Exposure Risk Assessment to Aflatoxins Through the Consumption of Peanut Among Children Aged 6-59 Months in Lusaka District

BY

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A dissertation submitted to the University of Zambia in partial fulfilment of the requirements for the award of the degree of Master of Science in FOOD SAFETY AND RISK ANALYSIS (FRS)

The University of Zambia
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DECLARATION

I, **Grace Musawa**, do hereby declare that the contents of the dissertation being submitted herein are my original work, and they have not been previously submitted to any University for the award of a degree or any other qualification.

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APPROVAL

This dissertation submitted by Grace Musawa is approved as fulfilling the requirements for the award of the degree of Masters of Science in Food Safety and Risk Analysis of the University of Zambia.

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DEDICATION

I dedicate this work to my mother and siblings, my family (husband and children), relatives and friends who love me and wish me well on this master's journey.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AF	Aflatoxin
AOAC	Association of Official Analytical Chemists
BMDL	Benchmark Dose Limit
DAD	Diode Array Detector
DE	Dietary Exposure
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-LinkedEnzyme-Linked Immunosorbent Assay
EFSA	European Food Safety Authority
ERES	Excellence in Research Ethics and Science Converge
FPIA	Fluorescence Polarization Polarization Immuno Assays
FD	Fluorescence Detector
GMP	Good Manufacturing Practices
HCC	Hepatocellular Carcinoma
HIV	Human Immunodeficiency Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
JECFA	Joint Expert Food Additives
L.C.	Liquid Chromatography
LFD	Lateral-Flow Devices
MoH	Ministry of Health
MoA	Ministry of Agriculture
MOE	Margin of Exposure
MS	Mass Spectrometry
SADC	Southern Africa Development Committee
SPSS	Statistical Package for Social Science
TFA	Trifluoroacetic Acid
TLC	Thin-Layer Chromatography
WHO	World Health Organisation

ABSTRACT

Aflatoxins are a family of toxins produced by certain fungi found on agricultural crops such as maize (corn), peanuts (groundnuts), cottonseed, and tree nuts. Based on the Codex Alimentarius framework for food control systems, this study assessed under five years old children's exposure risk to aflatoxins (AFs) through the consumption of peanuts in the Lusaka District. The objectives were threefold: to determine the consumption patterns of peanuts by under-five children in Lusaka; to estimate the likelihood of exposure to AFs through the consumption of peanuts in the study population; and assess the knowledge, attitudes and practices of the parents and guardians in the study population related to factors that might explain the development of aflatoxins in peanuts. The study was driven by the limited literature and knowledge on exposure risk estimation in under-five children, especially in Zambia, despite having several reports and studies on the high aflatoxin content in peanuts.

The study was a cross-sectional study that depended on secondary and primary data sources to achieve the objectives. Firstly, a desk review of scientific literature and government reports was conducted as a secondary data source. Secondly, a survey questionnaire was administered to collect primary data on the subject matter to close the data gaps. The questionnaire was administered to 795 respondents - mainly female parents and guardians for enrolled under-five in each of the health centres selected in Lusaka based on stratification to achieve a sample representative of the low, middle and high socio-economic status respondents. The respondents from each health centre comprised a 10 per cent sample from the total number of the under-five children enrolled at the respective health centre.

Questionnaire interviews were then administered tofemale parents or guardians of the selected children. The data were processed and analysed using IBM SPSS version 20. Theexposure risk were estimated by the carcinogenic risk of aflatoxins, margin of exposure and chronic dietary intake of aflatoxins. This study indicated increased exposure risk to aflatoxin in children under-five, which could result in health risks. This could be due to the presence of AFB1 AFB2, AFG1 AFG2 and AFs in peanuts sold in markets used to prepare meals for children. The overall exposure was highest in children who consumed a high level of aflatoxin contamination in peanuts and high-frequency consumption level. The study also established a possible double risk in exposure because most children have their peanuts mixed with maize meal which is also a susceptible crop to aflatoxin. Furthermore, there was poor knowledge of the presence of aflatoxin in peanuts among respondents, how aflatoxin gets in peanuts, and its health effects. The high levels of AFs in peanuts and high exposures accentuate the need for preventive measures. Particular attention should be given to raising awareness on the impact of aflatoxin risk exposure.

CHAPTER ONE: INTRODUCTION

1.0 Background

Aflatoxins (AFs) are toxic metabolites produced by *Aspergillus flavus* and *A. parasiticus*, which are abundant in warm and humid regions of the world (Moss, 1998; Ashiq, 2015). Aflatoxin-producing fungi can contaminate crops in the field, at harvest, and during storage. The most common types of AFs that are problematic to health are aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1, and aflatoxin M2, all of which are highly toxic and contain carcinogenic compounds that cause disease in livestock and humans (Binder *et al.,* 2007). The AFB1 has been classified as a group 1 human carcinogen by the International Agency for Research on Cancer (IACA, 1999). The AFs are mainly found in tropical countries with optimal temperature and humidity for mould growth and production of toxins. Generally, AFs are small stable molecules that cannot be destroyed by heat treatment or processing (Deshpande, 2002; Scudamore, 2009: Chen *et al.,* 2013).

Among the highly consumed commodities in Zambia by children are corn, wheat, rice, and peanuts, (ZDHS, 2014). Unfortunately, these foods are prone to AFs contamination (Ashiq, 2015; Bumbangi *et al.,* 2016). Among the foods mentioned above, peanuts and peanut products are cheap sources of proteins, especially for children under five years old. Protein is one of the macro-nutrients required for normal growth. However, the warm and humid environment entails a high likelihood of AFs presence in these food products, making them a threat to children's health.

In Zambia, studies have shown that women expose their children to various complementary foods at an early age (ZDHS, 2014). These foods include milk, banana, oranges, fruits, meats, fish, eggs, beans, and peanuts. Most protein source foods are expensive, making most mothers opt for peanuts. In this write-up, the peanut under discussion is bought raw and processed at home for children’s consumption; consequently, the use of peanuts exposes children to aflatoxin, a toxic substance. Against this background, this study aims at assessing the risk of exposure to AFs in children below the age of five through the consumption of peanuts in the Lusaka District.

1.1 Problem Statement

High aflatoxins have been reported in peanuts sold in markets and supermarkets of Lusaka District, Zambia (Flavien et al 2006). Despite these reports Mothers/guardians still feed peanut porridge to their children. This is done in order to meet the protein requirements of the children, as peanuts are a cheap source of protein. Therefore, the need for meeting the protein requirement versus the affordability of peanuts creates a double burden because the maize that are processed into meali meal and consequently used in porridge are also a susceptible crop to AF. Furthermore, Kachapula *et al,.* (2006) reports of common practices among people that sale peanuts who select the seemingly good looking peanut for sale while leaving behind the "bad nuts" for household use which includes preparing meals for children. Dietary diversity in Zambia is another major challenge whereby children do not consume food from enough food groups possibly increasing the frequency of peanuts consumption thus the equally possibly high risk of exposure to AF. Also the COVID-19 pandemic has had a negative impact on the price and availability of food and therefore affecting the food choices of families (UNICEF 2021).

It is difficult to eliminate the aflatoxins commonly present in peanuts even when using the available cooking methods. There are several reports worldwide on the harmful effects of aflatoxins in humans. Indeed, the consumption of these mycotoxins by children through consumption of peanuts can cause acute and chronic health effects (aflatoxicosis), including immune-system suppression, growth retardation, cancer, and death (Azziz-Baumgartner *et al.,* 2005; Gong *et al.,* 2004; Williams *et al.,* 2004; Wild and Turner, 2002). Aflatoxins are carcinogens and genotoxins agents that directly influence the structure of DNA (Williams *et al.,* 2004). Both in literature and practice Zambia has recorded high cases of stunting. According to ZDHS (2018), stunting levels in Zambia were at 35 percent and a proportion of these cases could be attributed to exposure to AF.

Chronic exposure to aflatoxin may result in liver cancer especially later in adulthood (IARC 1993). This has led the International Agency for Research on Cancer (IARC) to classify aflatoxin B1 as a group 1 carcinogenic agents to humans; and that the agent or mixture has sufficient evidence of carcinogenicity in humans. The risk of hepatocellular carcinoma is particularly

elevated in individuals with chronic hepatitis B virus infection who also are exposed to aflatoxins (IARC, 2002). Further, the IARC classifies the aflatoxin M as Group 2B (possibly carcinogenic to humans).

At present, the risk management strategies to reduce human exposure to AFs include enforcing legislation and regulation to guarantee that consumers are getting safe food (Nielen and Marvin 2008). Despite the country's effort to give recommendations to adhere to Codex Alimentarius, that is, by employing Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP), AFs in peanut remain a source of worry. Exposure to AFs should be as low as reasonably achievable, with the adoption of GAP and GMP being essential tools to achieve a considerable reduction in AF levels in food (Codex Alimentarius Commission 1995). Establishing regulatory limits and implementing monitoring programs can help keep AFs contamination under control in peanuts.

In some countries, maximum tolerable levels have been set for foods' AFs of human health concern (Wu *et al*., 2013). According to Kachapula (2017), he reports that the maximum tolerable level of AF in peanuts in Zambia is 10ng/kg. However, these regulations do not always agree with the required safety for the population, especially where large amounts of foods such as peanuts are consumed (Wu and Klensler, 2013). Despite evidence of the presence of AFs in peanuts (Chen *et al.*, 2013; Lien et al., 2019), there is a dearth of information on the exposure risk assessment of aflatoxin through the consumption of peanuts in children under five years in Zambia; hence this study was conducted to possibly fill the gap.

1.2 Significance of the Study

Despite the vast consumption of peanuts, the safety of consumers, especially children, is not guaranteed, given their susceptibility to the harmful effects of AFs. Therefore, this study will bring out insights and measures on reducing exposure to AFs in children through the consumption of peanuts. This study will also help contribute information to develop a dietary threshold to help prevent the risk of cancer in under-five children. Considering that the study on exposure risk assessment of under-five children to aflatoxin through peanut consumption Zambia is under-studied, findings will serve as baseline literature for future studies. Also, the study

findings will be important to the Ministry of Health (MoH) and the Ministry of Agriculture (MoA) of the Government of Zambia because it is poised to reveal new insights which may be a possible source of learning better ways of preventing the development of aflatoxins to harmful levels in peanut and also prevention of adverse ill health in under-five children. Therefore, policymakers may be guided in implementing sound policies that delivers better and quality health at the national level.

1.3 Study Objectives

1.3.1 Main Objective

The main objective of this study was to assess the risk of exposure to aflatoxins by under-five children in the Lusaka District through the consumption of peanuts.

1.3.2 Specific Objectives

1. Determine the consumption patterns of peanuts by under-five children in the Lusaka District.
2. To estimate the likelihood of exposure to aflatoxins through the consumption of peanuts in the study population.m
3. Assess the knowledge, attitude and practice of the guardian's study population related to factors that might explain the development of aflatoxins peanuts.

1.4. Study Questions

1. How often do children below the age of five in Lusaka consume peanuts?
2. Does the consumption of peanuts expose under-five children to aflatoxins?

CHAPTER TWO: LITERATURE REVIEW

2.0 Overview

This chapter presents the literature relevant to the study at hand. Specifically, it discusses the food consumption patterns of peanuts and the risks associated with exposure to AF through food consumption, the host range of aflatoxin, human exposure, aflatoxin standards for humans and animals, diagnosis, prevention, and control, also touches on dietary and clinical intervention.

2.1 Food Consumption frequency pattern and Aflatoxin Presence

Aflatoxins are mycotoxins produced by fungi of the genus Aspergillus that belong to grains storage flora. Aspergillus grows optimally at 25°C with a minimum necessary water activity of 0.75. It produces secondary metabolites at 10-12 ºC, but the most toxic ones are produced at 25°C with a water activity of 0.95 (Gimeno and Martins, 2003).

There are many Aspergillus species, basically grouped in three phylogenetically distinct sections. The leading producers of aflatoxins are *Aspergillus flav*us and *Aspergillus parasiticu*s (Cotty et al., 1994). However, it has been demonstrated that *Aspergillus nomius*, *Aspergillus pseudotamarii*, *Aspergillus parvisclerotigenus*, and *Aspergillus bombycis* of section Flavi; *Aspergillus ochraceoroseus* and *Aspergillus rambellii* from section Ochraceorosei; and *Emericella astellata* and *Emericella venezuelensis* from Nidulatans section also generate aflatoxins (IARC 2002).

There are 18 types of aflatoxins, but the naturally occurring and well-known ones are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), and aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Saleemullah et al., 2006). These names were given due to their blue (B) or green (G) fluorescence properties under ultraviolet light and their migration patterns during chromatography (Saleemullah et al., 2006).

There are also aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) hydroxylated derivatives of AFB1 and AFB2, respectively. These may be found in milk, milk products or meat (hence the designation M1). They are formed by the metabolism of B1 and B2 in the body of the animals following absorption of contaminated feeds (Wild & Gong, 2010). AFB1 is the most prevalent

aflatoxin usually found in cases of aflatoxicosis and is responsible for acute and chronic toxicity, carcinogenicity, teratogenicity, genotoxicity and immunotoxicity (Strosnider *et al.*, 2006).

Several studies have been conducted to determine the presence of AFs in food samples. In Tanzania, Kimanya *et al*. (2008) found the presence of AFs in 18% of the maize samples from four regions they studied with up to 158μg/kg. Of these contaminated samples, 12% had exceeded the Tanzania regulatory limit of 10μg/kg for total aflatoxins. Also, in Tanzania, a risk assessment study conducted by Shrimal *et al*. (2015) found a high prevalence of chronic aflatoxin exposures in young children. The exposure levels were associated with a maize and peanut diet, increased with age, and varied with season and location.

In Kenya, several mycotoxins were tested in milled rice from traders at different milling points within the Mwea Irrigation Scheme. This study involved traders providing details such as the name of the cultivars, village, sampling locality, etc. Overall, the study found that low amounts of toxins were observed in rice with a low frequency of samples above the regulatory limits for aflatoxin, 13.5%; ochratoxin A, 6%; and HT2 + T2, 0.5%. The maximum co-contamination was for 3.5% samples with six toxins in different combinations. The study found that the rice cultivar, paddy environment, time of harvest, and millers influenced the occurrence of different mycotoxins. Thus, the study recommended establishing integrated approaches to mitigate mycotoxin accumulation in rice in that area (Mutiga *et al.,* 2021).

Studies have shown that hazardous AF contaminations can occur at any point of the food chain, starting from field production to the final use of a wide variety of plant products, such as cereals, nuts, spices, and fruits (Shrimal et al., 2015: Lizárraga-Paulín *et al.*, 2013). AFs are transferred into different body parts of animals and humans after consumption and absorption from the gut. They can even be modified chemically, giving rise to further dangerous derivatives. These harmful compounds, such as AFM1, will be eventually excreted and appear even in milk (Serraino *et al.,* 2019). Dangerous indirect AFM1 contaminations of milk and dairy products have been reported in the literature in outstandingly high numbers. The direct AF contaminations of milk products by moulds and their mycotoxins have also been published (Warth *et al.,* 2019). Aflatoxins may also appear in human breast milk after conversion to AFM1, which is threatening for highly susceptible breastfeeding newborns. Aflatoxin also provokes developmental disorders in embryos in utero after passing through the placenta (Maleki *et al.,* 2015).

Modupeade *et al*. (2018) assessed the microbiological quality and risk assessment for aflatoxins in peanut and cashew nuts consumed in selected locations in Nigeria. Their study found that the nuts were contaminated with pathogenic bacteria and toxigenic fungi, resulting in aflatoxin contamination of the products, thereby posing health risks to their consumers.

In Zambia, reports of aflatoxin have also been reported. These have been reported at high levels of 0.7 to 108.9 ppb and 4 to 100 ppb in maize and peanuts, respectively. Even worse, in the Eastern province, Nyimba, where extremely high aflatoxin levels have been reported, at 4,980 ppb (Kankolongo *et al.,* 2009; Mukaka and Shipekesa, 2013; Njatau *et al.,* 1998). The fact that maize is a staple food and peanut is eaten widely in Zambia possesses a greater risk of human exposure to AF.

2.2 The Effect of the Risk of Exposure to Aflatoxin

Aflatoxins are a group of highly toxic, cancer-causing chemicals produced by several members of the fungal genus Aspergillus. This fungus and the toxins it produces reside typically in soil and plant materials, including grains or cereals, peanuts, seeds, and other legumes (Cotty *et al.*, 2008). Humans and animals are affected by contaminated dietary intake. The World Health Organization (WHO) characterises aflatoxins as an essential source of food-borne illnesses among chemicals (FAO/WHO 2006).

There are several reports worldwide on the harmful effects of aflatoxins in both humans and animals. Indeed, the consumption of these mycotoxins by a human through foods can cause acute and chronic health effects (aflatoxicosis), including immune-system suppression, growth retardation, cancer, and death (Azziz-Baumgartner *et al.*, 2005; Gong *et al.*, 2004; Williams *et al.*, 2004 Wild and Turner, 2002;). Aflatoxins are carcinogens and genotoxins agents that directly influence the structure of DNA (Williams *et al.*, 2004).

Chronic exposure to aflatoxin may result in liver cancer in both humans and animals (IARC 1993). This has led the International Agency for Research on Cancer (IARC) to classify aflatoxin B1 as a group 1 carcinogenic agents to humans. The agent or mixture has sufficient evidence of carcinogenicity in humans (IARC, 1993). The risk of hepatocellular carcinoma is particularly elevated in individuals with chronic hepatitis B virus infection exposed to aflatoxins (IARC,

2002). Further, the IARC classifies the aflatoxin M as Group 2B (possibly carcinogenic to humans).

In general, four potential impacts of aflatoxin have been identified: (i) deterioration of food and nutritional quality of agricultural products with an accompanying reduction in sensory characteristics, e.g., taste, odour, texture and colour, (ii) health-related productivity losses due to mutagenic and carcinogenic effects on humans who consume aflatoxin-contaminated food over an extended period, (iii) loss of income from livestock resulting from feeding aflatoxin-contaminated feedstuffs, e.g., higher mortality rates and lower feed to weight conversion ratios for chickens, ducks, egg-layers, and pigs, and (iv) losses of export markets and related economic gains due to regulations that restrict international trade of aflatoxin-contaminated grain. Such losses may cost up to 400-450 million U.S. dollars annually (Lubulwa and Davis, 1994). For these effects, aflatoxin increases morbidity and mortality in developing countries and particularly in Africa, where it represents an enormous economic and social burden.

