

**NON-CANCER HEALTH RISKS ASSOCIATED WITH PESTICIDE RESIDUES AND  
HEAVY METAL EXPOSURES IN LOCALLY MANUFACTURED CEREAL-BASED  
BABY FOODS IN KAMPALA CITY**

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FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF  
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**DECLARATION**

I declare that the dissertation, which I hereby submit for the degree of Master of Science in Food Technology of Kyambogo University, is entirely mine and has not been presented before to this or any other University or higher institution of learning for a similar award.

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## **DEDICATION**

To my dear mother, Mrs. Aidah Kagoya and my late father Al-hajji Abdu-Waheed Mukasa Ssentongo, who taught me the value of education and critical thinking. I am also grateful to Dad's unwavering effort to see me succeed.

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## LIST OF ACRONYMS

FAO	Food and Agricultural Organization
GC/MSD	Gas Chromatography, Mass Spectrophotometer Detector
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
MRL	Maximum Residue Limits
NEMA	National Environment Management Authority
OCP	Organochlorine Pesticide(s)
OPP	Organophosphate Pesticide(s)
WHO	World Health Organization
JECFA	Joint Expert Committee on Food Additive(s)
CDI	Climate and Development Initiative
ASTDR	Agency for Toxic Substances and Disease Registry
OECD	Organization for Economic Co-operation and Development of United Nations
HHRA	Human Health Risk Assessment
NSCDC	National Scientific Council on Developing Child
IARC	International Agency for Research on Cancer
NPIC	National Pesticide Information Center
POP	Persistent Organic Pollutant(s)
PARM	Platform for Agricultural Risk Management
GAI	Global Agricultural initiative
ECS	European Committee for Standardization
COHA	Cost Of Hunger in Africa

## ABSTRACT

Pesticides residues and heavy metals are major environmental pollutants and their toxicity is a problem of increasing significance for nutritional and environmental reasons. Data on the risk contribution of heavy metal contaminated cereal-based baby foods towards the disease burden in Uganda is inadequate. The study therefore assessed the awareness of exposure to pesticides, and heavy metals (HM) among manufacturers of baby foods, detected organochlorines and organophosphorus pesticides, determined the levels of selected HM (lead, cadmium, Arsenic, and mercury) and the non-cancer risks posed by these contaminants in cereal-based baby foods produced in Uganda. A set of pretested questionnaires was used to assess the level of pesticide and heavy metal responsiveness among manufacturers (n= 16) of baby foods. Samples of baby foods (n= 18) obtained from supermarkets, retail shops and groceries around Kampala City were analysed for organochlorines, organophosphate pesticides, and the heavy metals (As, Hg, Cd and Pb). Maximum levels of HM detected were 0.016 mg/kg, 0.016 mg/kg, 0.030 mg/kg, and 0.037 mg/kg for Pb, Cd, As, and Hg respectively. Pesticide residues were identified using Gas Chromatography coupled with Mass Spectrometry (GC/MS). Heavy metal concentration was determined using Atomic Absorption Spectrophotometry (AAS). Non-cancer risk posed to babies through heavy metal exposure in baby foods were determined for infants aged 6 to 24 months using the non-cancer hazard quotient (HQ) described by the United State Environmental Protection Agency (US EPA). The organochlorine 4, 4-Diphenyl DichloroTrichloro ethane (DDT) was the predominant pesticide detected in 88.8% of the baby foods tested. The concentration of As in baby foods ranged from 0.010 to 0.030 mg/kg, Hg content ranged from 0.003 to 0.037. The range concentration for Cd and Pb were 0.002 to 0.150 and 0.001 to 0.016 mg/kg, respectively. The hazard quotient of heavy metals in cereal based baby foods for infants was below 1 for Pb, Cd and Hg but was greater than 1 for As, indicating potential risk to the infants for As. Hazard index values of the heavy metals were above 1 for all baby foods. The non-cancer risk due to heavy metal exposure in locally manufactured baby foods in Kampala was unacceptable. Therefore, regular monitoring of baby foods for chemical contaminants is recommended to ensure safety.

## CHAPTER 1: INTRODUCTION

### 1.1 Background

A vital and productive society with a prosperous and sustainable future is built on a foundation of healthy child development (CDC, 2013; Levitt, 2015). Unfortunately, about 155 million children under 5 years of age are stunted (short for age), 52 million are wasted (thin for height), and 41 million are obese (WHO, 2017). Under nutrition is estimated to cause approximately 2.7 million child deaths in Uganda annually (UBOS, 2011; Uganda health and nutrition fact sheet, 2014). Health in the earliest years, beginning with the future mother's health before she becomes pregnant, lays the 'groundwork' for a lifetime of well-being (NSCDC, 2010). From the period of 6 to 24 months of age, children need to be fed on family foods in addition to breast milk. This is referred to as complementary feeding (Sirina, Strele, Siksna, & Gardovska, 2018). World Health Organization (WHO) defines complementary feeding as "a process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods and liquids are needed, along with breast milk" (WHO, 2001). Complementary foods can be a special preparation for the infant or usual family food that has been modified to make it easy to eat and digest (Michellini, et al., 2018). For energy, 200, 300, and 550 kcal per day is expected to be covered by complementary foods at 6–8, 9–11, and 12–23 months, respectively (Abeshu, Lelisa, & Geleta, 2016). The period of 6-24 is a vulnerable period, and is the time when malnutrition starts in infants (Abeshu, Lelisa, & Geleta, 2016). Complementary foods are introduced to an infant to provide nutrients and additional energy (Amagloh, et al., 2015). Babies prefer simple, uncomplicated foods and in Uganda, cereals are the most commonly used (Amagloh, et al., 2015). Complementary foods should be of sound nutritional quality, and in addition, should be free of physical, biological and chemical hazards.

There is a growing concern of environmental contamination by chemical agents produced by industrial, mining and agricultural activities (Rather, Koh, Paek, & Lim, 2017). Therefore, possible relationship with the increase in the prevalence of congenital malformations, asthma, cancer, and neurological and behavioral disorders in children all over the world (Song et al., 2017). Unfavorable situations regarding access to safe food and drinkable water, sewage disposal and housing conditions contribute to remarkable morbidity and mortality of children in developing countries (Song et al., 2017). Rapid economic development of Africa has led to increased pollution of the environment with heavy metals (HM) (Sarkodie & Owusu, 2017; Hernández et al., 2017). Reduced arable land availability, and the increased demand for food especially for local consumption, have also led to increased use of agrichemicals including fertilizers, pesticides, fungicides and herbicides (Pretty & Bharucha, 2014; Yongbo et al., 2015). Pesticide residues and heavy metals from these chemicals and other sources get into the environment and eventually find their way into the food chain (Song et al., 2017).

Food, milk, and water provide significant exposure routes for chemical contaminants such as heavy metals (WHO, 2000; EFSA, 2010). Children tend to retain a greater portion of a given dose of certain toxins than adults do, and are not as capable of detoxifying them in their bodies (Tasleem, et al, 2015). Regular consumption of food and water contaminated with pesticides residues and heavy metals elicits acute and or chronic toxicity among consumers especially among children (Macharia, 2015). HM metals and pesticide residues end up into baby foods via the raw materials used in their formulation. For instance fish is commonly used as an ingredient in baby foods regardless of the fact that fish has been reported to be contaminated with pesticide residues (such as 1, 1, 1-trichloro-2, 2-bis [p-chlorophenyl] ethane (DDT) and its metabolites) (Turusov et al., 2002; Ssebugere et al., 2009; Afful et al., 2010). Silver fish in Uganda, commonly used in baby complementary feeds has also been

reported contaminated with HM such as copper, cadmium, lead and zinc (Mbabazi and Wasswa, 2010). Lead is highly toxic and if consumed over a long period, it results in complications including neurotoxicity, immunosuppression, limb deformities, cancers and cognition issues in children less than five years (Burger et al., 2012). This therefore, predisposes baby foods to HM and pesticide contamination, which might lead to health concerns in babies. For instance, DDT metabolites are reported to be mutagenic, potentially carcinogenic and can mimic estrogen-like effects in humans (Robinson et al., 1985). However, there are limited studies documented on HM and pesticide residues in baby food in Uganda.

Based on the adverse effects of heavy metals, information on their levels in agricultural produce used as raw materials in baby foods, it is essential to analyze baby foods in healthcare and medical services. Some of the most prevalent classes of pesticides used in growing food crops are the carbamates, pyrethroids, and the organophosphates. After crops are harvested, they may retain residues of these pesticides. Organophosphates and persistent OCPs are classes of pesticides that are of concern for children's health. Examples of organophosphate pesticides include chlorpyrifos, azinphos methyl, methyl parathion, and phosmet. These pesticides are frequently applied to many of the foods important in children's diets, and certain organophosphate pesticide residues can be detected in small quantities on these foods. Lead and As are the frequently detected HM in baby foods. Many studies (Mbabazi and Wasswa, 2010; Hadiani, et al., 2014; Kolawole, Obueh, & Henrietta, 2015) have been conducted in reference to the concentration and potential health risks associated with pesticides residues and toxic metals, as well as necessary elements in baby foods around the world and found that infants are exposed to arsenic via diet since rice-based infant food products contained elevated levels of arsenic and pesticides residues in the baby foods and led to retarded brain development in babies and infants. The study carried out on milk and



beef in South Western Uganda found that the incremental lifetime cancer risk (ILCR) was high in beef as the levels of Pb played a major cumulative risk in increasing the cancer threat in the population. The hazard index (HI) was high ( $HI > 1$ ), thus showing that the cumulative effects of contaminants in the samples would be dangerous (Kasozi, Namubiru, Natabo, & Dickson, 2018). A Swedish study that tested a number of formula milks and weaning foods notably rice products for levels of arsenic. Some foods had relatively high levels of As (Ahoulé, Lalanne, Mendret, Brosillon, & Maïga, 2015). The concentration of Pb was found to be higher than the limits set by KEBS in all the brands tested except brand code T in the infant formula milk that marketed in the Nairobi county Kenya (Odundo, Nawiri, & Wanjali, 2014).

Worldwide, pesticides contribute to an estimated 26 million human poisonings and 220, 000 deaths each year (Gunnell, Eddleston, phillips, & Konradsen, 2007). According to the Institute for Health Metrics and Evaluation, in 2015, HM exposure accounted for nearly 0.5 million deaths and 9.3 million life years lost (DALYs), with the highest occurrence in developing regions. WHO estimated in 2012 that Pb was responsible for causing mild-to-moderate mental retardation of 0.6 million children annually. However, there is limited data on both the pesticide residues and HM contamination of baby food, as well as monitoring and human health risk assessment (HHRA) of pesticide and HM in baby foods in Uganda. Therefore, the objectives of this study were to determine the awareness of exposure to pesticides and HM among manufacturers of cereal-based baby foods, to detect the presence of organochlorines and organophosphorus pesticide compounds in cereal-based baby foods and determine the levels of Pb, Cd, As, and Hg in selected cereal-based baby foods.

Due to adverse effects of pesticides and HM consumed in foods, farmers may minimize contamination or stop environmental damage. Remediation may focus on reducing contaminants in the environment overall or reducing concentrations in foods specifically. A common method is to remove contaminated topsoil, which typically contains higher levels of contaminants than subsoil, alternatively, soil turnover and mixing in situ may be sufficient to dilute contaminant, such as metals, concentrations to an acceptable level (Thompson & Darwish, 2019). Thermal treatment or landfill and microbial bioremediation may also be used to reduce levels of metal contamination of soils in an environmentally friendly manner. For example, metal (cadmium, mercury, and chromium) accumulation in flowering Chinese cabbage was shown to be controlled by total metal concentrations in soil and available calcium (Liu, Kong, & Jia, 2007) and the predominant congener of technical DDT, p,p'-DDT, is susceptible to microbial metabolism and rarely accumulates in aerobic soils (Boul, 1995)

## **1.2 Statement of Problem**

Children aged 6 to 24 months need to be fed on complementary foods in addition to breast milk (WHO, 2000). The complementary foods include cereals, pureed fruits and vegetables and the food the rest of the family eat at home. However, due to continuous and increased use of pesticides in agriculture, there is growing concern of environmental contamination by chemicals and these substances also find their way in food. Food contamination has become more alarming in recent years (1960s to date) due to the development of industry and the consequent environmental pollution (Song et al., 2017). In 2008, pesticides were reported by Poison Control Centers as the ninth most common substance that caused poisoning to the population, and approximately 45% of all reports of pesticide poisoning were for children (Roberts & Karr, 2012). An estimated 400 to 500 children died of acute HM poisoning (Pb)

due to ingestion of food contaminated with HM –contained soil and dust in Nigeria (Tirima et al , 2018). High levels HM are also contained in the food processing chain because of disposal of industrial wastes, fertilizers, and use of pesticides.

Infants and young children are likely to suffer high effects due to continuous exposure as consumed however, there is scant data on pesticides compounds and heavy metals in cereal-based baby foods and their health effects on consumers in Uganda. There is also limited information on pesticide responsiveness among manufacturers of baby foods in Uganda. This knowledge gap presents risk management challenges. Therefore, this study seeks to find out the pesticide responsiveness among manufacturers of baby foods in Uganda. If the cereal-based baby food produced in Uganda, contain Organophosphates and organochlorine pesticides and HM. If they exist, they potentially cause a health risk because of consuming the foods. Frequent exposure to pesticides and HM may result in short-term (acute) and long-term (chronic) illnesses. Acute illnesses include headaches, stomach pains, vomiting, skin rashes, respiratory problems, eye irritations, sneezing, seizures, and coma (Hu, et al., 2015). The chronic illnesses, on the other hand, include cancer, asthma, dermatitis, endocrine disruption, reproductive dysfunctions, immunotoxicity, neurobehavioral disorders and birth defects (Amirah, Afiza, Faizal, Nurliyana, & Laili, 2013).

### **1.3 Justification of the study**

Lead, Cd, As, and Hg have no known biological functions and are number 1, 2, 3 and 7, respectively on the Agency for Toxic Substances and Disease Registry (ASTDR) priority list of the top 20 hazardous chemicals for toxicological profiling (ASTDR, 2015). They are found in drinking water, foods, dust, fish, and are active components of the pesticides (Onyedikachi, Belonwu, & Wegwa, 2018; Okereke, Essien, & Wegwu, 2016; Ahmad & Al-

Mahaqeri, 2015; Sears, Riina , and Kerry, 2012). Studies have shown that water, plants and animal products in Uganda contain elevated levels of heavy metals (Kasozi et al., 2018; Bamuwamye et al., 2017). Deaths resulting from direct exposure to pesticides are also common and HM (Ibrahim, 2015). Worldwide, pesticides contribute to an estimated 26 million human poisonings and 220, 000 deaths each year (Gunnell, Eddleston, phillips, & Konradsen, 2007). In a recent carefully conducted analysis, WHO have estimated that 4.9 million deaths (8.3 percent of total mortality worldwide) are attributable to environmental exposure and inappropriate management of selected metals (WHO, 2010). It is believed that lead, a heavy metal, for example, is thought to be responsible for 3 percent of cerebrovascular disease burden worldwide (WHO, 2010). Agrichemicals are nowadays widely used in Uganda and there is likelihood of their build up to harmful levels in baby foods (Semalulu, 2005). Therefore, there is a need for regular monitoring of pesticide residues and heavy metals in baby foods and/ or their raw materials since babies are tend to retain a greater portion of a given dose of certain toxins than adults do and babies are unable to detoxify such contaminants in their bodies (Tasleem, et al, 2015). This will ensure safety of baby food and their raw material supply consequently ensuring safety of infants (Bamuwamye et al., 2017; Amadi, Igweze, & Orisakwe, 2017). This is because infants and babies are more sensitive than adults to food contaminants due to higher rate of uptake by gastrointestinal track, an incompletely developed blood –brain barrier, an underdeveloped detoxification system and high food consumption relative to body mass (Vella & Attard, 2019). The study will be an informative to the health, Safety and Nutrition policies that focus on babies, infants and young children in Uganda.

