesis (Loutfy et al., 2012; Paiva et al., 2019). A wide range of enzymes uses copper as the co-factor in their biological reactions. Copper is related to the maintenance of normal important activities in the body such as the function of the immune system, nervous system, skin, hair pigmentation, iron transport, connective tissues, energy production in cells, estrogen metabolism in females, among others (Lanre-iyanda, 2012).

The deficiency of copper is associated with diverse effects such as anemia, suppression of growth, impaired reproductive performance, heart failure, and gastrointestinal disturbances (Das, 2016). Scoliosis and scorbutic-like changes have been reported in copper-deficiency in babies and young children (Das, 2016). Copper toxicity or overload mostly causes problems under certain conditions, notably genetic disorders such as Wilson disease (Jurowski et al., 2019). Copper toxicity is associated with conditions such as heart disease, gum diseases, arthritis, insomnia, osteoporosis, migraine, skin and hair problems, mental and emotional disorders (Codex Alimentarius Commission, 2011; Lanre-Iyanda, 2012).

2.3.7 Benefits and Toxicity of Chromium

Chromium is an essential trace metal. Chromium is found in most foods and hence the major route of exposure is through ingestion. Chromium may exist in the human body as Cr^{3+} or Cr^{6+} , in which the latter is more toxic (Prasad, 2008). Chromium is found in

most metal parts including milling plates and household kitchen equipment leading to its introduction by scratch. The wear and tear of the milling plates during food processing are the major sources of Chromium in cereal-based baby foods (Adeti, 2015). Chromium is necessary for insulin activities which help in the maintenance of effective glucose, lipid, and protein metabolism in the human body (Das, 2016; Loutfy et al., 2012; Pirsheh et al., 2016).

2.3.8 Benefits and Toxicity of Nickel

Nickel is an essential trace metal that acts as a cofactor for some enzymes, urease, and others. Nickel aids the metabolism of methionine, Vitamin B12, and folate (Prasad, 2008). Nickel is found in many tissues in the human body and this is mainly due to dietary intake. Most metallic and electronic products consist of nickel. Wearing and tearing of the metallic plates of the milling machine may facilitate the introduction of nickel into processed cereal-based foods (Adeti, 2015). The deficiency of nickel may lead to reduced growth, reproductive problems, and altered distribution of the other elements (Prasad, 2008). Toxicity of nickel can cause a variety of pulmonary adverse health effects such as lung inflammation, fibrosis, emphysema, tumor, anemia, and decreased growth (Fowler, 2015; Kumar et al., 2019; Ozdemir et al., 2019). Excessive intake of nickel has been associated with cancer, heart disease, and thyroid problems (Pirsheh et al., 2016).

2.3.9 Benefits and Harmful Effects of Cobalt

Cobalt is an essential element and forms an integral part of vitamin B12 (Reilly, 2002). It is therefore essential for the treatment of anemia, folate, and fatty acids metabolism

in the body (Das, 2016). Cobalt level in food is relatively very low compared to other trace element and its deficiency in animals is known to cause anemia. Cobalt toxicity arises from excessive intake through the main food and other contaminations. It is known to lead to cardiomyopathy, which damages the heart muscles due to anoxia and hypothyroidism (Prasad, 2008; Reilly, 2002).

2.4 Analytical Techniques for Metal Analysis

There has been an advancement in instrumentation and analytical techniques over the last two decades leading to improvement in analytical standards, effectiveness, cost-efficient, and productivity. Analytical techniques for metals analysis in baby foods include X-ray Fluorescence (XRF) and Inductively Coupled Plasma (ICP) (Gardener et al., 2019; Pandelova et al., 2012; Pirsahab et al., 2016; Tongesayi et al., 2013). Atomic absorption spectrometry (AAS) has also been used in a wide range of research for metal analysis. AAS has been used for metal analyses in baby foods (Das, 2016; Jurowski et al., 2019; Loutfy et al., 2012; Saracoglu et al., 2007; Winters, 2011).

2.4.1 Atomic Absorption Spectrometry (AAS)

AAS is one of the most widely used instruments for major and minor inorganic metal determination in a wide range of scientific disciplines. The method of analysis works on the basic principles that when a ground-state analyte is introduced into a flame, the atoms vaporize into gas to absorb electromagnetic radiation which emits electromagnetic radiation (light wavelength) characteristic of their ground-state energy levels (Fowler, 2015). There are different types of AAS but the most used types are the graphite furnace atomic absorption spectrometer (GFAAS) and flame atomic

absorption spectrometer (FAAS) (Fowler, 2015). The FAAS is rapid and sufficiently sensitive to permit the determination of most of the trace elements in food even to the level of ug/kg range for the modern models. The GFAAS allows for the determination of a wider range of elements.

In using the FAAS for metal analysis, the nebulizer sucks up the solution sample at a controlled rate and creates a fine aerosol into the spray chamber. The nebulizer aerosol is mixed thoroughly with the fuel and oxidant and introduced into the flame in the burner. The analytes are atomized by exposing the analyte to high temperatures in the flame. The hollow cathode lamp produces the source of light radiation directed through the flames. The monochromator then isolates a single atomic resonance line from the total spectrum of light emitted. The monochromator filters select the specific and narrow regions of the spectrum for the transmission into the detector within a specific wavelength region. In effect, the monochromator discriminates between different wavelengths. The photomultiplier tube detector measures the intensity of the atomic absorption or emission. The concentration of the analyte is calculated from the absorption using the Beer-Lambert law (Winters, 2011). The AAS is less expensive and easier to operate.

2.5 Analytical Techniques for PAHs Analysis

2.5.1 Extraction of PAHs in Foods

There are several analytical methods designed to determine PAHs in food matrices. However, with increasing research and a large number of samples using modern sophisticated and high sensitive instrumentations, some of these designed methods make it very difficult to achieve accurate and fast results. Several solvents are used for

extracting PAHs from various matrices, usually in a mixture of two or more solvents of different polarities since PAHs have a wide range of solubility. All solvents used are of a high quality due to the use of the GC or the HPLC.

2.5.1.1 The Methods of Extraction

The selection of the extraction method is dependent on factors such as the effectiveness, popularity of the method, simplicity of the application in the laboratory, cost, and prospects for development by further modification (Baranowska, 2016). Soxhlet extraction has been used for a long time for extraction but it is gradually being replaced with modern techniques. The Soxhlet procedure of extraction has a disadvantage of a long duration of 5–24 hours of extraction and the use of a large amount of solvent per batch of extraction. It is therefore not a suitable method of extraction for a large number of samples. The modern method which is an automated extraction technique helps to accelerate the solvent extraction procedures to limit time spent and amount of solvent for high recoveries. These methods include pressurized liquid extraction (PLE), Supercritical Fluid Extraction (SFE) which is very expensive, Ultrasound-Assisted Extraction (UAE), and Microwave-Assisted Extraction (MAE) (Baranowska, 2016). Another quick and easy method of extraction is QuEChERS extraction.

2.5.1.2 The QuEChERS Extraction Technique

The QuEChERS is the acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe. This method has several advantages over other conventional methods such as Soxhlet extraction which requires too much time and large volumes of solvent. Thus the advantages of the QuEChERS method which uses environmentally friendly solvents

include: quicker turnout of extraction, easy to follow steps, less cost, effective, rugged, low solvent consumption, high recovery rate, and safe (Urkude & Dhurvey, 2015). The QuEChERS method proves to be robust and has been used for PAHs analysis with high recoveries reported (Aldrighi et al., 2018; Escarrone et al., 2014; Anastassiades et al., 2003; Baranowska, 2016; Urkude & Dhurvey, 2015).

2.5.2. Clean-up of Extracts

Clean-up is done to remove non-specific (bulk matrix) interference or eliminate high molecular compounds, to protect the analytical system, and to effectively raise the concentration of the analyte for sensitivity reasons (Rashid et al., 2010). Solid-phase extraction (SPE) is probably the most commonly used sample preparative technique used in food samples. When working on matrices such as cereals, one would expect matrix co-extractives such as proteins, sugars, and other organic acids. These co-extracts may have certain properties that have them extracted alongside the PAHs. There is therefore the need to carry out clean-up to eliminate the co-extracts that can interfere with the results (El Hawari et al., 2017). For QuEChERS extraction the dispersive SPE is the most form of SPE suitable for cleanup.

2.5.3 Instrumental Analysis of PAHs

Instrumental analysis that would be used to identify and quantify PAHs in baby foods should be of high resolution, sensitivity, and selectivity because of the very low permissible level (Codex Alimentarius Commission, 2017). HPLC and GC have been the two main instrumentation used for the determination of PAHs in many food matrices and non-food matrices. HPLC coupled with ultra-violet (UV) or photodiode

array (PDA) detectors has been replaced with Fluorescence Detector (FLD) and the Gas Chromatography – Flame Ionization Detector (GC-FID) has also been replaced with Gas Chromatography – Mass Spectrometry (GC-MS) due to their poor selectivity and sensitivity (Swartz, 2010). Gas Chromatography –Mass Spectrometry, mass spectrometry (GC-MS/MS), and Gas Chromatography – High- Resolution Mass Spectrometry (GC-HRMS) are also some improvements in the sensitivity and selectivity of the GC-MS (Rozentale et al., 2017). The modern models of GC-MS such as the Agilent technologies 7000series Triple Quad GC-MS system are highly sensitive, selective, resolution, and very effective for this analysis.

CHAPTER THREE

METHODOLOGY

This chapter elaborates on all the activities carried out from the sampling of locally produced cereal-based baby foods in the Greater Accra region, through sample preparation, and the detections of metals and PAHs concentrations. Methods for the calculations of potential paediatric health risks are also detailed.

3.1 Sampling Area

The sampling area comprises major sales outlets of cereal-based baby foods. The outlets from where locally manufactured cereal-based baby foods were collected are located in: Accra Metropolis, Ga East Municipal, La Dadekotopon Municipal, and La Nkwatanang Madina Municipal, all in the Greater Accra Region, Ghana. These areas host a population of 2,560,985 which is >50 % of the total population of the Greater Accra region (Ghana Statistical Services, 2019). The 2010 population and housing census for the Accra Metropolis and the other three municipalities reported the total number of children within ages of 0–4 years as 226,811, the number of birth in the last 12 months as 42,453 and the number of women within 15–49 year as 650,459 with a total fertility rate of 2.38 children per woman (Ghana Statistical Service, 2012, 2014b, 2014a, 2014d, 2014c).

The sampling areas have most of their markets operated as open-market system and few regulated market system. Hence, there are different cereal-based baby food products; both branded and unbranded sold on the open markets compared to the regulated markets where only branded products are sold. A greater proportion of the

population in the selected metropolis and municipalities depend on the open-market system for most of their food supplies including cereal-based baby foods.

In this study, the cereal-based baby foods were sourced from hospitals, malls, and open markets within the selected metropolis and municipalities where patronage is high. The major hospitals: Korle Bu Teaching Hospital, 37 military hospital, Legon University hospital, and GAEC hospital are vantage sale points of cereal-based baby foods due to high patronage by nursing mothers whenever they attend hospital with their babies for checkups or treatment. These nursing mothers are often convinced that the cereal-based baby foods sold within the hospitals are wholesome compared to other outlets though without any scientific evidence. A map showing the sampling locations is presented in Fig. 3.1.

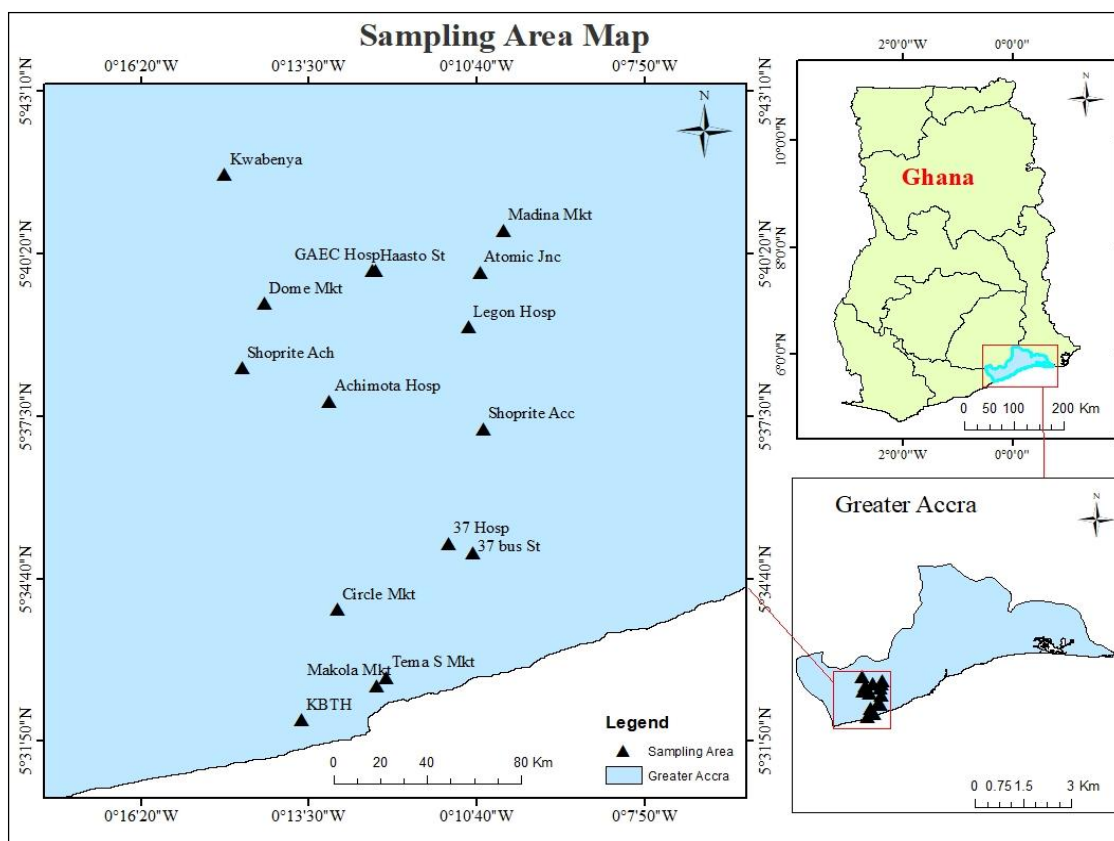


Fig. 3.1 Map showing the sampling locations within the Greater Accra region of Ghana

3.2 Sample Collection

The sampling of the cereal-based baby food products was done in accordance with the sampling protocols as described by Greenfield (2003) for retailed foods and Ghana Standard Authority guidelines on sampling (GS CAC/GL 50, 2004). Samples were collected in the original packaging from the markets, hospitals, and malls where there is high patronage. The original packaging materials were: paper, PET bottles, hard and soft sachets. Some of the PET bottled samples had inner seals, making them airtight. All the samples were in powdered form with their particle size of approximately less than 1 mm. Each of the samples was labelled with a unique identification code, transported to the laboratory for sample preparation and storage.

In all, 50 samples of cereal-based baby food were obtained from various hospitals, major and satellite markets in the Accra metropolis, and three other municipalities in the Greater Accra region. All the samples were cereal-based baby foods that were locally produced in Ghana. The samples were made up of seventeen (17) different brands and four (4) sets of unbranded cereal-based baby foods. The different unbranded samples that were from the same market and by the same producer were grouped into four sets as Set 1, Set 2, Set 3, and Set 4. Each sample is made of either one variety of cereal or a combination of two or more cereals with or without plant protein such as groundnut and /or soyabeans. Two samples were found to be mixed with animal protein, specifically milk. There were other two samples mixed with vegetables, that is, one mixed with carrot and the other with beetroot. The different types of cereals purchased including: maize (14 %), rice (14 %), wheat (10 %), and millet (4 %), with mixed cereals dominating with 58 % as illustrated in Fig. 3.2. The samples were of different weights ranging from 100 g to 900 g.

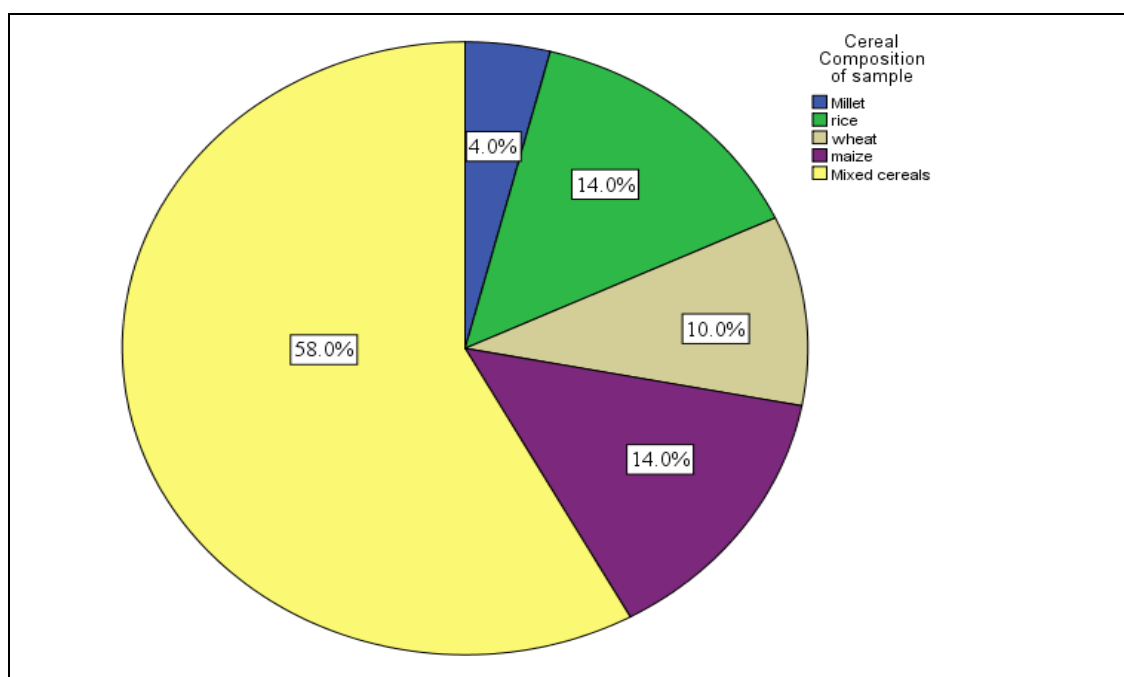


Fig. 3.2 Pie chart showing the distribution of cereals in the samples

A summary of information on the samples is shown in Table 3.1 while the detailed information on the samples is shown in Appendix 1. Only five samples had FDA registration numbers on their labels. A follow-up to Ghana Foods and Drugs Authority confirmed that those products were truly registered.

Table 3.1 Summary of Information on Sample Labels

Label information	Specified	Unspecified
Ingredient	32	18
FDA Registration Number	5	45
Contact Information	30	20
Expiry date	19	31
Other information *	26	24

*Information on weight, batch numbers, nutritional value, preparation, and storage.

3.3 Sample Preparation for Analysis

All the samples were in their fine powdered form, hence they were not macerated further. The samples were homogenized by mixing thoroughly using the laboratory homogenizer in accordance with the recommendations in GS EN 13804 (2002). Sub-samples of the homogenized powder were taken into pre-cleaned polyethylene containers and stored in a refrigerator at 4 °C for further analysis.

3.4 Determination of Trace Metals and PAHs Concentrations in the Samples

The metal analysis was carried out at the Inorganic Laboratory of the Nuclear Chemistry and Environmental Research Center of the Ghana Atomic Energy Commission (GAEC), Accra. The PAHs analysis were carried out at the Pesticide Residue Laboratory of the Ghana Standard Authority (GSA), Accra. All the 50 samples were analyzed for trace metal concentration and 30 samples out of the 50 samples were analyzed for polycyclic aromatic hydrocarbons. The general scheme of the analysis of trace metals and PAHs is presented in Fig. 3.3.