The burden of aflatoxin-induced liver cancer was assessed based on available aflatoxin biomarker data from a previous epidemiology study, hepatitis B virus infection prevalence, and Tanzania's population size in 2016. The study found about 1,480 (2.95 per 100,000 persons) new cases of aflatoxin-induced liver cancer in Tanzania and assumed all of them would die within a year (Kimanya *et al.,* 2021). The study was mainly in areas with large quantities of maize and peanuts.

A study by Bervis *et al*. (2021) detected 55% of AFB1 in the feed samples, while AFM1 was detected in 38.3% of milk samples. Mycotoxins are widely known harmful secondary metabolites produced by various moulds. The furanocoumarin derivative aflatoxins are among the most significant and most harmful mycotoxins contaminating feed and food and, consequently, imposing real threats on the health of both domestic animals and humans, initiating various highly pathological cellular and physiological processes (Toxins, 2021).

Studies have also confirmed the genotoxic and carcinogenic effects of A.F.s, primarily when the consequences of long-term exposures are evaluated (Strosnider et al., 2006; Walderhaug, 1992).

Also, dysfunctions of many organs of AF-exposed humans and animals have been reported, including the liver, kidneys, gastrointestinal tract, and reproductive and immune systems (Peles *et al.,* 2019). Additionally, AFsmay disturbs humans' early, even embryonic, development, resulting in growth and mental retardations and immune system dysfunctions.

2.3 Host Range

Aflatoxins contaminate a significant fraction of the world's food and feed commodities (Strosnider et al., 2006). The primary hosts of aflatoxins include maize, peanut and cottonseed (Cotty *et al.,* 2008; Kpodo *et al.,* 2000). From time to time, aflatoxins occur in tree nuts, including pecans, hazelnuts, walnuts, copra, and kola nuts (IARC, 2002). Aflatoxins contaminated foods were also reported in rice, sorghum, barley, rye, wheat, soya, milk, meat and other derivative products made from these primary feedstuffs (IARC, 2002; Saleemullah et al., 2006).

2.4 Scenario Pathway for Exposure

The exposure pathway of aflatoxin from farm to folk sets in the right at primary production due to a lack of good agricultural practices because peanut processing has less impact in the quest for elimination (Cotty *et al.,* 2008) (appendix 1).

2.5 Human exposure

Humans are exposed to aflatoxin through dietary intake (IARC, 2002). Two pathways of dietary exposure have been recognised:

(a) Direct ingestion of aflatoxins (mainly B1) in contaminated foods of plant origin such as maize, peanut and their products.
(b) Ingestion of aflatoxins (M1) carried over from feed into milk and milk products, including cheese and powdered milk. In addition to the carryover into milk, residues of aflatoxins may be present in the tissues of animals that consume the contaminated feed.

However, this exposure to aflatoxin explained above depends on the level of contamination in a given host commodity and the quantity of the commodity consumed. Hence, in some world

areas, aflatoxin levels in foods might be comparatively high, but exposures may be modest because of a diverse diet. However, similar food contamination levels in sub-Saharan Africa will translate to much higher exposure. This is owed to the staples of food consumed in this region (peanuts and maize), which are highly susceptible to being contaminated by aflatoxins due to poor grain storage conditions. In many countries and regions, these staples are consumed daily for the majority of the year and may constitute >50% of the diet (Wild and Turner, 2002). Human exposure to aflatoxin may secondarily result from exposure to air and dust containing toxins during the handling of contaminated products (Sorenson et al. 1984).

Some essential factors that affect aflatoxin contamination include the climate of the region, the genotype of the crop planted, the soil type, the minimum and maximum daily temperatures, and the daily net evaporation (Strosnider *et al.,* 2006). Aflatoxin contamination is also promoted by stress or damage to the crop. This is due to drought before harvest, insect activity, poor timing of harvest, heavy rains during and after harvest, and inadequate crop drying before storage. Levels of humidity, temperature, and aeration during storage are also essential factors intimately related to the actual problems of climate changes and environmental warming around the whole world (Cotty & Jaime-García, 2007; Paterson & Lima, 2010).

In addition, the fact that these toxins present high thermal stability is also a key factor, enabling them to remain in some cooked foods, meaning that freezing has minimal effect on their presence in foods (Sáez et al., 2011).

2.6 Aflatoxin standards for humans and animals

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has assessed aflatoxins B and G on several occasions since 1987 (JECFA, 1987, 1997, 1999, 2002, 2007). It has been recommended that, due to their carcinogenic potential, dietary exposure to aflatoxins should be minimised as much as possible.

Various studies have tried to determine the toxicity of the aflatoxin dose necessary to activate adverse effects in humans and animals. Nevertheless, several factors must be considered to ensure that aflatoxin becomes lethal to humans and animals. These include the amount of aflatoxin consumed daily, the frequency of consumption and the nutritional status of the

individual (FDA 2012). The toxic level of aflatoxin in humans is largely unknown, as evidenced by the different thresholds found in outbreaks reported in different countries (FDA 2012).

In northwest India, the aflatoxin level found in corn during the outbreak of 1974 was in the range of 0.25 to 15 mg/kg. In the outbreak of 1982 in Kenya, the level of aflatoxin intake was 38 μg/kg of body weight. While in 2004 and 2005, aflatoxin-contaminated homegrown maize with an average concentration of 354 ng /g was the source of the outbreak in Kenya. Thus, due to its high toxicity and carcinogenic properties, legal tolerance levels in different countries have been taken for aflatoxin in foods destined for human consumption. These limits vary between 4 - 20 parts per billion (ppb) (Egal *et al.,* 2005). Information is minimal concerning health effects associated with aflatoxin concentrations between 20 ppb and 300 ppb.

The Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program adopted a maximum level of 15 ppb for total aflatoxin in unprocessed peanuts and tree nuts and 10 ppb for ready-to-eat tree nuts (JECFA, 2008). The European Union has the most stringent standards for aflatoxins in the world. The limit is 4 ppb (Wagacha & Muthomi, 2008). The United States adopted 20 ppb as the maximum level for aflatoxin in food (Otsuki *et al.,* 2001*a*) and 0.5 ppb for milk (Kensler et al., 2010). The standard is set between 15-20 ppb for processed food products (DANYA and USAID, 2012). In China, as well as, the levels of aflatoxin B1 in peanut butter and sesame paste are set not to exceed 20 ppb (DANYA and USAID, 2012). However, for most African countries, aflatoxin is mainly unregulated. In 2003, aflatoxin regulations were present in five African countries, including Kenya and South Africa. The fixed standards of aflatoxin levels were between 10 ppb and 20 ppb for Kenya and South Africa, respectively (Mutegi *et al.,* 2009). The presence of *Aspergillus flavus* does not always indicate harmful aflatoxin levels; it does mean that the potential for aflatoxin production is present (USDA, 2012).

2.7 Diagnosis

2.7.1 Clinical Signs

Adult humans usually have a high tolerance of aflatoxin, and, in the reported acute poisonings, it is usually children who die (Williams *et al.,* 2004). The adverse effects of aflatoxins in humans and animals have been categorised in two general forms:

a) Acute aflatoxicosis

It is produced when moderate to high levels of aflatoxins are consumed. Specific, acute episodes of disease are characterised by haemorrhage and acute liver damage, which manifest as severe hepatotoxicity with a case fatality rate of approximately 25%, oedema of feet, absorption and metabolism of nutrients and alteration in indigestion. The early symptoms of hepatotoxicity from aflatoxicosis can include anorexia, malaise, and low-grade fever. Acute high-level exposure can progress to potentially lethal hepatitis with the rapid development of ascites, portal hypertension, vomiting, abdominal pain, jaundice, fulminant hepatic failure and death (Strosnider *et al.,* 2006; Walderhaug, 1992).

b) Chronic aflatoxicosis

Chronic effects result from ingestion of low to moderate levels of aflatoxins. These effects are usually subclinical and difficult to recognise at the beginning of the exposure.

It has been well documented that chronic aflatoxin exposure, particularly aflatoxin B1, is associated with an increased risk of developing hepatocellular carcinoma and disorders of immune function and nutrition problems such as growth retardation in children (Wu *et al.,* 2011; Wu & Khlangwiset. 2010). The aflatoxin exposure can also exacerbate problems of pre-existing health conditions. People infected with hepatitis B and exposed to aflatoxin are 30 times more likely to develop liver cancer than those who do not (Liu & Wu 2010). At the global level, estimated aflatoxin exposure contributes to between 4.6 per cent and 28.2 per cent of all cases of liver cancer. It should be noted that most of these cases have been observed in sub-Saharan Africa, south-east Asia and China. It should also be noted that these regions have the highest exposure to aflatoxin levels (Liu & Wu, 2010; Kirk *et al.*, 2006).

Aflatoxin B1 also chemically binds to DNA and causes structural DNA alterations resulting ingenomic mutation (Groopman *et al.,* 1985). Ingestion of aflatoxin, viral diseases, and hereditary factors has been suggested as possible aetiological agents of childhood cirrhosis (Dhanasekaran et al., 2011). Evidence indicates that children exposed to aflatoxin breast milk and dietary items such as unrefined peanut oil may develop cirrhosis. Malnourished children are also prone to childhood cirrhosis on the consumption of contaminated food (Dhanasekaran *et al.,*

2011). Several investigators have suggested aflatoxin as an etiological agent of Reye's syndrome in children in Thailand and New Zealand, though there is no conclusive evidence yet (Dhanasekaran et al., 2011). Recently, chronic aflatoxin exposure has also been linked to HIV and tuberculosis (Lizárraga-Paulín et al., 2011).

2.7.2 Laboratory

Analytical methods for mycotoxins in feeds and foodstuffs generally require toxin extraction from the matrix with a suitable extraction solvent, a clean-up step intended to eliminate interference from the extract, and, finally, detection/determination of the toxin by suitable analytical instruments/technologies. Clean-up is essential for analysing mycotoxins at trace levels and involves using solid-phase extraction and multifunctional or immunoaffinity columns (Giniani *et al.,* 2011).

The different analytical methods of aflatoxins can be divided into two groups:

a) Classical analytical technologies

This group includes:

Thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) is among the most widely-used analytical methods. This is a simple and cost-effective technique used for A.F. analysis in developing countries. It is used for screening purposes, multi-mycotoxin analysis and when low detection limits are not required (Pascale and Visconti, 2008).

Although TLC is a powerful tool for the simultaneous analysis of multiple samples for multiple mycotoxins, it cannot be used for sensitive or precise measurements unless densitometric analyses are performed (Pascale and Visconti, 2008). Highly reproducible, reliable results can be obtained if an autospotter is used to apply the samples. TLC can be used without cleaning up the extract, but extract purification prior to spotting increases sensitivity (Stroka *et al.,* 2000). However, it is labour-intensive and limited in the number of samples tested daily (Stroka *et al.,* 2000).

High-performance liquid chromatography (HPLC)

HPLC coupled with U.V., a diode array detector (DAD), or a fluorescence detector (F.D.) currently is the most widely used technique for the identification of the significant mycotoxins in food commodities (Giniani et al., 2011). HPLC/FD is highly sensitive, selective, accurate and repeatable, so specific labelling reagents have been developed and are commercially available for the derivatisation of non-fluorescent mycotoxins to form fluorescent derivatives. Either pre-column derivatisation, trifluoroacetic acid (TFA), or post-column derivatisation, with Br or I, can identify aflatoxins B1, B2, G1 and G2 (Pascale and Visconti, 2008).

The Association of Official Analytical Chemists (AOAC) International or the European Standardization Committee has adopted these methods as official or standard methods. In particular, methods for measuring aflatoxins in maize, raw peanuts and peanut butter; aflatoxin B1 and total aflatoxins in pistachios, figs and paprika; aflatoxin M1 in milk; aflatoxin B1 in baby food (Fu et al., 2008). In addition, HPLC/immune-affinity column methods have been validated to measure aflatoxins in hazelnut paste (Ibáñez-Vea *et al.,* 2011).

Liquid chromatography/mass spectrometry (LC/MS)

Liquid chromatography coupled with mass spectrometry (LC/MS) has been used for many years, mainly as a technique for A.F. quantification and confirmation of identities. Liquid Chromatography coupled with Mass Spectrometry is the most promising technique for simultaneously screening, identifying, and measuring many mycotoxins (Sorensen and Elbaek, 2005).

Accuracy, precision, and sensitivity of LC/MS methods may vary depending on the mycotoxin, matrix and instrument, with the method's sensitivity depending on the ionisation technique used. Quantitative measurement of mycotoxins by LC/MS often is unsatisfactory due to matrix effects and ion suppression. Purification of extracts by MycoSep® or immunoaffinity columns generally is needed prior to M.S. detection (Pascale and Visconti, 2008).

HPLC coupling to mass spectrometry has been the more commonly employed detection technique in the last years (Giniani *et al.*, 2011).

Enzyme-linked immunosorbent assays (ELISAs)

Since the late 1970s, AF-specific antibodies have been developed. The antibody development has led to the development of enzyme-linked immunosorbent assays (ELISAs) for AFs (Li *et al.*, 2009). The ELISAs are mainly used for qualitative, semi-quantitative and quantitative analysis of aflatoxin and other mycotoxins in several food matrices. In general, ELISAs do not require clean-up procedures, and the extract containing the mycotoxin is analysed directly (Li *et al.*, 2009). It provides fast and inexpensive screening assays but lacks accuracy at deficient deficient concentrations (competitive assays); it is limited in the range of matrices examined. Flow-through enzyme immunoassays for field use have been developed to rapidly detect aflatoxin B1 and aflatoxin M1 and provide consistent HPLC results. This assay was accurate and reliable, giving no false compliant and only a few false noncompliant results (Li *et al.*, 2009).

b) Emerging technologies for Aflatoxins analyses

Recently, various emerging methods based on novel technologies have been proposed for aflatoxins analyses. They include:

Lateral flow devices (LFDs)

A lateral-flow device also called an immunochromatographic test, is a rapid immunoassay based on the interaction between specific antibodies, immobilised on a membrane strip, and antibody-coated dyed receptors, *e.g.*, latex or colloidal gold, that reacts with the analyte to form an analyte-receptor complex (Tang *et al.*, 2009).

Competitive LFDs rely on the competition of the analyte, *e.g.*, aflatoxin, in solution for the binding sites of the labelled receptor. The test line contains an analyte-conjugate attached to the membrane that binds unbound receptors to form a coloured analyte-conjugate receptor complex. The control line includes a specific antibody attached to the membrane that binds with the labelled receptor. Upon binding, the control line changes to a coloured signal. If the signal in the test line is missing or weakly visible, the test indicates that the analyte is present in a sufficient amount (positive test). If the test line signal is visible, the test indicates that the analyte is not present in the extract (negative test) (Tang *et al.*, 2009).

LFDs are user-friendly format, cost-effective and provide a rapid response. These features make strip tests ideal for applications such as “on-site” detection of environmental and agricultural analytes (Tang et al., 2009). LFDs are often used to determine aflatoxin B1 in maize and peanuts and aflatoxin M1 in milk and other mycotoxins (Van der Spiegel *et al.,* 2013).

Fluorescence polarisation immunoassay (FPIA)

FPIA is a simple technique that measures interactions between a fluorescently labelled antigen and a specific antibody. Fluorescence Polarisation Immunoassayhave has been used to rapidly determine aflatoxins in solution though low accuracy and sensitivity were problems when these assays were used with cereal samples (Saha *et al.,* 2007).

Capillary electrophoresis

Capillary electrophoresis is an analytical technique that allows good separation of mycotoxins from potentially interfering species present in the extract based on electrical charge. These methods have been developed for various mycotoxins, including aflatoxins. Capillary zone electrophoresis with laser-induced fluorescence has been used to analyse aflatoxin B1 in maize (Pascale and Visconti, 2008). These methods are comparable in sensitivity, precision and accuracy to HPLC methods. The use of less expensive capillaries, the absence of organic solvents during the detection step, and shorter analysis times make capillary-zone electrophoresis methods viable alternatives to those requiring HPLC (Dong Yiyang, 1999).

Fibre-optic immunosensors

Evanescent wave-based fibre-optic immunosensors have been developed to detect fumonisin B1 and aflatoxin B1 in maize. A non-competitive assay was used for aflatoxin B1 that takes advantage of the native fluorescence of this mycotoxin. The sensor could detect 2 ng/ml of aflatoxin B1 in phosphate-buffered saline solution. Problems due to refractive index-related effects were observed in the presence of organic solvents, which reduced the assay's specificity (Maragos and Thompson, 1999).

Biosensors

A biosensor is an analytical device that incorporates a specific biological element, *e.g.*, an antibody, which creates a recognition event and a physical element that transduces the recognition event into an acoustic, electrical or optical signal. Immunochemical biosensors that use surface plasmon resonance, quartz crystal microbalance and screen-printed carbon electrodes have been described to detect mycotoxins.

Competitive surface plasmon resonance-based immunoassays have rapidly screened aflatoxin B1 and other mycotoxins in naturally contaminated matrices (Van der Gaag *et al.,* 2003). These methods can detect aflatoxins at very low levels with good accuracy and precision. The use of surface plasmon resonance equipment with four flow cells enables the detection of four mycotoxins in a single measurement (Van der Gaag *et al.,* 2003).

2.8 Prevention and Control

Aflatoxin contamination is a severe health concern rooted in the entire food chain, hence the need for a multidisciplinary approach to search for solutions. Interventions to reduce aflatoxin-induced illness can be roughly grouped into agricultural, dietary, and clinical.

2.8.1 Agricultural

Agricultural interventions are methods or technologies that could be applied either in the field (pre-harvest) or in drying, storage and transportation (post-harvest) to reduce aflatoxin levels in food (JECFA, 2008). Agricultural interventions can thus be considered "primary" interventions because they directly reduce aflatoxin in food.