## **1.4 Objectives of the study**

### **1.4.1 Major objective**

To assess the awareness of exposure to pesticide and heavy metals among manufacturers baby foods, detect the presence of organochlorines and organophosphorus pesticides residues and determine the levels of selected heavy metals (Pb, Cd, As, and Hg) in cereal-based baby foods produced in Uganda.

### **1.4.2 Specific objectives**

1. To determine the awareness of exposure to pesticides and HM among manufacturers of cereal-based baby foods.
2. To detect the presence of organochlorine (OC) and organophosphorus pesticide (OP) compounds in cereal-based baby foods on the market in Kampala.
3. To determine the levels of selected heavy metals Pb, Cd, As, and Hg and the non-cancer risks posed by them to the infants and babies via consumption of cereal-based baby food on the market in Kampala.

## **1.5 Hypotheses**

1. Cereal-based baby food manufactures in Uganda have little knowledge on the proper use, and health effect on pesticides and heavy metals to consumers.
2. Cereal-based baby foods produced in Uganda contain organochlorine and organophosphorus residues and high levels of Pb, Cd, As and Hg than permissible.
3. Consumption of cereal-based baby foods produced in Uganda can poses non-cancer health risks to babies and infants because of exposure to Pb, Cd, As, and Hg.

## **CHAPTER 2: LITERATURE REVIEW**

### **2.1 Pesticides**

A pesticide is any substance or mixture of substances intended for preventing, destroying, controlling repelling, or mitigating any pest (US/EPA, 2005). These includes vectors of human or animal disease, unwanted species of plants or animals causing harm during production, processing, storage, transport or marketing of agricultural produce food (FAO, 2005). Many pesticides are used in agriculture to increase yield, control microorganisms that may produce toxic or carcinogenic metabolites, and reduce the price of food production (Neff et al., 2015). Residues from these pesticides often remain on or in the produce, thus creating a source of human exposure (Neff, et al., 2012).

#### **2.1.1 Classification of pesticides**

Pesticides can be classified based on toxicity, target pest, mode of action and chemical structure (Yadav & Linthongambi, 2017). The classification of pesticides basing on toxicity is by WHO and made up of the following classes Ia, Ib, II, III and IV. (Table 1) (WHO, 2009).

**Table 1:** WHO Classification of pesticides

WHO class	Toxicity level	Lethal Dose (LD) 50 for the rat (mg/Kg body weight)		Examples
		Oral	Dermal	
	Extremely			
Class Ia	hazardous	<5	<50	Parathion, Dieldrin
Class Ib	Highly hazardous	5–50	50–200	Eldrin, Dichlorvos
	Moderately			
Class II	hazardous	50–2000	200–2000	DDT, Chlordane
Class III	Slightly hazardous	>2000	>2000	Malathion
	Unlikely to present acute hazard in			Carbetamide,
Class IV	normal use	5000		Cycloprothrin

Source: (WHO, 2009)

### 2.1.3.1 Classification based on target pest

In this mode of classification, pesticides are grouped according to the target pest they are intended to control (Table:2) (Yadav & Linthongambi, 2017).

**Table 2:** Pesticides classification by target pest

<b>Type of pesticides</b>	<b>Target Pests/Function</b>	<b>Example</b>
Insecticides	Kill insects and other arthropods	Aldicarb
Fungicides	Kill fungi (including blights, mildews, molds and rusts)	Azoxystrobin
Bactericides	Kill bacteria or acts against bacteria	Copper complexes
Herbicides	Kills weed (unwanted plants)	Atrazine
Acaricides	Kill mites that feed on plants and animals	Bifenazate
Rodenticides	Control mice and other rodents	Warfarin
Algaecides	Controls the growth of algae	Copper sulfate
Larvicides	Control the growth of larvae	Methoprene
Repellants	Repel pests by its taste or smell	Methiocarb
Desiccants	Act on plants by drying their tissues	Boric acid
Ovicides	Inhibits the growth of eggs of insects and mites	Benzoxazin
Virucides	Acts against viruses	Scytovirin
Molluscicides	Inhibit or kill mollusc's i.e. snail's	Metaldehyde
Nematicides	Kill nematodes that act as parasites of plants	Aldicarb
Avicides	Kill birds	Avitrol
Moth balls	Stop any damage to cloths by moth larvae and molds	Dichlorobenzene Trifluoromethylnitrophenol
Lampricides	Target larvae of lampreys which are jawless	3-trifluoromethyl-4-nitrophenol
Piscicides	Act against fishes	Rotenone
Sivicides	Acts against woody vegetation	Tebuthiuron
Termiticides	Kills termites	Fipronil

Source: (Yadav & Linthongambi, 2017).

### 2.1.3.2 Classification based on the mode of action

Based on the mode of action, pesticides are categorized according to the way they enter or affect the target pest (Yadav & Linthongambi, 2017). In this regard, pesticides are either contact or systemic. Contact pesticides control their targets by direct contact. For example,

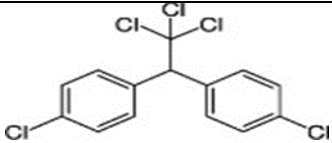

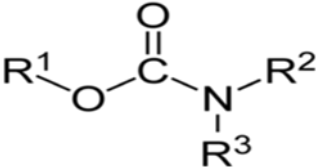
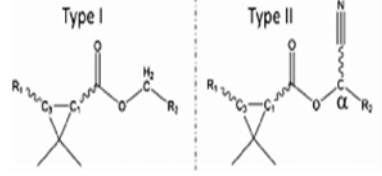
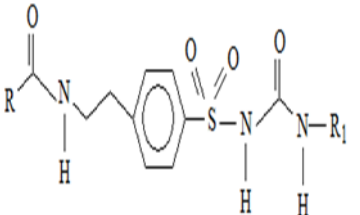


weeds are killed when enough of their surface area has been covered with the herbicide (Yadav & Linthongambi, 2017; IRAC, 2018). Insects may be killed when sprayed directly or when they crawl across surfaces sprayed with contact insecticides. Systemic pesticides control their targets by being translocated in treated plants (Yadav & Linthongambi, 2017; IRAC, 2018) and pose minimal risk of pesticide drift or worker exposure in agriculture (Sánchez-Bayo, Tennekes, & Goka, 2013). Examples that are used in Uganda include paraquat and diquat dibromide and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) and glyphosate for contact and systemic pesticides respectively.

### **2.1.3.3 Classification based on chemical Structure**

Under chemical structure classification, pesticides are categorized according to the chemical nature of the active ingredients. This mode of classification is by far the most accepted in the field of pesticides and environment (Yadav & Linthongambi, 2017). The classification gives clue of the efficacy as well as the physical and chemical properties of the respective pesticides. These aspects are very important in the mode of application during use of the pesticide. Based on chemical structure, pesticides are further divided into five main subgroups namely; organochlorines, organophosphorous, carbamates and pyrethroids and sulfonyleureas (Table ( USEPA, 2015; Yadav & Linthongambi, 2017)).

**Table 3: Pesticides Classification based on Structure**

Type of pesticide	Chemical structure	Characteristics	Application	Toxicity	Examples
Organochlorine	 <p>Dichlorodiphenyltrichloroethane Source: (Jayaraj, 2017)</p>	They have a long-term residual effect in the environment	They are widely used as insecticides for the control of a wide range of insects	They disrupt the nervous system	DDT, Lindane, endosulfan, aldrin
Organophosphorus	 <p>Source: (Jayaraj, 2017)</p>	They are biodegradable, minimum environmental pollution, and slow pest resistance	They are used as insecticides in agriculture and chemical warfare agents	They are Endocrine Disrupting chemicals (EDC)	Malathion, Temephos, Fenthion, Dichlorvos,
Carbamates	 <p>Source: (Raja, Sathiyaraj, Ali, &amp; Nasar, 2015)</p>	They can be easily degraded under natural environmental.	Huge family whose members are effective as insecticides, herbicides, and fungicides	They are EDC	Carbaryl, Carbofuran, Propoxur and aminocarb
Pyrethroids	 <p>Source: (Raja, Sathiyaraj, Ali, &amp; Nasar, 2015)</p>	They are non-persistent and get broken easily on exposure to light	They are commonly used as insecticides and recommended for in-home insect control because considered to be relatively non-toxic in all stages of life	They are safest insecticides for use in food.	Cypermethrin and Permethrin
Sulfonylureas	 <p>Source: (Joshi, Chadha, &amp; Rmchandran, 2014)</p>	They are easily degraded under natural environment	They are needed in agriculture as herbicides	They work by inhibiting the enzyme acetolactate synthase which is vital for weed growth. They exhibit extremely low acute and chronic mammalian toxicities in comparison with most herbicides	Pyrazosulfan ethyl and halosulfuron methyl

### **Organochlorine pesticides:**

Organochlorine pesticides (OCP) are organic compounds with five or more chlorine atoms and were the first synthetic organic pesticides to be used in agriculture and in public health (Yadav & Linthongambi, 2017; Jayaraj, 2017). These are compounds, which contain carbon, hydrogen, oxygen and chlorine. Most of them were widely used as insecticides for the control of a wide range of insects ( Jayaraj, 2017). Due to their low cost and the need against various pests, OCPs such as DDT, hexachlorocyclohexane (HCH), aldrin and dieldrin are among the most widely used pesticides in developing countries ( Jayaraj, 2017; Gupta et al, 2018). They are sub-divided into three (3) categories: DDT and its analogs, Cyclodienes (dieldrin, chlordane, and mirex) and hexachlorocyclohexane group (hexachlorobenzene and Lindane). The structure of OCPs in table 3.

### **Organophosphorus pesticides:**

Organophosphorus (OP) is the general name for organic derivatives of phosphorus (Okoli et al, 2017; (Gupta et al, 2018). Organophosphorus compounds were first developed shortly before the Second World War (Kazemi, Tahmasbi, Valizadeh, Naserian, & Soni, 2012). They were used as an insecticide in agriculture and later as chemical warfare agents (Gupta et al., 2018). The OPs are a group of both synthetic and biogenic compounds, characterized by the presence of carbon to phosphorus (C-P) bond (Jayaraj, 2017). This carbon to phosphorus bond replaces one of the four carbon-to-oxygen-to-phosphorus bonds of the more common phosphate ester (Table 3). Organophosphorus compounds are usually esters, amides, or thiol derivatives of phosphoric, phosphonic, phosphinic, or thiophosphoric acids with the following general formula for phosphates and phosphorothioates respectively and also additional side chains such as cyanide, thiocyanate, and phenoxy group (Raju & Kumar,

2016). There are pesticides on the other hand that contain a phosphate group in their basic structural framework as defined by Schrader's formula (Table 3).

The P=O containing structure is referred to as an oxon and the P=S structure as a thion. Schrader's formula is the basic structure of organophosphorous pesticides (Jayaraj, 2017). R1 is an alkyl and R2 may be an alkoxy, alkyl, or dialkylamido group, etc and nucleofuge atom or group (X) (Jayaraj, 2017). A nucleofuge is a leaving group and is the principle metabolite for specific identification, and may be a halogen, cyanide, or phenoxy. Organophosphorous samples degrade rapidly by hydrolysis on exposure to light, air and soil (Akpagu, Nnamani, & Chukwuebuka, 2015). However, small amounts of these pesticides may be detected in food and drinking water (Jayaraj, 2017). Symptoms of severe poisoning include unconsciousness, severe constriction of the pupil of the eye, muscle twitches, secretions from mouth and nose, breathing difficulty, and if not treated, death. Illness may occur quickly or be delayed a few hours. However, if signs or symptoms start more than 12 hours after exposure to the pesticide, it is probably some other illness (Jayaraj, 2017).

### **Carbamates:**

Carbamates are N-substituted esters of carbamic acid ( $\text{NH}_2\text{COOH}$ ) (Abbasi et al., 2017). They constitute a huge family whose members are effective as insecticides, herbicides, and fungicides, but they are most commonly used as insecticides. Examples of carbamates include aldicarb, asulam and bendiocarb (WHO, 1986). The general structure of carbamates (Table 3); If R1 is a methyl group, the carbamate is an insecticide, and if it is an aromatic moiety, it is a herbicide (Liu, Rong, Xiyan, Jinhua, & Lingxin, 2018). On the other hand, if R1 is a benzimidazole moiety, then the compound is a fungicide (Liu, Rong, Xiyan, Jinhua, & Lingxin, 2018). Carbamates function by the reversible inactivation of the enzyme

acetylcholinesterase (AChE). Acetylcholinesterase is involved in the termination of impulse transmission by rapid hydrolysis of the neurotransmitter acetylcholine in cholinergic pathways in the central and peripheral nervous systems into choline and acetate (Colovic, Krstic, Lazarevic-Pasti, Bondzic, & Vasic, 2013). Inhibition of this enzyme leads to increased parasympathetic tone (autonomic nervous system that opposes the effects of the sympathetic nervous system). This is due to increased activity at both muscarinic and nicotinic cholinergic receptors (Kazemi and Tahmasbi, 2012). Acute toxicity from carbamate exposure produces symptoms identical to OP pesticide toxicity. Symptoms of central nervous system toxicity can be significant in children; and seizures, coma, and death can occur (Roberts & Karr, 2012). Encephalopathy, headaches, photophobia, dizziness, and irritability following high-dose exposure have also been reported (Allister, 2015).

### **Pyrethroids:**

They are synthetic analogues of the naturally occurring pyrethrins derived from the flowers of the pyrethrum (*Chrysanthemum cinerariaefolium*) plant. The insecticidal components of pyrethrum flowers are the optically active esters derived from (+)-trans-chrysanthemic and (+)-trans-pyrethroic acids. Pyrethroids have been modified to increase their stability in the environment (Aggarwal, 2007). Pyrethroids are categorized according to their structure and toxicology, including those lacking the -cyano group on the phenoxy benzyl moiety (type I) and those with a -cyano group on the phenoxybenzyl moiety (type II) (Figure 4). In particular, three pyrethroid compounds, namely deltamethrin, permethrin, and alpha-cypermethrin, are commonly used as insecticides and are recommended for in-home insect control because they are considered to be relatively non-toxic to humans in all stages of life (Chrustek, et al., 2018). Pyrethrin I and II differ in their insecticidal properties, with pyrethrin I showing greater lethality and pyrethrin II showing greater knockdown (Peterson, 2016).