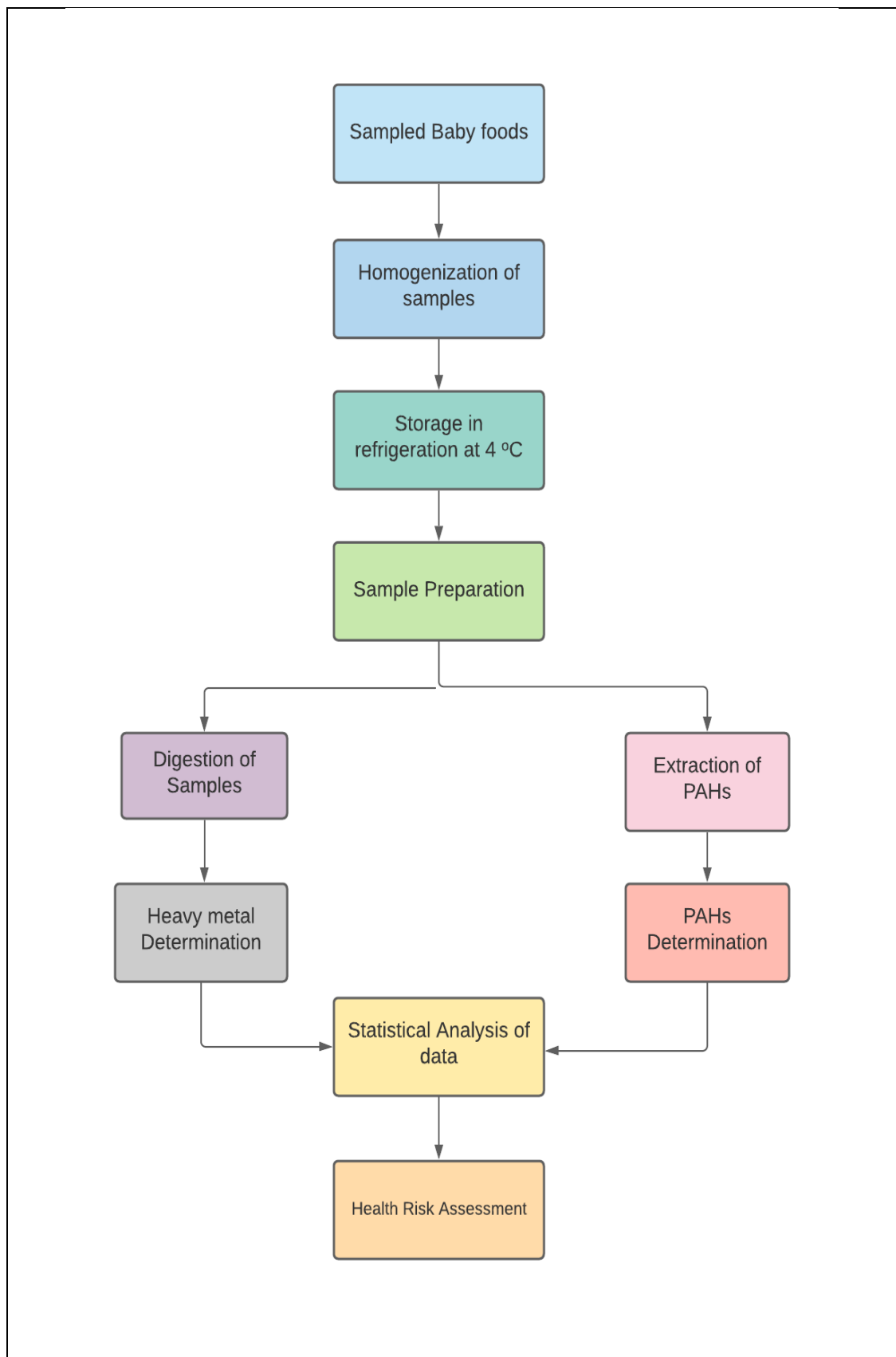


Fig. 3.3 Schematic of the research methodology

3.4.1 Analysis of Trace Metals

All the 50 samples were air-dried in a completely air-conditioned clean room. The concentrations of eight (8) different metals: iron, zinc, copper, cadmium, chromium, cobalt, lead, and nickel were determined. These metals can easily find their way into food either naturally or through contamination from the environment during cultivation or food processing (Adeti, 2015; Doamekpor et al., 2018; Kumar et al., 2019; Reilly, 2002).

3.4.1.1 Reagents for Sample Digestion and Metal Analysis

The Acids used for digestion of the cereal-based products include concentrated Trioxonitrate (V) acid (HNO_3 , 69 %), analytical grade supplied by British Drug House (BDH) limited and Hydrogen peroxide (H_2O_2 , 30 %), analytical grade supplied by MES Equipment Limited. The reference standards materials used for the metal analysis were from FLUKA ANALYTICAL, Sigma-Aldrich Chemie GmbH, and products of Switzerland. The double deionized water used in this analysis for dissolution, dilution, and rinsing of glassware was produced by the Nuclear Chemistry and Environmental Research Centre, GAEC.

3.4.1.2 Microwave Digestion of Samples

The acid digestion methods as previously described by Anim et al. (2011) and Ackah et al. (2013) were followed. Approximately 0.5 g each of the homogenized samples were weighed into labeled 100 mL polytetrafluoroethylene (PTFE) Teflon bomb, previously acid-washed and well dried, using the Mettler Toledo AB 204-S analytical balance. About 2.0 mL of double deionized water was added, followed by 7.0 mL of

concentrated nitric acid (HNO₃, 69 %), and allowed to stand for 20 minutes. After the pretreatment, 2.0 mL of concentrated nitric acid (HNO₃, 69 %) was added followed by 1.0 mL of hydrogen peroxide (H₂O₂, 30 %). The addition of the reagents to the samples was carried out in a fume chamber as a safety measure.

The samples in their Teflon bombs were then loaded onto the microwave carousel and secured tightly with vessel caps using a wrench. The complete assembly was then microwave irradiated for 50 minutes using the SINEO JUPITA-A microwave digestion as programmed in Table 3.2.

Table 3.2 **Digestion Program for metal analysis** (Shangai Sineo Microwave Chemistry Technology Co. Ltd. January 2015)

Step	Temp T/ °C	Time (t) /min	Power (W)
1	80	Ramp for 10 min and hold for 10 min	400
2	100	Ramp for 5 min and hold 5 min	400
3	115	Ramp for 15 min and hold for 5 min	400

After the digestion, the microwave digester was allowed to cool in the digestion vessels to about 40 °C. The Teflon bombs mounted on the microwave carousel were then cooled in a water bath to reduce the internal pressure and allow volatilized material to re-stabilize. The digestates were made up to 20 mL using double deionized water and filtered through 2.5 µm pore size Whatmann filter paper into 30 mL High-Density Polyethylene (HDPE) bottles for the metal analysis. The metals of interest were assayed using VARIAN AA 240FS – Fast Sequential Atomic Absorption Spectrometer. To

account for quality controls, reference standards, blanks, and sample duplicates were digested similarly as the samples.

3.4.1.3 Detection of Trace Metals Using AAS instrumentation

The digested cereal-based baby foods were analyzed for trace metals using a Varian AA 240FS Fast Sequential Atomic Absorption Spectrometer in an acetylene-air flame. The instrument parameters used, including the atomic absorption and the working conditions of the AAS together with the detection limits for the metals are shown in Table 3.3.

Table 3.3 Instrument parameters and working conditions for the AAS analysis (VARIAN Publication No 85-100009-00 Revised March 1989)

Metal	Lamp current (mA)	Slit width (nm)	Wavelength (nm)	Fuel	Support	Detection limit (mg/L)
Fe	5	0.2	248.3	Acetylene	Air	0.006
Zn	5	1	213.9	Acetylene	Air	0.001
Cu	4	0.5	324.7	Acetylene	Air	0.003
Cr	7	0.2	357.9	Acetylene	Air	0.001
Co	7	0.2	240.7	Acetylene	Air	0.005
Cd	4	0.5	228.8	Acetylene	Air	0.002
Pb	5	1	217	Acetylene	Air	0.001
Ni	4	0.5	341.4	Acetylene	Air	0.001

The recoveries expressed in the percentage of analytical standards together with the measured and expected values for the various metals are all shown in Table 3.4. The blanks and standard reference materials were analyzed alongside the samples in a similar manner to ensure reproducibility and quality assurance purposes.

Table 3.4 Results of analytical recoveries of the standards

Metal	Expected Conc. (mg/kg)	Measured Conc. (mg/kg)	Recoveries (%)
Fe	2	1.997 ± 0.0027	99.87
Zn	0.25	0.2496 ± 0.0005	99.84
Cu	2	1.998 ± 0.0005	99.92
Cd	0.5	0.499 ± 0.0013	99.86
Co	2	1.996 ± 0.0047	99.79
Cr	1	0.995 ± 0.0051	99.52
Pb	2	1.998 ± .0043	99.91
Ni	2	1.996 ± 0.0056	99.79

The final concentrations of the metal in mg/kg or ppm were calculated based on Equation 3.1 (USEPA, 1983).

$$C_f = \frac{C_i \cdot V}{m} \quad (\text{Eqn 3.1})$$

Where C_f = Final concentration of element (mg/kg)

C_i = concentration from AAS reading (mg/L)

V = Nominal Volume of analyte (L)

m = mass of sample used for analysis (kg)

Recoveries expressed in percentage were calculated using Equation 3.2 (EPA Contract Laboratory Program (CLP), Multi-Concentration, 2016).

$$R = \frac{Mv}{Ev} \cdot 100\% \quad (\text{Eqn 3.2})$$

Where R = Recovery

Mv = measured value

Ev = expected value

3.4.1.4 Quality Control/ Quality Assurance for Metal Analysis

The following Quality Control and Quality Assurance techniques were used during the determination of trace metals concentrations:

Regressions curves: The graphs showing the regression curves of the metals using standard reference materials are presented in Appendix 2 (a–h).

Analytical Blanks: Double deionized water was used as the blank to check contamination throughout sample preparation and analysis.

Repeatability: At least one sample in a batch of ten was repeated in the sample preparation and analysis.

Standards: Standard solutions were prepared from a standard stock solution and serially diluted to check the efficiency of the equipment being used. Standards were also included in the batch analysis. The concentrations of the standard solutions and their absorption were used to obtain the linear range for the AAS analysis.

Glasswares: The washing and rinsing of the glasswares were done per the procedure in GS EN 13804 (2002). The glasswares were thoroughly washed and rinsed with double deionized water and further acid-washed dried before each use.

3.4.2 Analysis of Polycyclic Aromatic Hydrocarbons (PAHs)

The samples were initially air-dried at room temperature in a completely air-conditioned clean room. In all, 30 cereal-based baby food samples were selected for PAHs analysis. The schematic diagram of the extraction, clean-up, concentration, detection, and quantification of the PAHs concentrations in the samples are presented in Fig. 3.4.

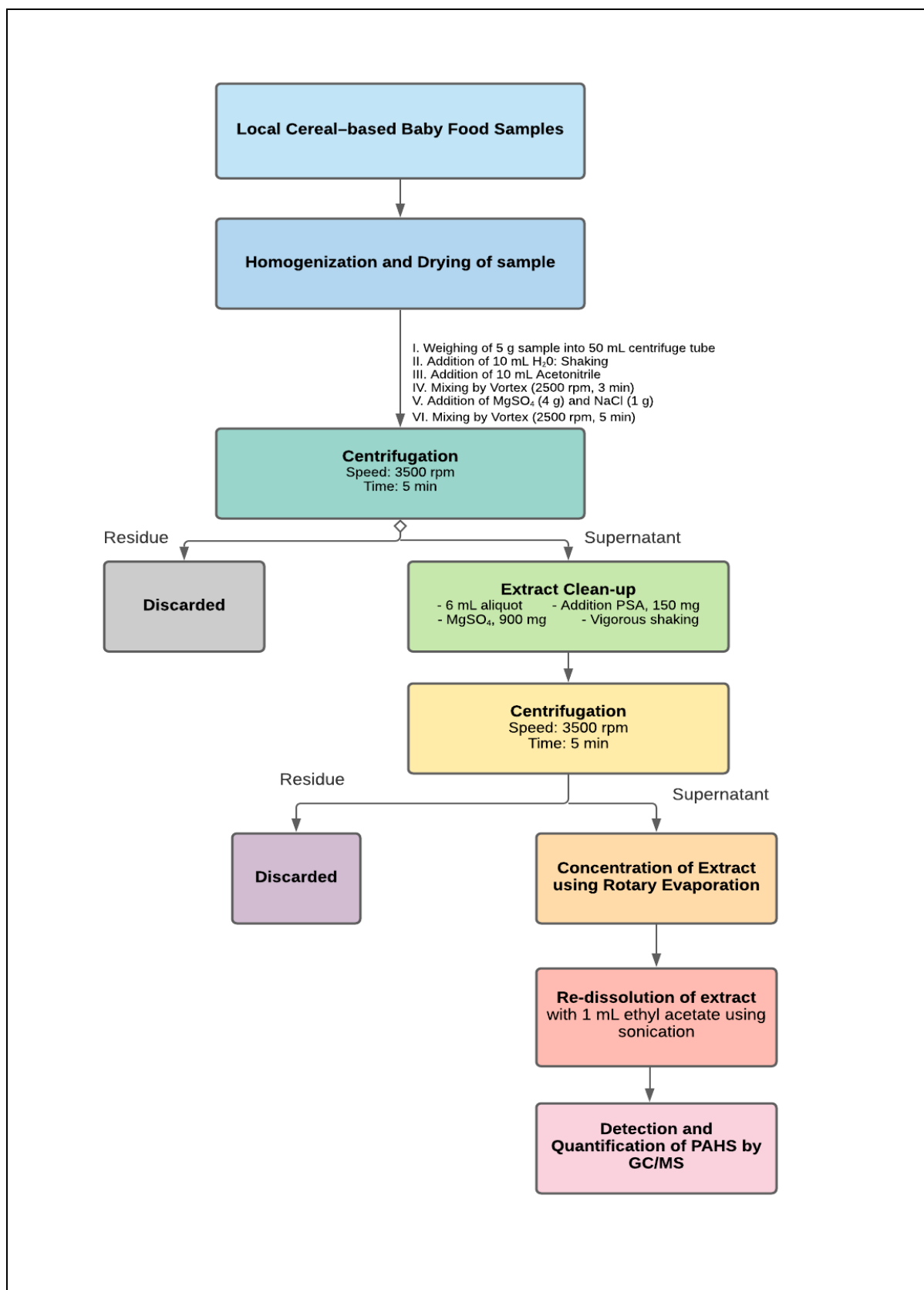


Fig. 3. 4 Schematic of the extraction and detection of PAHs.

3.4.2.1 Sample Selection Criteria

The selections of 30 out of 50 samples were based on the following considerations:

- i. The type of cereals: this was to ensure that all the cereal types were represented for the PAHs analysis. It is interesting to include rice-based baby food samples since the rice used for baby food preparation is usually not roasted. Thus the rice-based baby foods present a good sample to investigate other potential contamination sources apart from roasting.
- ii. Samples of the same brand: different samples (cereal composition) of the same brand were selected to account for differences in PAHs from the same brand.

3.4.2.2 Reagents Used for PAHs Extraction and Analysis

The 16-component PAH 45 standard used was obtained from Dr. Ehrenstorfer GmbH, Germany. The PAH standard used was made up 18 PAHs, namely: acenaphthylene, acenaphthene, fluorene, anthracene, naphthalene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[g,h,i]perylene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[e]pyrene, benzo[k]fluoranthene, indeno[1,2,3-c,d]pyrene, dibenzo[a,h]anthracene, and pyrene. The Acetonitrile ($\geq 99.8\%$) HPLC grade, used for extraction was from Honeywell (Muskegon, MI, USA), supplied by Fisher Chemicals. The Ethyl acetate ($\geq 99.8\%$) pesticide grade, supplied by CARLO ERBA Reagents was used for dissolving the cleaned extract. The extraction salts used were: magnesium sulphate anhydrous (MgSO_4) (99.0 %), extra pure supplied by Daejung Chemical Reagents and sodium chloride (NaCl) (99.0 %) analytical grade, supplied by Park Scientific Limited. The Bondesil PSA (Primary Secondary Amines) bulk sorbent 40 μm , supplied by Agilent Technologies was used for the clean-up of the

extracts. The acetone (99.5 %) analytical grade, used for rinsing glasswares was from VWR International (WestChester, PA, USA), supplied by the Park Scientific Limited. The double deionized water used for dissolution and rinsing of glasswares was prepared by the Pesticide Residue Laboratory of the Ghana Standard Authority.

3.4.2.3 Sample Extraction by QuEChERS Method

The PAHs were extracted using the QuEChERS protocols as described in the literature for solid grain samples by Escarrone et al. (2014). Approximately 5.0 g of the comminuted homogenous sample was weighed using the Mettler Toledo PG 1003-S analytical balance into a previously acetone-washed 50 mL centrifuge tube. About 10 mL of double deionized water was added and shaken for 30 seconds using the Thermolyne Maxi Mix plus to dissolve the sample and then 10 mL of acetonitrile ($\geq$ 99.8 %) was added and vortex using the Benchmark BenchMixer Multitube Vortexer at 2500 rpm for 3 minutes. An extraction salt made up of 4.0 g magnesium sulphate anhydrous (99.0 %) and 1.0 g sodium chloride (99.0 %) was added and immediately vortex for a further 5 minutes at 2500 rpm. The content was then centrifuged using the Hermle Z-300 centrifuge at 3500 rpm for 5 minutes. The supernatant was then decanted into a 15 mL centrifuge tube for further purification and clean-up.

3.4.2.4 Sample purification by dispersive SPE and concentration

About 6.0 mL aliquot of the extract was measured using the 10 mL adjustable pipette into a polypropylene centrifugation tube which already contained 150 mg PSA (40 μ m) and 900 mg magnesium sulphate (99.0 %). The tube was then closed and shaken vigorously for 1 minute using the Thermolyne Maxi Mix Plus and centrifuged at 3500

rpm for 5 minutes to remove residual water and other co-extracts such as proteins, sugars, and other organic acids. The supernatant was then decanted.

About 4.0 mL of the cleaned extracts were pipetted into acetone-washed 25 mL round bottom flasks and concentrated just to dryness below 40 °C using the rotary evaporator (Buchi Rotavapor R-210). About 1 mL ethyl acetate ($\geq 99.8\%$) was added to the extract and sonicated using the Clifton sonicator to re-dissolve the concentrated extract. The re-dissolved extracts were transferred using the Plugged disposal glass Pasteur pipette (230 mm) into a 2 mL standard opening vial for qualitative and quantitative detection of PAHs by the GC/MS. The internal standard and blanks all passed through the same processes of extraction, clean-up, and concentration.

3.4.2.5 Detection of PAHs using GC-MS Instrumentation

The PAHs in the sample extracts were analyzed using the Agilent technologies 7000 series Triple Quad GC-MS system with an auto-sampler. The GC-MS system was equipped with a Varian Inc. analytical column of 30 m x 0.25 mm internal diameter fused with silica capillary coated with 0.25 μm film VF – 5 ms together with EZ guard of 10 m long under the same condition. Helium gas (carrier gas) used was at a constant flow rate of 1.3 mL/min. The automatic injector used was operated in the splitless mode, at injection temperature of 250 °C and injection volume of 2 μL . The oven temperature program was initially set to 80 °C and held for 1 min, then ramp at a rate of 25 °C/min to 180 °C and at a rate of 5 °C/min to 300 °C and finally held at this temperature for 1 min. The mass spectrometer with an electron impact (EI) used, was operated in the ionization mode at ionization voltage of electron energy 70 eV and ion source temperature of 300 °C. The spectra were acquired using a mass range of 45 m/z to 450

m/z and the solvent delay automatic gain control. The solvent delay time was 4 minutes. The GC/MS calibration parameters for the detection of the PAHs are shown in Table 3.5.