Pre-harvest Handling Methods

This includes using biocontrol agents that establish a process of competitive displacement using non-toxigenic strains by different agricultural techniques and developing breeds stronger or more resistant crop strains. These methods are:

a) Biological Control

Instead of traditional chemical pesticides, biological strategies are environmentally friendly and come from natural resources. These include beneficial insects, plant extracts: essential oils of which aniseboldus the mountain thyme, clove, Griseb and Poleo (Reddy *et al.,* 2009), or the introduction of other natural organisms: Methyleugenol (Sudhakar *et al.,* 2009).

In preventing aflatoxin, biocontrol methods can be applied before harvest or in the fields when the plants grow and become mature. However, these methods are not as effective as those using chemical methods.

b) Competitive Displacement

The application of non-toxigenic strains of A. flavus on crops promotes competition with toxic ones, resulting in a significant reduction or elimination of toxigenic strains that produce aflatoxin (Probst et al., 2011). This method has proven to be very effective in the fight against aflatoxin contamination in various cultures (Probst *et al.,* 2011). The application of this method can be made in different ways, which influences the effectiveness of the results. It may involve: 1) inoculating the soil with a non-toxigenic strain, 2) spraying crops with conidia or spores of non-toxigenic strain and 3) spraying the plants with a product that comprises hydrosoluble non-toxigenic strain. The most effective means of delivering non-toxigenic strains to replace competitively toxigenic A. flavus is an aerial spray of formulated non-toxigenic strains (Lyn *et al.,* 2009).

c) Agricultural Techniques

Agricultural techniques, such as crop rotation (Jaime-Garcia & Cotty. 2010) and interventions to reduce exposure to environmental stress (Cotty and Jaime-Garcia. 2007), may also reduce aflatoxin contamination.

d) The genetic improvement of plants to provide resistance

Inserting the DNA of Bacillus thuringiensis (Bt) in maize directly enables the plant's production of the insecticidal toxin. Therefore, Bt maize is less susceptible to the penetration of insects (Mwangi & Ely. 2001).

Post-harvest handling Methods

These methods provide tools that eliminate or limit the spread of aflatoxins in agricultural products throughout the harvest. These include food processing, storage strategies such as drying and improved conditions and measures that are appropriate and well adapted to the agro-ecological zone (JECFA, 2008).

a) Food processing

Food processing does not wholly eliminate aflatoxin in harvested crops; however, it can significantly reduce aflatoxin levels in the final product (Scudamore, 2008). This procedure can be done using various techniques, including removing the shell of certain foods such as oats and pistachios (Scudamore, 2008); and torrefaction of foodstuffs such as groundnuts (Siwela *et al.*, 2011). However, it should be noted that cooking and canning have little effect on mycotoxins. Indeed, Scudamore (2008) showed that only 23 per cent of mycotoxins are lost when preparing meals at home, especially typical maize porridge in Africa. Canning contaminated food results in a loss of 15 per cent of mycotoxins.

b) Storage Strategies

These include:

Drying: The heat does not eliminate exposure to aflatoxins, but the gradual elimination of humidity in the crop is an effective method to prevent fungal growth and mould. The method often used is heated drying. It effectively limits the spread of A. flavus and aflatoxins (Magan & Aldred. 2007).

Storage Conditions: Measures should be taken to minimise fluctuations in temperature and humidity in silos and other storage buildings to prevent the increased risk of aflatoxin contamination of crops harvested. Furthermore, the presence of insects in warehouses must be avoided since their activity increases the level of temperature and humidity in the cultures. This can lead to fungal growth, resulting in the production of mycotoxins (Magan & Aldred. 2007).

Short-term silo bags can help guard against the infiltration of insects and moisture. However, impermeability may result in fungal growth (Udoh *et al.*, 2000). The storage of certain products, such as maize near or above the fireplace, is correlated to lower aflatoxin levels. Indeed, Udoh et al. (2000) reported that the dry smoke makes the stored crops less susceptible to fungal growth and insect infestation (Udoh *et al.* 2000).

c) Handling contaminated products

The aflatoxin-contaminated foods can be detoxified by the use of inorganic salts and organic acids such as sodium carbonate, sodium bicarbonate, potassium carbonate, ammonium

carbonate, acetic acid, the predominant sodium and ammonia (Shekhar *et al.,* 2009); and aflatoxin B1 binding agents (Oluwafemi & Da-Silva. 2009).

2.8.2 Dietary and Clinical Intervention.

Dietary and clinical interventions can be considered secondary interventions. They cannot reduce actual aflatoxin levels in food, but they can reduce aflatoxin-related illness.

Vaccinations against Hepatitis B

Vaccination against hepatitis B is not officially considered a control intervention against aflatoxins because the vaccine has no effect on aflatoxin levels in diets. However, knowing that aflatoxin is involved in the occurrence of hepatocellular carcinoma, together with hepatitis B and hepatitis C as significant risk factors, vaccination, therefore, reduces the synergistic impact of hepatitis B and the aflatoxin in inducing this liver cancer (Wild & Hall. 2000; Wu & Khlangwiset. 2010). Vaccination may also reduce cirrhosis caused by aflatoxin (Kuniholm *et al.,* 2008).

Awareness campaigns

Awareness campaigns aimed at improving the knowledge to change attitudes and practices of agriculture and food in the community are a major pillar in the fight and prevention against aflatoxins. This method was proven during an aflatoxicosis outbreak in Kenya in 2005. These campaigns should use systems already in place to disseminate information to the community (Strosnider *et al.,* 2006).

The use of Biomarker technology

Studies of how animals and humans metabolise aflatoxin provided opportunities to develop chemoprevention approaches (bio-score) in human populations. These methods cannot reduce the levels of aflatoxins in foods. However, they may improve disease related to aflatoxins by reducing the bioavailability of aflatoxin or reactive oxygen species that binds to DNA to initiate cancer (Wild & Turner. 2002).

CHAPTER THREE: METHODOLOGY

3.0 Study area

The study was conducted in Lusaka District, one of the Districts within Lusaka Province of Zambia's provinces (see Appendix 2). This District was purposively selected to build upon a similar study done in Lusaka District (Mukuka and Shipekesa, 2013).

3.1 Study design and data source

This was a two-tier study; the first part was a cross-sectional descriptive survey involving the collection related to peanut consumption. The second part was a risk modelling study conducted based on the Codex Alimentarius commission framework and utilized data from the consumption survey and secondary data from the literature search. The risk assessment method on data collection involving the four distinct steps of risk assessment, namely, hazard identification, hazard characterization, consumer exposure assessment and risk characterization, were employed. Point estimates for exposure risk were calculated using the Dietary Exposure, Margin of Exposure, and Carcinogenic Potency of Aflatoxin formulas, explained in detail **(3.4.1 Risk Modelling).** The exposure Risk Assessment model was developed right from the purchase of peanuts to the exposure of aflatoxin in under-five children through consumption. The study depended on both secondary and primary data sources as follows:

Secondary data: this included data on the exposure risk assessment to AFs in under-five children; assessment of AFs in peanuts; and data on the prevalence of hepatitis B in the study. Such data were mainly derived from reviewing scientific peer-reviewed papers and literature. Research questions guided this literature review.

Primary data: a survey was undertaken, and a questionnaire, as a data collection tool (see Appendix 3), was designed and administered to mothers/guardians. The data collected using the questionnaire included:

(a) Consumption frequency patterns of peanut for the child.

(b) Socio-demographic characteristics of the study population.

(c) The knowledge, attitudes and practices related to factors that might explain the development of aflatoxins peanuts.

(d) Food Safety practices for the household where these children reside.

The study also included risk modelling, which used data from the survey and secondary data

3.2 Sampling size and frame

The study anticipated finding of 1000 under-five children enrolled in all the selected health centres, markets where peanuts were being sold and in close proximity to health centres and some households. Although the selection from these locations was to ensure respondents with diverse socio-economic characteristics were included, those from markets were generally sampled as a proxy for under-five children enrolled at health centre. This was done because some health centres were visited on days when under-five clinics were not being held, and the time constraint coupled with the permission granted by the health facility authorities did not permit further visits, especially in view of COVID-19.

Against the planned sample size, the study successfully sampled 795 children represented mainly through their mothers and other female guardians. From each health centre where under-five clinics were being conducted during the research team, 10 per cent was obtained from the total number of the under-five children enrolled, and random interviews were then conducted. The selection of health centres was based on stratification to represent the respondents' low, middle, and high socioeconomic status. Therefore, the sampling frame was composed of respondents from three different socioeconomic statuses to necessitate a representative estimate of peanut consumption patterns of the under-five children of these areas.

3.3 Data Collection

The Researcher and Research Assistants administered a pretested questionnaire. The responses were digitally recorded using the Epicollect5 tool (http://www.epicollect.net/). Then the questionnaire was digitised using the Epicollect5 tool. Accordingly, it generated a form to capture social demographic data, knowledge, attitudes, and practices related to factors that might explain the development of in aflatoxins peanuts, consumption patterns, and food safety practices. The questionnaire (http://www.epicollect.net/project/consumption-survey-exposure-risk) captured individuals' knowledge, practices, attitudes, and awareness of the exposure risk of

aflatoxin through the consumption of peanuts in under-five children of the Lusaka District. The interview took an average of 10 minutes.

3.4 Data analysis

Data were processed and analysed using SPSS software with the correlation between the response variable (the level of aflatoxin exposure in under-five children) and the explanatory variables (age, weight, and frequency of peanut consumption per week).

3.4.1 Exposure Assessment (Risk Modelling)

Exposure Assessment Calculation

The intake of Afs was estimated using the average consumption of peanuts by children obtained from the Zambia Food-Based Dietary Guidelines (FBDG) Technical Recommendation of 2021. This gave dietary recommendations on the consumption of peanuts with different age groups of under-five children: 6- 8 months, 9-11 months, 12-23 months, and 24-59 months. The Af concentration was obtained from a study conducted in Lusaka District by Bumbangi (2016)
The dietary exposure analysis of A.F. was calculated using the following equation.

$$\textbf{Dietary exposure (ng/kg bw/day)} = \frac{\textbf{Consumption (g/day) X Afla. Conc. (ng/kg)}}{\textbf{bw (kg)}}$$

Afla. = Aflatoxin
Conc.= Concentration

Where B.W. is the average body weight of the age group, and C is the concentration (ng/kg) of the mean total aflatoxin concentration in the food item. According to Bumbangi (2016), C.R. is the food item's daily consumption rate (g/kg BWBW/day). This information was gotten from the Zambia Food-Based Dietary Guidelines (FBDG) Technical Recommendation in 2021.

3.4.2 Risk Characterisation

Carcinogenic risk of aflatoxins

The carcinogenic potency of Aflatoxins ($P_{estimated}$) and the cancer risk from exposure to Aflatoxins present in the diet was estimated using equations according to the procedure of the FAO/WHO Expert Committee on Food Additives (JECFA) (WHO 1999). These estimations

consider the carcinogenic potency of Aflatoxins for individuals with the hepatitis B virus ($PHBsAg^+$ = 0.3 $Cancers\ year^{-1}$ per 100 000 individuals) and non-infected individuals ($PHBsAg^-$ = 0.01 $Cancers\ year^{-1}$ per 100 000 individuals), the percentage of carriers ($PHBsAg^+$) and non-carriers ($PHBsAg^-$) of the hepatitis B virus, and the total intake calculated previously.

$P_{estimated}$ = ($PHBsAg^+$ × % population $PHBsAg^+$) + ($PHBsAg^-$ × % population $PHBsAg^-$)
Cancer risk = $P_{estimated}$ × total intake

Average potency (cancers/year/100,000 people) = (0.01×95%) + (0.30×5%)
×AFB1 or AFs intake (ng/kg bw/day)

Margin of exposure (MOE)

The margin of exposure (MOE) is the ratio between the total intake and a toxicological reference, usually, the lower bound of a benchmark dose level that causes 10% cancer incidence in rodents (BMDL10) (European Food Safety Authority (EFSA), 2005). A BMDL10 of 10% of 170 ng/kg BWBW/day estimated by EFSA (2005) based on carcinogenicity data in rats exposed to AFB1 was used to determine the most. The larger the MOE, the smaller the risk, and a value lower than 10 000 may indicate a human health concern (EFSA, 2005).

MOE = BMDL10/Exposure

3.5 Ethical considerations

Clearance to conduct the study was obtained from the School of the Veterinary University of Zambia, the National Health Research Authority (NHRA) and the Lusaka Health Provincial Office, while ethical clearance was obtained from Excellence in Research Ethics and Science Converge (ERES). The provincial and medical offices obtained authority to collect data from the short-listed clinics in the Lusaka District. Written or thumb printed voluntary informed consent (Appendix 4) was sought from participants. The participants' privacy and confidentiality were ensured with no names or national identities used in the questionnaires.

CHAPTER FOUR: RESULTS

4.1 Demographic Characteristics

4.1.1 Respondents' Demographics

As detailed in the methodology Chapter, 795 respondents were successfully interviewed. These were drawn from the market 16.2% (95% CI; 13.76-19.01), clinic 75.6% (95% CI: 72.42-78.51), and homes 8.2% (95% CI; 6.41-10.35). These places were mainly urban with 51% (95% CI; 47.78-54.84) and peri-urban with 49% (95% CI; 45.16-52.21). The selection of the areas was made by stratification to necessitate a balance of socio-economic classes; these were high, middle and low classes. This was to ensure representation from all three social classes. Females with a proportion of 91.1% (95% CI; 88.81- 92.92) were the largest group of respondents, while males accounted for 8.9% (95% CI; 7.08-11-18). The study had different age groups of guardians/ mothers of the under-five, these respondents' ages ranged from 18-60 years, and the majority were in the 21-30 years age group comprising 51% (95% CI;48.04-55.09). These respondents had under-five children in their households, with the majority, 46% (95% CI; 43.04-50.08), being children aged 24-59 months. The study also included people with different education levels. The majority is secondary level with 49%, followed by primary level with 34% (95% CI; 31.06-37.78).

Regarding the marital status of the respondents, the married were the largest group at 82% (95% CI; 79.39-84.82). The primary source of income was another key socio-economic characteristic of the respondents. A total of 49%, as the majority, reported that their primary source of income was through business. Their income ranged from less than ZMW 5,000 to above ZMK 10,000 per month. The primary source of fuel for cooking was charcoal at 67%. The households had various assets, with the majority, 25%, owning a Television set. People from different socioeconomic statuses had different sources of peanut. Those from a high and middle socio class got their peanuts from the supermarket, while those from a low social, economic status got from local markets. According to Bumbangi (2016), finding suggests that peanuts sold in supermarkets had a low aflatoxin level than those sold in local markets. Table 4.1 provides a statistical summary of the socio-demographic characteristics.

Table 4.1: Respondents' Demographic Characteristics

Demographic Variables		n(795)	%	95% C I	*P- Value*
Gender	Male	71	8.9	7.084-11.19	<0.001***
	Female	724	91.1	88.82-92.93	<0.001***
Age Group	18-20	45	5.67	4.32-7.56	<0.001***
	21-30	410	51.57	48.04-55.09	0.395
	31-40	295	37.12	33.76-40.58	<0.001***
	41-50	34	4.27	3.02-5.99	<0.001***
	51-60	11	1.38	0.73-2.54	<0.001***
Marital status	Divorced/ separated	8	1	0.44-2.05	<0.001***
	Married	654	82.6	79.39-84.82	<0.001***
	Single	118	14.8	12.48-17.55	<0.001***
	Widowed	15	1.88	1.1-3.17	<0.001***
Level of education	None	8	1	0.47-2.05	<0.001***
	Primary	273	34.34	31.06-37.78	<0.001***
	Secondary	387	48.68	45.16-52.21	0.478
	Tertiary	127	15.97	13.53-18.75	<0.001***
Household monthly income	Less than K5000	680	85.53	82.85-87.87	<0.001***
	K5000 – K10, 000	89	11.19	9.13-13.64	<0.001***
	Above K10,000	26	3.27	2.19-4.82	<0.001***
Household location	Peri-urban	387	48.68	45.16-52.21	0.478
	Urban	408	51.32	47.78-54.84	0.478

*** = Significant; n = Number of Respondents; CI= Confidence interval; Significant level < 0.05

4.1.2 Under-five children's Demographics and Peanuts Consumption Data

In Table 4.2, the children whose peanuts consumption data was obtained from the respondents comprised about 55% females as the largest category, and the rest were males. The study established that there were children from 6 months to 59 months on complementary foods. About 46% being the majority, of the children were in the 24-59 months old age range. Only four age categories - 6months, 6-12months; 12-18months; 18-24months - were statistically significant with 95% CI.

Regarding the children's consumption frequency pattern for peanuts, daily and weekly intakes were observed. Those who consumed peanuts twice per day constituted the majority at about 49% of the daily intake. These were followed by those who consumed the product once daily, being about 46%. As for weekly consumption, the majority comprising about 58% (95% CI; 54.84-61.80) of children consumed peanuts less than seven times weekly. Those consuming peanuts 7-14 times weekly accounted for about 38% (95% CI; 35.01-41.98) of the sample.

Although the children consuming peanuts 14 – 21 times per week was statistically significant (95% CI; 1.19-3.32), it only accounted for about 2% of all the children, as Table 4.2 shows.