Exposure to high doses of pyrethroids in air, food or water can cause giddiness, headache, vomiting, muscle twitching, low energy, convulsions and loss of consciousness (Jayaraj, 2017).

### **Sulfonylureas:**

Sulfonylureas are a class of organic compounds that are derivatives of sulphonamides and are used in medicine and agriculture. They are antidiabetic drugs widely used in the management of type 2 diabetes mellitus. They act by increasing insulin release from the beta cells in the pancreas (Seino, 2012). A number of sulfonylureas are also used as herbicides, because they interfere with plant biosynthesis of the amino acids, valine, isoleucine and leucine (Gerstein, et al., 2008). Most of the sulfonylurea agents are broad spectrum pesticides and work by inhibiting the enzyme acetolactate synthase, which is vital for weed growth (Gerstein et al., 2008). The enzyme that participates in protein synthesis of plants (Zheng, Yates, & Papiernik, 2008). The sulfonylureas are generally safer to humans and only a fraction of the sulfonylurea agent is needed in agriculture compared to other herbicides (Gerstein et al., 2008). They are traditionally divided into two groups or generations of agents' metformin and acarbose (Joshi, Chadha, & Ramachandran, 2014) (Figure 5).

The use of sulfonylurea herbicides has increased more than 100 times over the past 30 years. However, their presence, persistence and potential impact on the environmental aquatic ecosystems remain poorly studied (Lafontaine, et al., 2014). The occurrence of sulfonylurea herbicides in aquatic environments is receiving public attention (Battaglin, Furlong, Burkhardt, & Peter, 2000; Lafontaine et al., 2014). Residues of sulfonylureas have been detected in surface water and groundwater due to runoff and leaching after respectively (Battaglin, Furlong, Burkhardt, & Peter., 2000; Lafontaine et al., 2014). Because of their high

herbicidal activity sulfonylureas, some crops (e.g., legumes and pastures) are highly sensitive to trace-level residues of the herbicides in soils (Battaglin, Furlong, Burkhardt, & Peter., 2000; Lafontaine et al., 2014). An increased understanding of the environmental fate and behavior of sulfonylureas is imperative to reduce their potential negative effects on agronomic systems. Pyrazosulfuron ethyl (PE) and halosulfuron methyl (HM) are two new highly active sulfonylurea herbicides that are widely used for weed control in a variety of vegetables and other crops and have similar molecular structures, differing only in the substitutions on the pyrazole ring (Lafontaine, et al., 2014).

Animal studies showed low acute toxicity and little chronic, reproductive, or developmental toxicity or teratogenic effects (EPA, 2000 & 2001). At high sub Dossier Submitters (DS) of European Chemical Agency observed lethal, chronic oral doses, nonspecific effects such as weight loss and anemia (EPA, 2000 &2001). Chemical-specific effects were noted at high doses in animals. Primisulfuron-methyl produced chronic nephritis, testicular atrophy, tooth and bone growth disorders (Ongley, 1996; Kamrin, 1997). Halosulfuron and halosulfuron-methyl produced reproductive toxicity and fetotoxic malformations of central nervous system sulfosulfuron produced urinary calculi and bladder tumors (EPA, 2000 &2001).

### **2.1.2 Routes of exposure to pesticides**

The most common way most infants, children and adults are exposed to pesticides is by consuming them as residues on or in food (NRC, 1993). Children can further be exposed to pesticides present at various levels in the environment for example in soils, water and air (Fenske, Kedan, Lu, Fisker, & Andersena, 2002). Of much more concern is exposure to low levels of toxic chemicals in the diet over long periods which can lead to chronic illness (EFSA, 2011).

In Uganda, pesticide usage remains relatively unregulated and the use of counterfeited products inhibits monitoring as chemicals are frequently mislabeled (Nalwanga & Ssempebwa, 2011). The toxicological environment is compounded by the use of four classes of insecticides [organochlorines (DDT), pyrethroids, carbamates and organophosphates] to control disease vectors such as tsetse flies and *Anopheles funestus*, the highest prevalence of which occurs in Northern Uganda (Saleh, 2013). The tobacco and citrus agricultural systems of the region heavily rely on insecticide usage, particularly neonicotinoids (Srigiriraju, Semtner, & Bloomquist, 2010). Within this environmental context, many thousands of small-scale cereal food manufacturers, encouraged by global development organizations and the Government of Uganda, are struggling to produce sufficient cereal-based baby food to raise themselves out of poverty (). Studies investigating pesticide contamination levels of Ugandan cereal-based baby products are almost absent, although a Kenyan study reported the presence of fungicides (chlorothalonil), organophosphates (chlorpyrifos) and fluvalinate at low concentrations in pooled beeswax samples [16].

#### **2.1.4 Environmental effects of pesticides**

Pesticides have different distribution and stability patterns in the environment, even if all of them are distributed in the same way. The stability of a pesticide depends on its physical and chemical properties, and the characteristics of the environment (Fattore, Fanelli, & LaVecchia, 2007). Climate also plays a role in pesticide stability. Insecticides and herbicides persist 3 to 8 times longer in cold climates than in warm ones (Alharbi, Basheer, A.Khattab, & Ali, 2018). The most persistent pesticides are termed as “persistent organic pollutants” (POP). Persistent organic pollutants have low water solubility, persist in the environment, and bio-accumulate in the food chain (Jayaraj, Pankajshan, & Sreedev, 2016). They are lipophilic, travel long distances and produce toxic effects. Most POP are organochlorine pesticides and



include aldrin, endrin, chlordane, DDT, heptachlor, mirex, toxaphene and hexachlorobenzene. Persistent organic pollutants are banned for agricultural and domestic use in accordance with the Stockholm Convention (UNEP, 2009). However, DDT is one of the insecticides recommended by WHO for indoor residual spraying for malaria control, currently used to control malaria in developing countries including Uganda (WHO, 2011). Excessive use of pesticides may lead to the destruction of biodiversity. Many birds, aquatic organisms and animals are under the threat of harmful pesticides for their survival. Pesticides are a concern for sustainability of environment and global stability (Mahmood, Shazadi, Imadi, & Gul, 2015). Mahmood et al. (2015) reported that overuse of pesticides on aquatic ecosystems has led to a serious threat to species of fish including salmon. Pesticides are also seen to affect primary producers and macro-invertebrates (Mahmood, Shazadi, Imadi, & Gul, 2015).

### **2.1.5 Health effects of pesticides**

Pesticides are linked to a wide range of human health issues. They are implicated in inducing cancer e.g., organochlorine, creosote, and sulfallate, while others notably, the organochlorine agents Dichlorodiphenyltrichloroethane (DDT), chlordane, and lindane are tumor promoters, Young females who may be exposed to an endocrine disrupting agent such as DDT have been reported to have a higher incidence of breast cancer (Wallace, 2015), alterations in reproduction, as well as an array of symptoms associated with acute, or short-term, exposures (Nicolopoulou-Stamat, Maipas, Kotampasi, Stamatis, & Hens, 2016). Alterations in normal homeostatic endocrine functioning due to pesticide exposure occurring from fetal development through adulthood can affect the delicate balance of hormonal systems involved with normal development. Pesticides such as DDT, lindane and parathion are endocrine disrupting chemicals (EDC). An EDC is an exogenous substance or mixture that alters

functions of the endocrine system and consequently causes adverse developmental, reproductive, neurological, and immunological effects in an intact organism, or its progeny, or (sub)populations ( Palioura, Kandaraki, & Diamanti-Kandarakis, 2011; Yang, Kim, Weon, & Seo, 2015). Children under the age of 15 particularly exhibit significant risk because their central nervous system, respiratory, gastrointestinal, hepatic systems are still developing moreover they are the most likely targets for pesticide toxicity (ref). Children are also unable to eliminate or neutralize pesticides, which can result in numerous physiological deficits observed during development (Wallace, 2015).

Pesticides are endocrine disrupting chemicals (EDC). Endocrine disruptors may turn off, shut off, or modify signals that hormones carry and thus affect the normal functions of tissues and organs. Consequently, they interfere with fundamental sex steroid effects on the brain, the pituitary gland, the gonads and the accessory sex organs, such as the uterus and mammary gland in females and the prostate and seminal vesicles in males (Brander & Gabler, 2016). Pesticides are therefore associated with various human health effects such as effects on reproduction, endometriosis, precocious puberty, neuro- and immune functions, different types of cancers including cancers of the breast, endometrium, and prostate (Larebeke, 2012). Other negative consequences from pesticide exposure include birth defects and fetal death (Alarcon et al, 2005). Winston (2010), showed a link between pesticide use in rural areas of Costa Rica and incidence of childhood leukemia. Pesticides may also cause acute health effects to those exposed to them, which include blurred vision, muscle cramps, chest pains, excessive sweating, body tremors and shortness of breath (Hu et al., 2015). Chronic exposure to low-doses of pesticides may therefore create a problem of uncertainty among children (Alavanja et al, 2005). The studies on health risks of pesticides in food carried out by the University of Washington (2006) and on health outcomes in Farmworkers populations

exposed to pesticides, reported chronic, lower dose exposure is associated with respiratory problems, memory disorders, skin conditions, depression, miscarriage, birth defects, cancer and neurological conditions such as Parkinson's disease (Bolor, Boadi, Borquaye, & Afful, 2018).

### **2.1.6 Toxicity of pesticide**

Toxicity can be defined as the relative ability of a substance to cause adverse effects in living organisms (Damalas & Koutroubas, 2016; Egorova & Ananikov, 2017). Human health effects due to exposure to pesticides fall into two categories: short-term and long-term effects (Damalas & Koutroubas, 2016). Short-term or acute effects have a relatively quick onset usually minutes to days after exposure to relatively high concentrations of the pesticide. The effect may be local or systemic (Hu et al., 2015; Maipas, Kotampasi, Stamatis, & Hens, 2016). Local effects occur at the site of contact between the toxicant and the body. This site is usually the skin or eyes, and may include the lungs if irritants are inhaled or the gastrointestinal tract if corrosives are ingested. Effects include headaches, dizziness and fatigue (Hu et al., 2015; Maipas, Kotampasi, Stamatis, & Hens, 2016). Systemic effects are those that occur if the toxicant has been absorbed into the body from its initial contact point, transported to other parts of the body, and cause adverse effects in susceptible organs. Many chemicals can cause both local and systemic effects (Hu et al., 2015; Sarwar, 2015; Maipas, Kotampasi, Stamatis, & Hens, 2016). Infants and children pick up these contaminants via the complementary foods they are fed mainly derived from cereal products (UNIDO, 2004; Ismail, Taligoola, & Nakamya, 2012).

Chronic effects are those with a long period of time between exposure and injury (Hu, et al., 2015; Sarwar, 2015; Maipas, Kotampasi, Stamatis, & Hens, 2016). These effects may occur

after apparent recovery from acute exposure or because of repeated exposures to low concentrations of materials over a period of time (Hu, et al., 2015; Sarwar, 2015; Maipas, Kotampasi, Stamatis, & Hens, 2016). The three major causes of chronic toxicity are mutagenic toxicants, oncogenic toxicants, and teratogenic toxicants (Martins & Costa, 2015). Mutagens cause changes in the genetic material DNA. Oncogens cause the formation of tumors; a carcinogen causes the formation of malignant tumors (cancer). Teratogens cause birth defects. Pesticides can cause many types of cancer in humans. Some of the most prevalent forms include leukemia, non-Hodgkin's lymphoma, brain, bone, and breast, ovarian, prostate, testicular, and liver cancers (Bassil, et al., 2007). Chronic toxicity can also affect the reproductive system, nervous system, liver, and kidneys (Bassil, et al., 2007; Martins & Costa, 2015). Prolonged exposure to cholinesterase-inhibiting chemicals, such as organophosphates and carbamates can cause uncontrolled, rapid twitching of some muscles, paralyzed breathing, convulsions, and, in extreme cases, death (Anđelka, Tomašević and Gašić, 2012).

## **2.2 Heavy metals**

The term heavy metal refers to any metallic chemical element that has density greater than 5 g cm<sup>-3</sup> and is toxic at low concentrations (Raikwar, 2008). Heavy metals are trace elements because of their presence in trace concentrations (ppb range to less than 10ppm) in various environmental matrices (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). They are broadly categorized into four major groups based on their health importance (Table 4).

**Table 4: Classification of heavy metals**

<b>Trace (essential)</b>	<b>Non-essential</b>	<b>Less toxic</b>	<b>Highly toxic</b>
Iron (Fe)	Zirconium (Zr)	Cerium (Ce)	Cadmium (Cd)
Manganese (Mn)		Dysprosium (Dy)	Titanium (Ti)
Zinc (Zn)		Europium (Er)	Chromium(VI) (Cr <sup>6+</sup> )
Chromium(III) (Cr <sup>3+</sup> )		Gadolinium (Gd)	Cobalt (Co)
Copper (Cu)		Gallium (Ga)	Lead (Pb)
Nickel (Ni)		Tin (Sn)	Mercury (Hg)
Cobalt (Co)		Gold (Au)	Arsenic (As)

Source: (Raikwar, 2008)

In moderate quantities (60-70 µg/day, 0.15-1 mg/day, 2-3 mg/day, 2-3 mg/day, and 15mg/day), trace elements (such as Se, Co, Cu, Mn, and Zn respectively), are essential to maintain the metabolism of the human body (Bhargava, Gupta, Vats, & Goel, 2017). However, beyond these threshold levels, these elements become toxic. On the contrary, As, Cd, Hg and Pb have no known biological functions in the body and are highly toxic in very small doses (Singh, 2011; Saleh, 2013). Heavy metals, like pesticides, are endocrine disruptors. Because of their toxicity, and the fact they are ubiquitous in the environment, As, Cd, Hg and Pb, were the subject of this study.

Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources. Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations. The presence of heavy metals and other pollutants in the aquatic systems has become a serious problem. In the recent study by Bakayita, Norrstron, & Kulabako, 2019, the shallow groundwater from protected springs in Kampala city, surface water, wastewater, landfill leachates, and surface runoff from several sub-

catchments of the Lake Victoria catchment zone had elevated levels of metals above the WHO guideline values, Ugandan and Canadian standards (Bakyayita, Norrstron, & Kulabako, 2019). Heavy metals contamination in vegetables cultivated on a major urban wetland drainage system of Lake Victoria (Mbabazi, Kwetegyeka, Wasswa, & Bakyayita, 2010). Mercuric pollution of surface water, fish and yams in areas of Namukombe stream, Busia- Uganda (Omara, et al., 2019)

### **2.2.1 Arsenic**

Arsenic is ubiquitous in nature, it is found in air, water, fuels and marine life although the concentration of this element in air and water is generally low (Rasmussen & Andersen, 2003; Uthus, 2003). Arsenic is toxic to human cells at very low concentrations (Pant & Rao, 2010; Liu & Lu, 2010; Martinez, Vucic, Becker-Santos, Gil, & Lam, 2011; Flora, 2011). It induces mutagenesis, carcinogenesis and teratogenesis through alterations in cell differentiation and proliferation (Liu & Lu, 2010); the induction of chromosomal aberrations and sister chromatid exchange (Pant & Rao, 2010); and the mediation of increased cellular tyrosine phosphorylation, a mechanism associated with uncontrolled cell growth and cancer development, in exposed cells (Flora, 2011). Both exposure and genetic factors may influence susceptibility to arsenic-induced malignancies (Karagas et al., 2012). Once in the body, As binds to haemoglobin, plasma proteins and leukocytes and is redistributed to the liver, kidney, lung, spleen and intestines meaning that As may be associated with the aetiology of liver and lung cancers, and kidney failure (ref).