Table 3.5 GC/MS calibration parameters of PAHs standards

Compound	Retention time (min)	Ion (m/z)	Regression coefficient (r^2)	Slope	Intercept
Naphthalene	5.31	128.0	1.0000	1.000	-6.797E-4
Acenaphthylene	7.94	151.0 – 152.0	0.9878	0.974	-1.732
Acenaphthalene	8.38	152.0 – 154.0	0.8656	1.000	-5.828
Fluorene	9.85	165.0 – 166.0	0.9997	1.000	1.421E-3
Anthracene	14.05	178.0	0.9972	1.000	1.761E-3
Phenanthrene	14.31	178.0	0.9977	0.990	-6.736E-1
Fluoranthene	21.25	202.0	0.9995	1.000	1.648E-3
Pyrene	22.65	202.0	0.9973	1.000	2.667E-3
Benzo(a)anthracene	28.58	228.0	0.9998	1.000	6.282E-4
Chrysene	28.71	228.0	0.9987	1.011	-3.238E-1
Benzo(b)fluoranthene	32.28	252.0	0.9998	1.000	-2.781E-3
Benzo(k)fluoranthene	32.38	252.0	0.9990	1.000	3.399E-4
Benzo(e)pyrene	33.17	250.0 – 252.0	0.9999	1.000	1.699E-3
Benzo(a)pyrene	33.36	250.0 – 252.0	0.9778	0.965	-2.347
Pyrene	33.67	250.0 – 252.0	0.9982	1.003	2.099E-1
Benzo(g,h,i)perylene	38.29	276.0	0.9960	1.000	-1.246E-3
Dibenzo(a,h)anthracene	38.58	278.0	0.9985	1.000	-1.813E-3
Indeno(1,2,3-c,d)pyrene	39.68	276.0	0.9989	1.000	1.586E-3

NOTE: LOD limit of detection = 0.003mg/kg
LOQ limit of quantification = 0.01mg/kg

3.4.2.6 Quality Control and Quality Assurance for PAH Analysis

Recoveries: the recoveries were calculated as the differences in PAHs concentration in spiked samples and un-spiked samples relative to the concentration of spiked samples and expressed in percentages (Ciecierska and Obiedzin'ski, 2010).

Analytical Blanks: Blank samples were prepared with double deionized water and passed through all the stages of sample extraction, clean-up, concentration, and analysis on the GC/MS alongside the working samples. This was done for each batch of 15 samples.

Internal standard: About 0.1 µg/mL concentration was prepared from the certified standard material and used as the internal standards and regression curves obtained as shown in Appendix 3 (a - r).

Spiked samples: About 0.5 mL of the prepared internal standard was used to spike some of the samples and then analyzed alongside the samples passing through all the stages. This was very useful in the calculation of the recoveries for the various PAHs in the samples.

Certified Reference material: The certified reference material from Dr. Ehrenstorfer GmbH, Germany, was of high purity was used in the GC/MS analysis for qualitative and quantitative analysis.

Limit of quantification (LOQ): the LOQ values were calculated as 10 times the standard deviation of the regression value divided by the slope of the graph obtained for the concentrations of the least standard used.

Limit of Detection (LOD): the LOD value is determined by 1/3 the value of the LOQ calculated.

Repeatability: At least one sample in a batch of fifteen (15) was repeated throughout the analysis.

To avoid contamination of samples, one pipette each was used for each sample. Certified masses of 50 g, 5 g, and 0.5 g were used to calibrate the weighing instrument before weighing. All glasswares were well washed, rinsed with double deionized water and acetone, and allowed to dry in a desiccator in accordance with GS EN 13804 (2002).

3.5 Health Risk Assessment

The exposure pathway of toxic chemicals such as PAHs and trace metals among babies is through ingestion (US EPA, 2002). Health risk assessments were carried out for both the metals and PAHs contamination in the cereal-based baby foods to ascertain whether the measured concentrations pose health risks to babies in the proceeding sections.

The intake rate for babies used were: 0.267 kg/day for 6–8 months, 0.40 kg/day for 9–11 months, 0.73 kg/day for 12–23 months (Korley et al, 2019). The Exposure duration of 6 years, exposure frequency of 365 days, and the average life expectancy for toxicity of 2190 days (6 years x 365 days) were based on Nantongo (2019). The average body weights for the 6–8 months, 9–11 months, and 12–23 months babies used in the calculations were WHO median weight of males and females babies, and they were 7.9 kg, 8.8 kg, and 10.5 kg respectively (<https://www.who.int/childgrowth/standards/en/>).

3.5.1 Risk Assessment of Metals Contamination in Babies

For non-carcinogenic risk, the chronic daily intake (CDI) was calculated for the concentrations of metals obtained in the samples based on guidelines recommended by US EPA (1989) and using Equation 3.3.

$$CDI = \frac{Ci \cdot IR \cdot ED \cdot EF}{BW \cdot AT} \quad \text{Eqn (3.3)}$$

Where: CDI is the chronic daily intake in mg/kg/day

Ci is the concentrations of the metals in the samples in mg/kg

IR is the intake rate or the ingestion rate kg/day

EF is the exposure frequency day/year

Ed is the exposure duration per year

BW is the bodyweight of the babies in kg

AT is the average life expectancy of the toxicity in day

The potential human risks of babies due to the intake of metals measured in the samples were assessed based on the hazard quotient for the individual metals by using Equation 3.4. (US EPA, 1989) and Table 3.6.

$$HQ = \frac{CDI}{RfD} \quad \text{Eqn (3.4)}$$

Where: HQ is the hazard quotient

CDI is chronic daily intake as calculated in Equation 3.3 in mg/kg/day

RfD is the oral reference dose in mg/kg/day

The oral reference dose for the metals used in the calculations of the hazard index as stated by USEPA (2009) can be found in Table 3.6 (Adeti, 2015; USEPA 2009).

Table 3.6 The oral reference dose (RfD) for each metal. Adapted from the USEPA (2009)

Metals	RfD (mg/kg/day)
Iron	0.7
Zinc	0.3
Copper	0.04
Cadmium	0.0005
Chromium	0.0003
Cobalt	0.0003
Lead	0.0035
Nickel	0.02

The hazard index (HI) for all the measured metals were calculated using Equation 3.5 (US EPA, 1989). When the calculated hazard index is <1 , then the population will not experience significant non-cancer risks. On the other hand, if the calculated hazard index is ≥ 1 , then it means the population is likely to experience significant non-cancer risks and increasing values indicate increasing the probability of non-cancer health risks.

$$HI = HQ_{n1} + HQ_{n2} + HQ_{n3} + \dots + HQ_n \quad \text{Eqn (3.5)}$$

Where: HI is the hazard Index

$HQ_{n(1-n)}$ is the hazard quotients for the various metals

3.5.2 Risk Assessment of PAHs

Seven (7) PAHs are considered as probable carcinogenic and mutagenic by the US EPA (2002). The toxicity equivalent factors (TEF) by Nsibet and LeGoy, (1992) and the mutagenic equivalent factors (MEF) by Durant et al. (1996, 1999) have been defined for dibenzo(a,h)anthracene, indeno(1,2,3-c,d)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, chrysene, benzo(k)fluoranthene, and benzo(a)pyrene, with Benzo(a)pyrene having a value of 1.0 for both TEF and MEF (Durant et al., 1996, 1999; Nsibet and LeGoy, 1992). The TEF and MEF values for all the seven PAHs established by US EPA (2002) can be found in Table 3.7.

Table 3.7 Toxicity equivalent factor and mutagenic equivalent factors of the carcinogenic and mutagenic PAHs (Durant et al., 1996, 1999; Nsibet and LeGoy, 1992).

PAHs	TEF values	MEF values
Benzo(a)pyrene	1	1
Dibenzo(a,h)anthracene	1	0.29
Indeno(1,2,3-c,d)pyrene	0.1	0.310
Benzo(a)anthracene	0.1	0.082
Benzo(b)fluoranthene	0.1	0.250
Chrysene	0.001	0.017
Benzo(k)fluoranthene	0.01	0.11

The TEQ_{BAP} was calculated using Equation 3.6 Thi et al. (2016) and the TEF values for the carcinogenic PAHs are presented in Table 3.7.

$$TEQ_{BAP} = \sum (TEF_i \cdot C_i) \quad \text{Eqn (3.6)}$$

Where: TEF_i is the toxicity equivalent factors for the carcinogenic PAHs

C_i is the concentrations of the carcinogenic PAHs in the samples

The MEQ_{BAP} was calculated using Equation 3.7 by Thi et al. (2016) and the values of the MEF for the mutagenic PAHs in Table 3.7.

$$MEQ_{BAP} = \sum (MEF_i \cdot C_i) \quad \text{Eqn (3.7)}$$

Where: MEF_i is the mutagenic equivalents factors for the mutagenic PAHs

C_i is the concentration of the carcinogenic PAHs in the samples

The benzo(a)pyrene equivalent dose for carcinogenic PAHs were calculated by using Equation 3.8 with the TEQ values obtained from Equation 3.6 (Asamoah et al., 2019).

$$BAP-TEQ = \frac{TEQ \cdot IR \cdot EF \cdot ED}{BW \cdot AT} \quad \text{Eqn (3.8)}$$

Where: BAP-TEQ is the benzo(a)pyrene equivalent dose of carcinogenicity

TEQ is the toxicity equivalent

IR is the intake rate of the cereal-based baby food for babies in g/day

EF is the exposure frequency to carcinogenic PAHs (days/year)

ED is the exposure duration in years

BW is the bodyweight of the baby in kg

AT is the average life expectancy in years

The mutagenic PAHs were calculated by using Equation 3.9 with MEQ values obtained from Equation 3.7 (Asamoah et al., 2019)

$$\text{BAP-MEQ} = \frac{\text{MEQ} \cdot \text{IR} \cdot \text{EF} \cdot \text{ED}}{\text{BW} \cdot \text{AT}} \quad \text{Eqn (3.9)}$$

Where: BAP-MEQ is the benzo(a)pyrene equivalent dose for mutagenic PAHs

TEQ is the toxicity equivalent

IR is the intake rate of the cereal-based baby food for babies in g/day

EF is the exposure frequency to mutagenic PAHs (days/year)

ED is the exposure duration in years

BW is the bodyweight of the baby in kg

AT is the average life expectancy in years

The risks of carcinogenic or mutagenic were calculated based on Equation 3.10 and the oral carcinogenic or mutagenic slope factor for benzo(a)pyrene value (SF_{BAP}) was 7.3 per mg/kg/day as established by US EPA (1991).

$$\text{R}_{(\text{car or mut})} = \text{SF}_{\text{BAP}} \cdot \text{EQ}_{\text{BAP}} \quad \text{Eqn (3.10)}$$

Where: $\text{R}_{(\text{car or mut})}$ is the risk of carcinogenic or mutagenic in sample

SF_{BAP} is the oral carcinogenic or mutagenic slope factor for benzo(a)pyrene

EQ_{BAP} is the benzo(a)pyrene equivalent dose for carcinogenic or mutagenic PAHs

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Concentrations of Metals in the Samples

The results from this work show that the cereal-based baby foods sampled contain metals (both toxic and essential metals). Varying concentrations of iron (Fe), zinc (Zn), copper (Cu), cadmium (Cd), chromium (Cr), cobalt (Co), lead (Pb), and nickel (Ni) were measured in all the 50 samples. Validation of the results was done using reference materials from FLUKA ANALYTICAL, Sigma-Aldrich Chemie GmbH (Switzerland). The results showed recoveries between 99.52 % and 99.92 % (shown previously in Table 3.4 of Chapter 3) thus reflecting good agreement between expected and actual concentrations of respective metals.

Raw analytical results for the metal concentrations in the samples are also presented in Appendix 4. Generally, Fe was recorded in all samples, thus Fe had 100 % incidence ratio (Appendix 4). Also, the results showed relatively higher concentrations of Fe compared to the other metals. The lowest concentration of Fe (0.87 mg/kg) in the samples is greater than the highest concentrations for Cd (0.82 mg/kg), Cr (0.26 mg/kg), Co (0.23 mg/kg), Pb (0.58 mg/kg), and Ni (0.20 mg/kg), except Cu and Zn which measured highest concentrations of 0.92 mg/kg and 1.0 mg/kg respectively. The incidence ratios for Cd and Pb were 44% (Cd) and 24% (Pb) respectively (Appendix 4). Also, mean concentrations for Cd and Pb were 0.2 mg/kg and 0.06 mg/kg respectively. The mean concentration of Pb (0.06 mg/kg) in this study is lower than FAO/WHO (Codex Alimentarius Commission, 2011) maximum allowable concentration of 0.2 mg/kg in baby food substance. Although the concentration of Pb is lower, it is still a cause of worry since lead has the ability to bioaccumulate.

A statistical summary of the raw results is therefore presented in Table 4.1. As shown in Table 4.1, the mean concentrations of the metals across all samples were: 8.59 ± 9.36 mg/kg (Fe), 0.13 ± 0.14 (Zn), 0.16 ± 0.18 mg/kg (Cu), 0.20 ± 0.34 mg/kg (Cd), 0.02 ± 0.04 mg/kg (Cr), 0.01 ± 0.04 mg/kg (Co), 0.06 ± 0.12 mg/kg (Pb), and 0.01 ± 0.03 mg/kg (Ni). The high variability as indicated by the respective standard deviations (Table 4.1) could be attributed to variations in compositions of the cereal-based baby foods.

It should be noted that the samples were composed of different cereals; while some are composed of single cereals, others are composed of mixed cereals, ranging between mixtures of 2 to 4 cereal combinations (presented in Appendix 1). To identify the inherent patterns of metal distribution in the samples, the data were grouped according to the cereal compositions of the samples and the trends discussed in the following sections.

Table 4.1 Statistical summary of total metal concentration (mg/kg) across all samples.

Metals	Statistics							
	n	Mean (mg/kg)	Stdev.	Range	Median	Percentiles		
Fe	50	8.59	9.36	0.87 – 34.74	4.62	2.67	10.03	33.26
Zn	50	0.13	0.14	<0.001 – 1.00	0.12	0.08	0.15	0.22
Cu	50	0.16	0.18	<0.003 – 0.92	0.13	0.09	0.15	0.81
Cd	50	0.2	0.34	<0.002 – 0.82	<0.002	<0.002	0.29	0.81
Cr	50	0.02	0.04	<0.001 – 0.26	<0.001	<0.001	<0.001	0.08
Co	50	0.01	0.04	<0.005 – 0.23	<0.005	<0.005	<0.005	0.1
Pb	50	0.06	0.12	<0.001 – 0.58	<0.001	<0.001	0.01	0.35
Ni	50	0.01	0.03	<0.001 – 0.20	<0.001	<0.001	0.01	0.07

4.1.1 Distribution of the Metals in the Samples According to Cereal

Compositions

The samples were grouped as mixed cereals (a range of mixtures of 2 to 4 cereal combinations), rice-based, millet-based, wheat-based, and maize-based. Statistically, there were no significant differences ($p > 0.05$) in the metal concentrations across all types of cereal compositions (Appendix 5). Among the types of cereal compositions, there were no significant differences ($p > 0.05$) in the metal concentrations except cadmium which showed a significant difference ($p < 0.05$) for two cereal compositions: maize only and rice only (Appendix 5 to 9). A statistical summary of metal concentrations for the various groups of cereal compositions is presented in Table 4.2. The Fe concentrations between the groups of cereal compositions were higher in millet ($18.02 \pm 0.15.23$ mg/kg) and mixed cereals (10.96 ± 10.57 mg/kg) but were least in rice

only (2.62 ± 2.67 mg/kg). The concentrations of Zn were comparable for rice only (0.21 ± 0.35 mg/kg), millet only (0.19 ± 0.07 mg/kg) and the mixed cereals (0.13 ± 0.04 mg/kg) but least in maize only (0.06 ± 0.07 mg/kg). The Zn concentrations for the mixed cereals were lower than was expected, since some of the mixed cereal samples contained plants and animal proteins which can be good sources of Zinc. Copper, chromium, cobalt, and nickel were generally of low concentrations in all the different types of cereals. Cobalt was present in only the mixed cereals with a mean concentration of 0.02 ± 0.05 mg/kg.

Cadmium concentrations were: 0.40 ± 0.60 mg/kg (millet), 0.26 ± 0.38 mg/kg (rice), 0.17 ± 0.35 (wheat), 0.46 ± 0.42 (maize) and 0.12 ± 0.28 mg/kg (mixed cereals) as presented in Table 4.2. The measured concentrations of cadmium across all cereal types were notably above the FAO/WHO (Codex Alimentarius Commission., 2011) and Ghana FDA (GS Codex Stan 193, 2019) recommended concentration of 0.10 mg/kg for cadmium in cereal-based baby foods. The presence of cadmium in the samples could be due to contamination during the cultivation or milling stages. Szymczak et al. (1993) in a related study, attributed the measured cadmium concentrations of BDL – 0.20 mg/kg in cereals to contributions of industrial pollution of the farm soils. Adeti (2016) also reported cadmium concentrations of 0.21 mg/kg in maize, after first milling, which was higher than its previous cadmium concentration of 0.02 mg/kg before milling.

The mean concentrations of lead in the samples based on types of cereal compositions were generally below the FAO/WHO (Codex Alimentarius Commission, 2011) and Ghana FDA (GS Codex Stan 193, 2019) recommended concentration of 0.20 mg/kg for cereal-based baby foods and infant formulae. Notwithstanding, the maximum concentrations of Pb for each of the cereal types, apart from millet, were all above 0.20

mg/kg. The highest mean concentration of lead (0.16 ± 0.21 mg/kg) was measured in a maize-based sample. The observed highest mean concentrations of cadmium and lead which occurred in the maize-only (cereal type samples) could be partly attributed to contamination due to the milling of the maize as previously reported by Adeti (2015). Graphical representation of the metal distribution in the samples according to cereal types is presented in Fig. 4.1(a–f). The Fig. 4.1 (a–f) shows clearly the variation between Fe concentrations and the other metals (Zn, Cu, Cd, Cr, Co, Pb, and Ni). While Fe and Cd were relatively predominant across all cereal types (Fig. 4.1b–f) the other metals were always low in concentrations. It can be seen that the concentration of Fe across all samples (Fig. 4.1a) is greatly contributed by samples of the mixed cereal type as shown in Fig. 4.1b. Also worth noting is the greater contribution of Pb by the maize-only (Fig. 4.1f) and mixed cereal types (Fig. 4.1 b) to the general distribution of Pb across all samples (Fig. 4.1a). It is also encouraging to note that Pb and Cd were not present in the samples (BFC5, BFC9, BFC20, BFC21, and BFC22) that were certified by the FDA (Ghana) and samples (BFC 14 and BFC 15) that were formulated with vegetables whereas all other metals present in those samples were low in concentrations (Appendix 4).