Table 4.2: Demographic and consumption data for under-five children

Demographic Variables		**n(795)**	**%**	**95% C I**	***P- Value***
Gender	Male	354	44.53	41.05-48.06	0.002***
	Female	441	55.47	51.93-58.95	0.002***
Age Group	6months	29	3.6	2.50-5.26	<0.001***
	6-12months	122	15.34	12.95-18.08	<0.001***
	12-18months	186	23.39	20.52-26.53	<0.001***
	18-24months	88	11.07	9.01-13.51	<0.001***
	24-59months	370	46.54	43.04-50.08	0.055
Consumption/ day	Don't eat	8	1	0.478-2.05	<0.001***
	Once a day	367	46.16	42.66-49.70	0.033***
	Twice a day	386	48.55	45.03-52.09	0.435
	Thrice a day	34	4.28	3.02-5.99	<0.001***
Consumption/ week	Don't eat	8	1	0.47-0.205	<0.001***
	<than 7 times	464	58.36	54.84-61.80	0.001***
	7-14 times	306	38.49	35.01-41.98	0.001***
	14-21 times	16	2.01	1.19-3.32	<0.001***
	> 21 times	1	0.1	0.000-0.01	<0.001***

*****=** Significant**;** n = Number of Respondents; CI= Confidence interval; Significant level < 0.05

4.3 Size of peanut purchased and available storage

This study established that the respondents purchased peanuts in varying quantities or sizes. As Table 4.3 summarises, the majority, accounting for 34% (95% CI; 30.69-37.39) of the sample, reported having purchased a small peanut. Those who purchased medium peanuts at 30% (95% CI; 27.28-33.79) while 18% (95% CI; 15.53-21.01) purchased big peanuts. Other respondents purchased small, medium and big, small and medium, and medium-big medium-big peanuts. It is also crucial to note that some respondents do not purchase peanuts, and these were just 1% of the total sample.

Storage of the purchased peanuts is another crucial aspect of the study established. Almost 99% (95% CI; 97.62-99.36) stored their peanuts in a cool, dry place, and only 0.1% (95% CI; 0.04-1) utilised a "wet, cool place" for their peanut storage.

Table 4.3: Size and storage of peanut purchased

Variables		n(795)	%	95% C I	*P-values-Value*
Size of peanut purchased	Small	270	33.96	30.69-37.39	<0.001***
	Small; Medium	76	9.56	7.65-11.87	<0.001***
	Small; Medium; Big	1	0.1	0.00-0.01	<0.001***
	Small; Big	4	0.5	0.16-1.37	<0.001***
	Medium	242	30.44	27.28-33.79	<0.001***
	Medium; Big	50	6.29	4.75-8.27	<0.001***
	Big	144	18.11	15.53-21.01	<0.001***
	Do not Purchase	8	1	0.47-0.205	<0.001***
Storage of Peanut	Cool, dry place	785	98.74	97.62-99.36	<0.001***
	Wet cool place	2	0.25	0.04-1	<0.001***
	Don't store	8	0.1	0.47-0.205	<0.001***

n = Number of Respondents; CI= Confidence interval; Significant level < 0.05; *** = significant

4.2 Awareness of aflatoxin and its effects

Finding out how aware the respondents were of the presence of aflatoxin in food (particularly peanuts) and its effects was vital in this study. Only 18% (95% CI; 16.12-21.67) of the respondents were aware, while the majority - 81% (95% CI; 78.33-83.88) - were not aware of the presence of aflatoxin in peanuts. Furthermore, out of 795 total samples, only 12% (95% CI; 10.16-14.86) knew how aflatoxin gets in peanuts. The study also included information on whether respondents had bought bitter peanuts. About 91% (95% CI; 89.64-93.59) who were the majority acknowledged the purchase of bitter peanuts. Only 15% (95% CI; 13.18-18.35) went ahead to prepare a child's meal. Asked about the effects of aflatoxin in peanuts, 93% (95% CI; 91.17-94.81) of the respondents were not aware of the health effects of consuming aflatoxin, as **Table 4.4** portrays.

Table 4. 4: Data on aflatoxin awareness

Variables		n(795)	%	95% C I	*P-values-Value*
Aware of the presence of aflatoxin in peanut	No	646	81.26	78.33-83.88	<0.001***
	Yes	149	18.74	16.12-21.67	<0.001***
Aware of how aflatoxin gets in	No	697	87.67	85.14-89.84	<0.001***

peanut	Yes	98	12.33	10.16-14.86	<0.001***
Have you bought bitter peanut	No	65	8.18	6.41-10.36	<0.001***
	Yes	730	91.82	89.64-93.59	<0.001***
Have you gone ahead to give your child	No	671	84.4	81.65-86.82	<0.001***
	Yes	124	15.6	13.18-18.35	<0.001***
Aware of health effects of consuming aflatoxin	No	741	93.21	91.17-94.81	<0.001***
	Yes	62	7.8	6.07-9.94	<0.001***

n = Number of Respondents; CI= Confidence interval; Significant level < 0.05

4.3 Food safety practices

The study collected information on the food safety practices of the respondents as they handled food for their under-five-under children. This is because when food is being processed, utensils and hands can transfer the aflatoxin in peanuts to non-peanut foods. As Table 4.5 shows, the key among such practices was washing hands before and during food preparations. About 63% (95% CI; 59.29-66.12), constituting the majority, reported that they always washed their hands before and during food preparations. A statistically significant result was the less than 1% (95% CI: 0.09-1.19) who did not often wash their hands.

Another food safety practice investigated was cleaning surfaces and equipment for food preparation before use. The majority at 49% (95% CI; 45.53-52.59) always did so, while 35% (95% CI; 31.79-38.54) reported that they sometimes did so. Regarding whether the respondents separated utensils such as cutting boards when preparing raw and cooked foods, about 35% (95% CI; 31.79-38.54) as the majority did so sometimes. About 5% (95%; 3.99-7.28) reported that they never separated such utensils when preparing raw and cooked foods. Findings on separating raw and cooked foods during storage showed that about 68% (95% CI; 64.91-71.01) always did so, while about 1% (95% CI; 0.16-0.14) did not often do so.

Reheating cooked foods until it is boiling was also a practice investigated. About 37%, as the majority, upheld such a practice most times, while about 1% (95% CI; 0.55-2.22) did not often uphold the practice. Those who "most times" stored leftovers in a cool, dry place within two hours were about 31% (95% CI; 27.77-34.31) as the largest category doing so, while those in other response categories such as not often, sometimes, and always all recorded statistically

significant results with a confidence interval of 95%. The majority always practised checking and throwing away foods beyond their expiry date, accounting for 63%.

Table 4.5: Food safety practices of respondents

Variables		n(795)	%	95% C I	*P-Value*
Wash hands before and during preparations	Not often	3	0.38	0.09-1.19	<0.001***
	Sometimes	112	14.09	11.73-16.75	<0.001***
	Most times	181	22.77	19.93-25.87	<0.001***
	Always	499	62.77	59.29-66.12	0.001
I clean Surface and equip. For prep. Before use	Not often	14	1.76	1-3.01	<0.001***
	Sometimes	102	12.83	10.63-15.40	<0.001***
	Most times	289	36.35	33.02-39.82	0.001
	Always	390	49.06	45.53-52.59	0.619**
Separate utensils cutting boards when preparing raw & cooked food	Never	43	5.41	3.99-7.28	<0.001***
	Not often	130	16.35	13-88-19.15	<0.001***
	Sometimes	279	35.1	31.79-38.54	<0.001***
	Most times	136	17.11	14.59-19.95	<0.001***
	Always	207	26.04	23.05-29.26	<0.001***
Separate raw & cooked foods during storage	Not often	4	0.5	0.16-0.14	<0.001***
	Sometimes	51	0.5	0.16-0.137	<0.001***
	Most times	201	25.28	22.32-28.48	<0.001***
	Always	539	67.8	64.91-71.01	<0.001***
I reheat cooked foods until it is very hot	Not often	9	1.13	0.55-2.22	<0.001***
	Sometimes	209	29.29	23.29-29.52	<0.001***
	Most times	293	36.86	33.51-40.33	0.001
	Always	284	35.72	32.40-39.18	0.001
Leftovers are stored in a cool, dry place within 2hrs	Not often	106	13.33	11.08-15.94	<0.001***
	Sometimes	228	28.68	25.58-31.98	<0.001***
	Most times	246	30.94	27.77-34.31	<0.001***
	Always	215	27.04	24.01-30.30	<0.001***
Check & throw away food beyond its expiry date	Not often	40	5.05	3.66-6.85	<0.001***
	Sometimes	105	13.21	10.97-15.81	<0.001***
	Most times	148	18.62	16.0-21.54	<0.001***
	Always	502	63.14	59.67-66.49	0.001

n = Number of Respondents; CI= Confidence interval; Significant level < 0.05; ***= significant

4.4 Bivariate analysis of risk factors for exposure to aflatoxin

The variable used as an expected outcome was the consumption of bitter peanuts. This was a proxy to determine the risk factor for exposure to aflatoxin. Respondents' gender, age, level of education, household monthly income and location were cross-tabulated against the "bitter peanut" variable. Other variables included the under-five children's age, gender, weekly peanut

consumption, peanut form, and peanut consumption frequency pattern. More variables were drawn from the category of "awareness of aflatoxin and its effects" and the prevailing "food safety practices". Most such variables had a p-value less than 0.05, showing an association with the expected outcome. Table 4.6 provides a statistical summary of the risk factor bivariate analysis.

Table 4.6: Bivariate analysis of risk factors for exposure to aflatoxin

Factor	Value, n=795	*p-value*
Respondent Gender	7.695[a]	0.006
Respondent age	14.312[a]	0.006
Marital status	0.292[a]	0.961***
Respondent Level of education	26.692[a]	0.000
Household Monthly income	12.702[a]	0.002
Household Location	121.496[a]	0.000
Under-fiveUnder-five age	23.209[a]	0.000
Gender	4.036	0.45
Consumption/day	6.397[a]	0.094***
Consumption/week	65.187[a]	0.000
Peanut form	77.906[a]	0.000
State of peanut consumed	288.608[a]	0.000
Size of peanut	109.65[a]	0.000
Source of peanut	48.423[a]	0.000
Storage of peanut	2.355[a]	0.308***
Aware of the presence of aflatoxin	5.357[a]	0.021
Aware of how aflatoxin gets in peanut	2.887	0.089
Have you bought bitter peanut	8.429	0.004
Health effects of aflatoxin	2.899[a]	0.089***
Wash hands during food preparation	135.286[a]	0.000
Cleans surfaces and equipment	99.254[a]	0.000
Separates utensils	90.465[a]	0.000
Separate cooked and raw foods	6.276[a]	0.099***
Reheat cooked foods	47.056[a]	0.000
Leftovers food items	86.023[a]	0.000
Check and throw away expired food	44.915[a]	0.000

*** = Not Significant at 0.05; [a] = Value obtained by Pearson chi-Square; n = Number of Respondents; CI = Confidence interval; Significant level < 0.0

4.5 Multivariate analysis of factors for exposure risk to aflatoxin by under-five children

A stepwise binary logistic regression model was used to determine variables that could predispose factors for aflatoxin exposure in the under-five children of the Lusaka District. Variables with a *p-value* less than 0.05 in the bivariate analysis were included in the model. A significant Hosmer-Lemeshow goodness-of-fit test (p = 0.9605). The risk factors were significant when < 0.05 *p-value* and when the confidence interval did not include 1. The model fitted the data, thus increasing its reliability in predicting the exposure risk of aflatoxin in under-five children. The Receiver-operating characteristic curve analysis (**Figure 4.1**) demonstrated that the model was good in prediction (ROC=0.9508). The model had relatively high sensitivity and specificity in classifying what would contribute to the aflatoxin exposure in under-five children (**Figure 4.2**).

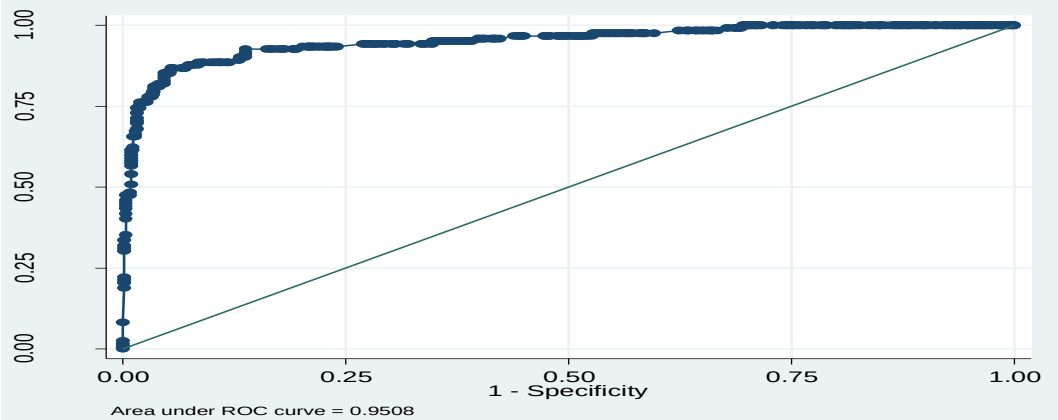


Figure 4.1: ROC curve demonstrating predictability of the model

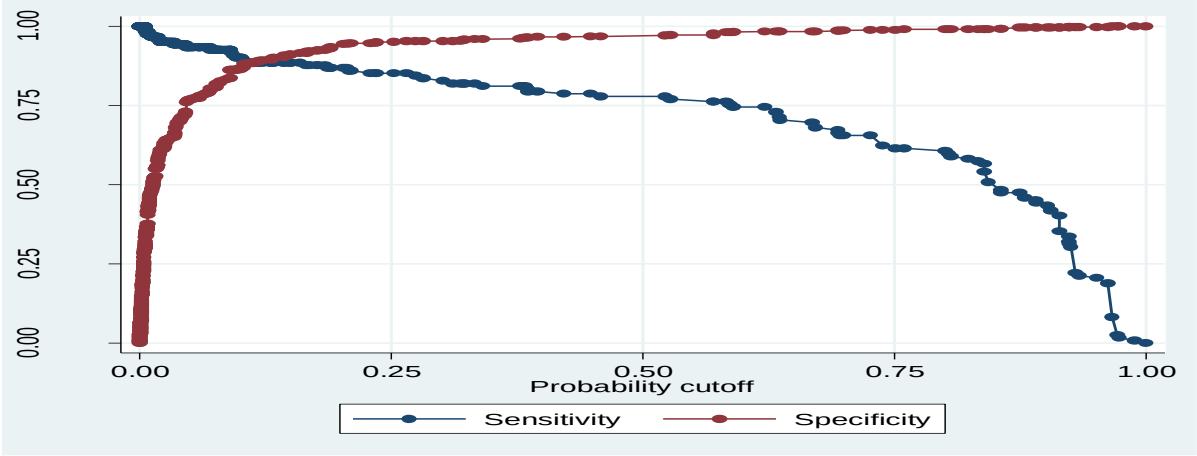


Figure 4.2: Graphs demonstrating probability cut-off versus sensitivity and specificity

The multivariate logistic regression model showed that Household location, Respondents' gender, Household monthly income, Peanut storage, Peanut state of Consumption, Consumption of peanuts weekly, separation of food that is cooked and uncooked, leftover food items, check and throwing away expired food and bought bitter peanut were predictors of exposure to aflatoxin. The food referred to above is food in general that comes into contact with raw peanuts, either by the utensils or hands of the food handlers.

The odds ratio for the household location that was peri-urban and urban was 12.435 (95% C.I.; 3.592-43.047) times more likely factor contributing to the exposure risk of aflatoxin compared to the reference. The respondent gender odds ratio for females was 8.634 (95% C.I.; 1.870-39.874). The risk is higher than that of the male respondents. The odds for the under-five gender was 2.035 (95% C.I.: 1.035-4.003). The exposure risk is higher in females compared to the reference in males. The odds ratio for the state of peanut consumed for those that had peanut butter (homemade) was 0.021 (95% C.I.; 0.005-0.087) risk was higher than the reference. Tables 4.7a-b provides a statistical summary of the likelihood estimates of risk factors.

Table 4.7a: Multivariate analysis of factors for exposure risk to aflatoxin

Variables	**Level**	**OR**	***p-value***	**95% CI**	
Household location	Peri-urban	R			
	Urban	12.435	0.000	3.592	43.047
Respondents' gender	Male	R			
	Female	8.634	0.006	1.870	39.874
Household monthly income	Less than K5000	R			
	K5000-K10, 000	1.136	0.798	0.427	3.024
	Above K10, 000	0.299	0.377	0.021	4.346
Under-five gender	Male	R			
	Female	2.035	0.040	1.035	4.003
Peanut storage	Do not store	R			
	At a cool, dry place	11.003	0.123	0.524	231.203
	AA wet, cool place	1.000	-	-	-

*** = Significant at 0.05; OR = Odds ratio; CI = Confidence interval; Significant level < 0.05; R = Reference category

Table 4.7b: Multivariate analysis of factors for exposure risk to aflatoxin

Variables	**Level**	**OR**	***p-value***	**95% CI**
Peanut state	Do not eat	R		

consumption state consumption	p.nut butter (homemade)	0.021	0.000	0.005	0.087
	P.nut butter (homemade), Raw form	1.000	-	-	-
	P.nut butter (homemade),Rawform, Snack roasted	0.049	0.016	0.004	0.575
	P.nut butter (purchased)	0.150	0.047	0.023	0.975
	P.nut butter (Purchased), P.nut butter (homemade)	0.057	0.000	0.023	0.140
	P.nut(Purchased), P.nut butter (homemade),Snack roasted	0.025	0.001	0.003	0.242
	P.nut (purchased),P.nut (homemade), Snack roasted,Rawform	0.096	0.050	0.009	0.998
	P.nut (purchased),Raw form	0.120	0.448	0.001	28.684
	P.nut (Purchased), Snack roasted	3.215	0.429	0.178	57.999
	P.nut butter (Purchased) Snack roasted, Raw form	0.047	0.015	0.004	0.556
	Raw form	0.688	0.861	0.010	45.504
often_consump_per_week	Do not eat	R			
	<7 times a week	0.119	0.056	0.013	1.052
	7-14 times a week	0.283	0.256	0.032	2.504
	14-21 times a week	1.000	-	-	-
	> 21 times a week	1.000	-	-	-
Separete_foods	Not often	R			
	Sometimes	0.125	0.018	0.022	0.700
	Most times	0.771	0.554	0.327	1.822
	Always	1.000	-	-	-
Leftover food items	Not often	R			
	Sometimes	1.304	0.823	0.126	13.447
	Most times most times	6.103	0.130	0.586	63.560
	Always	0.991	0.994	0.095	10.329
Check to throw away expired food	Not often	R			
	Sometimes	1.294	0.684	0.374	4.476
	Most times	0.425	0.196	0.116	1.556
	Always	1.000	-	-	-
Aware how aflatoxin gets peanuts.	No	R			
	Yes	0.420	0.087	0.155	1.135
Bought bitter peanut	No	R			
	Yes	150.954	0.000	30.340	751.062

*** = Significant at 0.05; OR = Odds ratio; CI = Confidence interval; Significant level < 0.05; R = Reference category

4.6 Dietary Exposure Analysis (Risk Modelling)

As detailed in the methodology chapter on the exposure assessment determination, Tables 4.8, 4.9 and 5.0 contain the numeric summary, which provides the basis for the calculations.