The daily human intake of As contained in food is in the range 0.5–1 mg, with the greatest concentrations coming from fish and the seafood (Taylor et al., 2017; Matsumoto-Tanibuchi, Sugimoto, Kawaguchi, Sakakibara, & Yamashita, 2018; Wolle & Conklin, 2018 ). None-the-

less, acute arsenic intoxication resulting in fatality is rare. According to literature, estimation of the true extent of the impact of chemical hazards in food on children's health is difficult due to the long latency periods that occur between exposure and outcome (Sly et al., 2016). Knowledge of the effects of exposure to hazardous chemicals such as As in food and water on children's health is therefore important.

### **2.2.2 Lead**

Lead is one of the most dangerous chemicals to children (Kumar & Clark, 2009; Wani, Ara, & Usmani, 2015). Aside from its acute toxicity, the most important effect of Pb exposure is chronic neurotoxicity, which is particularly severe during the first two to three years of life when early development of the central nervous system occurs (Kumar & Clark, 2009; Boeckelmann, Pfister, & Darius, 2011; Wani, Ara, & Usmani, 2015). Exposure to lead during this time increases the risk of mild mental retardation, attention deficit, hyperactivity disorder and other developmental disabilities (Koller, Brown, Spurgeon, & Levy, 2004). The Institute for Health Metrics and Evaluation (IHME) estimated that in 2016 lead exposure accounted for 540 000 deaths and 13.9 million years of healthy life lost (disability-adjusted life years (DALYs)) worldwide due to long-term effects on health (WHO, 2010). The highest burden was in low- and middle-income countries. IHME also estimated that in 2016, lead exposure accounted for 63.8% of the global burden of idiopathic developmental intellectual disability, 3% of the global burden of heart disease and 3.1% of the global burden of stroke (WHO, 2010). Lead is toxic to the peripheral and central nervous systems, reproductive system, the immune system, kidney and liver (Chandran & Cataldo, 2010; Jan, et al., 2015; Assi, Hezmee, Haron, Sabri, & Rajion, 2016; Fenga, Gangemi, Salvatore, Falzone, & Libra, 2017). The neurological and behavioral effects of lead are irreversible (Chandran & Cataldo, 2010).

There is no known safe blood lead concentration. Nevertheless, as lead exposure increases, the range and severity of symptoms and effects also increases. Even blood lead concentrations as low as 5 µg/dL may be associated with decreased intelligence in children, behavioral difficulties, and learning problems (Chandran & Cataldo, 2010; Jan, et al., 2015; Assi, Hezmee, Haron, Sabri, & Rajion, 2016; Fenga, Gangemi, Salvatore, Falzone, & Libra, 2017). Adults absorb approximately 15% of ingested lead, while children and pregnant women absorb nearly 50% of ingested lead (Keil, Berger-Ritchie, & McMillin, 2011). According to WHO, lead is a chemical of major public health concern, needing action by all Member States to protect especially children and women of reproductive age (WHO, 2010).

### **2.2.3 Cadmium**

Together with Hg and Pb, Cd is one of the ‘big three’ heavy metal poisons and has no known essential biological function (Tangahu, et al., 2011; Oves, Khan, Qari, Felemban, & Almeelbi, 2016). Cadmium is a soft, silver-white metal found naturally at low levels in rocks and soil (Aktar, Sengupta, & Chowdhury, 2009). It is used in nickel-cadmium batteries, electroplating as a component in metallurgical and brazing, soldering alloys, in pigments, and as stabilizer for plastics (Aktar, Sengupta, & Chowdhury, 2009; Sharma, Rawal, & Mathew, 2015). Further environmental sources are smelting of other metals like Zn, burning of fossil fuels and waste materials (often deposited as solid waste), use of high phosphate and sewage sludge fertilizers (ref). Cadmium is a toxic metal with elimination half-life of 10-30 years, accumulating in the body especially in the kidneys. Its urinary excretion is a biomarker of its body burden (Aktar, Sengupta, & Chowdhury, 2009; Sharma, Rawal, & Mathew, 2015).

Cadmium is a human carcinogen group I by the IARC (WHO, 2007). Intake of highly cadmium-contaminated foods causes acute gastrointestinal effects, such as vomiting and



diarrhoea. The main problem for chronically exposed populations is kidney damage and a possible higher risk of kidney stones (Bhattacharjee & Goswami, 2018). The amount of cadmium in the kidney tubular cells increases during a person's lifespan and makes up the major part of the cadmium body burden. Maternal exposure to cadmium is associated with low birth weight and an increase of spontaneous abortion (Bhattacharjee & Goswami, 2018). Cadmium is present at very low levels in a wide range of foods, and food products account for more than 90% of human exposure to cadmium (ref). The main food sources are the kidneys of animals, which are generally higher in cadmium than are other foods, as well as Cd-contaminated rice, soybeans and seafood ( Eticha & Afrasa, 2018 ). The packaging materials for pre-prepared and fresh foods may also contain considerable levels of Cd that may migrate into food.

#### **2.2.4 Mercury**

The environmental contamination of land, air, water, and wildlife in various ecosystems with Hg around the world due to the natural release and extensive anthropogenic use of Hg has been a global concern for decades (Gworek et al., 2015). Mercury is a global contaminant posing severe risks to the health of ecosystems and humans worldwide (Staufer, Pignolet, & Alvarado, 2016; Ye, et al., 2016). It occurs in nature in several physical and chemical forms, all of which can produce toxic effects in high doses. It is a highly reactive and toxic transition element. The different chemical forms of mercury include elemental mercury vapor ( $\text{Hg}^0$ ), inorganic mercurous ( $\text{Hg I}$ ), mercuric ( $\text{Hg II}$ ), and organic mercuric compounds (Keil, Berger-Ritchie, & McMillin, 2011). These forms have toxic effects in a number of organs such as brain, kidney, and lungs (Sharma, Singh, & Siddiqi, 2014). It is a teratogen, with no known mutagenic action; it trans-passes the placenta, interferes in key pathways in development; Notch pathway, modulation of key enzymatic antioxidant and oxidative stress

makers; it affects sperm phenotypes and various organ compartments during embryogenesis, and is neurotoxic (Manduca, Naim, & Signoriello, 2014). Mercury also affects spermatogenesis, causes malformations and reproductive damages in mammals.

The pathways of exposure to mercury include the following: (a) ingestion of food, (b) inhalation or absorption (via the skin) of mercury vapor at worksites, (c) exposure during the processing of industrial and household wastes, and (d) use of pharmaceutical drugs or cosmetics (Ye et al., 2016). Mercury, which passes into the human body through pathways such as the digestive system, respiratory system, and the skin, is absorbed within the body in varying rates, depending on the form of the mercury compound. The Korea Food and Drug Administration (KFDA) reported that the exposure contribution rate of inhalation from the air was 0.47–0.83 %, ingestion of drinking water (tap water) was 0.01–0.02 %, and ingestion of soil, 0.03–0.32 %; all of them were highest in infants and toddlers (Ye et al., 2016). The study also reported ingestion of food as the major medium of mercury exposure accounting for 98.85–99.48%. Mercury is present in fish and seafood products largely as methylmercury. Food sources other than fish and seafood products may contain mercury but mostly in the form of inorganic mercury. Methylmercury is highly toxic moreover at very low levels, particularly to the nervous system; the developing brain is the most sensitive target organ for methylmercury toxicity (Santos, et al., 2016).

The study shows on fish consumed on Lake Albert has mercury levels higher than FAO/WHO guidelines, and the belly fat of Nile perch bio-accumulated more mercury than Tilapia (Tamale, et al., 2016). Based on the above, it is clear that some fish species should not be eaten by the vulnerable groups (infants and children) due to levels of Hg found in the muscle and belly fat (Tamale, et al., 2016).

### **2.2.5 Environmental sources of contamination of arsenic, cadmium, mercury and lead**

Many heavy metals occur naturally in the earth's crust and are used in many industrial processes. Arsenic, Cd, Hg and Pb enter the environment primarily because of industrial emissions or via disposal of products containing these metals, including mercury-cadmium or cadmium-nickel batteries, lead-containing ceramics and glass, mercury thermometers, etc (Sharma, Kaur, & Nagpal, 2018). Metals are present in many consumer products as well (Turner, 2019). These metals end up in groundwater and soil, and finally in the food supply.

The major dietary sources of exposure are those foodstuffs in which, for particular reasons, high levels of the individual metals are present. Cadmium's greatest source of human exposure is phosphate fertilizers, followed by fossil fuel combustion, rechargeable batteries, certain cosmetics, iron and steel production, natural sources, cement production, cadmium-containing products, and cigarette smoke (Turner, 2019). In as far as, food is concerned; Cd is present in bread and other cereals, potatoes, root crops, and vegetables (Wallace, 2015). This is because Cd has been the most extensively studied and appears to be the most potent metalloestrogen at stimulating the estrogen receptor (Wallace, 2015).

Because of industrial releases of inorganic mercury into environments, followed by uptake by microorganisms, which convert the less toxic inorganic mercury into the more toxic methyl mercury, the latter bio-accumulates to potentially toxic levels in species at the top of the food chain (Santos, et al., 2016) which may then form part of the human diet (Santos, et al., 2016).

Lead contamination of food arises because of environmental emissions, such as mining and leaded petrol (Food Authority of Ireland, 2009). Lead can accumulate in fish and in addition can be at higher levels in the liver and kidney of animals (Food Authority of Ireland, 2009).

### 2.2.6 Heavy metal contamination in baby foods

Infant feeding deserves top priority in child healthcare and appropriate infant formula can prevent millions of deaths occurring from infantile gastroenteritis and malnutrition. The presence of As, Cd, Hg and Pb, in infant formula products has been reported in literature (Eticha, Afrasa, Kahsay, & Gebretsadik, 2018). Lead is n found in drinking water, certain kinds of fish, tilapia and Nile perch in Lake Albert, Uganda, contain high levels of Hg, and high levels of As are found in rice (Shirwaikar, 2013). This includes foods made for babies and toddlers, such as popular snacks, cereals, prepared entrées, and packaged fruits and vegetables. Exposure to hazardous chemicals during growth and development can result in long-term effects on the health of children. The strict regulations and measures applied in different countries mean that food is generally safe, but ingestion of contaminated food may still present an important route of exposure to chemical hazards. As their bodies are developing and they generally consume more food on per unit body weight than adults, children are at particular risk of illness from exposure to chemical hazards in food. The permissible limits focus on a few contaminants in food, mainly heavy metals. Unacceptably high exposures can be avoided when the levels of hazardous substances in food are monitored and controlled (Table 5)

**Table 5:** Permissible limits (mg/kg) for lead, cadmium, arsenic and mercury in baby foods

Agency	Pb	Cd	As	Hg
FAO/WHO (2003)	0.025	0.05	0.015	0.005
EU (2006)	0.02	0.02	0.1	0.5
USEPA (2014)	0.02	0.005	0.05	0.002
CAC(2018)/UNBS (2018)	0.01	0.1	0.2	0.001

### **2.3 Malnutrition rates and complementary feeding in Uganda**

Appropriate complementary feeding is important during the period of 6-23 months since it is also regarded as “critical window” for child’s health, growth, and development. A review by (Abeshu, Lelisa, & Geleta, 2016) reported that Complementary feeding should be timely starting at 6 months, adequate (in amount and variety). During these formative years, poor nutrition has immediate consequences of malnutrition. The malnutrition leads to low cognitive ability, morbidity, mortality, reproductive outcomes, reduced work capacity and health status during adolescence and adulthood (COHA, 2013)

The Ugandan infant and young child feeding policy guidelines and other policy guidelines such as the baby friendly health facility initiative, Essential nutrition actions and the micronutrient powders guidelines have been formulated. Despite these impressive efforts in promoting, protecting, and supporting optimal Infant Young Children fund (IYCF), it has been found that complementary foods are nutritionally inadequate, appropriate complementary feeding is still very low at 14 % (UBOS, 2017). This rather translates into high malnutrition levels. Malnutrition directly or indirectly is responsible for 45% of the under 5 year’s child mortality annually (WHO, 2017).

In eastern Uganda, 40% of children below 5 years suffer from iron deficiency anaemia (UNDP, 2005). Vitamin A deficiency was found to be a public health problem in the country according to WHO criteria with Xerophthalmia diagnosed in 5.38% of the children, 2.52% had night blindness, 1.74% had corneal scars, 1.04% had Bitot’s spots, and 0.26% had corneal xerosis (WHO, 2017). . Uganda has an infant mortality rate of 75/1,000 live births and an under 5 mortality rate of 137/1,000 (UNDP, 2005). However, a downward trend in infant and child mortality rates has been reported in the last 15 years. The mortality rate of children below 5 years between 2001 and 2006 dropped from 157 to 137deaths/1,000 live

births and to 130 in 2009 (UNDP, 2005). Malaria constitutes 25% of the child death, 19% are due to respiratory infections and 17% due to diarrhoea (UBOS, 2017).

### **2.3.1 Food and food products used for complementary feeding in Uganda**

Proper complementary feeding practice ensures that the foods given are rich in energy and in micronutrients (especially iron, zinc, calcium, vitamin A, vitamin C and folates), free of contamination (pathogens, toxins or harmful chemicals). Easy to eat and easily accepted by the infant, in an appropriate amount, easy to prepare from family foods, and at a cost that is acceptable to the families (Monte et al., 2004)

Foods should vary and be balanced with mixtures containing cereals, tubercles, foods of animal and vegetable origin, and fats should be offered since the consumption of these foods is relatively small among infants/children aged between 6 and 24 months. Only varied diets guarantee the supply of; energy, protein and micronutrient, enhance good eating habits, and prevent the development of anorexia caused by monotonous foods. Due to low intake of micronutrient rich foods, low bioavailability and frequent infections, micronutrient requirements are often not met. Infants aged between 6 and 12 months cannot eat enough foods to meet their micronutrient requirements and fortified foods are inaccessible to most rural families. Therefore micronutrient supplementation, e.g. of vitamin A are recommended in endemic deficit areas.

### **2.3.2 Pesticide use in grain /food storage**

Food security in sub-Saharan Africa largely depends upon improved food productivity with sustainable agricultural practices and the reduction of post-harvest losses caused by pests and diseases (Dahiru, Abdullahi, & Bukar, 2014). Xiaosong and Weston (1995) reported that, pests and diseases pose the greatest threat to increased food production, storage and handling with insects causing 15 – 100% pre-harvest, and 10 – 60% post- harvest food grain losses

respectively. In particular, storage insect pests cause substantial damage to the stored grain (Mvumi and Stathers, 2003). However, Kennedy (1998) pointed out that to ensure high food quality and Dahiru et al, 2014 standards, which are acceptable to the consumer, quality control, including good storage and handling practices must be observed at all times. Managing stored grains requires the use of various techniques to ensure that the quality of the stored grains does not deteriorate over time. These measures include the use of sanitation; storing sound, dry grain; managing temperature and aeration; and using chemical protectants, regular sampling, and fumigation (Dahiru, Abdullahi, & Bukar, 2014).