Table 4.2 Statistical summary of metals concentrations in the samples based on their cereal compositions

Metals	Statistics according to cereal compositions																			
	Millet only				Rice only				Wheat only				Maize only				Mixed cereal			
	Mean, mg/kg	n	Stdev.	Range	Mean, mg/kg	n	Stdev.	Range	Mean, mg/kg	n	Stdev.	Range	Mean, mg/kg	n	Stdev.	Range	Mean, mg/kg	n	Stdev.	Range
Fe	18	2	15.23	7.25–28.79	2.62	7	2.67	0.87–8.56	5.78	5	2.61	3.68–10.02	4.09	7	3.96	1.23–12.63	10.96	29	10.57	1.98–34.74
Zn	0.19	2	0.07	0.14–0.24	0.21	7	0.35	0.02–1.0	0.13	5	0.03	0.10–0.16	0.06	7	0.07	<LOD–0.18	0.13	29	0.04	0.07–0.21
Cu	0.03	2	0.04	<LOD–0.06	0.15	7	0.04	0.09–0.18	0.11	5	0.03	0.07–0.14	0.11	7	0.05	0.01–0.17	0.19	29	0.24	<LOD–0.92
Cd	0.4	2	0.56	<LOD–0.79	0.26	7	0.38	<LOD–0.81	0.17	5	0.35	<LOD–0.80	0.46	7	0.42	<LOD–0.80	0.12	29	0.28	<LOD–0.82
Cr	<LOD	2	-	-	0.03	7	0.03	<LOD–0.08	0.01	5	0.03	<LOD–0.07	0.04	7	0.1	<LOD–0.26	0.01	29	0.02	<LOD–0.08
Co	<LOD	2	-	-	<LOD	7	-	-	<LOD	5	-	-	<LOD	7	-	-	0.02	29	0.05	<LOD–0.23
Pb	0.03	2	0.04	-	0.06	7	0.13	<LOD–0.35	0.06	5	0.12	<LOD–0.27	0.16	7	0.21	<LOD–0.58	0.03	29	0.09	<LOD–0.35
Ni	0.03	2	0.04	<LOD–0.05	0.03	7	0.08	<LOD–0.20	0.01	5	0.03	<LOD–0.06	0.03	7	0.03	<LOD–0.07	0.01	29	0.02	<LOD–0.06

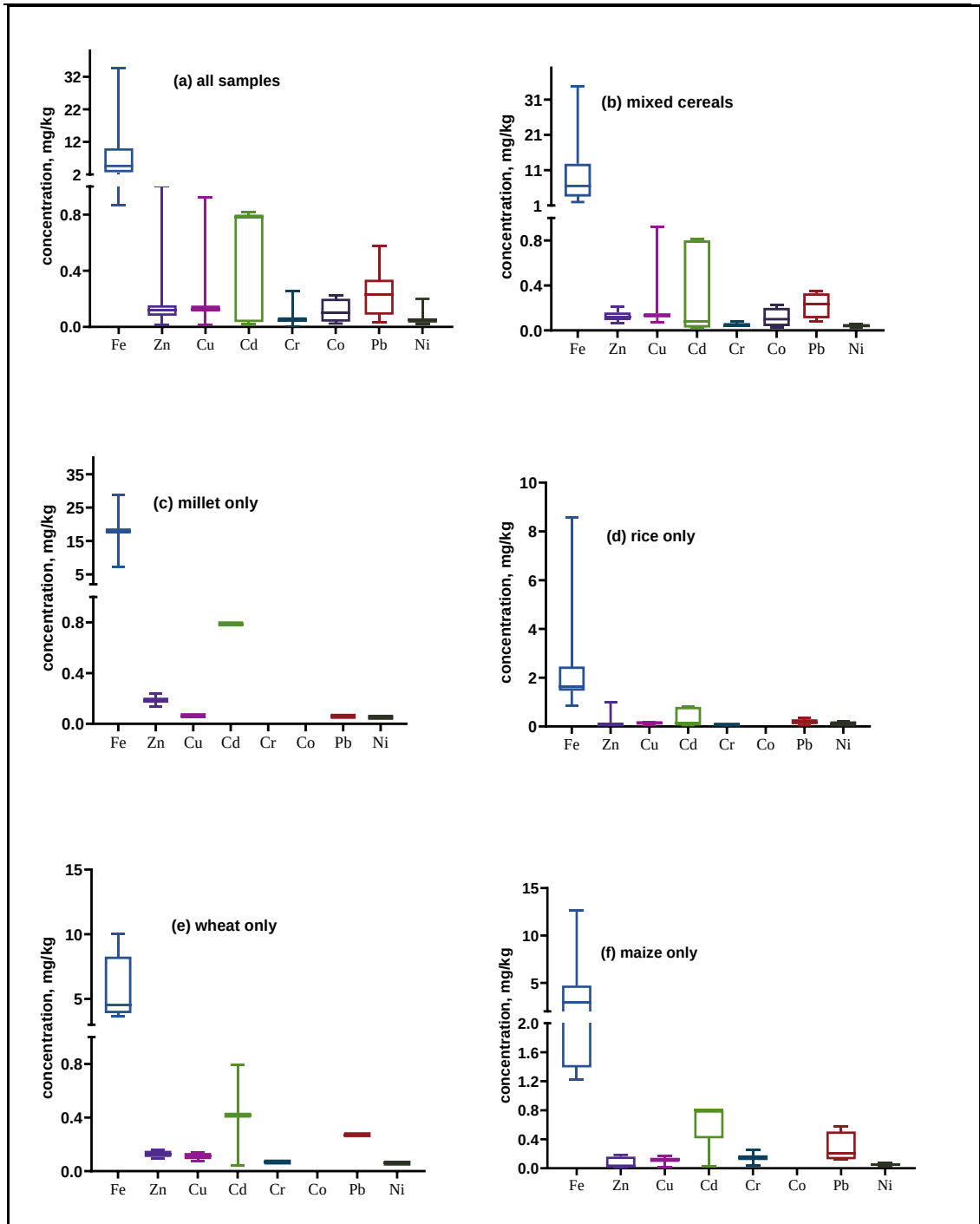


Fig. 4.1 1Box and Whiskers plot of metal concentrations for: (a) all samples, (b) mixed cereals only, (c) millet only, (d) rice only, (e) wheat only, and (f) maize only. Whiskers indicate the maximum and minimum concentrations.

4.1.2 Comparison of Metal Concentrations with Previous Studies

A survey of literature reports on metal concentrations in cereal-based baby foods in comparison with this study is presented in Table 4.3. The concentrations of Fe measured in this study were higher than those reported for Libya by Elbagermi et al. (2017) and Nigeria by Iwegbue et al. (2010) but lower when compared to observations from studies in Saudi Arabia by Al Khalifa & Ahmad, (2010) and Turkey by Saracoglu et al. (2007). The concentration of Fe in the baby food samples reported for Saudi Arabia, was greater than the measured concentrations of Fe in this study by a factor of 4 (Table 4.3). This notable difference, could be due to the fact that, baby foods in Saudi Arabia were reportedly fortified with iron as a dietary iron requirement (Al Khalifa & Ahmad, 2010). Zinc concentrations in this work were also low when compared to previous works as shown in Table 4.3. According to the Codex Alimentarius Commission (2011), cereals generally have low concentrations of zinc. Thus to meet Zn requirements for babies via cereal-based foods, the cereal products can be fortified using both plant and animal sources of Zn.

The cadmium concentrations measured in this study were however higher than all the previous studies shown in Table 4.3. The highest cadmium concentration was more than the FAO/WHO as reported by Codex Alimentarius Commission, (2011) and Ghana FDA recommended concentration of 0.10 mg/kg by a factor of about 8 (GS CODEX STAN 193, 2019). The high concentration of cadmium in this study could be as a result of contamination at the milling stage due to wear and tear of the milling plates. Although Cd and Pb are both toxic metals, the mean concentrations of Pb reported in this study were notably lower than the recommended concentration

guidelines provided by FAO/WHO (Codex Alimentarius Commission, 2011) and FDA (GS CODEX STAN 193, 2019) for baby foods but that was not in the case of Cd.

The maximum concentration of Pb measured in this study was consistent with a similar study in Iran by Sadeghi et al. (2014) but higher when compared with results of similar studies in India by Tripathi et al. (1999), Libya by Elbagermi et al. (2017), Nigeria by Iwegbue et al. (2010), Pakistan by Kazi et al. (2010), Ethiopia by Eticha et al. (2018), Saudi Arabia by Al Khalifa & Ahmad, (2010), and Romania by Edward et al. (2013) as illustrated in Table 4.3.

Table 4.3 Comparison of concentrations (mg/kg) of metals in the cereal-based baby foods with previous studies and international standards

Country	n	Fe	Zn	Cd	Pb	reference
Ghana	50	8.59 ± 9.36* (0.87 – 34.74)**	0.13 ± 0.14 (<0.001 – 1.0)	0.20 ± 0.34 (<0.002 – 0.82)	0.06 ± 0.12 (<0.001 – 0.58)	This work
FAO/WHO	-	-	-	0.10	0.20	(Codex, 2011)
FDA (Ghana)	-	-	-	0.10	0.20	(GS Codex Stan 193, 2019)
India	48	-	(9.37 – 34.60)	(0.001 – 0.018)	(0.04 – 0.08)	(Tripathi et al., 1999)
Libya	9	(1.47 – 4.67)	-	(0.01 – 0.03)	(0.01 – 0.02)	(Elbagermi et al., 2017)
Iran	240	-	118.6 ± 14.7	0.05 ± 0.01	0.45 ± 0.01	(Sadeghi et al., 2014)
Nigeria	19	(4.3 – 26.6)	(1.72 – 7.81)	(<0.001 – 0.02)	(<0.001 – 0.10)	(Iwegbue et al., 2010)
Pakistan	500	-	-	(0.026 – 0.09)	(0.05 – 0.09)	(Kazi et al., 2010)
Ethiopia	-	-	(27.90 – 71.55)	Not detected	(BDL – 0.10)	(Eticha et al., 2018)
Saudi Arabia	5	130.1 ± 76.1	24.6 ± 6.54	0.02 ± 0.003	0.02 ± 0.003	(Al Khalifa & Ahmad, 2010)
Romania	63	-	-	(0.0003 – 0.01)	(0.001 – 0.01)	(Edward et al., 2013)
Turkey	19	(1.02 – 67.5)	(0.92 – 37.2)	-	-	(Saracoglu et al., 2007)
* Mean concentration ()** range BDL – below detection limit						

4.1.3 Comparison of Metal Concentrations in the Samples Based on Brands.

The metal concentrations of all the brands including Set 1 to Set 4 (sets of unbranded samples from the same producer) are represented graphically in Fig.4.2 (a–h). The metals Fe, Zn, Cu, Co, Cr, Cd, Pb, and Ni were present in the brands at varying concentrations. Among the samples that were branded (per the labelling details), there was no significant difference ($p > 0.05$) in concentrations for the metals: Fe, Zn, Cu, Co, Cr, and Pb. However, there was an observed significant difference ($p < 0.05$) in the concentrations of Cd and Ni among the various brands (Appendix 10). The observed variability for Cd and Ni in the branded samples could be due to differences in the composition of raw materials (cereal types) used, difference in milling technology and equipment, difference in cooking expertise or difference in cooking materials.

Fe and Zn were the most dominant metals present in the branded samples as shown in Fig 4.2 (a & b) while cobalt was the least measured in only two brands: FSC (0.02–0.12 mg/kg) and JF (<LOD – 0.23 mg/kg) (Fig. 4.2e). The brands FSC (3.80 – 32.47 mg/kg), VFC (2.69 – 28.01 mg/kg), SF (34.22 mg/kg), JF (3.72 – 34.74 mg/kg), and Set 2 (1.46 – 28.84 mg/kg) exhibited exceptionally high iron concentration from the other brands (Fig. 4.2 a). Cr, Co and Ni occurred at low concentrations with their maximum concentrations below 0.30 mg/kg (Fig 4.2 d–f). Only 5 brands: BCF (<LOD – 0.26 mg/kg), JWG (0.20 mg/kg), Set 2 (<LOD – 0.35 mg/kg), Set 3 (0.12 – 0.35 mg/kg) and Set 4 (0.04 – 0.58 mg/kg) recorded values for lead with the highest concentration in Set 4 (Fig 4.2 h). These same 5 brands also recorded for nickel but of lower concentration (Fig 4.2 f).

The following brands BCF (<LOD – 0.82 mg/kg), JWG (0.81 mg/kg), Set 2 (<LOD – 0.80 mg/kg), Set 3 (0.80 – 0.81 mg/kg) and Set 4 (0.80 mg/kg) recorded high maximum

concentration of cadmium above the FAO/WHO by Codex Alimentarius Commission, (2011) and Ghana FDA (GS Codex Stan 193, 2019) recommended concentration of 0.10 mg/kg by factor of approximately 8 (Fig 4.2 g). These brands could be responsible for the high mean cadmium concentrations in this study.

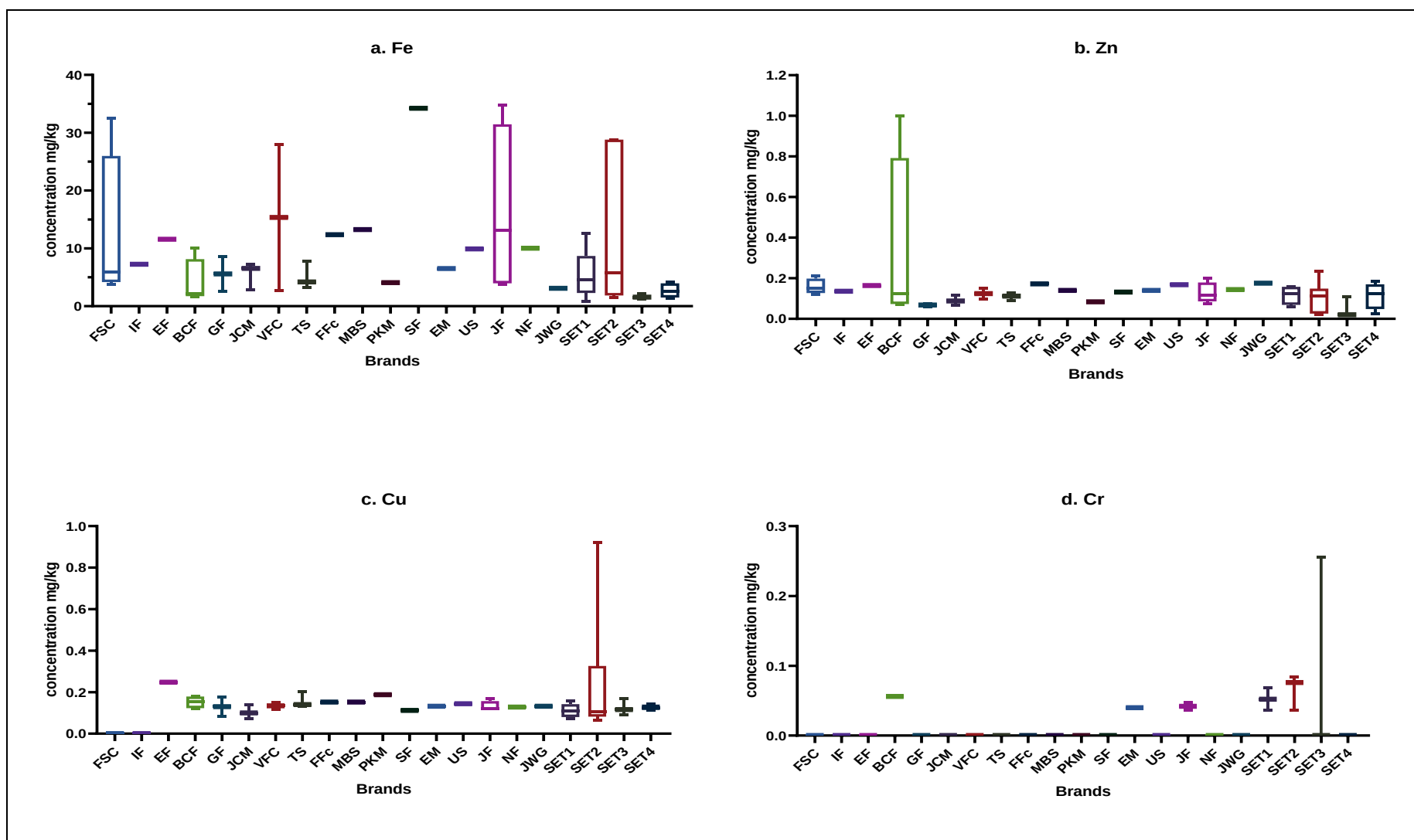


Fig. 4.2 (a-d) Box and Whiskers plot of metal concentrations in the samples based on brands for: (a) iron, (b) Zinc, (c) Copper, and (d) Chromium. Whiskers indicate the maximum and minimum concentrations

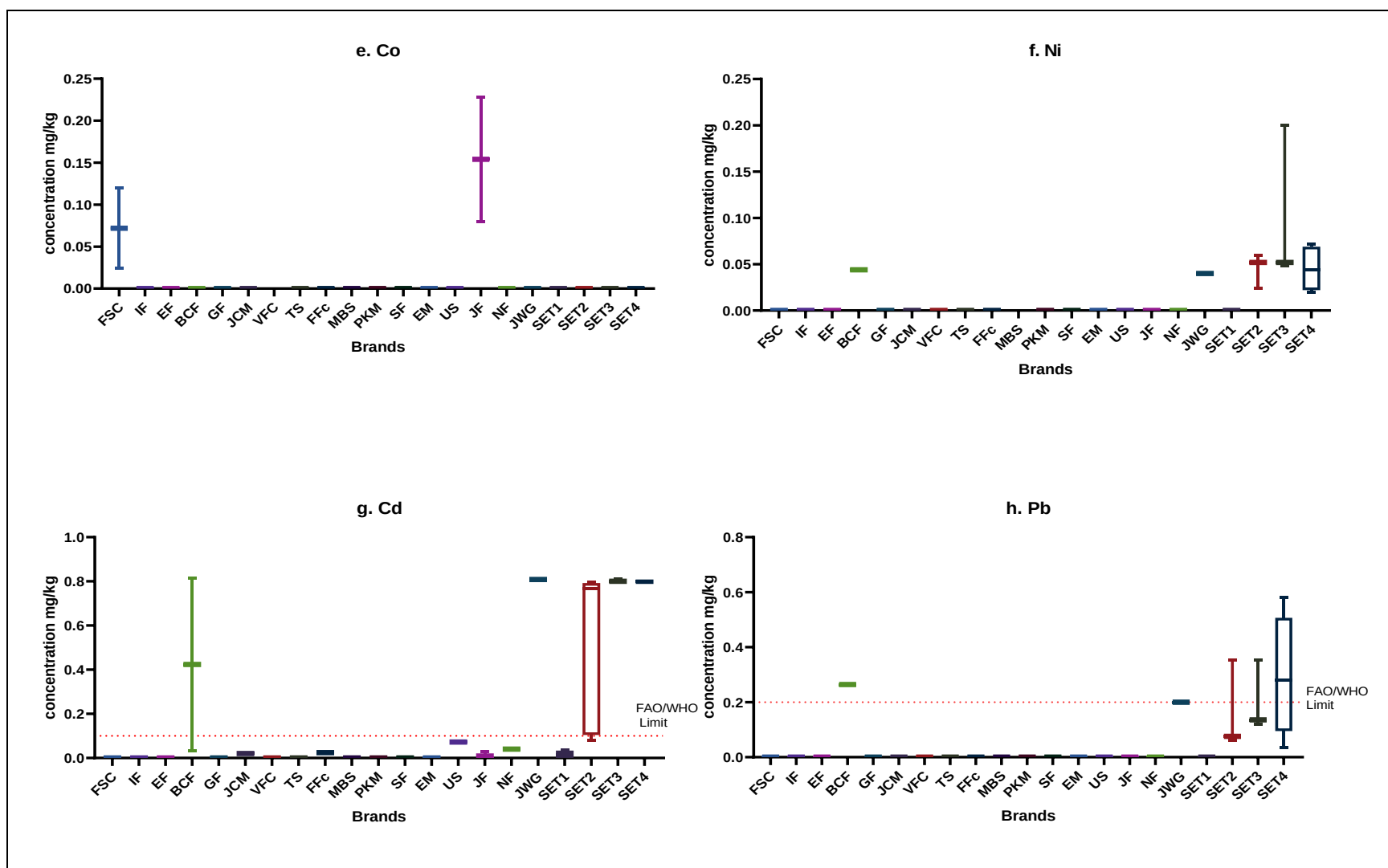


Fig. 4.2 (e-h) Box and Whiskers plot of metal concentrations in the samples based on brands for: (e) cobalt, (f) nickel, (g) cadmium, and (h) lead. Whiskers indicate the maximum and minimum concentrations

4.1.4 Potential Human Health Risk Assessment Associated with Metal

Contamination

The potential human health risks associated with exposure to the metals: iron, cadmium, and lead were estimated in this work based on formulae from literature and recommended values (Korley et al., 2019; Nantongo, 2019; USEPA 1989). The concentrations of zinc, copper, cobalt, chromium, and nickel were generally low and were not included in the assessment of health risk. The estimated chronic intake and the risk hazard indices computed for the age ranges of 6–8 months, 9–11 months, and 12–23 months are presented in Appendix 11. Summary of the potential health risks is shown in Table 4.4 and discussed under this section.