Table 4.8: Summary of the Food Frequency Consumption by Age

Childs Age in Months	Frequency of Consumption	Amount of Food
6-8	2-3	2-3 tablespoons tablespoons
9-11	3-4	2-3 tablespoons tablespoons
12-23	3-4	3-4 tablespoons tablespoons
24-59	4-5	3-4 tablespoons

Source: Zambia Food-Based Dietary Guidelines Technical Recommendation 2021. Tablespoon = 10g

Table 4.9: Standard and average weight of under-five by age

Age (months)	Weight			
	Female	Ave.	Male	Ave.
6-8	7.3-8.2	7.75	7.9-8.9	8.4
9-11	8.7-9.4	9.05	9.5-10.2	9.85
12-23	9-11.3	10.15	9.7-12	10.85
24-59	12.1-18	15.05	12.7-18.5	15.6

Source; WHO standard guideline for growth monitoring for under-five children

Table 4.10: Summary of aflatoxin concentration in raw peanut samples from Lusaka District

Variables	Positive observations%	Min (ng/kg)	Max (ng/kg)
AFB1	n= 41(44.6)	0.000015	0.0466
AFB2	n=41(44.6)	0.000006	0.01317
AFG1	n=21(22.8)	0.000005	0.00051
AFG2	n=7(7.6)	0.000006	0.00004
AF	n=51(55.)	0.000014	0.04867

Source: Bumbangi et al (2016).

4.7 Exposure Assessment and Risk Characterisation

The Dietary Exposure (DE) of AFB1 (Min & max)) Furthermore, the resulting Hepatocellular Carcinoma (HCC)and Margine of Exposure (MOE) values of the raw peanut using the food consumption frequency data from the survey conduct shown in table 4.2. The DE of the AFB1 (min) for daily and weekly consumption for all the age groups results showed no significance to be of concern to cause exposure risk. The HCC also showed small values, indicating a very low concern of exposure risk. The MOE results for all the food frequencies were above ten thousand (10 000), which indicated no exposure risk. Table 5.1a provides the statistical summary.

The DE of AFB1 (max) for the daily consumption for all the age groups ranged from 0.150-0.108 for females and 0.139-0.105 for males. For twice a day, the D.E. for females 0.3-0.217, males 0.277-0.209, three-time three-time per day females 0.45-0.325 and males 0.416-0.314, less the seven times a week female 0.9-0.648 and males 0.832-0.63, fourteen times per week females 2.1-1.512 and males 1.942- 0.105, twenty times per week females 3-2.16 and for twenty-one times per week females 3.15-2.268. The values above of the D.E. indicated concern for the exposure risk. This risk increased as the frequency of consumption increased from once per day to twenty-one times per week. Having significant values of the D.E. suggested an HCC and low MOE that was below 10,000, which is shown in Table 5.1b. This indicated concern on the exposure risk to aflatoxin. The statistical results for AFB2, AFG1 and A.F. are attached in the (Appendix 4)

Table 11a: D.E. of AFB1 (min) and Risk characterisation based on MOE and HCC risk approach

AFB_1 0.000015 ng/kg (Min)						
	Female			Male		
Age	D.E.	HCC	MOE	D.E	HCC	MOE
FFC= 1/day						
6-8	0.000048387	0.000001106	3513340.36	0.000044643	0.000001021	3807987.81
9-11	0.000041436	0.000000948	4102712.62	0.000038071	0.000000871	4465341.07
12- 23	0.000051724	0.000001183	3286675.43	0.000048387	0.000001106	3513340.36
24-59	0.000034884	0.00000079	4873294.35	0.000033654	0.00000077	634664.64
FFC = 2/ days						
6-8	0.00096774	0.000002213	1756670.18	0.000089286	0.000002042	1903993.91
9-11	0.000082872	0.000001895	2051356.31	0.000076142	0.00001741	2232670.54
12-23	0.000103448	0.000002366	1567571.55	0.000096774	0.000002213	1756670.18
24-59	0.000069768	0.00000159	2436647.17	0.000067308	0.00000539	2525702.74
FFC= 3/days						
6-8	0.000145161	0.000003556	1171113.45	0.000133929	0.000003281	1269329.27
9-11	0.00124308	0.000030455	13675.71	0.000114213	0.000002798	1488447.02
12-23	0.000155172	0.000003802	1095558.48	0.000145161	0.000003556	1171113.45
24-59	0.000104652	0.000002564	1624431.45	0.000100962	0.000002474	1683801.83
FFC < 7 times/ week						
6-8	0.000290322	0.000007113	585556.73	0.000267858	0.000006563	634664.64
9-11	0.000248616	0.000006091	683785.44	0.000038071	0.000038071	4465341.07
12-23	0.000331034	0.00000811	513542.42	0.0000290822	0.000007113	585556.73
24-59	0.000209304	0.000005128	812215.72	0.000201924	0.000004947	841900.91
FFC = 14 times/ week						
6-8	0.000677418	0.000016591	250952.88	0.000625002	0.000015313	271999.13
9-11	0.000580104	0.000014213	293050.90	0.000532994	0.000013056	318952.93
12-23	0.000724136	0.000017741	234762.53	0.000677418	0.000016591	250952.88
24-59	0.000488376	0.000011965	348092.45	0.000471156	0.000011543	360814.68

FFC = 20 times/ week						
6-8	0.00096774	0.00002371	175667.02	0.00089286	0.000021875	190399.39
9-11	0.00082872	0.000020304	205135.63	0.000761142	0.000018648	223348.59
12-23	0.00103448	0.000025345	164333.77	0.00096774	0.00002371	175667.02
24-59	0.00069768	0.000017093	243664.72	0.00067308	0.00001649	252570.27
FFC = 21 times / week						
6-8	0.001016127	0.000024895	167301.92	0.000937503	0.000022969	181332.75
9-11	0.000870156	0.000021319	195367.27	0.000799491	0.000019588	212635.29
12-23	0.001086204	0.000026612	156508.35	0.001016127	0.000024895	167301.92
24-59	0.000732564	0.000017948	232061.64	0.000706734	0.000017315	240543.12

DE: Dietary Exposure; MOE- Margin of Exposure: Female & Male; HCC cases/year/10individuals (1.2% of HBsAg+); - HCC cases/ year/whole population group (1.2% of HBsAg+) (ZDHS); FFC- Food Frequency Consumption;

Table 12: D.E. of AFB1 (min) and Risk characterisation based on MOE and HCC risk approach

AFB_1 0.0466 ng/kg (Max)						
	Female			Male		
Age	D.E.	HCC	MOE	D.E.	HCC	MOE
FFC= 1/day						
6-8	0.150	0.003675	1133.33	0.1387	0.00339815	1225.66
9-11	0.1287	0.000315315	1320.90	0.118	0.002891	1440.67
12- 23	0.1606	0.0039347	1058.53	0.150	0.003675	1133.33
24-59	0.1083	0.00265385	1569.71	0.10455	0.002561475	1626.02
FFC = 2/ days						
6-8	0.3	0.00735	566.67	0.2774	0.0067963	612.83
9-11	0.2574	0.0063063	660.45	0.236	0.005782	720.34
12-23	0.3212	0.0078694	529.26	0.3	0.00735	566.67
24-59	0.2166	0.0053067	784.86	0.2091	0.0051229	813.01
FFC= 3/days						
6-8	0.45	0.011025	377.78	0.4161	0.01019445	408.56
9-11	0.3861	0.00945945	440.30	0.354	0.008673	480.23
12-23	0.4818	0.0118041	352.84	0.45	0.011025	377.78
24-59	0.3249	0.00996005	523.24	0.31365	0.007684425	542.01
FFC< 7 times/ week						
6-8	0.9	0.02205	188.89	0.8322	0.0203889	204.28
9-11	0.768	0.018816	221.35	0.708	0.017346	240.11
12-23	0.9636	0.0236082	176.42	0.9	0.02205	188.89
24-59	0.648	0.015876	262.35	0.63	0.015435	269.84
FFC= 14 times/ week						
6-8	2.1	0.05145	80.95	1.9418	0.0475741	87.55
9-11	1.792	0.043904	94.87	1.652	0.040474	102.91
12-23	2.484	0.060868	68.44	2.1	0.05145	80.95
24-59	1.512	0.037044	112.43	0.105	0.0025725	1619.05
FFC= 20 times/ week						
6-8	3	0.0735	56.67	2.774	0.067963	61.28

9-11	2.56	0.06272	66.41	2.36	0.05782	72.03
12-23	3.212	0.078694	52.93	3	0.0735	56.67
24-59	2.16	0.05292	78.70	2.1	0.05145	80.95
FFC = 21 times / week						
6-8	3.15	0.077175	53.97	2.9127	0.07136115	58.37
9-11	2.688	0.065856	63.24	2.478	0.060711	68.60
12-23	3.3726	0.0826287	50.41	3.15	0.077175	53.97
24-59	2.268	0.055566	74.96	2.205	0.0540225	77.09

DE: Dietary Exposure; MOE- Margin of Exposure: Female & Male; HCC cases/year/10individuals (1.2% of HBsAg+); - HCC cases/ year/whole population group (1.2% of HBsAg+) (ZDHS); FF-Food Frequency Consumption.

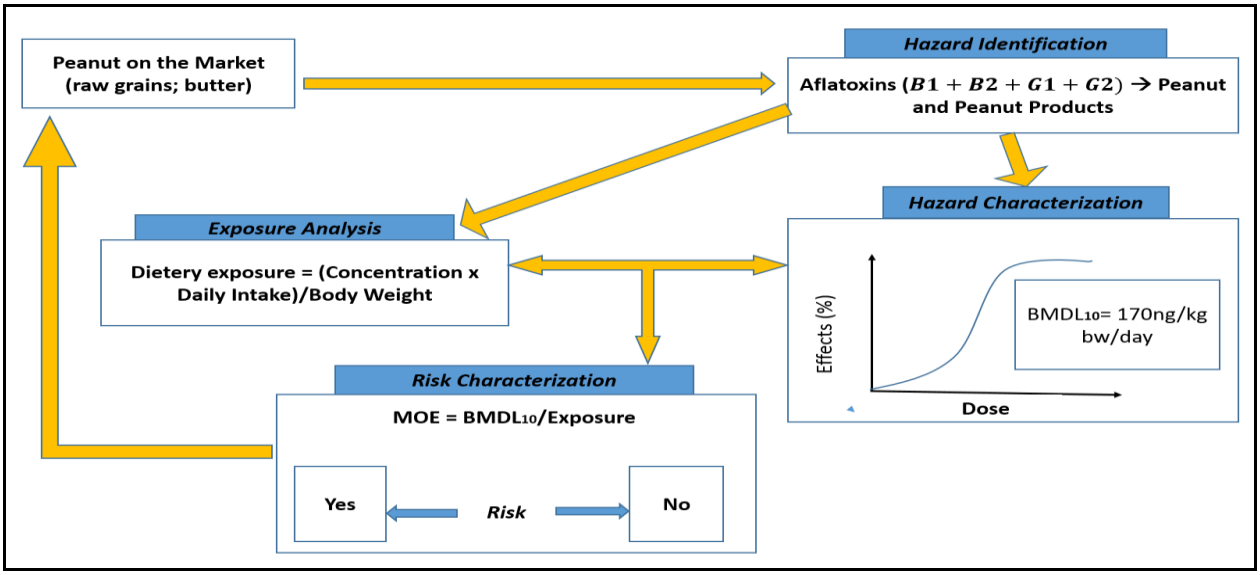


Figure 4.3: Description Scenario of Exposure Analysis of Aflatoxin in Peanut and Peanut product

Source: Author's

CHAPTER FIVE: DISCUSSION

5.0 Introduction

This study was conducted to assess the risk of exposure to aflatoxin in under-five children through the consumption of peanuts in the Lusaka District. The key questions were: how often do children below the age of five in Lusaka consume peanuts? Moreover, does the consumption of peanuts expose under-five children to aflatoxins?

5.1 Consumption Survey

5.1.1 Demographic Information

Most of the respondents in the study were females with a proportion of 91.1% (95% CI; 88.81-92.92), while males accounted for 8.9% (95% CI; 7.08-11-18). This could be attributed to the fact that there is poor male involvement in nutrition issues for children's nutrition. This is in line with the Catholic Relief Service Rwanda (2016) observation, which reported that men generally see themselves as engaged in their children's food consumption mainly via financial support, making them have no information on food consumption because the activity part is left to females. This cultural attitude in Rwanda is prevalent among most cultures in Africa and particularly in Zambia, where men generally perceive children's feeding as a female activity.

5.1.2 Under-five children's Demographics and Peanuts Consumption Data

Children who consumed peanuts comprised about 55% of females as the largest category, and 45% were males. Regarding their consumption frequency pattern for peanuts, daily and weekly intakes were observed. Those who consumed peanuts twice per day constituted the majority at about 49%. These were followed by those who consumed the product once daily, being about 46%. As for weekly consumption, most children comprising about 58% (95% CI; 54.84-61.80) consumed peanuts less than seven times weekly. Those consuming peanuts 7-14 times weekly accounted for about 38% (95% CI; 35.01-41.98) of the sample. The study established that those who had peanut 7- 14 and above were more likely to be exposed to aflatoxins, unlike those whose consumption frequency was less than 7 times in a week. This is in line with the study done by Egal *et al.* (2004), who reported that dietary exposure is due to the cumulative consumption of a diet of peanuts. This results in an increased likelihood of aflatoxin exposure.

5.1.3 Form of the peanut consumed in- relation to heat effect on peanut

The exposure assessments among the children were based on peanuts in raw form. However, the consumption survey established that the forms of peanut consumed included consuming the peanut in porridge, in relish, and as a roasted or boiled snack. Therefore, this means the peanut was subjected to heat for some of the meals. Heating could suggest a possible reduction in aflatoxin concentration. However, the process is ineffective considering the heating temperatures as per the local recipe used at household levels to prepare the - foods mentioned above. It is reported that aflatoxins are heat-stable such that when peanuts are cooked, they undergo a 23% reduction in aflatoxin, while the roasting method accounted for about 15% reduction (Scudoamore, 2008).

Other studies, such as Ogunsanwo (2004), contrast the foregoing conclusion by reporting that peanuts prepared with variations in roasting conditions show a positive correlation between the loss of aflatoxins in the products and the roasting conditions. Peanuts roasted at 140^0C for 40 minutes resulted in 58.8% and 64.5% reductions in AFB1and AFG1, respectively, while those roasted at 150^0C for 25 minutes resulted in 68.5% and 73.3% reductions in AFB1and AFG1, respectively. Roasting at 150^0C for 30 minutes led to 70.0% and 79.8% reductions in AFB1 and AFG1, respectively. Ogunsanwo (2004) further adds that roasting peanuts at such a high temperature is not achievable under domestic cooking conditions; peanut lose the pleasant sensory taste, and most of it is burnt and not fit for human. Although these contrasting findings exist, they enrich the understanding of the subject matter, whereas, in the current study, our focus was on raw peanuts that are used in meal preparation for children. Therefore, the raw peanut-based exposure risk calculations add another dimension to existing knowledge.

5.1.4 Storage of Peanuts

Storage of the purchased peanuts was another crucial aspect the study established because storage plays a significant role in preventing or arresting further growth of aflatoxin at the household level, which relates to raw peanuts. Almost 99% (95% CI; 97.62-99.36) of the respondents stored their peanuts in a cool, dry place. This shows that most respondents were aware of the correct storage of peanuts despite having 0.1% (95% CI; 0.04-1) that utilized a

"wet, cool place" for their peanut storage. In support of these storage practices, JECTA (2008) reported that the most effective way to prevent aflatoxin development in post-harvest crops susceptible to aflatoxin is by storing them in a cool, dry place and ensuring that they are stored with the correct moisture content.

Good practices such as the correct way of storing peanuts among the respondents, however simple the act is, require commendation. In designing interventions that address bad practices, it could help communities if promotion campaigns started by highlighting the existing good works to serve as an example for reversing the challenge. This would encourage communities to do better in areas where they were lacking whilst not neglecting the small but important aspects of food storage and preservation.

5.1.5 Awareness of aflatoxin and its effects

The study also established awareness (or lack of it) of the presence of aflatoxin in peanuts, how it gets in and its health effects when consumed. The majority (81%) of the respondents were not aware of the presence of aflatoxin (Table 4.4). Similarly, most (87.6%) had no idea of how aflatoxin gets in peanuts, and 93% did not know the health effects of consuming aflatoxin. These statistics could explain the increased exposure risk to aflatoxin through the consumption of contaminated peanuts and further contamination at the household level.

The lack of awareness of the presence of aflatoxin may also be explained by the challenges people have in identifying aflatoxin. In most local languages the respondents spoke, aflatoxin as a concept did not seem to exist – there was no clear word for referring to aflatoxin. Mostly, respondents just associated aflatoxin with bitterness in the peanuts they consumed. Therefore, even if bitterness may not accurately describe aflatoxin because different contaminants may make the peanuts bitter, it was still a relevant proxy because when peanuts are actually aflatoxin-contaminated, they taste bitter. However, there are other possible contaminants which could make peanuts taste bitter. For instance, during peanuts processing, it could be possible that bitterness arises from the peanuts being infested with insects and hence consumed together. However, given the thoroughness in most respondents' preparation of the peanuts, especially when roasting or pounding and sieving for mixing with porridge, the possibility of aflatoxin-related bitterness may be more prevalent than from other sources. This in line with a study done

by (GIZ 2019), they also report on poor awareness about aflatoxins, let alone the dissemination of appropriate control measures to monitor contamination at the field level, during storage, and in commercialization. Primary healthcare centers in Africa rarely relate liver cancer or other negative health effects to food consumption and aflatoxins.