Of all the pesticides released into the environment every year by human activity, persistent pesticides are among the most dangerous (FAO/WHO, 2005). They are highly toxic, causing an array of adverse effects, notably death, diseases and birth defects among human and animals. These additives and chemicals are also effective at creating a toxic storage environment, lowering the pH, or leaving an unpleasant smell. Pesticides can effectively control pest and insect infestations and prevent grain losses; however, their effectiveness can be reduced over time, after which the grain is susceptible to losses (Mlambo, Mvumi, Stathers, Mubayiwa, & Nyabako, 2017)

### **2.3.3 Post-harvest handling, warehouse handling and storage of cereals?**

Harvesting is the first step in the grain supply chain and is a critical operation in deciding the overall crop quality. In the developing countries, crop harvesting is performed manually using hand cutting tools such as sickle, knife, scythe, cutters.

In Africa, Post-harvest losses remain a persistent challenge in the cereal sub-sector. According to the World Resources Institute, approximately 23% of available food in Sub-

Saharan Africa is lost or wasted (GKI, 2014). Post-harvest loss of grains lowers the income and standards of living of the farmers (Okoruwa, Ojo, Akintola, Ologhobo, & Ewet, 2009) due to poor practicing of post-harvest handling processes of harvesting, drying, shelling, treatment and storage which reduces the grain not only in quality but also in quantity (Gustavsson, Cederberg, Sonesson, Otterdijk, & Meybeck, 2011; Tokida, Haneishi, Tsubi, Asea, & Kikuchi, 2014). Poor post-harvest handling such as poor drying and improper storage conditions lead to losses due to storage pest and aflatoxin contamination (MAAIF & UBOS, 2013). Maize is usually harvested with moisture content in the range of 18–26%, which is considerably higher than the 12–14% commercial standard for East Africa (US EAS 2: 2018), therefore, drying is important to reduce the moisture level to accepted level of 13.5%. Most farmers in Africa, both small and large, rely almost exclusively on natural drying of crops from a combination of sunshine and movement of atmospheric air through the product, so damp weather at harvest time can be a serious cause of post-harvest losses (De Lima, 1982). Grains should be dried in such a manner that damage to the grain is minimized. Farmers should avoid contamination of the grain by using heavy polythene or Tarpaulin or use concrete slab to maintain the maize quality (MAAIF & UBOS, 2013).

Shelling or threshing is a process that frees the grain from the cob, seed head or pod. This process involves the removal of grains husks to check for damage. During this process, a lot of care is needed in order to avoid breakage of grain as a way of reducing risk of pests (US EAS 2: 2018), Shelling (hand-threshing) can be done with a hand-held sheller or using hands (US EAS 2: 2018).

This process should be carefully done because it can assist in the development of insects that may actually be seen during the storage season (FAO, 2009). Cereals can be prone to aflatoxin contamination, particularly when they come into contact with infested soil during



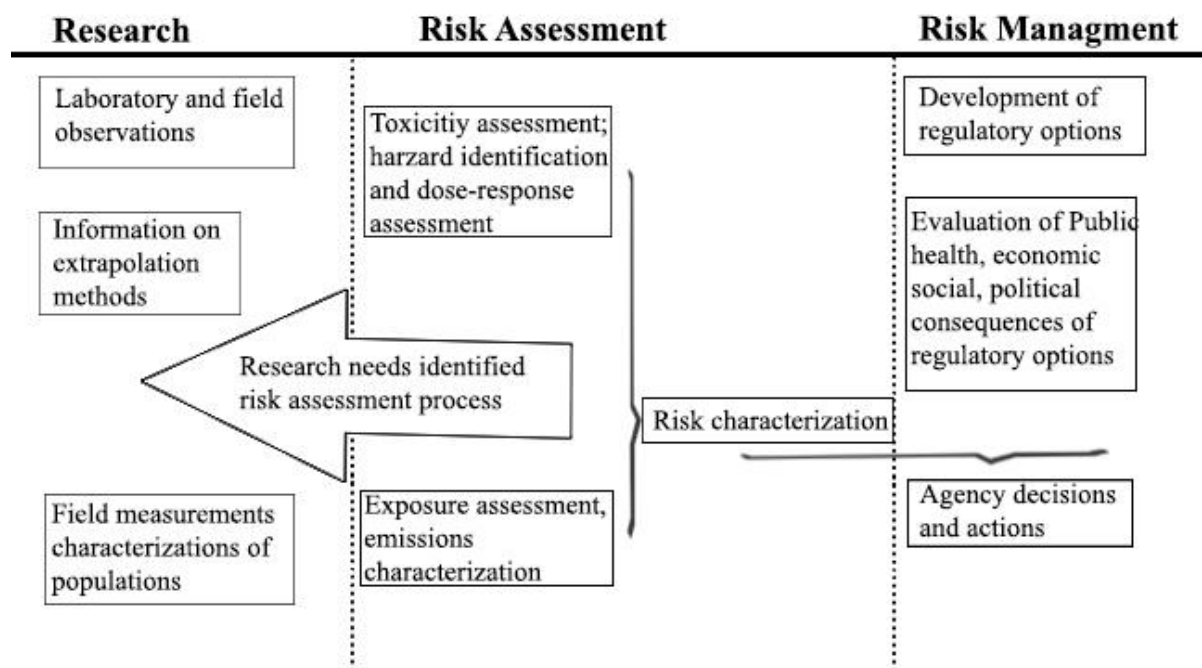
harvesting, threshing, and drying, therefore during this process, farmers should ensure that maize should not get into contact with soil and water (Kimatu et al, 2012).

The main objective of grain storage is to maintain the quality of the produce for a long time (Okoruwa et al, 2012). Due to inadequate storage practices, farmers in the region including Uganda lose up to 40% of their harvest to insects, pests, moulds and moisture (ref). Traditionally, clay-lined grain silos are used for storage in Africa. In each instance, subsistence farmers must take into account the difficulties of storing maize at optimal conditions and balance humidity, the moisture content of the kernels, and the potential for pest infestations (Meridian Institute, 2005). Temperature and moisture content of the cereal grains are the two key features affecting the resulting quality of the grain, biochemical reactions, dry matter losses, allowable storage times and overall storage management of the grain (Lawrence and Maier, 2010). Much as farmers do not have storage space and containers, they struggle to protect the crop from mice and other pests (AGRA, 2013). Farmers in Africa increasingly store grains in polypropylene bags, but the poor aeration in these bags may encourage fungal growth and aflatoxin production, if the grains are not dried to a safe level (Hell, et al., 2000). Poor conditions and lack of adequate storage facilities result in significant post-harvest losses at various stages of the supply chain (World Bank, 2010). Traditionally in Uganda, maize is stored in different locally constructed storage structures such as granaries, Mud-silos, Tua, cribs and commercial stores or living rooms for a period of 2 to 6 months (Kaaya & Kyamuhangire, 2010). In one study, aflatoxin levels were determined in 480 food samples which included maize, sorghum, groundnuts, millet, and rice grains, the frequency of aflatoxin contamination was particularly high (Kaaya & Warren, 2005).

Measures such as integrated pest management to control the infestations are being carried out (PARM, 2017). Integrated pest management involves using fertilizers that can help to produce a healthy plant that is resistant to pests. Counterfeit pesticides coupled with poor application methods by farmers (either poor equipment, poor dosage, non-selective application of pesticides) have led to pesticide resistance in some instances (ref). This means that existing pesticides in the market cannot easily manage certain pests. For example, studies in Uganda have demonstrated the development of resistances by some strains of *Phytophthora infestans* to Metalaxyl fungicide (Mukalazi et al., 2001).

#### **2.4 Health risk assessment due to heavy metals and pesticide residues**

Within the area of food safety, risk is defined as the analysis and prioritization of the combined probability of food contamination, consumer exposure and the size of the anticipated public health impact of especially chemical, and microbiological something missing here related to food (Van-der-Fels-Klerx, et al., 2017). Risk assessment is a four-step analytical process comprising hazard identification, dose-response assessment, exposure assessment, and risk characterization. Health risk assessment on the other hand, is the process used to evaluate the toxic effects of a chemical by monitoring human exposure to the chemical, and characterizing the nature and probability of adverse health effects in the humans who may be exposed to the chemical in contaminated biological media (CAC., 2001; USEPA, 2016). Health risk assessment can be used to characterize the potential adverse health effects of human exposure to environmental hazards and is an effective tool for supporting decision making and corrective actions in risk management (Figure 7). It provides scientific references to different health authorities, related government departments and environmental protection organisations for helping their decision making (Wu, Liu, & Chen, 2018).



**Figure 1:** Elements of risk assessment and risk management

Source: (FAO/WHO, 1995)

Hazard identification is the determination of whether exposure to a heavy metal, when feeding the baby can cause an increase in the incidence or severity of an adverse health effect such as cancer, birth defect, neurotoxicity, etc. Dose–response assessment is the process of characterizing the relationship between the dose of a chemical and the incidence or severity of an adverse health effect in the exposed population. Exposure assessment is the process of specifying the exposed population, identifying potential exposure routes, and measuring or estimating the magnitude, duration, and frequency of exposure. Exposure can be assessed by direct measurement or estimated with a variety of exposure models. Exposure assessment can be quite complex since exposure frequently occurs to a mixture of chemicals from a variety of sources (air, water, soil, food, etc.). It consists of characterization of the exposure setting, identification of the exposure pathway and quantification of exposure (Wu, Liu, & Chen, 2018). Risk characterization is the integration of information obtained in the first three steps to develop a qualitative or quantitative estimate of the likelihood that any of the hazards

associated with the chemical (s) of concern will be realized. This step includes a descriptive characterization of the nature, severity, and route dependency of any potential health effects, as well as variation within the population (s) of concern. Any uncertainties and limitations in the analysis are described in the risk characterization, so that the strengths, weaknesses, and overall confidence in the risk estimates can be understood. In order to calculate the risk, firstly, the intake dose should be obtained from the exposure calculation (Wu, Liu, & Chen, 2018).

#### **2.4.1 The risk determination process**

There are various risk analysis and assessment techniques available in literature. However, a basic classification of the risk analysis and assessment methodologies based on the literature include deterministic and probabilistic approaches (Marhavidas, Koulouriotis, & Gemeni, 2011). Deterministic risk or “point” estimate approach consists of assigning a single representative value to each exposure parameter, which appears in a risk equation leading to a unique risk value (Rivera-Velasquez, Fallico, Guerra, & Straface, 2013). This approach is based on a selection of a fixed level in the distribution of consumption multiplied by a fixed value (mean, median, mode etc.), chosen from the distribution of concentration usually obtained by field sampling and measurement. In practice, the fixed levels utilized to calculate a “point estimate” are generally chosen assuming a conservative scenario, thus being on the safe side when determining the absence of safety concern. This method may not reflect the exposure of the overall population, but it is often considered the most appropriate for screening purposes.

Probabilistic risk analysis considers the degree of variability and uncertainty of each parameter in the risk equation through an estimation based on stochastic methods such as, for

instance, Monte Carlo simulations to derive a distribution of a risk based on multiple sets of values sampled for random variables (Liesefeld, 2015). In the probabilistic approach, each parameter in the risk equation is assigned a probability density function that describes the behavior of the risk in probabilistic terms. Thus, the probabilistic risk analysis may provide more information than the traditional deterministic approach through the curves of probability distributions, which evaluate intervals of possible values of the risk, each one with a specified probability (Rivera-Velasquez, Fallico, Guerra, & Straface, 2013).

#### **2.4.2 Health risks due to heavy metal exposures in baby foods**

Health risk assessment classifies risks as cancer or non-cancer. The classification determines the procedure to be followed when calculating potential risks (Yu, Wang, & Zhou, 2014). Cancer risks associated with exposure to a measured dose of chemical contaminant can be estimated using the incremental lifetime cancer risk (ILCR). The United States Environment protection Agency (USEPA) suggests the chronic daily intakes (CDI) of toxic chemicals, which represents the lifetime average daily dose (ADD) as the exposure metric (Yu, Wang, & Zhou, 2014). The ILCR is obtained using equation 1.

$$\text{ILCR} = \text{CDI} \times \text{the cancer slope factor (CSF)} \quad (1)$$

The CDI is calculated according to equation 2 (USEPA, 1989).

$$\text{CDI} = (\text{C} \times \text{IR} \times \text{EF} \times \text{ED}) / (\text{BW} \times \text{AT}) \quad (2)$$

Where: CDI is the chronic daily intake (mg/kg/day); C is the concentration of the contaminant in the food, (mg/L); IR is the ingestion rate per unit time; ED is the exposure duration (6 years for children; EF the exposure frequency (365 days/year); BW is the body

weight (15 kg body weight for children); AT is the average time for carcinogens,  $AT=70 \times 365 = 25550$  days for both children and adults; for non-carcinogens,  $AT=ED \times 365$  which equals 2190 days for children (USEPA,1989).

Non-cancer risks (such as diabetes, cardiovascular diseases) are estimated as the non-cancer hazard quotient (HQ), which is computed using equation 4.

$$HQ = CDI/RfD \tag{3}$$

Where: RfD (RfD;  $mgkg^{-1}day^{-1}$ ) is the oral reference doze of the chemical hazard, is an estimate of the maximum permissible risk on human population through daily exposure, taking into consideration a sensitive group during a lifetime (Li et al., 2013). For instance the RfD for lead, cadmium, arsenic and mercury are 0.00036 (mg/kg/day), 0.0005 (mg/kg/day), 0.003 (mg/kg/day), and 0.0003 (mg/kg/day) respectively (USEPA, 1989).

## **CHAPTER 3: MATERIALS AND METHODS**

### **3.1 Materials**

Baby foods used in this study, were purchased from supermarkets and shops in Kampala City. All chemicals used were of analytical grade. Baby foods were coded with letters A, B, C, D, E and F in order to conceal their source and identity. Each sample had replicates from different batches, representing with the producer.

### **3.2 Equipment**

Pesticides screening was determined using a GC-MS 5973 Agilent (Japan) with column DBS-MS (30 m x 0.25 mm ID, film thickness 0.25  $\mu$ m). Heavy metal concentration was determined on a Shimadzu Electro-thermal Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS; model AA-6300, Japan) equipped with High-speed Deuterium (BGC-D2) and Self-Reversal Method Background Correction (BGC-SR) along with an ASC-6100 auto-sampler (Shimadzu Corporation, Japan).

### **3.3 Methods**

#### **3.3.1 Evaluation of heavy metal and pesticide awareness among manufacturers of baby foods in Uganda**

In order to determine the level of pesticides residues and heavy metals contamination awareness among manufacturers of baby foods, sixteen (16) raw material stores of these companies were assessed using a set of questionnaires. The assessment included the level of education of the working staff, availability of pallets, organization of the store, humidity control in the store and preservation of the grains.