Table 4.4 Estimated health risk indices associated with metals (Fe, Cd and Pb) contamination of the cereal-based baby food samples (n = 50)

	Age	Statistics			% above 1.0
		mean	St. dev.	Range	
HQ (Fe)	6 - 8 months	0.41	0.45	0.04 – 1.68	14
	9 - 11 months	0.56	0.60	0.06 – 2.26	14
	12 - 23 months	0.86	0.92	0.09 – 3.47	26
HQ (Cd)	6 - 8 months	13.61	22.73	<LOD – 55.16	44
	9 - 11 months	18.30	30.56	<LOD – 74.18	44
	12 - 23 months	28.12	46.97	<LOD – 114.01	44
HQ (Pb)	6 - 8 months	0.53	1.18	<LOD – 5.60	18
	9 - 11 months	0.71	1.58	<LOD – 7.53	18
	12 - 23 months	1.09	2.44	<LOD – 11.58	22
HI	6 - 8 months	14.55	23.52	0.10 – 59.84	54
	9 - 11 months	19.95	32.23	0.13 – 84.53	54
	12 - 23 months	30.07	48.61	0.20 – 123.69	58

The calculated hazard quotient for Fe, Cd, and Pb, and the hazard index for all the samples showed that consumption of some cereal-based baby foods may pose health risks to the respective target groups since hazard quotients and hazard indices were > 1.0 in some cases. Generally, potential health risk indices increased with increasing age group, and this was due to the increasing intake rate with age. It was observed that cadmium was the major contributing factor to the high hazard index across the specified age groups. Hazard quotients for cadmium were in the range: <LOD – 55.16, <LOD – 74.18, and <LOD – 114.01 for age groups 6–8 months, 9–11 months, and 12–23 months respectively. Although the concentrations of iron in all the samples were relatively higher than cadmium and lead, iron posed the least health risk compared to Cd and Pb.

4.2 PAHs Contamination in the Cereal-based Baby Food Samples

The results of this work showed the presence of 15 out of 18 target PAHs in the samples at varying concentrations. The analytical calibration curves of the PAHs showed a good linearity coefficient (r^2) ranging from 0.8656 to 1.000 for acenaphthene and naphthalene respectively. The average percentage recoveries of 82 – 96 % were consistent with the acceptable range of 70 – 120 % (CORESTA Guide No. 5, 2018). Summary of the concentrations of PAHs in the samples was presented in Table 4.5 while the raw results of the PAHs were presented in Appendix 12. Naphthalene was dominant in all the samples with 100% detection while acenaphthene, fluorene, and dibenzo(a,h)anthracene were all below the respective detection limits. Statistically, there was no significant difference ($p > 0.05$) between the PAHs concentration based on the type of cereal compositions (Appendix 13).

The concentrations of total PAHs in the samples were in a range of <LOD – 890 µg/kg with a mean of 276.7 ± 256.2 µg/kg. Naphthalene and acenaphthylene both recorded the highest concentrations of 700 µg/kg, but had mean concentrations of 90.7 ± 125.3 and 64.3 ± 162.1 respectively, followed by anthracene with the maximum concentration of 300 µg/kg and a mean of 66.3 ± 94.8 µg/kg.

The benzo(a)pyrene and PAH4 [sum of benzo(a)pyrene, chrysene, benzo(b)fluoranthene and benzo(a)anthracene], which have been set as markers by the European Commission for assessing carcinogenic and mutagenic PAHs in foods European Commission Regulation No 1881 (2006) were both present in 33.3 % of the total samples. Benzo(a)pyrene and PAH4 were in the range of <LOD – 40 µg/kg and <LOD – 140 µg/kg with mean concentrations of 5.0 ± 9.0 µg/kg and 16.0 ± 32.2 µg/kg respectively. These concentrations could pose health concerns since they exceeded the recommended maximum limit of 1.0 µg/kg in processed baby foods including cereal-based foods (Aldrighi et al., 2018b; European Commission Regulation No 1881, 2006; Rozentale et al., 2017; Toxicological Factsheet Series, 2015). The high concentrations of benzo(a)pyrene and PAH4 in the samples could be partially due to unregulated food processing temperatures, and the type of fuel used in the preparation. Rose et al. (2015) reported on different PAHs levels for different fuel used and Abdel-shafy & Mansour, (2016) also reported that food cooked at high temperature through grilling, roasting, or frying form major sources of PAHs generation.

Previous studies from other countries indicate benzo(a)pyrene concentration in baby foods from Romanian by Soceanu et al. (2016), UK survey by White et al. (2004), and Poland by Ciecierska and Obiedzin'ski, (2010), were 0.12 – 0.26 µg/kg, <0.01 – 1.0 µg/kg, and 0.17 – 0.21 µg/kg respectively. The concentration of benzo(a)pyrene in this

study was about a factor of 40 higher than results obtained from Romania, Poland, and UK.

Table 4.5 Summary of PAHs concentrations ($\mu\text{g/kg}$) in the samples

PAHs	N	Range	Mean	Std. Deviation
Naphthalene	30	10 – 700	90.7	125.3
Acenaphthylene	30	<LOD – 700	64.3	162.1
Acenaphthlene	30	-	-	-
Fluorene	30	-	-	-
Anthracene	30	<LOD – 300	66.3	94.8
Phenanthrene	30	<LOD – 40	4.7	9
Fluoranthene	30	<LOD – 60	13	18.6
Pyrene	30	<LOD – 60	11.3	17.4
Benzo(a)anthracene	30	<LOD – 50	5.3	12
Chrysene	30	<LOD – 30	3.7	8.5
Benzo(a)pyrene	30	<LOD – 40	5	9
Benzo(b)fluoranthene	30	<LOD – 20	2	4.8
Benzo(e)pyrene	30	<LOD – 10	0.3	1.8
Pyrene	30	<LOD – 20	2	4.8
Benzo(k)fluoranthene	30	<LOD – 50	2.7	9.8
Indeno(1,2,3-c,d)pyrene	30	<LOD – 90	3	16.4
Dibenzo(a,h)anthracene	30	-	-	-
Benzo(g,h,i)perylene	30	<LOD – 70	2.3	12.8
ΣPAH4^*	30	<LOD – 140	16	32.2
ΣPAHs^{**}	30	<LOD – 890	276.7	256.2

* ΣPAH4 refers to the sum of benzo(a)anthracene, chrysene, benzo(a)pyrene, and benzo(b)fluoranthene

** ΣPAHs refers to the sum of all the PAHs in the sample.

4.2.1 Comparison of PAHs Concentrations between Samples Based on Cereal Compositions

Summary of PAHs concentration in the samples based on their cereal compositions: rice, millet, wheat, maize and mixed cereals are presented in Table 4.6. There were no significant difference ($p > 0.05$) in PAHs concentration among the mixed cereals, except the naphthalene that showed significant difference ($p < 0.05$) among the mixed cereals as presented in Appendix 14. The significant difference ($p < 0.05$) observed for naphthalene in the mixed cereal samples could be due to its smaller molecular weight and easy to form even at lower temperatures.

Naphthalene and acenaphthylene were the only two PAHs measured in the samples with single cereal composition viz. millet, rice, and maize. Details on the sample labels, showed that the rice samples were not roasted in the course of preparation but yet recorded naphthalene and acenaphthylene at concentrations in the range of 120 – 180 $\mu\text{g/kg}$ and 10 – 20 $\mu\text{g/kg}$ respectively. Thus, the PAHs measured in the rice samples in particular, could be due to other factors such as cultivation or during processing and packaging. This observation however agrees with findings by Aldrighi et al. (2018) where PAHs were also measured in rice samples that were not subjected to roasting. The samples containing maize or millet only were roasted and they recorded total PAHs of 60 – 80 $\mu\text{g/kg}$ and 120 $\mu\text{g/kg}$ respectively. The presence of PAHs in the maize and millet could be due to food processing factors such as unregulated roasting temperatures, cooking duration or type of fuel.

The concentrations of benzo(a)pyrene, sum of PAH4 and total PAHs in samples composed of wheat only were in the range of $<\text{LOD}$ – 10 $\mu\text{g/kg}$, $<\text{LOD}$ – 40 $\mu\text{g/kg}$ and 120 – 340 $\mu\text{g/kg}$ respectively. The benzo(a)pyrene measured in the samples composed

of wheat only could be as a result of prolonged cooking. This result was higher than the concentration reported by Rozentale et al. (2017) for wheat bread: 0.22 – 1.07 µg/kg (PAH4), 0.49 µg/kg (mean PAH4) and 0.064 µg/kg (benzo(a)pyrene).

The concentrations of benzo(a)pyrene, the PAH4 and the total PAHs measured in the mixed cereals in this work, were in the range: <LOD – 40 µg/kg, <LOD – 140 µg/kg and 20 – 890 µg/kg respectively. The observed concentrations of PAHs in the samples of mixed cereal compositions could be as a result of the presence of other supplements such as groundnut and/or soyabean. The content of PAHs in such cereal-based baby foods could be increased when oil-containing cereals or legumes are subjected to excessive heating processes such as roasting at high temperatures (Toxicological Factsheet Series, 2015).

Table 4.6 Summary of PAHs concentrations ($\mu\text{g/kg}$) across all samples types based on cereal composition.

PAHs	Composition of cereals in $\mu\text{g/kg}$																			
	Millet				Rice				Wheat				Maize				Mixed Cereals			
	Conc.	N	Std. Dev.	Range	Mean	N	Std. Dev.	Range	Mean	N	Std. Dev.	Range	Mean	N	Std. Dev.	Range	Mean	N	Std. Dev.	Range
NAP	80	1	-	-	150	2	42	120–180	55	2	49	20–90	60	2	-	60–60	92	23	141	10–700
ACA	40	1	-	-	15	2	7	10–20	20	2	14	10–30	10	2	14	ND–20	78	23	184	ND–700
ACE	ND	1	-	-	ND	2	-	-	ND	2	-	-	ND	2	-	-	ND	23	-	-
FLU	ND	1	-	-	ND	2	-	-	ND	2	-	-	ND	2	-	-	ND	23	-	-
ANT	ND	1	-	-	ND	2	-	-	95	2	134	ND–190	ND	2	-	-	78	23	99	ND–300
PHE	ND	1	-	-	ND	2	-	-	ND	2	-	-	ND	2	-	-	6	23	10	ND–40
FLT	ND	1	-	-	ND	2	-	-	20	2	28	ND–40	ND	2	-	-	15	23	19	ND–60
PYR	ND	1	-	-	ND	2	-	-	15	2	21	ND–30	ND	2	-	-	13	23	18	ND–60
BAA	ND	1	-	-	ND	2	-	-	5	2	7	ND–30	ND	2	-	-	7	23	13	ND–50
CHR	ND	1	-	-	ND	2	-	-	10	2	14	ND–20	ND	2	-	-	3	22	7	ND–30
BAP	ND	1	-	-	ND	2	-	-	5	2	7	ND–10	ND	2	-	-	6	23	10	ND–40
BBF	ND	1	-	-	ND	2	-	-	ND	2	-	-	ND	2	-	-	3	23	5	ND–20
BEP	ND	1	-	-	ND	2	-	-	ND	2	-	-	ND	2	-	-	ND	23	2	ND–10
PYL	ND	1	-	-	ND	2	-	-	5	2	7	ND–10	ND	2	-	-	2	23	5	ND–20
BKF	ND	1	-	-	ND	2	-	-	ND	2	-	-	ND	2	-	-	3	23	11	ND–50
IND	ND	1	-	-	ND	2	-	-	ND	2	-	-	ND	2	-	-	4	23	19	ND–90
DAA	ND	1	-	-	ND	2	-	-	ND	2	-	-	ND	2	-	-	ND	23	-	-
BGP	ND	1	-	-	ND	2	-	-	ND	2	-	-	ND	2	-	-	3	23	15	ND–70
ΣPAH_4	ND	1	-	-	ND	2	-	-	20	2	28	ND–40	ND	2	-	-	19	23	36	ND–140
ΣPAHs	120	1	-	-	165	2	35	140–190	230	2	156	120–340	70	2	14	60–80	277	23	256	20–890

ND means concentrations were not detected

4.2.2 Distribution of Carcinogenic and Non-carcinogenic PAHs in the Samples

The results obtained from this study showed varying concentrations of both carcinogenic and non-carcinogenic PAHs in the cereal-based baby food samples. The concentrations of non-carcinogenic PAHs were higher than the carcinogenic PAHs by a factor of about 10. All the samples recorded at least two of non-carcinogenic PAHs which include naphthalene (10 – 700 µg/kg), acenaphthylene (<LOD – 700 µg/kg), and anthracene (<LOD – 300 µg/kg) shown in Appendix 12.

The carcinogenic PAHs which include benzo(a)pyrene (<LOD – 40 µg/kg), indeno(1,2,3-c,d) pyrene (<LOD – 90 µg/kg), benzo(a)anthracene (<LOD – 50 µg/kg), benzo(b)fluoranthene (<LOD – 20 µg/kg), chrysene (<LOD – 30 µg/kg), and benzo(k)fluoranthene (<LOD – 50 µg/kg) occurred in 1/3 of the total samples (Appendix 12). The carcinogenic PAHs were present in samples of wheat only and mixed cereals compositions, but were not measured in samples composed of maize only, rice only and millet only (Table 4.6). Although the concentrations of carcinogenic PAHs exceeded the FAO/WHO recommended limits in baby foods, concentrations of the PAHs were notably lower compared to the non-carcinogenic PAHs (Table 4.6). To investigate whether the concentrations of the carcinogenic PAHs which were above the FAO/WHO limits pose any cancer risk to babies, further risk assessments were carried out and reported in the next sub-section (4.2.3).

4.2.3 Potential Human Health Risk Assessment Associated with PAHs

Contamination

The results of the human risk assessment of PAHs contaminations in babies between the ages of 6–8 months, 9–11 months, and 12–23 months are shown in Table 4.7. The

BAPEQ were calculated for carcinogenicity and mutagenicity using the TEQ values and MEQ values respectively as shown previously in Chapter 3. The results show that for benzo(a)pyrene equivalent as BAPEQ and also carcinogenic and mutagenic risks for all the age groups occurred in 33.33% of the samples analysed.

The carcinogenic risk based on $BAPEQ_{\text{carcinogenic}}$ (Table 4.7) were: $2.47 \times 10^{-6} - 1.40 \times 10^{-5}$ (for 6-8 months), $3.32 \times 10^{-6} - 1.88 \times 10^{-5}$ (9-11 months), and $5.10 \times 10^{-6} - 2.88 \times 10^{-5}$ (for 12-23 months). This implies that for every one million (1.0×10^6) babies feeding on the products that recorded carcinogenic PAHs, approximately 2 – 14 babies (for ages 6–8 months), 3 – 19 babies (for ages 9–11 months) and 5 – 29 babies (for 12–23 months) may pose cancer and other non-cancer related health risks. Similarly, the mutagenic risk based on $BAPEQ_{\text{mutagenic}}$ (Table 4.7) were: $2.47 \times 10^{-6} - 2.05 \times 10^{-5}$ (for ages 6-8 months), $3.32 \times 10^{-6} - 2.75 \times 10^{-5}$ (for ages 9-11 months), and $5.10 \times 10^{-6} - 4.23 \times 10^{-5}$ (for ages 12-23 months). Thus out of every 1 million (1.0×10^6) babies: 2 – 20 (for ages 6–8 months), 3 – 28 (for ages 9–11 months), and 5 – 42 (for ages 12–23 months) may have mutagenic effect and other non-carcinogenic health effects emanating from PAHs contaminated samples (food).

The result of the health risk assessment obtained in this work is consistent with findings reported elsewhere by Asamoah et al. (2019). In a study on health risks associated with breastfeeding in children, it was reported that 1 out of 1×10^5 and 2 out of 1×10^5 infants can be susceptible to cancer and non-cancer effects (Asamoah et al., 2019).

Table 4.7 Results of carcinogenic and mutagenic risk assessment due to exposure to PAHs in the samples.

Sample	BAPEQ (carcinogenic) µg/kg/day			BAPEQ (mutagenic) µg/kg/day			risk (carcinogenic) x10 ⁻⁶			risk (mutagenic) x10 ⁻⁶		
	6–8 months	9–11 months	12–23 months	6–8 months	9–11 months	12–23 months	6–8 months	9–11 months	12–23 months	6–8 months	9–11 months	12–23 months
BFC1	1.91	2.57	3.95	2.81	3.77	5.80	13.95	18.76	28.83	20.48	27.54	42.33
BFC2	0.82	1.10	1.69	0.92	1.24	1.90	5.97	8.03	12.34	6.70	9.01	13.85
BFC3	0.44	0.60	0.92	0.52	0.70	1.08	3.24	4.35	6.70	3.80	5.11	7.86
BFC5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC11	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC12	0.37	0.50	0.77	0.38	0.51	0.78	2.72	3.66	5.62	2.75	3.70	5.70
BFC13	0.37	0.50	0.77	0.37	0.50	0.77	2.72	3.65	5.612	2.71	3.65	5.60
BFC14	0.81	1.09	1.68	0.86	1.16	1.78	5.93	7.97	12.25	6.28	8.45	12.98
BFC15	0.37	0.50	0.77	0.37	0.50	0.77	2.72	3.65	5.62	2.71	3.65	5.60
BFC16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC17	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC18	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC19	0.34	0.46	0.70	0.34	0.46	0.70	2.47	3.32	5.10	2.47	3.32	5.10
BFC20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

BFC22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC25	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC27	0.37	0.50	0.77	0.42	0.57	0.87	2.71	3.65	5.61	3.08	4.15	6.37
BFC28	0.34	0.46	0.70	0.34	0.46	0.70	2.47	3.32	5.10	2.47	3.32	5.10
BFC30	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC41	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC44	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC46	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC49	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BFC50	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
mean	0.21	0.28	0.42	0.24	0.33	0.51	1.50	2.01	3.09	1.78	2.40	3.68
max	1.91	2.57	3.95	2.81	3.77	5.80	13.95	18.76	28.83	20.48	27.54	42.33

4.2.4 Preliminary Source Apportionment of PAHs

A simple diagnostic ratio of the results of the entire samples was run to ascertain whether the PAHs measured in the samples were coming from external pollution or contamination emanating from processing methods. The diagnostic tools used for tracking the source of PAHs were described by researchers elsewhere (Okedeyi et al., 2012; Ravindra et al., 2008; Okedeyi et al., 2012; Katsoyiannis & Breivik, 2014) based on the fact that different sources of PAHs exhibit different molecular composition (Cao et al., 2019).

The results of the diagnostic ratios of the PAHs are represented in Table 4.6. The ratios obtained were PHE/ANT (0.08), ANT/(ANT +PYR) (0.86), ANT/(ANT+PHE) (0.84), FLT/(FLT +PYR) (0.72) and BAA/(BAA + CHR) (0.63). The results were consistent with the set values established to be associated with pyrolytic sources of PAHs as shown in Table 4.6 (Katsoyiannis & Breivik, 2014; Okedeyi et al. 2012; Ravindra et al., 2008). The pyrolytic sources of PAHs indicate that the PAHs contamination in the cereal-based baby foods were predominantly due to the food processing protocols which involved heating (roasting) of the cereals. This could be due to the roasting of the cereals at high temperatures and/or the long duration of the roasting of the cereals. Studies by Rose et al. (2015) have confirmed that roasting of foods could introduce and/or induce more PAHs, and that increased roasting duration is relative to the formation of more PAHs in food.

Table 4.8 Diagnostic ratio results showing the source apportionment of PAHs in the cereal-based baby food samples.