5.1.4 Bivariate analysis of risk factors for exposure to aflatoxin

The variable used as an expected outcome was the consumption of bitter peanuts. This, as explained earlier, was assumed as a proxy for the presence of aflatoxins in peanuts and therefore used to determine the risk factor for exposure to aflatoxin. Respondents' gender, age, level of education, household monthly income and location were cross-tabulated against the "bitter peanut", the outcome variable. Other variables included the under-five children's age, gender, weekly peanut consumption, peanut form, and peanut consumption state. Most of the variables used were significant, indicating they had a positive association with exposure to aflatoxin in children. The above-mentioned variables could be linked to household income, therefore suggesting a lack of variety or diverse diet of food given to the children, hence their diet being restricted too much more of peanut-based meals that is maize meal mixed with peanut. Children are deprived of a diverse diet due to the aforementioned in view of proving a cheap source of protein in under-five children. This is in line with the study done by (Wu, 2014). They report on the importance of a diverse diet, recommended increasing consumption of fresh products like vegetables and fruits. Certain vegetables may have a protective effect; e.g. consumption of green, leafy vegetables seems to have some protective effect by impeding aflatoxin absorption. Cruciferous vegetables, onions, and garlic contain protective phytochemicals that impede the processes through which aflatoxins lead to liver cancer.

The factors identified in multivariate analysis as increasing the risk of consuming ‘bitter peanuts’ were: household location; respondent's gender; monthly household income; under-five gender; peanut storage; state of peanut at consumption; weekly consumption of peanut per week; separate foods; eating leftover food items; checking to throw away expired food; awareness of how aflatoxin gets peanuts; bought bitter peanut. As can be seen, most of the variables identified as risk factors for exposure to aflatoxin were those associated with income levels and awareness

levels. Respondents from low--income households and reduced knowledge of aflatoxin were more likely to eat bitter peanuts, probably because they could not afford good quality peanuts.

5.2 Exposure Assessment Risk Modelling

From the results obtained from 795 respondents, most under-five children were on complementary foods. The study focused on children because they were among the vulnerable group and their diet was much more composed of peanuts as a cheap source of protein in the Lusaka District. Furthermore, the fact that maize was the principal staple consumed in large quantities alongside the peanut by the under-five-under-five children made a case to study the subject matter. This situation is more critical considering that peanuts powder is often added to maize porridge for infants because of its high protein value. Maize is another crop that is more susceptible to AFs contamination (Kpodo *et al.,* 2000; Cotty *et al.*, 2008). Maize meal -peanut porridge was consumed almost every day among the children in the Lusaka District, thus increasing the risk of double exposure to A.F. from both the peanut and maize sources. These results are comparable to those obtained by Egal *et al.* (2004) in Benin and Togo in West Africa, who reported that maize and peanut consumption was an essential source of aflatoxin exposure even if there are other food sources of dietary exposure to aflatoxin. This is because the alternative foods are consumed less than the maize and peanuts.

5.2.1 Hazard identification

In this study, the aflatoxin concentration was adopted from a study done by Bumbangi (2016). This is because the peanut samples he used in his study were from markets and supermarkets in the Lusaka District, where the respondents in this study purchased their peanuts for consumption. The concentration levels of AFB2, AFG1 and AFG2 were low compared to the levels of AFB1. AFB1, the most carcinogenic type, were detected in the range of 0.000015-0.0466 ng/kg in forty-one raw peanuts samples (44.6% of incidence) and had the highest concentration levels (0.0466 ng/kg). This corroborates with what has been reported in other studies where the three types of AFs in peanuts samples were observed at low levels compared to the B1 (Huang *et al.*, 2010; Siwela, Mukaro, & Nziramasanga, 2011). Similar studies conducted in the Democratic Republic of Congo (DRC) (Kamika & Takoy, 2011), Sudan (Shami Elhaj &Ahmed Altayeb, 2011), and

Malaysia (Sulaiman *et al.,* 2007) reported a proportion of aflatoxin concentration to be 53%, 53.3%, and 50%, respectively.

Regarding the children's consumption frequency pattern for peanuts, daily and weekly intakes were observed. Those who consumed peanuts twice per day constituted the majority at about 49% of the daily intake. These were followed by those who consumed the peanut once daily, being about 46%. As for weekly consumption, the majority comprising about 58% (95% CI; 54.84-61.80) of children consumed peanuts less than seven times weekly (Table 4.2).

5.2.2 Risk Characterisation

To draw understanding and meaning on the exposure risk based on the consumption frequency given above, Tables 5.1a and 5.1b give dietary exposure (D.E.) and risk characterisation based on the initial aflatoxin concentration in peanuts. It shows that consumption of peanuts once per day has less exposure risk to aflatoxin. This also results in having a high margin of exposure (MOE) above 10,000 and reducing the hepatocellular carcinoma (HCC) risk. In addition, they increase consumption, increase the exposure risk and reduce the margin of exposure values below 10,000. This shows a high dietary exposure, resulting in a high risk of HCC, which poses health effects in the long run in children.

To support those above, several reports worldwide have reported on the harmful effects of aflatoxins in humans, and children are among the vulnerable group. Azziz-Baumgartner *et al.* (2005); Gong *et al.* (2004); Wild and Turner (2002); Williams *et al.* (2004) also report that indeed, the cumulative consumption of these mycotoxins by a human through foods can cause acute and chronic health effects (aflatoxicosis) including immune-system suppression, growth retardation, cancer, and death. A study by Williams *et al.* (2004) explains that Aflatoxins are carcinogens and genotoxins agents that directly influence the structure of DNA. Furthermore, the literature gives information on the chronic exposure to aflatoxin that may result in human liver cancer (IARC 1993). This has led the International Agency for Research on Cancer (IARC) to classify aflatoxin B1 as a group 1 carcinogenic agent to humans. The agent or mixture has sufficient evidence of carcinogenicity in humans (IARC, 1993). The risk of hepatocellular

carcinoma is particularly elevated in individuals with chronic hepatitis B virus infection exposed to aflatoxins (IARC, 2002).

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of this study indicate increased exposure risk to aflatoxin in children under-five, which could result in health risks, especially among low-income households. This could be due to the presence of AFB1 AFB2, AFG1 AFG2 and A.F. in peanuts sold in markets used to prepare meals for children. The overall exposure was highest in children who consumed peanuts 6 times to not less or 21 times in a week. The study also established a possible double risk in exposure simply because most children have their peanuts mixed with maize meal which is also a susceptible crop to aflatoxin. Furthermore, there was poor knowledge of aflatoxin among respondents, how aflatoxin gets in peanuts and its health effect. The high levels of A.F. in peanuts and high exposures accentuate the need for preventive measures. Particular attention should be given to raising awareness on the impact of aflatoxin risk exposure.

6.2 Recommendations

- Through ministries of Health and Agriculture, the Government of Zambia and collaborating partners could develop, implement, and scale-up diverse diet awareness campaigns for children in the Lusaka District (and elsewhere) to curb the peanut aflatoxin exposure risk.
- There is a need to create awareness among farmers on good agricultural practices that prevent the growth of aflatoxin at a primary production level of peanuts and all other foods in general.
- Standards bodies such as Zambia Compulsory Agency (ZCA) and Zambia Bureau of Standards (ZABS) could establish measures to restrict the sale of peanuts with high aflatoxin levels on the market embark on respective awareness campaigns.
- There is a need for multi-stakeholder participation in creating and scaling-up community awareness on aflatoxin and its effect in communities of Lusaka District and elsewhere through targeted events and others such as child health weeks and their respective media programmes.
- There need to study the compounding effect of aflatoxin from maize and grounds used in making baby porridge.

6.3 Limitations

Although the study successfully interviewed a large sample, the challenge of accessing more male respondents as parents and guardians did not necessitate the enrichment of a gender-balanced perspective. However, mothers often have more information on family nutrition. Furthermore, due to limited resource (funds and time) availability, it was impossible to obtain actual peanut portions from the field. Also the absence of the local word for Aflatoxin made it difficult to explain the hazard that was being investigated. Despite these limitations, the said study's scientific method makes the findings, analysis, interpretations, and recommendations reliable.

REFERENCES

Azziz-Baumgartner E., Lindblade K., Gieseker K., Schurz Rogers H., Kieszak S., Njapau H., Schleicher R., McCoy L. F., Misore A., DeCock K., Rubin C., Slutsker L(2005). Aflatoxin Investigative Group. Case-Control Study of an Acute Aflatoxicosis Outbreak, Kenya, 2004. Environmental Health Perspectives 113(12):1779-1783.

Ashiq, S. (2015). Natural occurrence of mycotoxins in food and feed: Pakistan perspective. Comprehensive Reviews in Food Science and Food Safety, 14, 159–175 http://dx.doi.org/10.1111/crf3.2015.14.issue-2

Binder, E.M.; Tan, L.M.; Chin, L.J.; Handl, J.; Richard, J. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim. Feed Sci. Tech. 2007, 137, 265–282.

Bankole S.A. and Adebanjo A. (2003). Mycotoxins in food in West Africa: current situation and possibilities of controlling it. Afr. J. of Biotechnol. Vol. 2 (9), pp. 254-263.

Bennett J. W. and Klich M. (2003). Mycotoxines. Clinical Microbiology Reviews 16, 497–516.

Bumbangi N.F, Muma J.B, Choongo K, Mukanga M, Velu M.R, Veldaman F, Hatloy A and Mapatono M. A. (2016). Occurrence and factors associated with aflatoxin contamination of raw peanut from Lusaka Districts market Zambia.

Catholic Relief Services (2016): Father Engagement in nutrition: A qualitative analysis in Muhanga and Karongi District in Rwanda. https://www.crs.org/sites/default/files/tools-research/father-engagement-in-nutrition.pdf

Castegnaro M. and Pfohl-Leszkowicz A. (1999) Aflatoxins. In: Les Mycotoxines dans l'Alimentation: Evaluation et Gestion du Risque [Mycotoxins in Food: Risk Evaluation and Management], Paris, Technique et Documentation, pp. 199–247.

Caceres, I.; El Khoury, R.; Bailly, S.; Oswald, I.P.; Puel, O.; Bailly, J.D. Piperine inhibits aflatoxin B1 production in Aspergillus

Chen, Y.C.; Liao, C.D.; Lin, H.Y.; Chiueh, L.C.; Shih, D.Y.C. Survey of aflatoxin contamination in peanut products in Taiwan from 1997 to 2011. J. Food Drug Anal. 2013, 21, 247–252.

Cotty P. J., Probst C. and Jaime-Garcia R. (2008). Etiology and management of aflatoxin contamination. Mycotoxins: detection methods, management, public health, and agricultural trade. CABI, Wallingford, United Kingdom, 287-299.

Cotty P.J. and Jaime-Garcia R. (2007) Influences of climate on aflatoxin producing fungi and aflatoxin contamination. International Journal of Food Microbiology, 119(1-2):109- 115.

Cotty P.J., Bayman P., Egel D.S. and Elias K.S. (1994). Agriculture, aflatoxins and Aspergillus. In Powell K.A., Renwick A. and Peberdy J.F. (eds.). The Genus Aspergillus: From Taxonomy and Genetics to Industrial Application. Plenum Press, New York, pp. 1-27.

DANYA International and USAID. (2012). Aflatoxine: une synthèse de la recherche en santé, agriculture et commerce. Nairobi.

Deshpande, S.S (2002), Handbook of Food Toxicology; CRC Press: New York, NY, USA.

Deutache Gesallschaft fur Internationale Zusammenarbeit (GIZ) Gmbtt (2019) Aflatoxin and Nutrition: Retrived on march 14, 2022. https://www.giz.de/de/downloads/giz2019_en_aflatoxins-and-nutrition.pdf

F. Wu, S. L. Stacy, and T. W. Kensler, "Global risk assessment of afatoxins in maize and peanuts: Are regulatory standards adequately protective?"Toxicological Sciences, vol.135,no.1,pp.251–259, 2013

Dhanasekaran D., Shanmugapriya S., Thajuddin N. and Panneerselvam A. (2011). Aflatoxins and Aflatoxicosis in Human and Animals. Aflatoxins - Biochemistry and Molecular Biology, Dr. Ramon G. Guevara-Gonzalez (Ed.), ISBN: 978-953-307-395-8, InTech.

Dong Yiyang. (1999). Capillary electrophoresis in food analysis. Trends in Food Science and Technology 10: 87-93.

Egal S., Hounsa A., Gong Y.Y., Turner P.C., Wild C.P., Hall A.J., Hell K. and Cardwell K.F. (2005). Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. International Journal of Food Microbiology 104: 215–224.

FAO/WHO. (2006). Food safety risk analysis: A guide for national food safety authorities. FAO Food and Nutrition Paper No 87. Rome 2006.

Filazi Ayhan and Sireli Ufuk Tansel. (2013). Occurrence of Aflatoxins in Food, Aflatoxins - Recent Advances and Future Prospects, Prof. Mehdi Razzaghi-Abyaneh (Ed.), ISBN: 978 953-51-0904-4, InTech.

Frisvad, J.C.; Hubka, V.; Ezekiel, C.N.; Hong, S.B.; Nováková, A.; Chen, A.J.; Arzanlou, M.; Larsen, T.O.; Sklenář, F.; Mahakarnchanakul, W.; et al. Taxonomy of Aspergillus section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. Stud. Mycol. 2019, 93, 1–63.

Food and Drug Administration. (2012). Aflatoxins. Bad Bug Book, Food-borne Pathogenic Microorganisms and Natural Toxins. Handbook, Second Edition. U.S., pp.231-236.

Fu Z., Huang X. and Min S. (2008). Rapid determination of aflatoxins in corn and peanuts, Journalof Chromatography A, Vol.1209, No.1, pp. 271–274, ISSN 0021-9673.

Galvano F., Galofaro V. and Galvano G. (1996). Occurrence and stability of aflatoxin M1 in milk and milk products: A worldwide review. J. Food Protect., 59, 1079–1090

Gimeno Alberto and Martins Maria Ligia. (2003). Mycotoxins and Mycotoxicosis in Animals and Human. Special Nutrients Inc, Miami, USA.

Giniani C. D., Sergiane S. C., Vivian F., Renata H. B., Helen C. S. H., Michele M. S., Melissa dos Santos O., Jaqueline G-B., Ednei G. P. and Eliana B.-F. (2011). Aflatoxins: Contamination, Analysis and Control, Aflatoxins - Biochemistry and Molecular Biology, Dr. Ramon G. Guevara-Gonzalez (Ed.), ISBN: 978-953-307-395-8, InTech.

Glaston K.M., Mvula M.A., Koaze H. and Baba N. (2000). Aflatoxin contamination of Kenyan maize flour and malted Kenyan and Malawian grains. Sci Rep. Fac. Agric. 89: 1-7. Okayama University.

Gong Y.Y., Hounsa A., Egal S., Turner P.C., Sutcliffe A.E., Hall A.J., Cardwell K. and Wild C.P. (2004). Post-weaning exposure to aflatoxin results in impaired child growth: A longitudinal study in Benin, West Africa. Environmental Health Perspectives 112, 1334-1338.

Groopman J.D., Donahue P.R., Zhu J., Chen J. and Wogan G.N. (1985). Aflatoxin metabolism in humans. Detection of metabolites and nucleic acid adducts in urine by affinity chromatography. Proc. Natl. Acad. Sci., USA, Vol.82, pp.6492-6497.

Hussain, A.; Ali, J.; Akther, S.; and Qatullah, S. (2011). Degradation of aflatoxins by roasting in contaminated peanuts. Pak. J. Biochem. Mol. Biol. 44(2): 56-59.

International Agency for Research on Cancer [IARC]. (1999). some industrial chemicals and dyestuffs. Retrieved April 20, 2015, from http://monographs.iarc.fr/ENG/Monographs/vol29/volume29.pdf

IARC (International Agency for Research on Cancer). (1993). Aflatoxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins.56, 245-395.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 82, 1-556.

Ibáñez-Vea M., Corcuera L., Remiro R., Murillo-Arbizu M., González-Peñas E. and Lizarraga E. (2011). Validation of a UHPLC-FLD method for the simultaneous quantification of aflatoxins, ochratoxin A and zearalenone in barley. Food Chemistry, Vol.127, No.1, pp. 351–358, ISSN 0308-8146.

JAICAF (Japan Association for International Collaboration of Agriculture and Forestry). (2008). The Maize in Zambia and Malawi. Association for International Collaboration of Agriculture and Forestry, Minato-ku, Tokyo, Japan. pp. 3-23.

Jalili, M. A review on aflatoxins reduction in food. Iran. J. Health Saf. Environ. 2015, 3, 445 459.

Jaime-Garcia R. and Cotty P. J. (2010). Crop rotation and soil temperature influence the community structure of Aspergillus flavus in soil. Soil Biology & Biochemistry, 42: 1842-1847.

Joint Food and Agricultural Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA). (1998). Forty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Safety evaluation of certain food additives and contaminants in food: Aflatoxins. Food Additives Series No. 40. Geneva (Switzerland): WHO. p. 359–469.

Kankolongo M.A., Hell K. and Nawa I.N. (2009). Assessment for fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia. J. Sci. Food Agric., 89, pp. 1366–1375.

Kimanya ME, De Meulenaer B, Tiisekwa B ,Ndomo Sigonda M , Devlieghere F, vancamp J, (2008):Occurrence of fumonisins with aflatoxins in home stored maize for human consumption in rural villages of Tanzania. Food Addit Contam. 25:13531364. https://doi.org 10.1080/02652030802112601PMI:19680843

Kensler T. W., Roebuck B. D., Wogan G. N., and Groopman J. D. (2010). Aflatoxin: A 50-Year Odyssey of Mechanistic and Translational Toxicology. Toxicological Sciences, 120(1): S28–S48.

Kirk G. D., Bah E., and Montesano R. (2006). Molecular epidemiology of human liver cancer: insights into etiology, pathogenesis and prevention from The Gambia, West Africa. Carcinogenèses 27: 2070-2082.