The questionnaire consisted of interrelated questions on the dimensions of post-harvest and warehouse handling of cereal grains before being processed (Appendix 2) and self-administered. Opinions, perceptions, attitudes and beliefs from 32 respondents were gathered. The questionnaire method was used to collect this type of data because it is an efficient data collection mechanism (Sekaren, 2003; Creswell, 1994). It also enables collection of substantial information over a short time interval at low cost moreover free from bias of the interviewer (Kothari, 2004).

### **3.3.2 Sampling and sample treatment**

A total of eighteen (n=18) samples of locally produced cereal-based baby foods were purchased from local supermarkets in Kampala city. Samples A, B, C, D, E, and F were millet, rice, rice, maize, rice and pure rice-based products respectively. Samples were stored in clean polyethylene bags according to type to the procedures of Directorate of Government Analytical Laboratories (DGAL) chemistry laboratory for further preparation and analysis for pesticide residue types and heavy metals. The samples were kept under cool dry conditions until analysis.

### **3.3.3 Pesticide analysis**

The European Standard Method EN 15662:2008 was modified to obtain an appropriate extraction and clean-up procedure for baby food samples (Harmoko, Kartasasmita, & Tresnawati, 2015). The sample was homogenized and 5.0g weighed into a 50 mL centrifuge tube. Then, 10.0 mL aliquot of purified deionised water was added into the above tube. Then it was placed in a ceramic homogenizer. The tube was shaken by hand for a few seconds to hydrate the sample and allowed to stand for 30 minutes. Extraction was carried using 15.0 mL acetonitrile with 1% acetic acid added to the sample matrix. Ten (10) µg/mL ISTD1 of



internal standard solution was added and shaken for 30 seconds. This was followed by sample clean-up using 4.0 g anhydrous  $\text{MgSO}_4$  and 1.0 g NaCl and added the following buffer salts for pH sensitive analytes targeted; 1.0 g trisodiumcitratetrihydrate and 0.5 g disodium hydrogen citrate sesquihydrate. Immediately, the contents were manually shaken for 1 minute and then centrifuged at 8000 rpm for 5 minutes. Then, 8 ml of the supernatant was transferred into a 15-mL QuEChERS dispersive centrifuge tube containing of anhydrous magnesium sulphate (900 mg) and PSA (150 mg) and vortex the extract with the sorbent for 1 minute. This was centrifuged tube for 5 minutes at 8000 rpm and transferred 3 ml of the supernatant using a pipette to a clean glass centrifuge tube (10-mL capacity). The tube was sealed and shaken vigorously for 30 seconds and added 75  $\mu\text{L}$  of internal standard solution ISTD2 (concentration, 1  $\mu\text{g}/\text{mL}$ ).

Concentration was performed by adding 0.5 mL toluene to the supernatant and evaporated the supernatant to less than 0.5 mL under a stream of nitrogen in a 35°C water bath. The extract was reconstituted in 1.5 mL of toluene and run on Gas Chromatography-Mass Spectrophotometer (GC-MS). Representative matrix-matched calibration curves were applied to compensate for matrix effects. The analysis was done to identify the pesticides contained in the samples. This method was validated according to the requirements of European method EN 15662 2007 (ECS, 2008).

### **3.3.4 Determination of heavy metal content**

The sample (5 g) was weighed and dried in an oven (Japan) at 105°C until constant weight was attained. To each dried sample, was added magnesium nitrate solution (15% w/v) followed by charring on a hot plate (Japan) to ensure carbonization. It was then ashed in a muffle furnace (Japan) at 450°C. The ash was dissolved in nitric acid,  $\text{HNO}_3$  (15% w/v) with

warming to ensure total dissolution and the mixture filtered through an acid washed filter paper into a 50 ml volumetric flask. The resulting solution was diluted to the mark of the flask with deionized water. The solution was analyzed for Pb, Cd, As, and Hg and the concentration of blank corrected. Standard solutions of the respective metals were also prepared at 5 different concentrations and their absorbance (A) determined. A calibration curve for each metal was generated and used to extrapolate the metal concentrations in the sample analyte. The calibration curves were linear within the concentration range, with regression coefficients ( $R^2$ ) > 99% (Appendix 4). Heavy metals were determined at wavelengths ( $\lambda$ ) 283.3, 228.8, 193.7, and 253.7 nm for Pb, Cd, As, and Hg, respectively.

### 3.3.5 Heavy metal exposure assessment

The health risk posed to babies and infants was determined by the specific dietary intake of each metal contaminant. The daily intake of HM from the consumption of baby foods was estimated using equation 1 (Copat et al., 2013):

$$\text{Estimated Chronic Daily Intake (CDI)} = (C \times IR \times EF \times ED) / BW_a \times AT \quad (1)$$

Where C, IR, ED, BW<sub>a</sub> and AT represent the HM mean concentrations in baby food ( $\text{mgkg}^{-1}$ ), ingestion rate for food (Kg), Exposure frequency, Exposure duration, Body weight and average time, respectively. The average body weight of a child according to age was obtained from WHO/FAO body weight tables (Appendix 3).

### 3.3.6 Non-cancer risks posed to children due to heavy metal exposures in baby foods

Non-cancer risks were evaluated by the non-cancer hazard quotient (HQ) (Eqn. (2)).

$$HQ = CDI / RfD \quad (\text{Eqn. 2})$$

$$\text{Hazard index (HI)} = HQ \quad (\text{Eqn. 3})$$

Table of energy expenditure for both 6 to 12 and 12 to 24 months (Appendix 3) was used to work out the amount of food the baby would require. The ingestion rate was determined using the energy terms in the product label for each baby food, The RfD values for Pb, Cd, As, and Hg are 0.0035, 0.001, 0.0003 and 0.0003 (mgkg<sup>-1</sup>day<sup>-1</sup>), respectively (Harmanescu, Alda, Bordean, Gogoasa, & Gergen, 2011). To evaluate the potential risk to human health through more than one heavy metal, the chronic hazard index (HI) was obtained as the sum of all HQ calculated for each element (RajeshKumar, et al., 2018). It was assumed that the magnitude of the effect is proportional to the sum of the multiple metal exposures and that similar working mechanism linearly affects a target organ (Sultana, Rana, Yamazaki, & Aono, 2017). The results were then interpreted as shown in Table 6.

**Table 6:** Interpretation of results non-cancer health risks determination

Interpretation	Risk level
Population will not experience significant non-cancer risks.	HQ or HI < 1
Population will experience significant non-cancer risks.	HQ or HI ≥ 1
Population in a level of concern.	1 < HQ or HI < 5
Requires additional data gathering	10 < HQ or HI < 100
Reduce the risk immediately.	HQ or HI > 100

Source: (USEPA, 1989)

### 3.6 Statistical Analysis

Data were analyzed using Stata statistical package versions 12 to generate parametric statistics. Analysis of variance was performed to compare levels of heavy metals and pesticide residues between the different baby foods and respondents from manufacturers. Means were separated using the least significance difference (LSD) at  $p < 0.05$ .

## **CHAPTER 4: RESULTS AND DISCUSSION**

### **4.1 Heavy metal and pesticide awareness among manufacturers of baby foods in Uganda**

Out of the 16 stores sampled, 9 (55.6%) of the stores had supervisors with university level of education while 44.4% had A-level as their highest level of education. Forty percent of the technical staff in all stores had had university as their highest level of education compared to 60% who had high school certificate qualification as their highest level of qualification. Regarding the condition of the store, 88.9% of the sampled facilities stored their cereals on wooden in jute bags supported on wooden pallets. About 11.1% did not store their cereals on pallets. In addition, the organization of store contents revealed that all the respondents interviewed stored cereals separately from other produce. Moreover, 90% of the respondents stored their cereals under dry conditions. These stores had humidity meters to determine the conditions within the stores. Only 10% of the stores appeared damp but not moist meaning that cereals were stored under moisture proof conditions. Maize was the cereal identified to be most stored by the respondents (Table 7).

The majority of the respondents (90%) could easily detect the spoilage of grains, leaving 10% unable to detect spoilt grains (Table 7). With respect to pest control, fumigation was done by skilled personnel in 60% of the facilities while in the remaining 40%, fumigation was done by unskilled personnel. However, all the respondents indicated that fumigation was done in line with quality management systems. In 80% of the facilities, grains were kept for 2 weeks after the fumigation, while 20% kept the grains for 4 weeks before processing. Maize was the cereal identified to be the most prone to attack by pests (Table 8).

**Table 7:** Cereals most stored in Kampala city

Cereals stored	Frequency	Percentage
Beans	5	16.1
Maize	9	29
Millet	6	19.3
Rice	2	6.5
Sorghum	3	9.7
Soya	4	12.9
Wheat	2	6.5
Total	31	100

Only 20% of the manufacturers had UNBS certification, on average, the stores demonstrated adequate awareness of pesticides and heavy metals (Table 8). However, it was observed that these manufacturers purchased their produce early at the beginning of the harvesting and held their stock in the stores for a year.

**Table 8:** Cereals identified to be prone to spoilage.

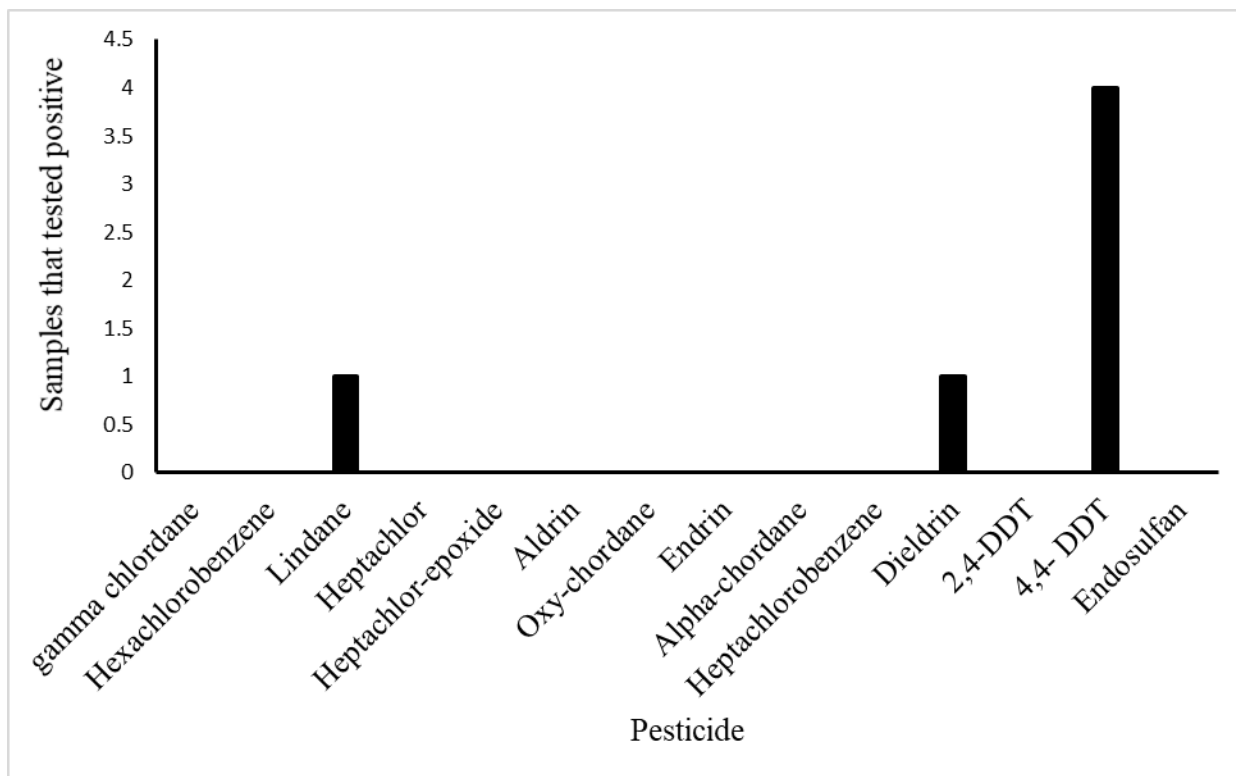
Cereals prone to spoilage	Frequency	Percentage
Beans	7	26.9
Groundnuts	2	7.7
Maize	9	34.6
Millet	3	11.5
Rice	1	3.8
Sorghum	4	15.4
Total	26	100

In Uganda, storage plays a vital role in the food supply chain, and several studies reported that maximum losses happen during this time (Kumar & Kalita, 2017; Manandha, 2018). According

to Manandha (2018) 1.4% to 5.9% losses occur at the farm level and insect infestation is the major reason of storage losses in most of the cases (Manandha, 2018). As much as 50%–60% cereal grains can be lost during the storage stage due only to the lack of technical inefficiency (Kumar & Kalita, 2017). Synthetic insecticides dudu cyper 5% EC, striker, malathion from suppliers for instance Bukoola chemicals are used in Uganda and play an important role in controlling the pests and reducing losses during storage of grains (Mugisha-Kamatenes, 2008). The use of synthetic pesticides has raised a number of both ecological and medicinal problems yet their use has not substantially reduced the pest losses. There is a lot of hope that botanical pesticides will take us a long way in fighting the dangers associated with conventional pesticides, however, botanical pesticides also need risk assessment and hazard characterization in relation to human intake for a given time (Mugisha-Kamatenes, 2008).

#### **4.2 Pesticide residues detected in baby foods**

Baby foods were screened for organochlorine and organophosphorous pesticide residues using HPLC/MS. Of the 14 organochlorine pesticides (gamma-chlordane, hexachlorobenzene, lindane, heptachlor, heptachlor-epoxide, aldrin, oxy-chordane, endrin, alpha-chlordane, heptachlorobenzene, dieldrin, 2, 4-DDT, 4, 4-DDT and endosulfan considered in the study, only three were detected in baby foods (certificate of analysis from DGAL). The organochlorine (4, 4-DDT), was detected in all foods except A and D. Lindane and dieldrin were each detected in only one batch of baby foods B (rice-based product) and F (pure rice product), respectively (Figure 8).



**Figure 2:** Organochlorine pesticide residues detected in baby foods sold in Kampala city

Several chemical contaminants including pesticides and other agrochemicals have been reported in food products in Uganda (Ssebugere and Wasswa, 2010). Five organochlorine pesticides (OCP), namely; aldrin, dieldrin, endosulfan, lindane, DDT and its metabolites were detected in milk in Kampala (Kampire & Kiremire, 2011). Organochlorine pesticide residues were also reported in skin, flesh and whole carrots from markets around Lake Victoria basin (Nannyonga et al., 2013). High levels of DDT were reported in breast milk of mothers nursing their first child and residing in urban areas in Uganda (Ejobi, Kanja, Kyule, Nyeko, & Opuda-Asibo, 1998). In Kenya, high levels of OCPs were reported in breast milk of mothers in rural areas, and p,p'DDT and p,p'DDE were the most frequently encountered contaminants. There were also traces of p,p'DDE in 99.5% and p,p'DDT in 78.2% of the samples. Other residues detected were dieldrin (27%), beta-HCH (18.5%), lindane (12%), and alpha-HCH (8.8%) (Kinyamu, Kanja, Skaare, &

Maltho, 1998). Organochlorine pesticide residue levels were also reported to be high in Zimbabwe (Chikuni, Skare, Nyazema, & Polder, 1991). The major metabolite of p,p'-DDT, p,p'-DDE, and the 3 lindane isomers were detected in high levels with beta-HCH being the most persistent and Dieldrin and heptachlor epoxide levels were quite low (Chikuni, Skare, Nyazema, & Polder, 1991).