Diagnostic ratio	This study	Petrogenic	Pyrolytic
^a PHE/ANT	0.08	>15	<10
^b ANT/(ANT + Pyr)	0.86	<0.1	>0.1
^c ANT/(ANT + PHE)	0.84	<0.1	>0.1
^d FLT/(FLT + Pyr)	0.72	<0.4	>0.4
^d BAA/(BAA +CHR)	0.63	<0.2	>0.35

^aOkedeyi et al. (2012); ^bRavindra et al. (2008); ^cOkedeyi et al. (2012); ^dKatsoyiannis & Breivik (2014)

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

5.1.1 Metals

This study measured a suite of metals in cereal-based baby foods. The concentrations of the metals across all samples were: 0.87 – 34.74 mg/kg (Fe), <0.001 – 1.00 mg/kg (Zn), <0.003 - 0.92 mg/kg (Cu), <0.002 – 0.82 mg/kg (Cd), <0.001 – 0.26 mg/kg (Cr), <0.005 – 0.23 mg/kg (Co), <0.001 – 0.58 mg/kg (Pb), and <0.001 – 0.20 mg/kg (Ni). Although the concentrations of all the metals were below the recommended limits, the mean concentration of Cd was notably above the FAO/WHO limit of 0.1 mg/kg. Nonetheless, the calculated hazard quotients indicate that exposure to metals in some of the cereal-based baby foods may affect the health of babies. The results showed that the hazard quotient for Fe (for both ages 6–8 months and 9–11 months) in 14% of the samples were > 1 and therefore indicative of potential health risk whereas for ages 12–23 months, hazard quotient for Fe in 26% of the samples were also >1. Similarly, the hazard quotient for Pb showed that 18 % of the samples were > 1 (for the ages: 6–8 months and 9–11 months), whereas 22 % were >1 for ages 12–23 months. Across all the studied age groups, 44% of the samples had hazard quotients >1 for cadmium. Pb and Cd are toxic metals that can bio-accumulate, thus their hazard quotients >1 in at least one sample is a cause for health concern. The calculated hazard indices based on the hazard quotients were > 1 for the ages 6–8 months and 9–11 months in approximately 54 % of the samples whereas for ages 12–23 months, hazard indices were > 1 in 58 % of the samples analysed. Generally, non-cancer

risk due to Fe, Pb, and Cd exposure is observed for all the age groups in more than 50 % across all samples.

5.1.2 PAHs

The results of this study also showed varying concentrations of both carcinogenic and non-carcinogenic PAHs in the samples. Naphthalene showed a 100 % incidence ratio in all the samples analysed. Out of 18 target PAHs: acenaphthylene, fluorene, and dibenzo(a,h)anthracene were not detected in any of the samples. The total PAHs were in a range of <LOD – 890 µg/kg with a mean of 276.7 ± 256.2 µg/kg. Concentrations of Benzo(a)pyrene and sum of PAH4, measured in 33.3% of the samples were in the ranges of <LOD – 40 µg/kg and <LOD – 140 µg/kg with mean concentrations of 5.0 ± 9.0 µg/kg and 16.0 ± 32.2 µg/kg respectively, above FAO/WHO recommended value of 1.0 µg/kg. All the carcinogenic PAHs were observed in samples composed of wheat only and mixed cereals. Naphthalene and acenaphthylene were measured in samples of single cereal composition: maize, rice, and millet only. The preliminary source apportionment predicted pyrolytic sources of PAHs contamination in the samples.

BaPEQ calculation for both carcinogenic and mutagenic risk using the TEQ and MEQ showed potential health risk to babies upon exposure. The results showed that for every 1×10^6 (one million) babies, a maximum of 14 (6–8 months), 19 (9–11 months), and 29 (12–23 months) babies may have cancer and non-cancer related health implications. The mutagenic risk also showed that for every 1×10^6 (one million) babies a maximum of 20 (6–8 months), 28 (9–11 months), and 42 (12–23 months) babies may have mutagenic health-related implications and other non-cancer related health concerns.

The result from the study shows that mutagenic, carcinogenic, and non-carcinogenic risks can be associated with some locally produced cereal-based baby foods available on the markets in the Greater Accra region. Thus, the consumption of such samples can be potentially harmful to babies within the studied age groups.

5.2 Recommendations

Based on the results of the study and the conclusions drawn from the study, the under-listed recommendations are proposed:

- (i)** There should be increased surveillance on the wholesomeness of cereal-based baby foods by regulatory authorities since cadmium, lead and PAHs showed potential carcinogenic, mutagenic, and other non-carcinogenic health effects to babies.
- (ii)** Production of cereal-based baby foods should start with quality raw materials and the production should be done in a well hygienic environment. The use of stainless steel mills can help eradicate the metals contamination in cereal-based baby food products.
- (iii)** Education of the producers about routes of PAHs contamination with emphasis on the roasting of cereals in safely regulated temperatures can curb PAHs contamination in the cereal-based baby foods produced in Ghana.
- (iv)** Future studies in this area should be expanded to cover the entire country particularly in the rural areas where regulatory activities are often relaxed due to lack of logistics.

- (v) An in-depth assessment of trace metals and PAHs contamination in baby foods should be carried out and establish appropriate program to improve baby food quality and safety in Ghana.

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APPENDICES

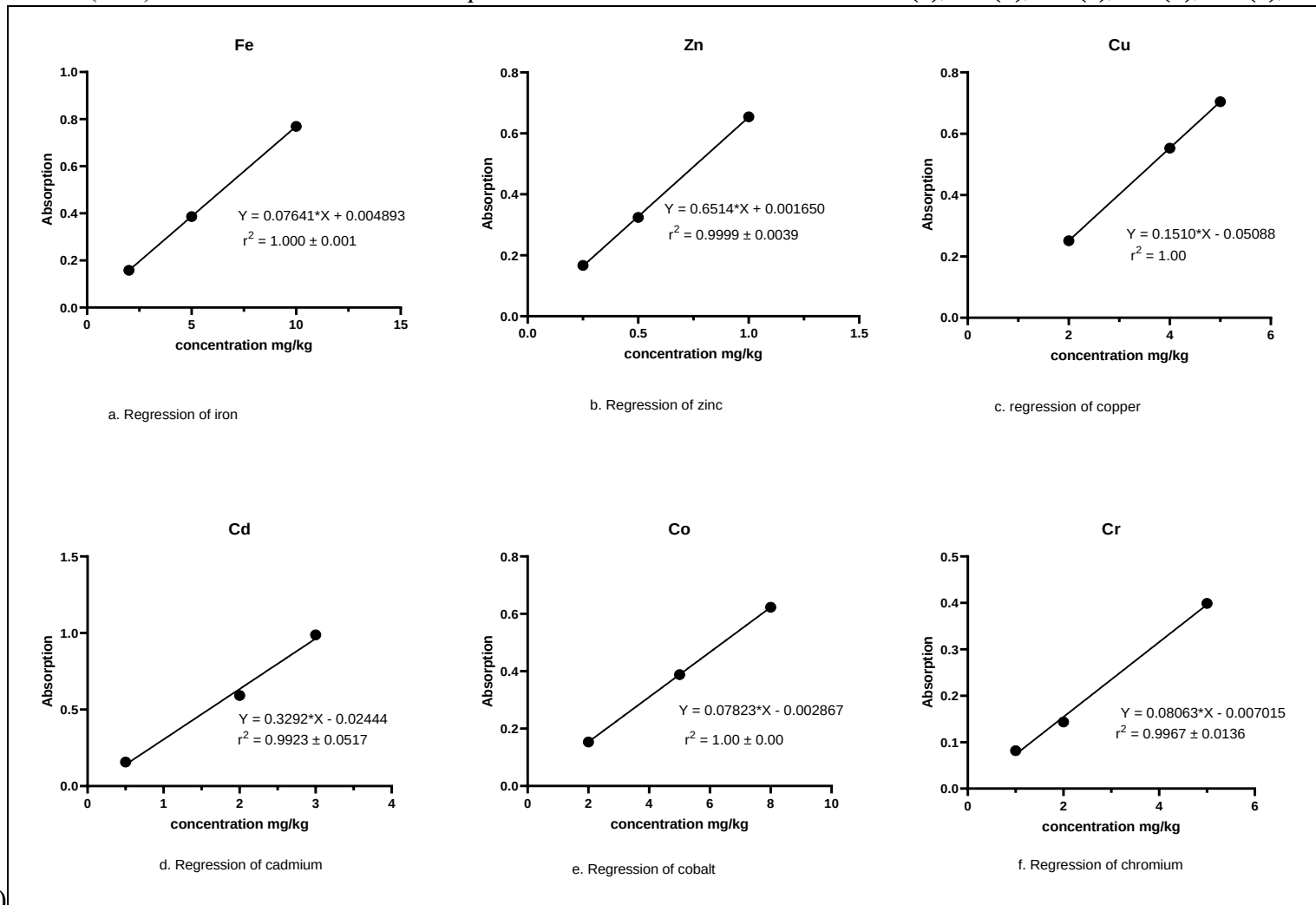
Appendix 1. Detailed information of the cereal-based baby food samples (Chapter 3)

Sample ID	Sale outlet/location	Brand name	Composition	Manf. Date	Expiry date	BATCH NO	FDA NO
BFC1	Achimota Hospital	Franca`s Special Cereamix (FSC)	Rice and soyabean	30/11/2019	29/11/2020	N/A	N/A
BFC2	Achimota Hospital	Franca`s Special Cereamix (FSC)	Wheat and soyabean	N/A	N/A	N/A	N/A
BFC3	Achimota Hospital	Franca`s Special Cereamix (FSC)	Millet and soyabean	23/11/2019	22/11/2020	N/A	N/A
BFC4	Achimota Hospital	Franca`s Special Cereamix (FSC)	Wheat, oat and soyabean	N/A	N/A	N/A	N/A
BFC5	Legon Hospital	Island Foods (IF)	Millet	06/10/2019	05/10/2020	N/A	FDA/CE 17-012
BFC6	Legon Hospital	Express Foods (EF)	Rice, wheat and soyabean	N/A	Jul-22	N/A	N/A
BFC7	Kwabanya	Blessed Child Foods (BCF)	Rice	N/A	Oct-21	09 219	N/A
BFC8	Kwabanya	Blessed Child Foods (BCF)	Rice	N/A	Nov-20	100119	N/A
BFC9	Kwabanya	Blessed Child Foods (BCF)	Rice, soyabean and groundnut	09/08/2019	10/09/2021	08 0119	FDA/Ce 15-005
BFC10	Kwabanya	Ghatty Foods (GF)	Rice and wheat	N/A	Oct-20	26219	N/A
BFC11	Kwabanya	Ghatty Foods (GF)	Rice	N/A	Jul-20	11990	N/A
BFC12	Korle Bu Hospital	Justina Cereal Mix (JCM)	Wheat	N/A	N/A	N/A	N/A
BFC13	Korle Bu Hospital	Justina Cereal Mix (JCM)	Rice, wheat, oat and soyabean	N/A	N/A	N/A	N/A
BFC14	Korle Bu Hospital	Justina Cereal Mix (JCM)	Rice and beetroot	N/A	N/A	N/A	N/A
BFC15	Korle Bu Hospital	Vic-Flow Cereal Mix (VFC)	Rice, soyabean and carrot	N/A	N/A	N/A	N/A
BFC16	Korle Bu Hospital	Vic-Flow Products (VFC)	Maize and groundnut	N/A	N/A	N/A	N/A
BFC17	Korle Bu Hospital	Theomens Soya Bean Prod. (TS)	Wheat and soyabean	N/A	N/A	N/A	N/A
BFC18	Korle Bu Hospital	Theomens Soya Bean Prod. (TS)	Rice and soyabean	N/A	N/A	N/A	N/A
BFC19	Korle Bu Hospital	Theomens Soya Bean Prod.	Maize and soyabean	N/A	N/A	N/A	N/A
BFC20	37- Millitary Hospital	Finer Foods (Ceresoya) (FFC)	Wheat, soyabean and milk	13/11/2019	13/11/2020	N/A	FDB/CE 10-007

BFC21	37 bus stop	Mummy Bernice Sweets (MBS)	Rice, wheat, millet, soyabean and groundnut	N/A	N/A	N/A	FDA/tb 1021
BFC22	37 station	Pagefive koko mix (PKM)	Wheat, maize, barley and skimmed milk	07/02/2019	12/06/2021	00 7	FDA-T044
BFC23	Accra Mall -(Shoprite)	Selasie (SF)	Millet and soyabean	Jan-19	Jan-22	02 659	N/A
BFC24	Tema Station	Enjoy Me (EM)	Rice, oat, millet soyabean and groundnut	N/A	N/A	N/A	N/A
BFC25	Madina market	Unick Soya –Rich (US)	Wheat and soyabean	Dec-18	Dec-20	unk1000	N/A
BFC26	Atomic hospital	Jay`s foods (Enermeal) (JF)	Wheat, oat and soyabean	Nov-19	Nov-20	OA#021	N/A
BFC27	Atomic hospital	Jay`s foods (Enermeal) (JF)	Rice, oat and soyabean	Sep-19	Sep-20	RI#013	N/A
BFC28	Atomic hospital	Jay`s foods (Enermeal) (JF)	Rice, wheat and soyabean	Nov-19	Nov-20	WH#020	N/A
BFC29	Atomic hospital	Jay`s foods (Enermeal) (JF)	Millet and soyabean	Nov-19	Nov-20	RI#021	N/A
BFC30	Atomic roundabout	NAAB foods (NF)	Wheat	N/A	N/A	N/A	N/A
BFC31	Madina market	N/A (SET1)	Wheat	N/A	N/A	N/A	N/A
BFC32	Madina market	N/A (SET 1)	Rice	N/A	N/A	N/A	N/A
BFC33	Madina market	N/A (SET1)	Maize	N/A	N/A	N/A	N/A
BFC34	Madina market	N/A (SET1)	Wheat	N/A	N/A	N/A	N/A
BFC35	Madina market	N/A(SET1)	Maize	N/A	N/A	N/A	N/A
BFC36	Makola market	N/A (SET2)	Maize	N/A	N/A	N/A	N/A
BFC37	Makola market	N/A (SET2)	Rice	N/A	N/A	N/A	N/A
BFC38	Makola market	N/A (SET2)	Millet	N/A	N/A	N/A	N/A
BFC39	Makola market	N/A (SET2)	Wheat and oat	N/A	N/A	N/A	N/A
BFC40	Makola market	N/A (SET2)	Rice and oat	N/A	N/A	N/A	N/A
BFC41	Makola market	N/A (SET2)	Rice, wheat and oat	N/A	N/A	N/A	N/A
BFC42	Tema Station Market	N/A (SET3)	Maize	N/A	N/A	N/A	N/A
BFC43	Tema Station Market	N/A (SET 3)	Rice	N/A	N/A	N/A	N/A

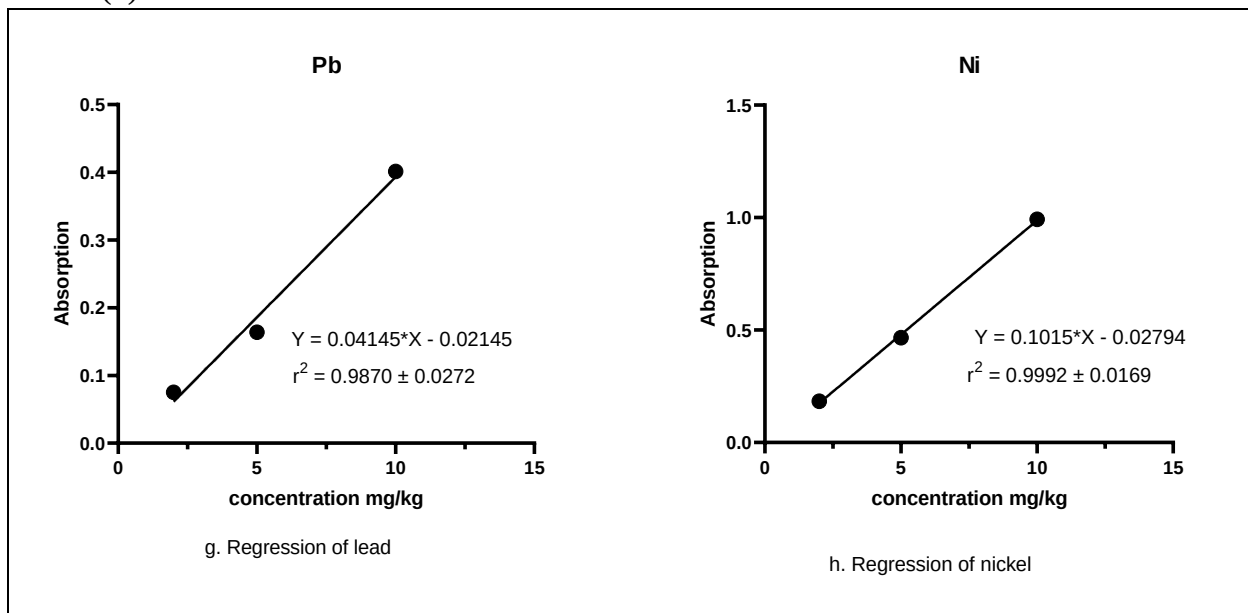
BFC44	Tema Station Market	N/A (SET 3)	Maize	N/A	N/A	N/A	N/A
BFC45	circle market	N/A (SET 4)	Rice	N/A	N/A	N/A	N/A
BFC46	circle market	N/A (SET 4)	Maize	N/A	N/A	N/A	N/A
BFC47	circle market	N/A (SET 4)	Wheat	N/A	N/A	N/A	N/A
BFC 48	circle market	N/A (SET4)	Maize	N/A	N/A	N/A	N/A
BFC 49	Kwabanya	Blessed Child Foods (BCF)	Maize, soyabean and groundnut	Nov-19	Dec-21	2	N/A
BFC 50	Kwabanya	Julia`s whole Grain Meal (JWG)	Wheat, millet, sorghum, soyabean and groundnut	25/01/2019	25/01/2020	N/A	N/A

Appendix 2 (a - f): Calibration curves for the quantitation of metal concentrations: Fe (a), Zn (b), Cu (c), Cd (d), Co (e), and

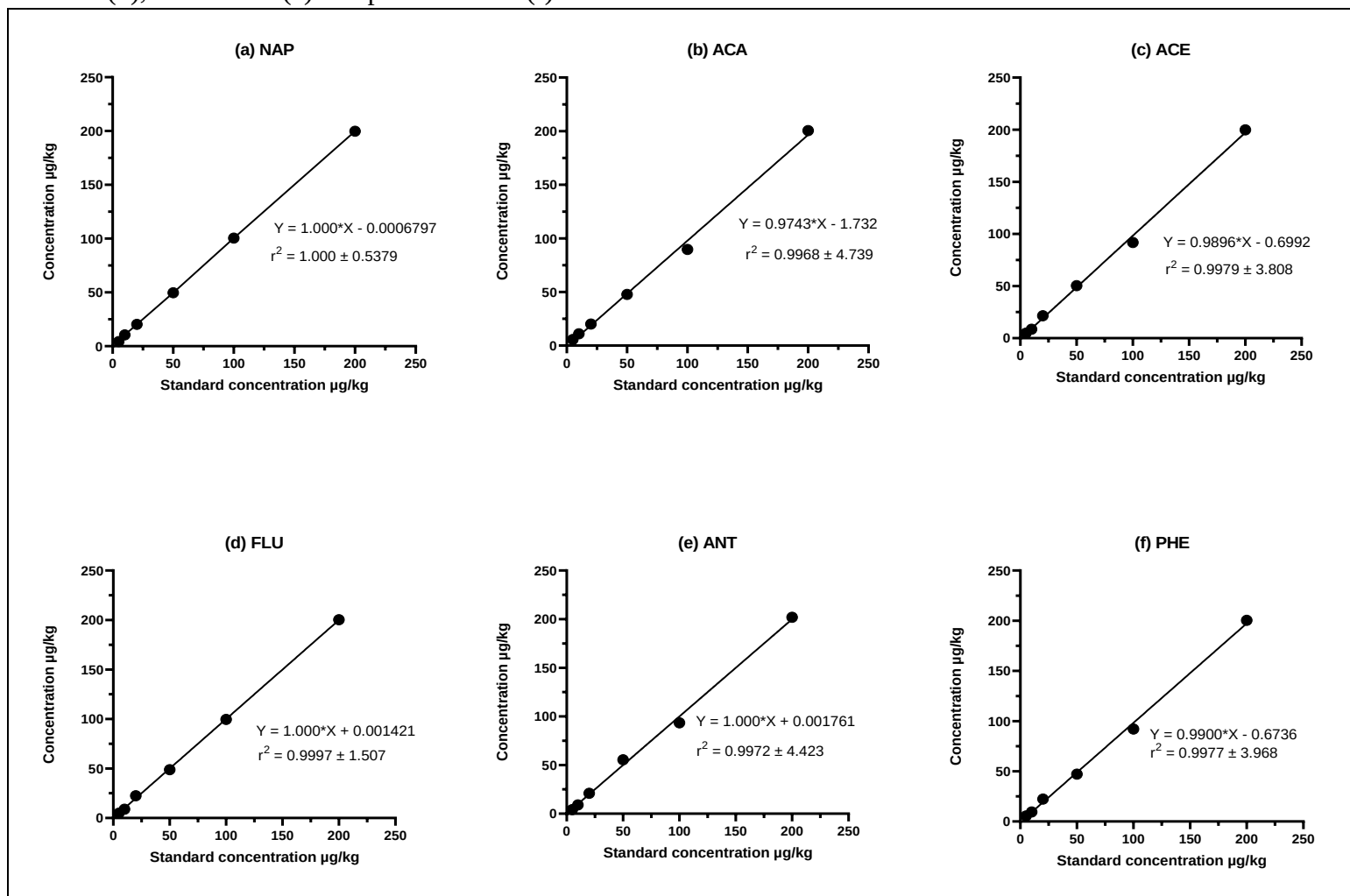


Cr (f)