Kpodo K., Thrane U. and Hald B. (2000). Fusaria and fumonisins in maize from Ghana and their cooccurrence with aflatoxins. International Journal of Food Microbiology 61, 147-157.

Kuniholm M. H., Lesi O. A., Mendy M., Akano A. O., Sam O., Hall A. J., Whittle H., Bah E., Goedert J. J., Hainaut P., and Kirk G.D. (2008). Aflatoxin Exposure and Viral Hepatitis in the Etiology of Liver Cirrhosis in the Gambia, West Africa. Environ Health Perspect 116, 1553–1557.

Lizárraga-Paulín, E.G.; Miranda-Castro, S.P.; Moreno-Martínez, E.; Torres-Pacheco, I.; Lara Sagahón, A.V. Novel methods for preventing and controlling aflatoxins in food: A worldwide daily challenge. In Aflatoxins—Recent Advances and Future Prospects; Razzaghi-Abyaneh, M., Ed.; InTech: Rijeka, Croatia, 2013; pp. 93–128.

Lewis L., Onsongo M., Njapau H., Schurz-Rogers H., Luber G., Kieszak S., Nyamongo J., Backer L., Dahive A., Misore A., DeCock K., Rubin C., and the Kenya Aflatoxicosis Investigation Group (2005). Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya, Environ Health Perspect. December; 113(12): 1763–1767.

Li P., Zhang Q., Zhang W., Zhang J., Chen X., Jiang J., Xie L. and Zhang D. (2009). Development of a class-specific monoclonal antibody-based ELISA for aflatoxins in peanut. Food Chemistry, Vol.115, No.1, pp. 313–317, ISSN 0308-8146.

Liu Y., and Wu F. (2010). Global Burden of Aflatoxin-Induced Hepatocellular Carcinoma: A Risk Assessment. Environ Health Perspect 118 (6): 818-824.

Lizárraga-Paulín E. G., Moreno-Martínez Ernesto and Miranda-Castro Susana P. (2011). Aflatoxins and Their Impact on Human and Animal Health: An Emerging Problem. Aflatoxins - Biochemistry and Molecular Biology, Dr. Ramon G. Guevara-Gonzalez (Ed.), ISBN: 978-953-307-395-8, InTech.

Lubulwa A. S. G. and Davis J.S. (1994). Estimating the social costs of the impacts of fungi and aflatoxins. In Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R. (eds.) Stored Products protection, Proceedings of the 6th International Working Conference in Stored product Protection, 17- 23 April 1994, Canberra, Australia. CAB International, Wallingford, UK.

Lyn M. E., Abbas H. K., Zablotowicz R. M., and Johnson B. J. (2009). Delivery systems for biological control agents to manage aflatoxin contamination of pre-harvest maize. Food Additives and Contaminants, 26(3): 381–387.

MAFF (Ministry of Agriculture, Fisheries and Food). (1999). Survey of Aflatoxins, Ochratoxin A, Fumonisins and Zearalenone in Raw Maize (Food Surveillance Information Sheet No.192), Edinburgh, Scottish Executive Department of Health, UK.

Magan N. and Aldred D. (2007). Post-harvest control strategies: minimizing mycotoxins in the food chain. International Journal of Food Microbiology, 119(1-2): 131–139.

Maleki, F.; Abdi, S.; Davodian, E.; Haghani, K.; Bakhtiyari, S.(2015): Exposure of infants to aflatoxin M1 from mother's breast milk in Ilam, Western Iran. Osong Public Health Res. Perspect, 6, 283–287.

Moss, M.O. Recent studies of mycotoxins. J. Appl. Microbiol. 1998, 84, 62S.

Maragos C.M. and Thompson V.S. (1999). Fiber-optic immunosensor for mycotoxins. Natural Toxins 7, 371-376.

Mukuka R. M. and Shipekesa A. M. (2013). Value Chain Analysis of the Groundnuts Sector in the Eastern Province of Zambia. The Indaba Agricultural Policy Research Institute. Working Paper No. 78.

Mutegi C. K., Ngugi H. K., Hendriks S. L. and Jones R. B. (2009). Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya International Journal of Food Microbiology, 130(1): 27–34.

Mwangi P. N. and Ely A. (2001). Assessing risks and benefits: Bt maize in Kenya. Biotechnology and Development Monitor, 48: 6–9.

Njapau H., Muzungaile M.E. and Changa R.C. (1998). The effect of village processing techniques on the content of aflatoxins in corn and peanuts in Zambia. J Sci Food Agric 76:450–456.

Peles, F.; Sipos, P.; Gy˝ori, Z.; Pfliegler, W.P.; Giacometti, F.; Serraino, A.; Pagliuca, G.; Gazzotti, T.; Pócsi, I. (2019): Adverse effects, transformation, and channeling of aflatoxins into food raw materials in livestock. Front. Microbiol. 10, 2861

Oluwafemi F. and Da-Silva F.A. (2009). Removal of aflatoxins by viable and heat-killed Lactobacillus species isolated from fermented maize. Journal of Applied Biosciences, 16: 871–876.

Ogunsanwa B.M, faboya O.O.P , Idowawu O.R, Lawal O.S and Bankole S.A (2004). Effects of roating on the aflatoxin contents of Nigerian peanut seeds.

Otsuki T., Wilson J. S., and Sewadeh M. (2001a). What price precaution? European harmonization of aflatoxin regulations and African groundnut exports. European Review of AgriculturalEconomics, 28(2): 263–283.

Otsuki T., Wilson J.S. and Sewadeh M. (2001b). Saving two in a billion: Quantifying the trade effect of European food safety standards on African exports. Food Policy 26, 493-514.

Pascale Michelangelo and Visconti Angelo. (2008). Overview of Detection Methods for Mycotoxins. Mycotoxins Detection Methods, Management, Public Health and Agricultural Trade. pp. 171-183.

Paterson R.R.M. and Lima N. (2010). How will climate change affect mycotoxins in food? Food Research International, 43(7):1902-1914.

Probst C., Bandyopadhyay R., Price L. E., and Cotty P. J. (2011). Identification of Atoxigenic Aspergillus flavus Isolates to Reduce Aflatoxin Contamination of Maize in Kenya. Plant Disease, 95(2): 212–218

Reddy K. R. N., Reddy C. S., and Muralidharan K. (2009). Potential of botanicals and biocontrol agents on growth and aflatoxin production by Aspergillus flavus infecting rice grains. Food Control, 20: 173–178.

Ráduly, Z.; Szabó, L.; Madar, A.; Pócsi, I.; Csernoch, L. (2019). Toxicological and medical aspects of Aspergillus-derived mycotoxins entering the feed and food chain. Front. Microbiol. 10, 2908.

Sáez A. C., Fernández A. M. C., Marteache A. H., Larrañaga M. R. M., Losada P. P., and Casamayor P. B. (2011). Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) in relation to the effect on the Spanish population of the derogation of national regulation on maximum allowed limits for aflatoxins B1, B2, G1 and G2 in food.

Saha D., Acharya D., Roy D., Shrestha D. and Dhar T. (2007). Simultaneous enzyme immunoassay for the screening of aflatoxin B1 and ochratoxin A in chili samples. Analytica Chimica Acta, Vol.584, No.2, pp. 343–349, ISSN 0003-2670

Saleemullah A.I., Khalil I.A. and Shah H. (2006). Aflatoxin contents of stored and artificially inoculated cereals and nuts. Food Chemistry, 98(2006):699-703.

Sauer D.B., Storey C.L. and Walker D.E. (1984). Fungal populations in US farm-stored grain and their relationship to moisture, storage time, regions and insect infestation. Phytopathology, 74, 1050–1053.

Scudamore K. A. (2008). Fate of fusarium mycotoxins in the cereal industry: Recent UK studies. World Mycotoxin Journal, 1(3): 315–323.

Scudamore, K.A.; Hazel, C.M.; Patel, S.; Scriven, F. (2009). Deoxynivalenol and other Fusarium mycotoxins in bread, cake, and biscuits produced from UK-grown wheat under commercial and pilot scale conditions. Food Addit. Contam. 26, 1191–1198.

Serraino, A.; Bonilauri, P.; Kerekes, K.; Farkas, Z.; Giacometti, F.; Canever, A.; Zambrini, A.V.; Ambrus, Á, (2019). Occurrence of aflatoxin M1 in raw milk marketed in Italy: Exposure assessment and risk characterization. Front. Microbiol. 10, 2516.

Shirima C.P, Kimanya M.E, Routledge M.N, Srey . C, Kinabo J.L, Humpf H.U (2015); A Prospective Study of Growth and Biomarkers of Exposure to Aflatoxin and Fumonisin during Early Childhood in Tanzania.EnvironHealthPersp. 123:173–178

Shekhar M., Singh S., Khan A. A. A. and Kumar, S. (2009). Efficacy of Inorganic salts and Organic acids against Colony Growth of Aspergillus flavus and their use to control Aflatoxin level in Post-Harvest Maize. Internet Journal of Food Safety, 11: 4–10.

Sitko N.J, Chapoto A., Kabwe S., Tembo S., Hichaambwa M., Lubinda R., Chiwawa H., Mataa M., Heck S., and Nthani D. 2011. Technical Compendium: Descriptive Agricultural Statistics and Analysis for Zambia in Support of the USAID Mission's Feed the Future Strategic Review. Food Security Research Project Working Paper No. 52. Lusaka, Zambia: FSRP.

Siwela A. H., Mukaro K. J. and Nziramasanga N. (2011). Aflatoxin Carryover during Large Scale Peanut Butter Production. Food and Nutrition Sciences, 2: 105–108.

Sorensen L.K. and Elbaek T.H. (2005). Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry. Journal of Chromatgraphy B 820, 183-196

Sorenson W.G., Jones W., Simpson J. and Davidson J.I. (1984). Aflatoxin in respirable airborne peanut dust. J Toxicol Environ Health; 14(4):525-33

Stroka J., Van Otterdijk R. and Anklam E. (2000). Immunoaffinity column clean-up prior to thin layer chromatography for the determination of aflatoxins in various food matrices.Journal of Chromatography A 904, 251-256

Strosnider H., Azziz-Baumgartner E., Banziger M., Bhat R. V., Breiman R., Brune M., DeCock K., Dilley A., Groopman J., Hell K., Henry S. H., Jeffers D., Jolly C., Jolly P., Kibata G. N., Lewis L., Liu X., Luber G., McCoy L., Mensah P., Miraglia M., Misore A., Njapau H., Ong C., Onsongo M. T. K., Page S. W., Park D., Patel M., Phillips T., Pineiro M., Pronczuk J., Schurz Rogers H., Rubin C., Sabino M., Schaafsma A., Shephard G., Stroka J., Wild C., Williams J. T., Wilson D. (2006). Workgroup Report: Public Health Strategies for Reducing Aflatoxin Exposure in Developing Countries. Environmental Health Perspectives, 114(12):1989-1903.

Sudhakar P., Latha P., Sreenivasulu Y., Bhaskar Reddy B. V., Hemalatha T. M., Balakrishna M., and Raja Reddy K. (2009). Inhibition of Aspergillus flavus colonization and aflatoxin (AFB1) in peanuts by methyleugenol. Indian Journal of Experimental Biology, 47: 63-67.

Tang D., Sauceda J.C., Lin Z., Ott S., Basova E., Goryacheva I., Biselli S., Lin J., Niessner R., and Knopp D. (2009). Magnetic nanogold microspheres-based lateral-flow immunodipstick for rapid detection of aflatoxin B2 in food. Biosensors and Bioelectronics, 25: 514–518.

Udoh, J. M., Cardwell, K. F., and Ikotun, T. (2000). Storage systems for maize (Zea mays L.) in Nigeria from five agroecological zones. Journal of Stored Products Research, 36: 187 -201.

United States Department of Agriculture (USDA). 2012. Aflatoxin Hanbook. Washington, DC.

USAID and Southern Africa Trade Hub. (2012). Technical Report: Value Chain Approach Aflatoxin (Groundnuts) Final Report. Gaborone, Botswana.

Van der Gaag B., Spath S., Dietrich H., Stigter E., Boonzaaijer G., Van Osenbruggen T. and Koopal K. (2003). Biosensors and multiple mycotoxin analysis. Food Control 14, 251 -254

Van der Spiegel M., Sterrenburg P., Haasnoot W., and Van der Fels-Klerx H.J. (2013). Towards a decision support system for control of multiple food safety hazards in raw milk production. Trends in Food Science & Technology 34: 137-145

Wagacha J. M. and Muthomi J. W. (2008). Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. International Journal of Food Microbiology, 124(1), 1–12.

Walderhaug M. (1992). Ciguatera. Food-borne Pathogenic Microorganisms and Natural Toxins Handbook. Food and Drug Administration, U.S.

Wang J.S., Huang T., Su J., Liang F., Wei Z., Liang Y., Luo H., Kuang S.Y., Qian G.S., Sun G., He X., Kensler T.W. and Groopman J.D. (2001) Hepatocellular carcinoma and aflatoxin exposure in Zhuqing Village, Fusui County, People's Republic of China. Cancer Epidemiol. Biomarkers Prev., 10, 143–146.

Wild C. and Gong Y. (2010). Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis, 31:71-82.

Wild C. P. and Hall A. J. (2000). Primary prevention of hepatocellular carcinoma in developing countries. Mutat. Res. 462: 381–389.

Wild C.P. and Turner P.C. (2002). The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis 17, 471– 481.

Williams J.H., Phillips T.D., Jolly P.E., Stiles J.K., Jolly C.M. and Aggarwal D. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences and interventions. American Journal of Clinical Nutrition 80, 1106-1122.

Wu F. and Khlangwiset P. (2010). Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions. Food Additives & Contaminants 27: 496–509.

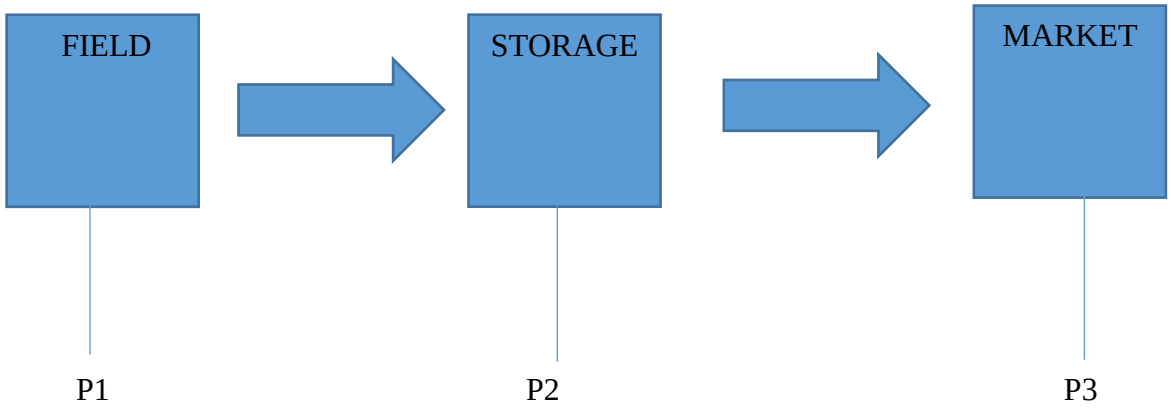
Wu F., Narrod C., Tiongco M., and Liu, Y. (2011). The health economics of aflatoxin: Global burden of disease. AflaControl, ifRPRi. Working Paper 4. february 2011.

Wu F. Mitchell, Nicole J., Denis., Kensler., Thomas W. (2014). Reduced Foodborne Toxin exposure, benefit of improving dietary diversity. Toxocological Sciences 141 (2), 329 -334.

APPENDICES

Appendix 1: Map of Zambia showing Lusaka District

Appendix 2: Exposure pathway



Appendix 3: Letter of introduction and informed consent

My name is Grace Musawa, and I am a student at The University of Zambia (UNZA) inthe School of Veterinary Medicine pursuing a Master of Science in Food Safety and Risk Analysis. I am conducting a study on "Exposure risk Assessment through the consumption of Peanut in Under-five children in Lusaka Districts". The information you may contribute to this study may be helpful to helpfuldeveloping policies on regulating aflatoxin in peanut health and improve the primary production care of peanuts.

1. Procedure to be followed

Participation in this study will require that I ask you some questions, which I will record in a questionnaire. I will collect information on:

(e) Food consumption for each member of the household.

(f) Socio-demographic characteristics of the study population.

(g) The study population's knowledge, attitude, and practice related to factors that might explain the development of aflatoxins peanuts.

Weights will be taken using scales. This study will use non-invasive methods to collect information.

You have the right to refuse participation in this study. Please remember that participation in this study is voluntary. You may ask questions related to the study at any time. You are free to refuse to respond to any question, and you can stop the interview at any time. You may also withdraw from the study at any time without any consequences.

2. Benefits

If you participate in this study, you will help us aboutlearn about the exposure risk assessment of afflatioxin aflatoxin through the consumption of peanuts,which may help improve the quality of life of the under-five children.

3. Discomforts and risks

Some of the questions you will be asked are on the intimate subject and may be embarrassing and make you uncomfortable. If this happens, you may refuse to answer these questions if you so

choose. You may also stop the interview at any time. The interview and nutritional status assessment may take approximately 20 minutes of your time.

4. Compensation

There are no rewards or incentives offered for participation in this study. Participation is purely voluntary.

5. Confidentiality

Your name will not be recorded on the questionnaire. The questionnaires will be kept in a locked cabinet for safekeeping. All the information you give will not be disclosed to a third party and will be used for research purposes only.

6. Voluntary participation

Participation is voluntary, and should you want to discontinue or decline to answer any questions, feel free to do so. I, however, encourage you to participate in the study as the findings will be important for improving the health of the under-five children.

7. Contact information

If you have any further questions about the study, you may contact;

- Prof. John Muma is based at UNZA-VET (jmuma@unza.zm).
- Dr. Chisoni Mumba at UNZA-VET
- Dr. Flavien Bumbang at UNZA-VET

8. Participant's statement

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions, and they have been answered to my satisfaction. The information provided in this research may help understand the exposure risk assessment of aflatoxin through peanut consumption by under-five children. I have been assured of confidentiality on any information that will be shared. My participation in this study is entirely voluntary.