Most organochlorine pesticides were banned in the world under the Rotterdam and Stockholm conventions due to their persistence in the environment and toxicity to non-target organisms (ref). However, residues of these compounds are still found in the environment (Olutona & Livingstone, 2018). The public health sector in Uganda heavily depends on pesticides to control vector borne diseases such as malaria, sleeping sickness, bilharziasis and filariasis (Thompson et al., 2017). Organochlorine pesticides, p,p'-DDT, dieldrin and lindane, are the major pesticides used in the control of mosquitoes and tsetse-flies in Uganda (Semalulu, 2005). Lindane is used in seed and soil treatment (Ezemonye, Ikpesu, & Tongo, 2008), DDT and lindane are commonly used on cereal plantations, and for the control of stem borers in maize and endosulfan marketed as thiodan, is widely used for controlling aphids, thrips, beetles, foliar feeding larvae, mites, borers, cutworms, bollworms, bugs, whiteflies, leaf-hoppers, and slugs on grains (Janssen, 2011; Boadu, 2013). Your discussion should link with the objectives of the study. Where do infant formula and related risks come into the discussion

#### **4.3 Heavy metal concentrations in baby foods**

Baby foods were assessed for their As, Cd, Hg, and Pb contents using AAS. Food E had the highest total amount of the metals among all baby foods (Table 9). This was followed by D, F,



A, and C had 15% at 17.8, 17.0, 15.7 and 15.0%, respectively. Food B had the least amount of metals; 11.8% of the total. Arsenic had the highest presence in baby foods with a prevalence of 46% (Table 9). Mercury followed at 20.3% and then cadmium at 17.6%. Lead was least prevalent in baby foods and accounted for only 16.1% of the total. The concentration of As in baby foods ranged from 0.010 to 0.030 mg/kg, with a mean of  $0.017 \pm 0.007$  mg/kg (Table 9). Mercury content ranged from 0.003 to 0.037. All the metals were detected in the cereal-based foods. There was no significant difference ( $P > 0.05$ ) between the different foods in terms of heavy metal contamination. However, baby foods A and E had relatively higher levels of As and Hg than other samples (Table 9).

**Table 9:** Concentration of lead, cadmium, arsenic and mercury in baby foods (mg/kg) sold in Kampala city

Metal	Baby foods						Min	Max	Mean	SD
	A	B	C	D	E	F				
Pb	0.004	0.002	0.003	0.003	0.012	0.013	0.001	0.016	0.006	0.006
Cd	0.003	0.004	0.009	0.009	0.010	0.005	0.002	0.016	0.007	0.003
As	0.025	0.015	0.015	0.013	0.020	0.015	0.010	0.030	0.017	0.007
Hg	0.004	0.005	0.007	0.015	0.099	0.006	0.003	0.037	0.008	0.003

All the four objected metals were detected in the food samples under study. Mercury had the highest concentrations (0.037 mg/kg) while lead had the lowest concentrations (0.001 mg/kg) among all samples analyzed. Total As concentration as high as 0.03 mg/kg was determined in ready-to-eat rice-based gruels and porridges intended for infants from 4 months in Sweden (Concha, Eneroth, Hallström, & Sand, 2013). However, Öhrvik, Engman, Kollander, & Sundström (2013) measured 0.04 mg/kg as the highest concentration of arsenic in rice-based products. According to Signes-Pastor, Carey, & Meharg (2016), 14% of the rice in baby foods

within the United Kingdom is above the JECFA. As maximum level for rice (0.200 mg/kg). Arsenic is ubiquitous in nature; it is found in rocks, water, air, animals, and plants (Ahoulé, Lalanne, Mendret, Brosillon, & Maïga, 2015; Cubadda, Jackson, Cottingham, Horne, & Kurzius-Spencer, 2017). Rice is a major contributor to inorganic arsenic in human diets (Meharg, 2006). Rice and rice-based products have been reported with elevated inorganic arsenic concentrations in many studies, in parts exceeding the regulations set by the European Commission (Wünsche, Lambert, Gola, & Biesalski, 2018). Wheat, maize and oats are aerobically grown and do not translocate arsenic from soil or water to the grain like seen in rice, and show lower arsenic concentrations (Awasthi, Chauhan, Srivastava, & Tripathi, 2017). Arsenic was reported in rice-based infant food obtained from supermarkets and pharmacies in Sweden, the United Kingdom and Spain (Concha, Eneroth, Hallström, & Sand, 2013). The means of total As and inorganic As in Iranian rice were 0.12 and 0.082 mg/kg, respectively (Cano-Lamadrid, Munera-Picazo, Burló, Hojjati, & Carbonell-Barrachina, 2015). Marín et al. (2018) assessed metal levels in different foods and obtained an inorganic As average value of 0.0133 mg/kg fresh weight in cereals, pulses, tubers and nuts. Rice had the high level of As with an average of 0.0740 mg/kg.

Rice-based products are widely used to feed young children during weaning due to its availability, bland taste, high nutritional value and relatively low allergic potential (Signes-Pastor, Carey, & Meharg, 2016). Rice accumulates significantly higher levels of inorganic As from soil and water than other crops due to anaerobic paddy soil culture, which renders inorganic As highly available for plant uptake, leading to *ca.*10-fold higher concentrations in the grain compared to aerobically grown grains such as maize, wheat or barley (Signes-Pastor, Carey, & Meharg, 2016). Thus, arsenic released into the upstream environment from industrial activities,

sewage treatment works, pesticide application and fertilizer use may result in elevated levels in paddy soils. The arsenic content in rice varies depending on the type of the rice cultivar, the place where it is cultivated, and how it is processed; brown rice contains higher concentrations than white rice (Hojsak et al., 2015). Rice-based products also have high levels of As and show a positive correlation between rice content and their As concentration (Signes-Pastor, Carey, & Meharg, 2016). Children, especially toddlers, aged 1–3 years, are at risk concerning arsenic dietary exposure (Guillod-Magnin, Brüscheiler, Aubert, & Haldimann, 2018).

A wide concentration range of Cd from not detected to 0.4 mg/kg in infant foods was reported in different countries (Eticha, Afrasa, Kahsay, & Gebretsadik, 2018).. Muntean, Muntean, Creta, & Duda (2013) reported Cd in baby foods from local supermarkets in Romania, to be in the range from limit of detection to 0.00475 mg/kg. These authors also observed Cd more frequently in cereal based samples as compared to other baby foods. After long exposure periods, Cd accumulates to toxic concentrations in the kidney cortex and this process starts very early in life (Schümann, 1990). In 3-year-old children, Cd concentrations in the kidney can reach up to one-third of those found in adults. Chronic exposure to low levels of Cd can produce tubular damage in the kidney through oxidative stress in humans (Huang et al., 2009). Eticha, Afrasa, Kahsay, & Gebretsadik (2018) reported levels of lead in the range of not detected to 0.103 mg/kg in infant formula in Ethiopia. The levels of Pb and Cd found in breakfast cereals and rice products ranged from < 0.01 to 0.25 mg/kg for Pb and < 0.01 to 0.11 mg/kg for Cd (Tinggi & Schoendorfer, 2018). Rice-containing formulas and baby foods in the United States were reported to be high in Pb and Cd (Gardener, Bowen, & Callan, 2019). Median contents of mercury were 0.0005, 0.0005 and 0.0004 mg/kg for processed cereal-based food infant formulae in this study, and baby

foods, respectively, with a maximum value of 0.0195 mg/kg in a baby food containing fish. Processed cereal based foods contained the largest amounts of cadmium (up to 0.01 mg/kg).

#### 4.4 Non-cancer risks of heavy metals and pesticides in baby food

The Hazard Quotient (HQ) for Pb, Cd, As and Hg in the baby foods was 0.11, less than 1 (0.11) for both girls and boys in the 6, 12 and 24-month categories. This implies that, the risk assessment of Pb taken with cereal based baby food consumption were within safe limits (HQ <1). Nonetheless, foods E and B had the highest HQ (Table 10).

**Table 10:** Hazard quotient for lead in locally manufactured baby foods in Kampala

Element	6 months		12 months		24 months	
	Boys	Girls	Boys	Girls	Boys	Girls
Pb	0.11	0.11	0.11	0.11	0.11	0.11
Cd	0.11	0.11	0.11	0.11	0.11	0.11
As	1.84	1.87	1.83	1.81	1.86	1.87
Hg	0.39	0.40	0.39	0.39	0.40	0.40

Cereal based-products proved to be contributing most to infants' and small children's exposure with the lead and cadmium; however, none of the obtained data was higher than the maximum allowed levels according to Commission Regulation (EC) No 1881/2006 in Romania (Muntean, Muntean, Creta, & Duda, 2013). Anemia and reduced intelligence scores are observed in children after exposure to Pb (Schümann, 1990). The key to preventing lead toxicity in children is to reduce or eliminate persistent sources of lead exposure in their environment (Wani, Ara, & Usmani, 2015). Lead from food and other sources contributes about 400,000 deaths each year

(Levin, et al., 2008). Ingested lead gets into the blood and causes harm to a child's developing brain, contributing to learning and behavioral problems (NTP, 2012).

The HQ for Cd ranged from 0.10 to 0.13 for both girls and boys in the 6, 12 and 24-months' categories due to consumption of locally manufactured baby foods. Like in the case of Pb, foods E and B had the highest hazard quotient (HQ) for Cd (Table 14). These results mean that babies in all categories, would not experience non-cancer risks due Cd in the baby foods.

Cereal based-products contribute most, to Cd exposure in infants and small children (Edward, Muntean, Creta, & Duda, 2013; Mania et al., 2015). Chronic Cd exposure has negative effects on kidneys, heart, lungs, and bones. The main long-term effect of exposure to low Cd concentration being emphysema and kidney insufficiency, reproduction problems, cardiovascular diseases and hypertension. Teratogenic and mutagenic effects have also been reported for Cd (Waalkes, 2000; Donkin, Ohison, & Teaf, 2000; Krizova, Salgovicova, & Kovac, 2005).

The HQ for As was in the range 1.60 to 2.12 for both girls and boys in all age groups meaning that As contributed most towards exposure to non-cancer risks in the exposed populations. Food E and B had the highest HQ for As (Table 15).

There is increasing concern regarding arsenic contaminants in rice, which is one of the most common first solid infant foods in Uganda. Daily dietary intake of food and water contaminated with As is highly unsafe as it may cause a high possibility of appearance of cancerous and non-cancerous health risks in human population (ref). Hazard quotient values  $> 1$  were determined in

respect of As in mustard seeds and maize grains for producing baby food samples in Ropar wetland India (Sharma, Kaur, & Nagpal, 2018). Exposure assessment in the US showed that the largest source of As for infants and toddlers between 4 and 24 months old was rice cereal (55%), followed by other infant solid foods (19%), and drinking water (18%) in that order (Shibata, Meng, Umoren, & West, 2016). Arsenic is a toxic metalloid that naturally occurs in soil and groundwater, and accumulates in rice at higher levels than in other crops. A metalloid refers to an element which is intermediate of a metal and a nonmetal; especially one that exhibits the external characteristics of a metal, but behaves chemically more as a nonmetal. (Oxford Dictionary, 2017)

Hazard quotients for Hg ranged from 0.34 to 0.45 for both girls and boys in the 6, 12 and 24 - months' categories. Therefore, none of the foods would pose significant non-cancer risks due to mercury exposure. In spite of this, foods B and E registered the higher HQ values for Hg than the other baby foods

Consumption of commercial infant food in Portugal did not pose a risk to infants when the provisionally tolerable weekly intake (PTWI) for food sounds incomplete (Martins, Vasco, Paixão, & Alvito, 2013). Acute or chronic mercury exposure can cause adverse effects during any period of development. Mercury is a highly toxic element; there is no known safe level of exposure. Ideally, neither children nor adults should have any mercury in their bodies because it provides no physiological benefit. Mercury is neuro-, nephro-, and immunotoxic. The development of the child in utero and early in life is at particular risk. Mercury is a global pollutant, bioaccumulating, mainly through the aquatic food chain, resulting in a serious health hazard for children (Bose-O'Reilly, McCarty, Steckling, & Lettmeier, 2011). This article provides an extensive review of mercury exposure and children's health.

#### 4.5 Cumulative hazard quotient for lead, cadmium, arsenic and mercury in baby food

To evaluate the potential risk to human health through more than one heavy metal, the chronic hazard index (HI) was obtained as the sum of all HQ calculated for Pb, Cd, As and Hg in baby foods (Sultana, Rana, Yamazaki, & Aono, 2017). It was assumed that the magnitude of the effect is proportional to the sum of the multiple metal exposures and that similar working mechanisms linearly affect the target organ (Guerra, Trevizam, Muraoka, Marcante, & Canniatti-Brazaca, 2012). Hazard index was computed as the sum of HQ calculated. The HI values ranged from 2.13 to 2.82 indicating that non-cancer risks due to heavy metals consumed in locally manufactured baby foods in Kampala was in a level of concern ( $1 < HI < 5$ ; Table 11).

**Table 11:** Hazard index for heavy metals in baby foods

Baby food	6 months		12 months		24 months	
	Boys	Girls	Boys	Girls	Boys	Girls
A	2.42	2.46	2.41	2.38	2.45	2.46
B	2.78	2.82	2.76	2.74	2.82	2.82
C	2.27	2.3	2.25	2.23	2.3	2.3
D	2.28	2.32	2.27	2.25	2.32	2.32
E	2.78	2.82	2.76	2.74	2.82	2.82
F	2.16	2.19	2.15	2.13	2.19	2.19

A study in Nigeria, reported HQ for Pb, and HI for Pb, As, Ni, Hg, Cu, Cd, Al, and Cr in brands of instant noodles to be greater than 1. This meant higher probability of non-carcinogenesis among consumers due to heavy metal exposures in the foods (Charles, Ogbolosingha, & Afia, 2018).

## **CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS**

### **5.1 Conclusions**

Human chronic exposure to toxic pesticide residues and metals can lead to their bioaccumulation in the body thereby causing deleterious effects. Knowledge on the health effects of pesticide residues and heavy metal exposure in food is still low in Uganda. Knowledge and awareness on pesticide and HM contamination in cereal-based baby foods is still lacking in Uganda. Quantification of health effects due to pesticides residues and heavy metal exposure in baby foods is therefore an important strategy to improve public health. Arsenic was a predominant contaminant followed by Pb, Cd and Hg in that order. The hazard quotient of heavy metals in cereal based baby foods for infants was below 1 for Pb, Cd and Hg but was greater than 1 for As, indicating potential risk to the babies and infants for As. Hazard index values of the studied heavy metals were above 1 for all baby foods implying Non-cancer risks due to heavy metal exposure in locally manufactured baby foods in Kampala was unacceptable hence baby foods were unsafe for consumption. Lindane, organochlorine pesticide residues, and 4, 4-DDT were detected in baby foods B and E, and these foods also registered the highest levels of heavy metals. I would prefer making conclusions emanating from the data presented in relation with national/ international standards. Where is the concluding statement on awareness studies?