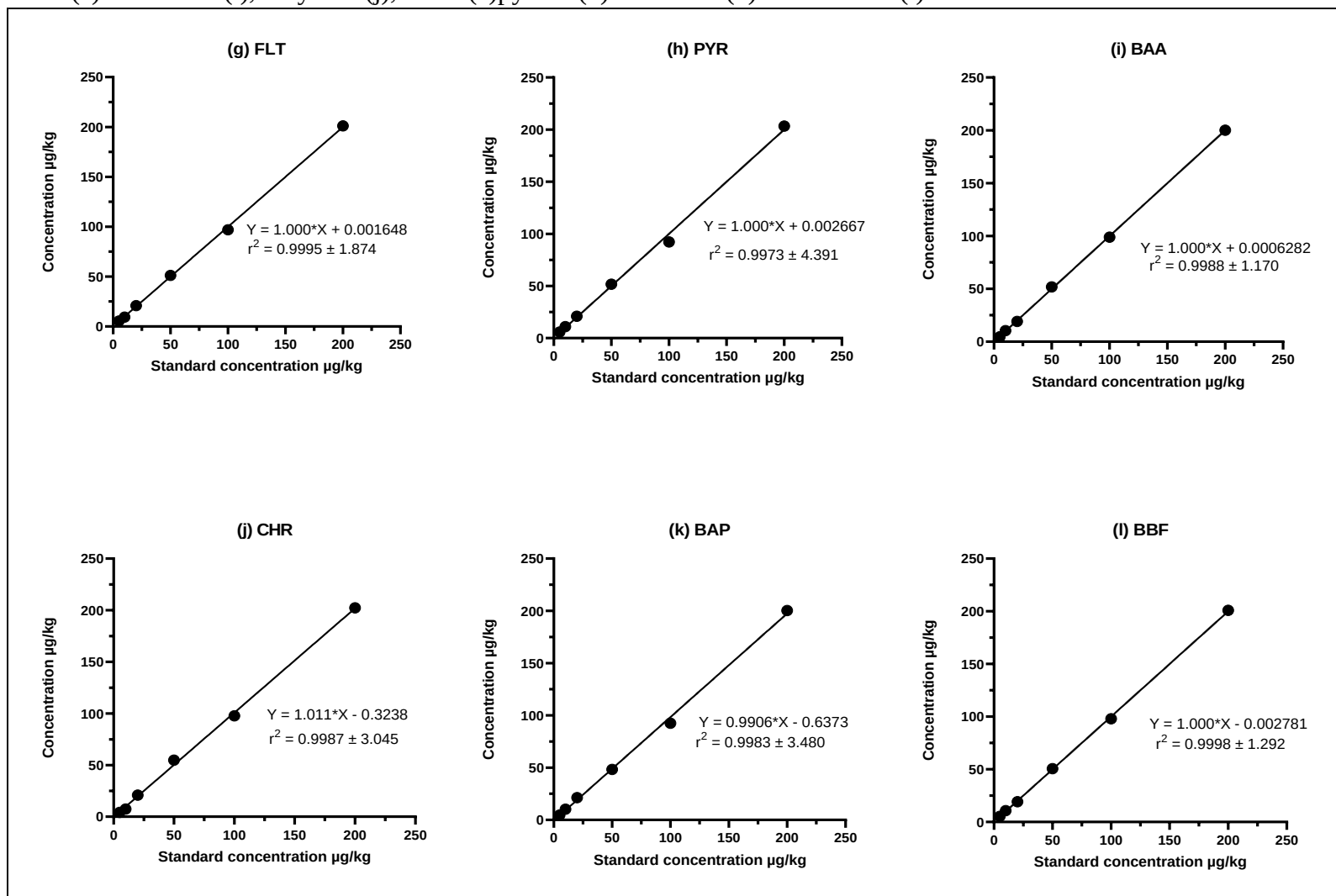
Appendix.2 (g-h): Calibration curves for the quantitation of metal concentrations: Pb (g) and Ni (h)



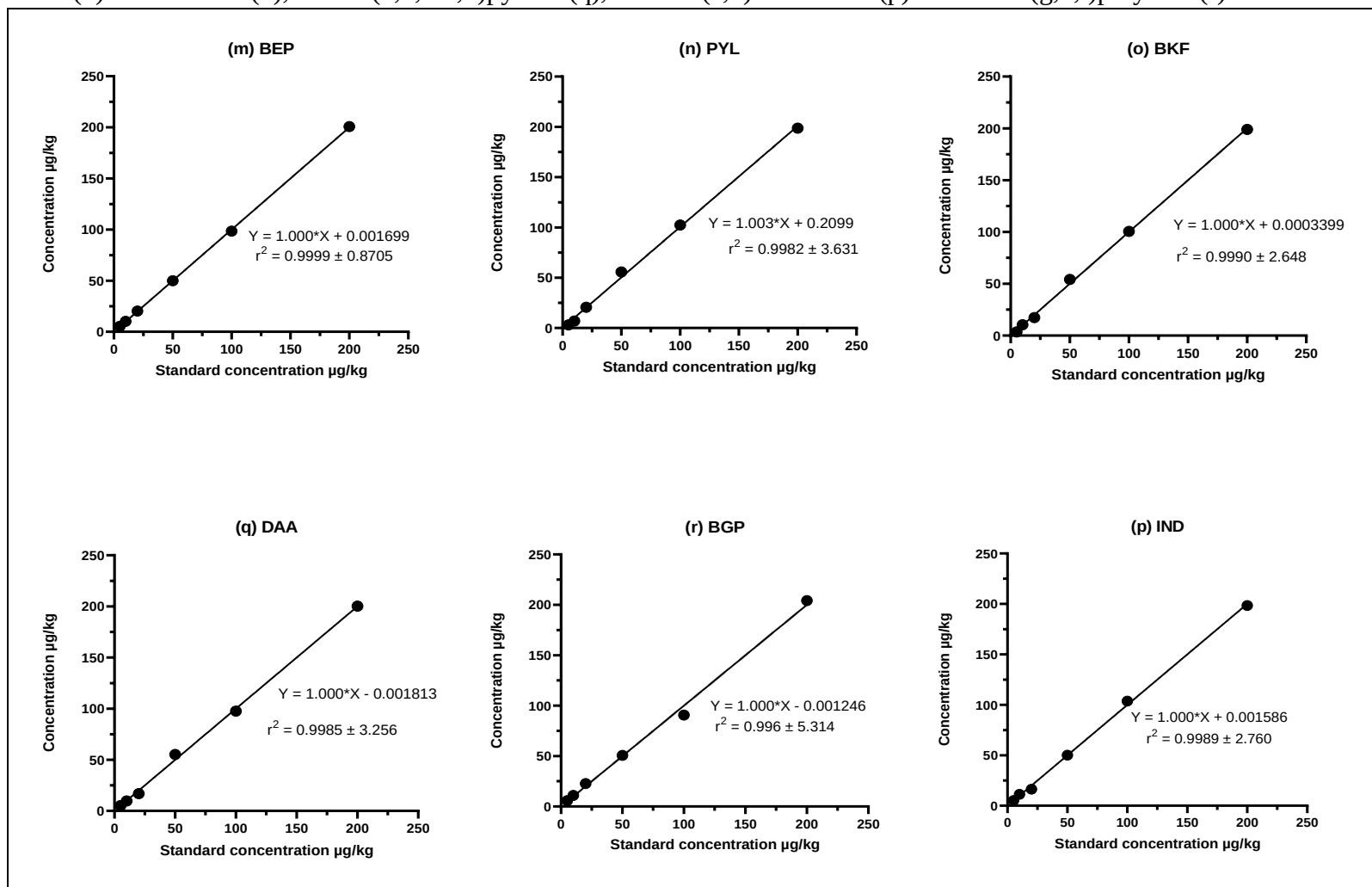
Appendix 3. (a -f): Calibration curves for the quantitation of PAHs: naphthalene (a), acenaphthylene (b), acenaphthene (c), fluorene (d), anthracene (e) and phenanthrene (f)



Appendix 3 (g – i): Calibration curves for quantitation of PAHs in the samples: fluoranthene (g), pyrene (h), benzo(a)anthracene (i), chrysene (j), benzo(a)pyrene (k) and benzo(b)fluoranthene (l)



Appendix 3 (m – r): Calibration curves for quantitation of PAHs in the samples: benzo(e)pyrene (m), pyrene (n), benzo(k)fluoranthene (o), indeno(1,2,3-c,d)pyrene (q), dibenzo(a,h)anthracene (p) and benzo(g,h,i)perylene (r)



Appendix 4: Results of metals concentrations (mg/kg) in the samples

Metal concentration in mg/kg								
Sample ID	Iron	Zinc	Copper	Cadmium	Chromium	Cobalt	Lead	Nickel
Std 1	1.998	0.25	1.998	0.501	0.998	1.989	2.001	1.989
BFC1	3.8	0.12	<0.003	<0.002	<0.001	0.024	<0.001	<0.001
BFC2	6.524	0.148	<0.003	<0.002	<0.001	<0.005	<0.001	<0.001
BFC3	32.468	0.212	<0.003	<0.002	<0.001	0.12	<0.001	<0.001
BFC4	5.284	0.156	<0.003	<0.002	<0.001	<0.005	<0.001	<0.001
BFC5	7.252	0.136	<0.003	<0.002	<0.001	<0.005	<0.001	<0.001
Std 1	1.998	0.25	1.999	0.501	0.999	1.989	2.001	1.989
BFC6	11.576	0.164	0.248	<0.002	<0.001	<0.005	<0.001	<0.001
BFC7	1.632	0.072	0.18	0.032	<0.001	<0.005	<0.001	<0.001
BFC8	2.46	1	0.176	<0.002	0.056	<0.005	<0.001	<0.001
Blank 1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
BFC9	2.028	0.076	0.132	<0.002	<0.001	<0.005	<0.001	<0.001
Std 1	1.998	0.25	1.998	0.499	0.989	1.998	1.999	1.999
BFC10	2.6	0.076	0.084	<0.002	<0.001	<0.005	<0.001	<0.001
STDQC 1	4.989	0.499	5.001	1.998	2.001	4.999	5.001	5.001
Rpt BFC2	6,524	0.148	<0.003	<0.002	<0.001	<0.005	<0.001	<0.001
BFC11	8.556	0.06	0.176	<0.002	<0.001	<0.005	<0.001	<0.001
Std 1	1.999	0.25	1.999	0.501	0.999	1.989	2.001	1.999
BFC12	6.528	0.116	0.143	<0.002	<0.001	<0.005	<0.001	<0.001
BFC13	7.204	0.088	0.1	0.02	<0.001	<0.005	<0.001	<0.001
BFC14	2.808	0.068	0.072	<0.002	<0.001	<0.005	<0.001	<0.001
BFC15	2.688	0.096	0.116	<0.002	<0.001	<0.005	<0.001	<0.001
Std 1	1.998	0.25	1.998	0.499	0.999	1.999	1.999	1.998
BFC16	28.008	0.152	0.152	<0.002	<0.001	<0.005	<0.001	<0.001
BFC17	7.804	0.112	0.132	<0.002	<0.001	<0.005	<0.001	<0.001
BFC18	3.276	0.088	0.204	<0.002	<0.001	<0.005	<0.001	<0.001
BFC19	4.192	0.128	0.14	<0.002	<0.001	<0.005	<0.001	<0.001
Std 1	1.998	0.25	1.998	0.498	0.999	1.998	2.001	2.001
Blank 2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
BFC20	12.372	0.172	0.152	0.024	<0.001	<0.005	<0.001	<0.001
STDQC 2	4.989	0.5	4.989	1.998	2.001	4.989	4.989	4.999
Rpt BCF 12	6.528	0.116	0.14	<0.002	<0.001	<0.005	<0.001	0.001
BFC21	13.256	0.14	0.152	<0.002	<0.001	<0.005	<0.001	<0.001

BFC22	4.064	0.084	0.188	<0.002	<0.001	<0.005	<0.001	<0.001
Std1	1.989	0.25	1.998	0.499	0.989	1.999	2.001	2.001
BFC23	34.224	0.132	0.112	<0.002	<0.001	<0.005	<0.001	<0.001
BFC24	6.508	0.14	0.132	<0.002	0.04	<0.005	<0.001	<0.001
BFC25	9.912	0.168	0.144	0.072	<0.001	<0.005	<0.001	<0.001
Std 1	1.998	0.25	1.999	0.498	1	1.998	1.999	2.001
BFC26	4.684	0.116	0.168	0.028	0.036	<0.005	<0.001	<0.001
BFC27	21.604	0.116	0.124	<0.002	0.048	<0.005	<0.001	<0.001
BFC28	3.724	0.076	0.116	<0.002	<0.001	0.08	<0.001	<0.001
BFC29	34.736	0.2	0.116	<0.002	<0.001	0.228	<0.001	<0.001
Blank 3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
BFC30	10.024	0.144	0.128	0.04	<0.001	<0.005	<0.001	<0.001
STDQC 1	5.001	0.5	5.001	1.999	1.998	4.989	5.001	5.001
Rpt BCF 22	4.064	0.084	0.188	<0.002	<0.001	<0.005	<0.001	<0.001
BFC31	3.68	0.096	0.108	<0.002	<0.001	<0.005	<0.001	<0.001
Std 1	1.995	0.249	1.999	0.498	0.989	1.999	1.989	1.999
BFC32	0.868	0.06	0.156	0.036	0.052	<0.005	<0.001	<0.001
BFC33	12.628	<0.001	0.088	0.028	0.036	<0.005	<0.001	<0.001
BFC34	4.548	0.16	0.072	<0.002	0.068	<0.005	<0.001	<0.001
BFC35	4.728	0.152	0.013	<0.002	<0.001	<0.005	<0.001	<0.001
Std 1	1.999	0.249	1.998	0.498	0.989	1.999	1.989	1.989
BFC36	2.92	0.028	0.12	<0.002	<0.001	<0.005	<0.001	<0.001
BFC37	1.464	0.02	0.088	0.128	0.076	<0.005	<0.001	<0.001
BFC38	28.788	0.236	0.064	0.788	<0.001	<0.005	0.06	0.052
BFC39	28.84	0.12	0.128	0.796	0.036	<0.005	<0.001	<0.001
Blank 4	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
BFC40	1.988	0.116	0.092	0.08	0.084	<0.005	0.352	0.024
STDQC 2	4.989	0.501	4.998	1.999	1.999	4.999	5.001	5.001
Rpt BFC32	0.868	0.06	0.156	0.36	0.052	<0.005	<0.001	<0.001
BFC41	8.552	0.112	0.92	0.768	<0.001	<0.005	0.076	0.06
Std 1	1.998	0.249	1.998	0.498	0.989	1.999	1.999	1.989
BFC42	1.228	0.02	0.168	0.796	0.256	<0.005	0.136	0.048
BFC43	1.568	0.108	0.116	0.812	<0.001	<0.005	0.352	0.2
BFC44	2.232	0.016	0.092	0.8	<0.001	<0.005	0.12	0.052
BFC45	1.76	0.12	0.12	0.796	<0.001	<0.005	0.036	0.02
Std 1	1.998	0.25	1.998	0.5	0.989	1.999	2.001	2.001
BFC46	1.392	0.024	0.128	0.8	<0.001	<0.005	0.288	0.028
STDQC 3	5.001	0.499	4.989	1.999	1.998	4.998	5.001	4.998

BFC47	4.112	0.128	0.112	0.796	<0.001	<0.005	0.272	0.06
Std 1	1.998	0.249	1.999	0.499	0.999	1.999	2.998	1.999
BFC48	3.484	0.184	0.144	0.8	<0.001	<0.005	0.58	0.072
Blank 5	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
BFC49	10.028	0.186	0.816	0.816	<0.001	<0.005	0.264	0.044
BFC50	3.092	0.176	0.808	0.808	<0.001	<0.005	0.2	0.04
Std 1	1.999	0.249	1.999	0.501	0.999	1.989	2.001	1.999
Rpt BFC42	1.232	0.024	0.172	0.796	<0.001	<0.005	0.136	0.044
Freq. (%)	100	98	90	44	20	8	24	24

Appendix 5: Results of ANOVA showing variations of metal concentrations across all samples

			ANOVA				
			Sum of Squares	df	Mean Square	F	Sig.
Iron mg/kg * Cereal Composition of sample	Between Groups	(Combined)	772.102	4	193.025	2.466	0.058
	Within Groups		3521.802	45	78.262		
	Total		4293.904	49			
Zinc mg/kg * Cereal Composition of sample	Between Groups	(Combined)	0.08	4	0.02	1.086	0.375
	Within Groups		0.827	45	0.018		
	Total		0.907	49			
Copper mg/kg * Cereal Composition of sample	Between Groups	(Combined)	0.093	4	0.023	0.667	0.618
	Within Groups		1.573	45	0.035		
	Total		1.667	49			
Cadmium mg/kg * Cereal Composition of sample	Between Groups	(Combined)	0.774	4	0.193	1.789	0.148
	Within Groups		4.867	45	0.108		
	Total		5.641	49			
Chromium mg/kg * Cereal Composition of sample	Between Groups	(Combined)	0.008	4	0.002	1.116	0.361
	Within Groups		0.076	45	0.002		
	Total		0.084	49			
Cobalt mg/kg * Cereal Composition of sample	Between Groups	(Combined)	0.003	4	0.001	0.494	0.74
	Within Groups		0.064	45	0.001		
	Total		0.067	49			
Lead mg/kg * Cereal Composition of sample	Between Groups	(Combined)	0.096	4	0.024	1.67	0.174
	Within Groups		0.647	45	0.014		
	Total		0.743	49			
Nickel mg/kg * Cereal Composition of sample	Between Groups	(Combined)	0.006	4	0.001	1.326	0.275
	Within Groups		0.049	45	0.001		
	Total		0.055	49			

Appendix 6: Results of ANOVA for metal concentrations in samples composed of Wheat only

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Fe	Between Groups	26.904	3	8.968	23.806	0.149
	Within Groups	0.377	1	0.377		
	Total	27.281	4			
Zn	Between Groups	0	3	0	0.065	0.971
	Within Groups	0.002	1	0.002		
	Total	0.002	4			
Cu	Between Groups	0.002	3	0.001	1.135	0.583
	Within Groups	0.001	1	0.001		
	Total	0.003	4			
Cd	Between Groups	0.494	3	0.165	.	.
	Within Groups	0	1	0		
	Total	0.494	4			
Cr	Between Groups	0.001	3	0	0.2	0.889
	Within Groups	0.002	1	0.002		
	Total	0.004	4			
Co	Between Groups	0	3	0	.	.
	Within Groups	0	1	0		
	Total	0	4			
Pb	Between Groups	0.059	3	0.02	.	.
	Within Groups	0	1	0		
	Total	0.059	4			
Ni	Between Groups	0.003	3	0.001	.	.
	Within Groups	0	1	0		
	Total	0.003	4			

Appendix 7: Results of ANOVA for metal concentrations in samples composed of maize only

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Fe	Between Groups	60.066	3	20.022	1.772	0.325
	Within Groups	33.897	3	11.299		
	Total	93.963	6			
Zn	Between Groups	0.009	3	0.003	0.369	0.783
	Within Groups	0.024	3	0.008		
	Total	0.033	6			
Cu	Between Groups	0.009	3	0.003	1.594	0.356
	Within Groups	0.006	3	0.002		
	Total	0.015	6			
Cd	Between Groups	1.067	3	0.356	2865.25	0.0
	Within Groups	0	3	0		
	Total	1.068	6			
Cr	Between Groups	0.021	3	0.007	0.636	0.64
	Within Groups	0.033	3	0.011		
	Total	0.054	6			
Co	Between Groups	0	3	0	.	.
	Within Groups	0	3	0		
	Total	0	6			
Pb	Between Groups	0.229	3	0.076	5.344	0.101
	Within Groups	0.043	3	0.014		
	Total	0.271	6			
Ni	Between Groups	0.004	3	0.001	4.304	0.131
	Within Groups	0.001	3	0		
	Total	0.005	6			

Appendix 8: Results of ANOVA for metal concentrations in samples composed of rice only

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Fe	Between Groups	42.147	5	8.429	24.59	0.152
	Within Groups	0.343	1	0.343		
	Total	42.49	6			
Zn	Between Groups	0.312	5	0.062	0.145	0.953
	Within Groups	0.431	1	0.431		
	Total	0.743	6			
Cu	Between Groups	0.008	5	0.002	199.343	0.054
	Within Groups	0	1	0		
	Total	0.008	6			
Cd	Between Groups	0.845	5	0.169	351.816	0.04
	Within Groups	0	1	0		
	Total	0.846	6			
Cr	Between Groups	0.005	5	0.001	0.667	0.725
	Within Groups	0.002	1	0.002		
	Total	0.007	6			
Co	Between Groups	0	5	0	.	.
	Within Groups	0	1	0		
	Total	0	6			
Pb	Between Groups	0.103	5	0.021	.	.
	Within Groups	0	1	0		
	Total	0.103	6			
Ni	Between Groups	0.033	5	0.007	.	.
	Within Groups	0	1	0		
	Total	0.033	6			

Appendix 9. Results of ANOVA for metal concentrations between samples of mixed-cereal composition

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Fe	Between Groups	1138.262	15	75.884	0.496	0.902
	Within Groups	1987.906	13	152.916		
	Total	3126.168	28			
Zn	Between Groups	0.022	15	0.001	0.913	0.571
	Within Groups	0.021	13	0.002		
	Total	0.044	28			
Cu	Between Groups	0.867	15	0.058	1.109	0.43
	Within Groups	0.678	13	0.052		
	Total	1.545	28			
Cd	Between Groups	1.488	15	0.099	1.95	0.117
	Within Groups	0.661	13	0.051		
	Total	2.149	28			
Cr	Between Groups	0.006	15	0	0.981	0.519
	Within Groups	0.005	13	0		
	Total	0.011	28			
Co	Between Groups	0.021	15	0.001	0.421	0.944
	Within Groups	0.043	13	0.003		
	Total	0.064	28			
Pb	Between Groups	0.108	15	0.007	0.907	0.576
	Within Groups	0.103	13	0.008		
	Total	0.211	28			
Ni	Between Groups	0.004	15	0	1.218	0.364
	Within Groups	0.003	13	0		
	Total	0.007	28			

Appendix 10: Results of ANOVA for metal concentrations between brands

			ANOVA				
			Sum of Squares	df	Mean Square	F	Sig.
Iron mg/kg * Brand	Between Groups	(Combined)	1708.887	20	85.444	0.959	0.53
	Within Groups		2585.017	29	89.139		
	Total		4293.904	49			
Zinc mg/kg * Brand Name	Between Groups	(Combined)	0.223	20	0.011	0.473	0.957
	Within Groups		0.684	29	0.024		
	Total		0.907	49			
Copper mg/kg * Brand	Between Groups	(Combined)	0.753	20	0.038	1.195	0.324
	Within Groups		0.914	29	0.032		
	Total		1.667	49			
Cadmium mg/kg * Brand	Between Groups	(Combined)	4.379	20	0.219	5.03	0
	Within Groups		1.262	29	0.044		
	Total		5.641	49			
Chromium mg/kg * Brand	Between Groups	(Combined)	0.025	20	0.001	0.606	0.876
	Within Groups		0.059	29	0.002		
	Total		0.084	49			
Cobalt mg/kg * Brand	Between Groups	(Combined)	0.024	20	0.001	0.799	0.696
	Within Groups		0.043	29	0.001		
	Total		0.067	49			
Lead mg/kg * Brand	Between Groups	(Combined)	0.415	20	0.021	1.834	0.067
	Within Groups		0.328	29	0.011		
	Total		0.743	49			
Nickel mg/kg * Brand	Between Groups	(Combined)	0.033	20	0.002	2.175	0.028
	Within Groups		0.022	29	0.001		
	Total		0.055	49			

Appendix 11: Hazard index and Hazard quotients for Fe, Cd and Pb concentrations in all the samples.

	HQ (Fe)			HQ (Cd)			HQ (Pb)			HI		
Sample	6 -8 months	9- 11 months	12- 23 months	6 -8 months	9- 11 months	12- 23 months	6 -8 months	9- 11 months	12- 23 months	6 -8 months	9- 11 months	12- 23 months
BCF1	0.18	0.25	0.38	-	-	-	-	-	-	0.18	0.25	0.38
BCF2	0.31	0.42	0.65	-	-	-	-	-	-	0.31	0.42	0.65
BCF3	1.57	2.11	3.24	-	-	-	-	-	-	1.57	2.11	3.24
BCF4	0.26	0.34	0.53	-	-	-	-	-	-	0.26	0.34	0.53
BCF5	0.35	0.47	0.72	-	-	-	-	-	-	0.35	0.47	0.72
BCF6	0.56	0.75	1.16	-	-	-	-	-	-	0.56	0.75	1.16
BCF7	0.08	0.11	0.16	2.16	2.91	4.47	-	-	-	2.24	3.02	4.63
BCF8	0.12	0.16	0.25	-	-	-	-	-	-	0.12	0.16	0.25
BCF9	0.10	0.13	0.20	-	-	-	-	-	-	0.10	0.13	0.20
BCF10	0.13	0.17	0.26	-	-	-	-	-	-	0.13	0.17	0.26
BCF11	0.41	0.56	0.85	-	-	-	-	-	-	0.41	0.56	0.85
BCF12	0.32	0.42	0.65	-	-	-	-	-	-	0.32	0.42	0.65
BCF13	0.35	0.47	0.72	1.35	1.82	2.79	-	-	-	1.70	2.29	3.51
BCF14	0.14	0.18	0.28	-	-	-	-	-	-	0.14	0.18	0.28
BCF15	0.13	0.17	0.27	-	-	-	-	-	-	0.13	0.17	0.27
BCF16	1.35	1.82	2.80	-	-	-	-	-	-	1.35	1.82	2.80
BCF17	0.38	0.51	0.78	-	-	-	-	-	-	0.38	0.51	0.78
BCF18	0.16	0.21	0.33	-	-	-	-	-	-	0.16	0.21	0.33
BCF19	0.20	0.27	0.42	-	-	-	-	-	-	0.20	0.27	0.42
BCF20	0.60	0.80	1.23	1.62	2.18	3.35	-	-	-	2.22	2.99	4.59
BCF21	0.64	0.86	1.32	-	-	-	-	-	-	0.64	0.86	1.32
BCF22	0.20	0.26	0.41	-	-	-	-	-	-	0.20	0.26	0.41
BCF23	1.65	2.22	3.42	-	-	-	-	-	-	1.65	2.22	3.42
BCF24	0.31	0.42	0.65	-	-	-	-	-	-	0.31	0.42	0.65

BCF25	0.48	0.64	0.99	4.87	6.55	10.06	-	-	-	5.35	7.19	11.05
BCF26	0.23	0.30	0.47	1.89	2.55	3.91	-	-	-	2.12	2.85	4.38
BCF27	1.04	1.40	2.16	-	-	-	-	-	-	1.04	1.40	2.16
BCF28	0.18	0.24	0.37	-	-	-	-	-	-	0.18	0.24	0.37
BCF29	1.68	2.26	3.47	-	-	-	-	-	-	1.68	2.26	3.47
BCF30	0.48	0.65	1.00	2.70	3.64	5.59	-	-	-	3.19	4.29	6.59
BCF31	0.18	0.24	0.37	-	-	-	-	-	-	0.18	0.24	0.37
BCF32	0.04	0.06	0.09	2.43	3.27	5.03	-	-	-	2.48	3.33	5.12
BCF33	0.61	0.82	1.26	1.89	2.55	3.91	-	-	-	2.50	3.37	5.17
BCF34	0.22	0.30	0.45	-	-	-	-	-	-	0.22	0.30	0.45
BCF35	0.23	0.31	0.47	-	-	-	-	-	-	0.23	0.31	0.47
BCF36	0.14	0.19	0.29	-	-	-	-	-	-	0.14	0.19	0.29
BCF37	0.07	0.10	0.15	8.65	11.64	17.88	-	-	-	8.72	11.73	18.03
BCF38	1.39	1.87	2.87	53.26	71.64	110.09	0.58	0.78	1.20	55.23	74.70	114.16
BCF39	1.39	1.87	2.88	53.81	72.36	111.21	-	-	-	55.20	74.24	114.09
BCF40	0.10	0.13	0.20	5.43	7.31	11.23	3.40	4.57	7.03	8.93	14.46	18.46
BCF41	0.41	0.56	0.85	51.91	69.82	107.30	0.73	0.99	1.52	53.06	71.89	109.67
BCF42	0.06	0.08	0.12	53.81	72.36	111.21	1.31	1.77	2.71	55.18	75.16	114.05
BCF43	0.08	0.10	0.16	54.89	73.82	113.45	3.40	4.57	7.03	58.36	80.95	120.63
BCF44	0.11	0.14	0.22	54.08	72.73	111.77	1.16	1.56	2.40	55.34	75.27	114.39
BCF45	0.08	0.11	0.18	53.81	72.36	111.21	0.35	0.47	0.72	54.24	73.20	112.11
BCF46	0.07	0.09	0.14	54.08	72.73	111.77	2.78	3.74	5.75	56.92	78.57	117.66
BCF47	0.20	0.27	0.41	53.81	72.36	111.21	2.63	3.53	5.43	56.63	78.06	117.05
BCF48	0.17	0.23	0.35	54.08	72.73	111.77	5.60	7.53	11.58	59.84	84.53	123.69
BCF49	0.48	0.65	1.00	55.16	74.18	114.01	2.55	3.43	5.27	58.19	80.10	120.28
BCF50	0.15	0.20	0.31	54.62	73.45	112.89	1.93	2.60	3.99	56.70	77.65	117.19

Appendix 12: Results of PAHs concentrations (µg/kg) in the samples with calculated PAH4 and total PAHs

ID	NAP	ACA	ACE	FLU	ANT	PHE	FLT	PYR	BAA	CHR	BAP	BBF	BEP	PYL	3BKF	IND	DAA	BGP	PAH4	Total PAHs
5 ppb	4.3	5.7	4.7	4.9	4.2	5.5	5.5	5.8	4.6	4.2	4.7	5.4	5.3	3.1	3.5	5.1	5.3	5.7	-	-
10 ppb	10.5	11.0	8.5	8.8	9.0	9.6	9.3	11.1	10.4	7.41	10.3	10.8	10.3	6.9	10.4	11.2	9.8	11.0	-	-
20 ppb	20.3	20.1	21.6	22.5	21.0	22.3	20.9	20.9	19.1	20.9	21.3	19.3	20.2	20.6	17.4	16.4	17.0	22.7	-	-
50 ppb	49.6	47.8	50.4	48.9	55.3	47.2	51.2	51.7	51.8	54.8	48.3	50.6	50.0	55.7	54.2	50.2	55.2	50.6	-	-
100 ppb	100.5	79.7	91.8	99.7	93.5	92.1	96.9	92.2	98.9	97.7	72.6	98.0	98.5	102.4	100.5	103.7	97.5	90.7	-	-
200 ppb	199.8	200.5	199.8	200.3	201.9	200.5	201.2	203.3	200.2	202.3	200.3	200.9	200.7	198.7	199.0	198.4	200.2	204.1	-	-
BFC1	60	10	ND	<LOD	300	20	60	60	50	30	40	20	10	20	50	90	ND	70	140	890
BFC2	70	<LOD	<LOD	ND	170	10	30	30	30	<LOD	20	10	<LOD	10	20	<LOD	ND	ND	60	400
BFC3	70	20	<LOD	<LOD	240	20	40	30	20	10	10	10	<LOD	10	10	<LOD	ND	ND	50	490
BFC6	140	10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	<LOD	<LOD	<LOD	ND	<LOD	<LOD	<LOD	0	150
BFC10	10	10	ND	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	ND	0	20
RPT BFC																				
10	10	10	ND	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	ND	-	-
BFC13	30	<LOD	ND	ND	210	10	40	40	10	10	10	<LOD	<LOD	<LOD	ND	<LOD	ND	ND	30	360
BFC14	30	10	ND	ND	200	10	50	40	30	30	20	0.010	<LOD	10	<LOD	<LOD	ND	ND	90	440
BFC15	70	10	ND	ND	150	40	30	30	10	10	10	<LOD	<LOD	<LOD	ND	<LOD	ND	ND	30	360
BFC16	20	<LOD	ND	ND	20	<LOD	10	<LOD	<LOD	ND	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	ND	0	50
BFC17	20	<LOD	ND	ND	20	ND	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	<LOD	ND	ND	ND	ND	0	40
BLK	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
BFC18	10	<LOD	ND	ND	10	10	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	<LOD	ND	ND	ND	ND	0	30
BFC19	40	<LOD	ND	ND	<LOD	ND	10	<LOD	ND	ND	10	<LOD	<LOD	<LOD	ND	<LOD	ND	ND	10	60
BFC20	90	570	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	0	660
BFC21	90	180	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	0	270
BFC22	160	700	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	0	860

BFC24	10	<LOD	ND	ND	20	<LOD	<LOD	<LOD	ND	ND	ND	<LOD	<LOD	<LOD	ND	<LOD	ND	ND	0	30
BFC25	40	30	<LOD	ND	<LOD	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	<LOD	0	70
Rpt																				
BFC25	40	30	<LOD	ND	<LOD	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	<LOD	-	-
BFC26	40	<LOD	ND	ND	120	10	20	20	ND	ND	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	ND	0	210
BFC27	50	<LOD	ND	ND	170	<LOD	30	30	<LOD	<LOD	10	10	<LOD	<LOD	<LOD	<LOD	ND	ND	20	300
BFC28	50	10	ND	ND	170	10	30	30	<LOD	ND	10	<LOD	<LOD	<LOD	ND	<LOD	ND	ND	10	310
BLK	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
BFC41	190	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	0	190
BFC 49	700	170	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	<LOD	0	870
BFC 50	120	70	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	0	190
BFC5	80	40	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	<LOD	0	120
BFC7	180	10	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	0	190
BFC11	120	20	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	0	140
BFC12	20	10	ND	ND	190	<LOD	40	30	10	20	10	<LOD	<LOD	10	<LOD	<LOD	ND	ND	40	340
BFC30	90	30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	120
RPT BFC																				
30	90	30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-
BLK	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
BFC44	60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	<LOD	0	60
BFC46	60	20	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	<LOD	<LOD	<LOD	ND	<LOD	ND	<LOD	0	80
Freq. %	100	63.33	0	0	46.67	30	40	33.33	23.33	20	33.33	16.67	3.33	16.67	10	3.33	0	3.33	33.33	-

ND = Not Detected

LOD = Limit of Detection

Appendix 13: Results of ANOVA for PAHs concentrations across the samples

ANOVA							
			Sum of Squares	df	Mean Square	F	Sig.
Conc. NAP * Composition of Cereal	Between Groups	(Combined)	0.012	4	0.003	0.164	0.955
	Within Groups		0.444	25	0.018		
	Total		0.455	29			
Conc. ACA * Composition of Cereal	Between Groups	(Combined)	0.02	4	0.005	0.166	0.954
	Within Groups		0.743	25	0.03		
	Total		0.762	29			
Conc. ANT * Composition of Cereal	Between Groups	(Combined)	0.027	4	0.007	0.72	0.587
	Within Groups		0.234	25	0.009		
	Total		0.261	29			
Conc. PHE * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.579	0.681
	Within Groups		0.002	25	0		
	Total		0.002	29			
Conc. FLT * Composition of Cereal	Between Groups	(Combined)	0.001	4	0	0.736	0.576
	Within Groups		0.009	25	0		
	Total		0.01	29			
Conc. PYR * Composition of Cereal	Between Groups	(Combined)	0.001	4	0	0.608	0.661
	Within Groups		0.008	25	0		
	Total		0.009	29			
Conc. BAA * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.275	0.891
	Within Groups		0.004	25	0		
	Total		0.004	29			
Conc. CHR * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.694	0.604
	Within Groups		0.001	24	0		
	Total		0.001	28			
Conc. BAP * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.433	0.784
	Within Groups		0.002	25	0		
	Total		0.002	29			
Conc. BBF * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.355	0.838
	Within Groups		0.001	25	0		

	Total		0.001	29			
Conc. BEP * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.066	0.991
	Within Groups		0	25	0		
	Total		0	29			
Conc. PYL * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.377	0.823
	Within Groups		0.001	25	0		
	Total		0.001	29			
Conc. BKF * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.149	0.962
	Within Groups		0.003	25	0		
	Total		0.003	29			
Conc. IND * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.066	0.991
	Within Groups		0.008	25	0		
	Total		0.008	29			
Conc. BGP * Composition of Cereal	Between Groups	(Combined)	0	4	0	0.066	0.991
	Within Groups		0.005	25	0		
	Total		0.005	29			

Appendix 14: Results of ANOVA for PAHs concentrations in samples of mixed cereal composition

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
NAP	Between Groups	0.431	13	0.033	35.588	0.0
	Within Groups	0.008	9	0.001		
	Total	0.439	22			
ACA	Between Groups	0.385	13	0.03	0.745	0.695
	Within Groups	0.357	9	0.04		
	Total	0.742	22			
ANT	Between Groups	0.155	13	0.012	1.762	0.199
	Within Groups	0.061	9	0.007		
	Total	0.216	22			
PHE	Between Groups	0.001	13	0	0.538	0.85
	Within Groups	0.001	9	0		
	Total	0.002	22			
FLT	Between Groups	0.006	13	0	1.813	0.187
	Within Groups	0.002	9	0		
	Total	0.008	22			
PYR	Between Groups	0.005	13	0	1.555	0.256
	Within Groups	0.002	9	0		
	Total	0.008	22			
BAA	Between Groups	0.002	13	0	1.059	0.478
	Within Groups	0.002	9	0		
	Total	0.004	22			
CHR	Between Groups	0.001	13	0	1.399	0.324
	Within Groups	0	8	0		
	Total	0.001	21			
BAP	Between Groups	0.001	13	0	0.649	0.768
	Within Groups	0.001	9	0		
	Total	0.002	22			
BBF	Between Groups	0	13	0	0.714	0.718

BEP	Within Groups	0	9	0		
	Total	0.001	22			
	Between Groups	0	13	0	0.191	0.996
PYL	Within Groups	0	9	0		
	Total	0	22			
	Between Groups	0	13	0	0.945	0.551
BKF	Within Groups	0	9	0		
	Total	0.001	22			
	Between Groups	0.001	13	0	0.654	0.765
IND	Within Groups	0.001	9	0		
	Total	0.003	22			
	Between Groups	0.002	13	0	0.191	0.996
BGP	Within Groups	0.006	9	0.001		
	Total	0.008	22			
	Between Groups	0.001	13	0	0.191	0.996
	Within Groups	0.004	9	0		
	Total	0.005	22			