## **5.2 Recommendations**

Regular inspection of baby foods and agricultural produce used in the formulation of complementary baby foods for HM and pesticide residues since all the objected HM were present in the selected baby foods. Regular chemical residue monitoring especially in baby foods and control of heavy metal contents during food production is recommended. Government policies emphasizing the minimal amounts of pesticide residues present in agricultural produce used in baby foods should be reinforced. Even much better, organic farming is by far a better way of minimizing and eliminating pesticide residues in food products since several of these (4, 4-DDT, Lindane and dieldrin) were detected in the selected baby foods under the study. An in-depth risk assessment of pesticides residues in all baby foods in Uganda and establish necessary programs to improve baby food quality and safety is needed.

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## APPENDIX 1: LETTER OF INTRODUCTION



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DEPARTMENT OF FOOD TECHNOLOGY

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7<sup>th</sup> July 2017

To whom it may concern

Dear Sir/Madam

**RE: MS. SARAH NANTONGO**

This is to introduce to you the above referenced person who is a Masters' student in Kyambogo University in the Department of Food Technology. Sarah is conducting a research on chemical food safety on cereal based baby foods in Uganda.

Researches like this are very crucial for our developing nation Uganda and we would like to encourage researches in our universities to help us fight ignorance and diseases so that we can forge development.

Any assistance rendered to Sarah will highly be appreciated.

Yours sincerely



Okullo Apita Aldo  
**Senior Lecturer, Faculty of Science**  
Kyambogo University.

## APPENDIX 2: RESEARCH QUESTIONNAIRE

### Practices that occur in the stores/warehouse/factories feeding stores

The purpose of this questionnaire is to obtain your views and opinions about the practices that go on where unprocessed cereals are stored before being taken for production.

Your responses are voluntary and will be confidential responses, will not be identified by individual. All responses will be compiled together and analyzed as a group.

This work is being gathered by a student in Kyambogo University-Masters in Food Science and Processing.

Please tick the response that you think is most appropriate to each statement (where applicable). If you wish to make any comments in addition to these ratings please do so at the back page.

1. Area where the store/factory/warehouse is located:

(a) Central (b) Western (c) Northern (d) Eastern (e) Southern

2. Give a serial number and the name of store/factory/warehouse (where applicable)

.....

3. Level of education of the supervisor of the storage facility: (a) University level (b) A-level

(c) O-level (d) Below O-level

4. Level of education of the lining technical staff: (a) University (b) A-level (c) O-level

(d) Below O-level

5. Identify at most 3 cereals prone to food spoilage that are stored in the warehouse:

(i).....(ii).....(iii).....

6. Are the cereals stored on the Pallets? (a) Yes (b) No
  
7. How are the store contents organized: (a)Cereals separated (b) mixed together (c) Kept near other food stuffs (d) Near non-food stuffs
  
8. Conditions of storage of the cereal(a) Dry (b) Dump & wet (c)Humid (d) Moisturized
  
9. Packaging and storage of cereals stored: (a)Appropriate (b).Inappropriate If appropriate please specify the packaging materials.....  
.....  
.....
  
10. Type of cereals Stored:.....
  
11. How do you preserve the grains? (a) By drying only (b) Fumigate them (c) Fumigation & drying (d) Just leave them Drying
  
12. Is the type of grains stored here are at high risk of being infected with the pests? (a) Yes (b) No
  
13. Can the production people easily detect the spoilage of grains? (a) Yes (b) No
  
14. If the answer in 11 above in fumigation is done by qualified and skilled personnel?(a)Yes (b) No
  
15. How often do they fumigate?.....
  
16. Common pesticides used for fumigation.....  
.....  
.....
  
17. Mention the company /person (designation) responsible for fumigation.....

- .....
18. Are quality management systems followed when carrying out fumigation?(a) Yes (b) No
19. How many days after fumigation are the grains kept before them for processing? (a) After 2 weeks (b) Immediately after (c) 1 month
20. If others please specify.....

**Checklist showing the level of organization, of the store and personal habits in the stores**

1. Number of staff: (a) Males..... (b).....
2. Knowledge of food laws regarding pesticides handling : (a) Yes (b) No
3. Regularly monitored by public health officials: (a)Yes (b) No
4. Do you have medical examination cards: (a) Yes (b) No
5. What are the most common behavior and habits of your staff? (a) Coughing and sneezing (b) Nose picking (c) Smoking (d) Non -smoking
6. Do you put on/use personal protective gear when carrying out daily work and when may be fumigating the grains? (a) Yes (b) No
7. Do you know the dangers of pesticides used in fumigation? (a) Yes (b) No
8. Do you carry out tests after fumigation and before processing? (a)Yes (b) No
9. If yes, Please specify.....  
.....
10. What common problems are encountered when carrying out fumigation of the cereals  
.....  
.....
11. Do you have UNBS certification? (a) Yes (b) No
12. If yes, how do you gain from it?

.....  
.....

13. Suggestions from the operators on how government may assist to solve the problems identified in 10 above

.....  
.....

14. Are you willing to attend training workshops organized to educate him/her about fumigation and organizing stores of cereals for producing baby food products. (a) Yes  
b) No

**END**

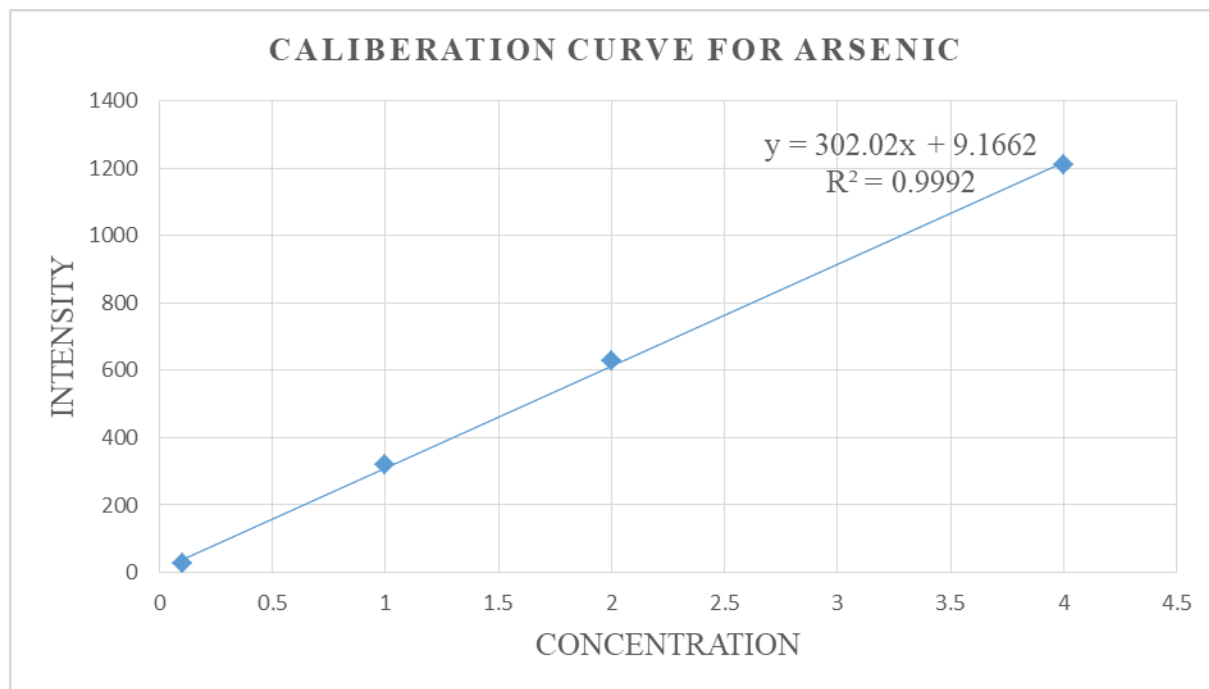


**APPENDIX 3: ENERGY REQUIREMENTS FOR INFANTS AND  
CHILDREN**

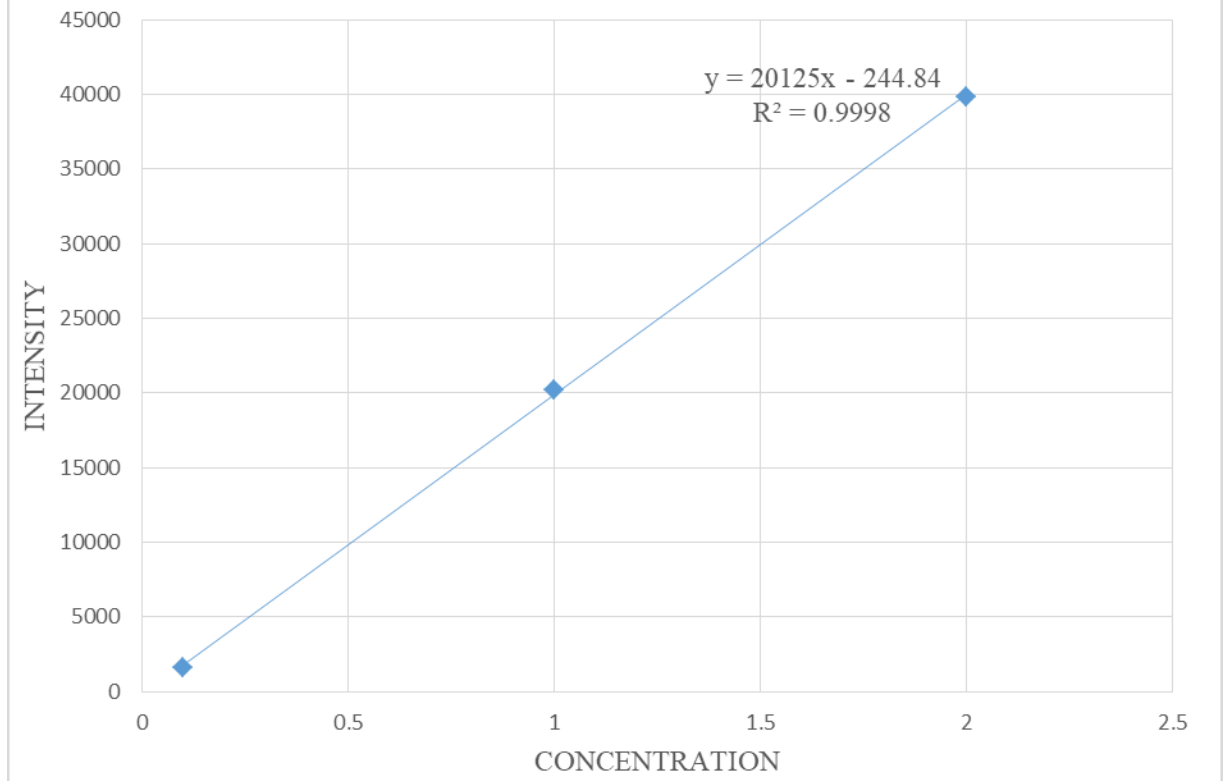
Age (months)	1985 FAO/WHO/ UNU		2004 FAO/WHO/UNU			age months	MJ/ day
	kJ/kg/ day	MJ/ day	kcal/ day	kJ/kg/ day	kcal/ kg/day		
0-1	519	2.166	518	473	113	1	1.975
1-2	485	2.387	570	434	104	2	2.372
2-3	456	2.494	596	397	95	3	2.393
3-4	431	2.38	569	343	82	4	2.293
4-5	414	2.546	608	340	81	5	2.494
5-6	404	2.674	639	337	81	6	2.699
6-7	397	2.73	653	329	79	7	2.795
7-8	395	2.845	680	330	79	8	2.971
8-9	397	2.936	702	330	79	9	3.121
9-10	414	3.058	731	335	80	10	3.318
10-11	418	3.145	752	336	80	11	3.418
11-12	437	3.243	775	337	81	12	3.531

## APPENDIX 4: CALIBERATION CURVES FOR SELECTED HEAVY METALS

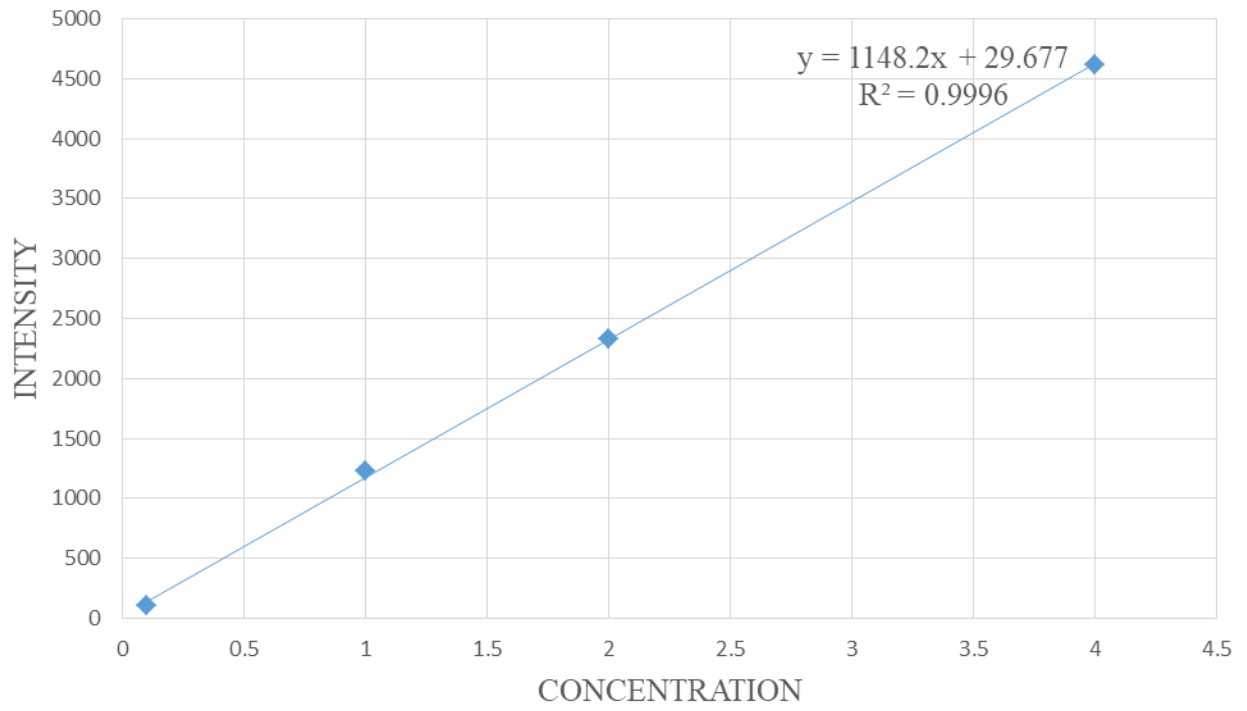
### METALS



### CALIBRATION CURVE FOR CADMIUM



### CALIBRATION CURVE FOR LEAD



### CALIBERATION CURVE FOR MERCURY