Signature of Participant…… **Date**……

Signature of Witness…… **Date**……

9. Investigator's statement

I, the undersigned, have explained to the volunteer the study procedure, benefits, risks, compensation, confidentiality and contact information in a language she or he understands.

Name of the investigator……

Signature of the investigator…… **Date**……

Appendix 4: Questionnaire

This is a questionnaire on a study on "Exposure risk Assessment through the consumption of Peanut in Under-five children in Lusaka Districts" being conducted in partial fulfilment of the requirements of the Master of Science in Food Safety and Risk Analysis programme at The University of Zambia (UNZA) under the School of Veterinary Medicine. For further details and consent forms, please refer to the "Letter of Introduction and Informed Consent."

Questionnaire Code No._____			
Name of the interviewer_____			
Date of Interview_____/_____/_____/		**Start time_____**	
Questionnaire Checked_____		**End time**	**_____**
Instruction: Complete the questionnaire by writing the response code in the last column.			
Section A: Demographic characteristics (Mother/guardian)		**Code**	
A1	Age (Mother/guardian)	1	<20 years
		2	20-29 years
		3	30-39 years
		4	40-49 years
		5	>50 years
A2	Sex.	1	Male
		2	Female
A3	Marital status.	1	Married
		2	Single
		3	Divorced
		4	Separated
A4	Religion.	1	Christian
		2	Muslim
		3	Other (specify)_____
A5	Place of residence.	1	Urban – formal
		2	Urban –Informal
		3	Rural

Section B: Socio-economic characteristics (mother/guardian)			
B1	Have you ever attended school?	1	Yes
		2	No
B2	If yes, in B1, let me know the highest level of education attained?	1	No formal education
		2	Completed Primary
		3	Not Completed primary
		4	Completed Secondary
		5	Not Completed Secondary
		6	Completed certificate education
		7	Completed Diploma
		8	Completed Degree
		9	Not completed tertiary
		10	Other (specify)_____
B3	Please let me know your occupation?	1	Casual worker
		2	Housewife
		3	Regular Job_____
		4	Self employed_____
		5	Student
		6	Other_____
B4	Please let me know your primary source of income in the household?	1	Salaried job
		2	Husband
		3	Self-employed
		4	Casual income
		5	Other (specify)_____
B5	How much do you approximately earn per month in Zambian Kwacha?	1	Below 1000
		2	2000 - 3000
		3	4000-5000
		4	Above 5000

B6	If married, how much does your spouse approximately earn per month in Zambian Kwacha?	1	Below 1000
		2	2000 - 3000
		3	4000-5000
		4	Above 5000
B7	How much do you approximately spend on groundnuts monthly?	1	Below 50
		2	50 -100
		3	100- 200
		4	200-300
		5	Above 300
Section C: Socio-demographic characteristic, consumption frequency pattern of peanut, and the child's medical information.			
C1	Age	1	0-6 months
		2	6-12 months
		3	12-18months
		4	18-24months
		5	24-59months
C2	Sex	1	Female
		2	Male
Consumption frequency pattern of peanut			
C3	Does your child take peanuts?	1	Yes
		2	No
C4	What is the source of the peanut?	1	Local market
		2	Supermarkets
		3	Other (specify)_____
C5	In what form does your child take the peanut?	1	In porridge
		2	In relish
		3	As a snack(raw)
		4	As a snack (cooked)
		5	Other (specify)_____
C6	In what state do you use the peanut?	1	Peanut butter (homemade)
		2	Raw Form
C7	How frequently does your child eat peanuts per day?	1	Once a day
		2	Twice a day
		3	3 times a day

		4	>4 times a day
C8	How often does your child eat peanuts per week?	1	<7 times a week
		2	7-14 times a week
		3	14-21 times a week
		4	>21 times a week.
Medical Information			
C8	Has your child received the Hepatitis B vaccine	1	Yes
		2	No
C9	What is the weight of the child?		

SECTION I: HOUSEHOLD FOOD SAFETY PRACTICES

Instruction: Kindly select the option that applies to you

I.1 I wash my hands before and during food preparation and eating.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

I.2 I clean surfaces and equipment used for food preparation before re-using on other food.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

I.3 I use separate utensils and cutting boards when preparing raw and cooked food.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

I.4 I separate raw and cooked food during storage.

a) Always
b) Most times

c) Sometimes
d) Not often
e) Never

I.5 I reheat cooked food until it is very hot throughout.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

I.6 After I have cooked a meal, I store any leftovers in a cool place within two hours.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

I.7 I check and throw away food beyond its expiry date.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

SECTION J: KNOWLEDGE, ATTITUDE AND PRACTICE OF GUARDIANS RELATED TO FACTORS THAT MIGHT EXPLAIN THE DEVELOPMENT OF AFLATOXINS GROUNDNUTS.

Instruction: Kindly select the option that applies to you

J.1 Are you aware of the presence of aflatoxin in groundnuts

1 Yes
2 No

J.2 Are you aware of how aflatoxin gets in groundnuts

1 Yes
2 No

J.3 Have you bought groundnuts that are bitter?

1. Yes
2. No

J.4 If so, have you gone ahead to use it for baby consumption?

1. Yes
2. No

J.3 What is the main channel you knew about the presence and how aflatoxin gets in groundnuts?

1 TV
2 Radio
3 Colleague
4 Others (specify)…….

J.4 Are you aware of any health effects that might arise as a result of exposure to aflatoxin through consumption of groundnuts in under-five children

1 Yes
2 No

Appendix 5: Dietary Exposure and Risk Characterization

4.7 Exposure Assessment and Risk Characterisation.

The DE of AFB2, AFG1, AFG2 and A.F (maximum conc.) for the daily, three-time and three-time per day consumption for all the age groups was generally low for both males and females. This was also seen by having a high MOE above 10,000 and low HCC. When considering the weekly consumption, the DE and the HCC erehigh. At the same time, the MOE was low, below 10,000, indicating a high exposure risk of aflatoxin when the concentration of aflatoxin is high.

The DE, HCC of AFB2, AFG1, AFG2 and A.F (min conc.) was low, suggesting less exposure risk even when the frequency of consumption increased from once per day to twenty-one times per week. The MOE was above 10,000. This shows that a low aflatoxin concentration possesses lesser concern on the exposure risk to aflatoxin. The statistical results for AFB2, AFG1, AFG2 and A.F for both maximum and minimum are shown below:

Table 5.2a: DE of AFB2 (max) and Risk characterization based on MOE and HCC risk approach

AFB_2 0.01317 (Max)						
	Female			Male		
Age	D.E	HCC	MOE	D.E	HCC	MOE
FFC = 1/day						
6-8	0.0425	-	4000.00	0.823	-	206.56
9-11	0.0364	-	4670.33	0.0334	-	5089.82
12- 23	0.454	-	374.45	0.0425	-	4000.00
24-59	0.0306	-	5555.56	0.0295	-	5762.71
FFC = 2/ days						
6-8	0.085	-	2000.00	1.646	-	103.28
9-11	0.0728	-	2335.16	0.0668	-	2544.91
12-23	0.908	-	187.22	0.085	-	2000.00
24-59	0.0612	-	2777.78	0.059	-	2881.36
FFC = 3/days						
6-8	0.1275	-	1333.33	2.469	-	68.85
9-11	0.1092	-	1556.78	0.1002	-	1696.61
12-23	1.362	-	124.82	0.1275	-	13333.33
24-59	0.0918		1851.85	0.0885	-	925.93
FFC = < 7 times/ week						
6-8	0.255	-	666.67	4.938	-	34.41
9-11	0.2184	-	778.39	0.2004	-	848.30
12-23	2.724	-	62.41	0.255	-	666.67
24-59	0.1836	-	925.91	0.177	-	960.45
FFC = 14 times/ week						
6-8	0.595	-	285.71	11.522	-	14.75
9-11	0.5096	-	333.59	0.4676	-	363.56

12-23	6.356	-	26.75	0.595	-	285.71
24-59	0.4284	-	396.83	0.413	-	411.62
FFC = 20 times/ week						
6-8	0.85	-	200.00	16.46	-	10.33
9-11	0.728	-	233.52	1.002	-	169.66
12-23	9.08	-	18.72	0.85	-	200.00
24-59	0.612		277.78	0.59	-	288.14
FFFC = 21 times / week						
6-8	0.8925	-	190.48	17.283	-	9.84
9-12	0.7644	-	222.39	0.7014	-	242.37
12-23	9.534	-	17.82	0.8925	-	190.48
24-59	0.6426	-	264.55	0.6195	-	274.41

Table 5.2b: DE of AFB1 (min) and Risk characterization based on MOE and HCC risk approach

AFB_2 (0.000006 min)						
	Female			Male		
Age	D.E	HCC	MOE	D.E	HCC	MOE
FFC = 1/day						
6-8	0.000019355	-	8783260.14	0.000017857	-	9520076.16
9-11	0.000016575	-	10256410.26	0.000015228	-	111634.59
12- 23	0.00002069	-	8216529.73	0.000019355	-	8783260.14
24-59	0.000013953	-	12183759.76	0.000013462	-	12628138.46
FFC = 2/ days						
6-8	0.00003871	-	4391630.07	0.00035714	-	4760038.08
9-11	0.00003315	-	5128205.13	0.000030456	-	558122.96
12-23	0.00004138	-	4108264.862	0.00003871	-	4391630.07
24-59	0.000027906		6091879.88	0.000026924	-	6314069232
FFC = 3/days						
6-8	0.0000058065	-	2927753.38	0.000053571	-	3173358.72
9-11	0.000049725	-	3418803.42	0.000045684	-	2927753.38
12-23	0.00006207	-	2738843.24	0.000058065	-	2927753.38
24-59	0.000041859	-	4061253.26	0.000040386	-	4209379.49
FFC = < 7 times/ week						
6-8	0.00011613	-	1463876.69	0.000107142	-	1586679.36
9-11	0.00009945	-	1709401.71	0.000091368		1860607.65
12-23	0.00012414	-	1369421.62	0.00011613	-	1463876.69
24-59	0.000083718	-	2030626.63	0.000080772	-	2104689.744
FFC = 14 times/ week						
6-8	0.00027097	-	627375.72	0.000249998	-	680005.44
9-11	0.00023205	-	732600.733	0.000213192	-	797403.28
12-23	0.00028966	-	586894.98	0.00027097	-	627375.72
24-59	0.000195342	-	870268.55	0.000188468	-	902009.89
FFC = 20 times/ week						
6-8	0.0003871	-	439163.01	0.00035714	-	476003.81

9-11	0.0003315	-	512820.51	0.00030456	-	558182.29
12-23	0.0004138	-	410826.49.	0.0003871	-	439163.01
24-59	0.00027906		609187.99	0.00026924	-	631406.92
FFFC = 21 times / week						
6-8	0.000406455	-	418250.48	0.000374997	-	194286.38
9-11	0.000348075	-	488400.49	0.000319788	-	531602.19
12-23	0.00043449	-	391263.32	0.000406455	-	418250.48
24-59	0.000293013	-	580179.04	0.000282702	-	601339.93

Table 5.3a: DE of AFG1 (max) and Risk characterization based on MOE and HCC risk approach

AFG_1 (0.00051 Max)						
	Female			Male		
Age	D.E	HCC	MOE	D.E	HCC	MOE
FFC = 1/day						
6-8	0.00164	-	103658.54	0.01275	-	13333.33
9-11	0.01408	-	12073.86	0.00129	-	131782.91
12- 23	0.001758	-	96700.79	0.00164	-	103658.54
24-59	0.001186	-	143338.95	0.001144	-	148601.39
FFC = 2/ days						
6-8	0.00328	-	51829.27	0.0255	-	6666.67
9-11	0.02816	-	6036.93	0.00258	-	65891.47
12-23	0.003516	-	48350.39	0.00328	-	51829.27
24-59	0.002372	-	71669.48	0.002288	-	74300.69
FFC = 3/days						
6-8	0.00492	-	34552.84	0.03825	-	4444.44
9-11	0.04224	-	4024.62	0.00387	-	43927.65
12-23	0.005274	-	32233.59	0.00492	-	34552.84
24-59	0.003558	-	47779.65	0.003432	-	20161.29
FFC < 7 times/ week						
6-8	0.00984	-	17276.42	0.0765	-	2222.22
9-11	0.08448	-	2012.31	0.00774	-	21963.82
12-23	0.010548	-	16116.79	0.00984	-	17276.42
24-59	0.007116	-	23889.83	0.006864	-	24766.89
FFC = 14 times/ week						
6-8	0.02296	-	7404.18	0.1785	-	952.38
9-11	0.19712	-	862.42	0.01806	-	9413.07
12-23	0.024612	-	6907.19	0.02296	-	7404.18
24-59	0.016604	-	10238.49	0.016016	-	10614.38
FFC = 20 times/ week						
6-8	0.328	-	518.29	0.255	-	666.67
9-11	0.2816	-	603.69	0.0258	-	6589.15
12-23	0.03516	-	4835.04	0.328	-	518.29
24-59	0.02372	-	7166.95	0.02288	-	7430.07
FFFC = 21 times / week						
6-8	0.03444	-	4936.12	0.26775	-	634.92

9-11	0.29568	-	574.95	0.02709	-	6275.37
12-23	0.036918	-	4604.79	0.03444	-	4936.12
24-59	0.024906	-	6825.66	0.024024	-	7076.26

Table 5.3b: DE of AFG1 (min) and Risk characterization based on MOE and HCC risk approach

AFG_1 (0.000005 min)						
	Female			Male		
Age	D.E	HCC	MOE	D.E	HCC	MOE
FFC = 1/day						
6-8	0.000016129	-	10540021.08	0.000014881	-	11423963.44
9-11	0.000013812	-	12308137.85	0.00001269	-	13396375.1
12- 23	0.000017241	-	9860216.93	0.000016129	-	10540021.08
24-59	0.000011628	-	14619883.04	0.000011218	-	15154216.44
FFC = 2/ days						
6-8	0.000032258	-	5270010.54	0.000029762	-	5711981.72
9-11	0.000027624	-	6154068.93	0.00002538	-	6698187.55
12-23	0.000034482	-	4930108.46	0.000032258	-	5270010.54
24-59	0.000023256	-	7309941.52	0.000022436	-	7577108.22
FFC = 3/days						
6-8	0.000048387	-	3513340.36	0.000044643	-	3807987.81
9-11	0.000041436	-	4102712.62	0.00003807	-	4405458.37
12-23	0.000051723	-	3286738.98	0.000048387	-	3513340.36
24-59	0.000034884		4873294.35	0.000033654	-	5051405.48
FFC = < 7 times/ week						
6-8	0.000096774	-	1756670.18	0.000089286	-	1904548.51
9-11	0.000082872	-	2051356.31	0.00007614	-	2232729.83
12-23	0.000103446	-	1643369.49	0.000096774	-	1756670.18
24-59	0.000069768	-	2508558.61	0.000067308	-	2525702.74
FFC = 14 times/ week						
6-8	0.000225806	-	752858.65	0.000208334	-	815997.39
9-11	0.000193368	-	879152.70	0.00017766	-	956885.94
12-23	0.000241374	-	704301.21	0.000225806	-	752858.65
24-59	0.00022792	-	745875.75	0.00015705	-	1082444.03
FFC = 20 times/ week						
6-8	0.00032258	-	527001.054	0.00029762	-	571198.17
9-11	0.00027624	-	615406.89	0.0002538	-	669818.75
12-23	0.00034482	-	4930010.85	0.0032258	-	527001.05
24-59	0.00023256	-	730994.15	0.00022436	-	757710.82
FFFC = 21 times / week						
6-8	0.000338709	-	501905.77	0.000812501	-	209230.51
9-11	0.00029002	-	586101.80	0.00026649	-	637922.62
12-23	0.000362061	-	469534.14	0.000388709	-	501905.77
24-59	0.0002441	-	696184.91	0.000235578	-	721629.35

Table 5.4a: DE of AFG2 (max) and Risk characterization based on MOE and HCC risk approach

AFG_2 (0.00004 Max)						
	Female			Male		
Age	D.E	HCC	MOE	D.E	HCC	MOE
FFC = 1/day						
6-8	0.00012903	-	1317523.06	0.00011904	-	1428091.39
9-11	0.00011049	-	1538600.79	0.00010152	-	1674546.89
12- 23	0.00013793	-	1232509.24	0.00012903	-	1317523.06
24-59	0.00009302	-	1827563.97	0.00008974	-	1894287.61
FFC = 2/ days						
6-8	0.00025806	-	658761.53	0.00022381	-	759579.64
9-11	0.00022098	-	76930.04	0.00020304	-	837273.44
12-23	0.00027586	-	616254.62	0.00025806	-	658761.53
24-59	0.00018604		913781.98	0.00017887	-	950410.91
FFC = 3/days						
6-8	0.00038709	-	439174.35	0.00035712	-	476030.47
9-11	0.00033147	-	512866.926	0.00030456	-	558182.29
12-23	0.00041379	-	410836.41	0.00038709	-	439174.35
24-59	0.00027906	-	609187.99	0.00026923	-	631428.03
FFC = < 7 times/ week						
6-8	0.00077419	-	219586.61	0.00071424	-	238015.23
9-11	0.00066294	-	256433.46	0.00060912	-	279091.15
12-23	0.00082758	-	205418.21	0.00077419	-	219586.61
24-59	0.00055812	-	304593.99	0.00053846	-	315714.60
FFC = 14 times/ week						
6-8	0.00180642	-	94108.78	0.00166656	-	102006.52
9-11	0.0014686	-	115756.50	0.00142128	-	119610.49
12-23	0.00193102	-	88036.37	0.00180642	-	94108.78
24-59	0.00130228	-	130540.28	0.00125641	-	135306.26
FFC = 20 times/ week						
6-8	0.0025806	-	65876.15	0.0023808	-	71404.56
9-11	0.002098	-	81029.55	0.0020304	-	83727.34
12-23	0.0027586	-	61625.46	0.0025806	-	65876.15
24-59	0.0018604	-	91378.19	0.00179487	-	94714.38
FFFC = 21 times / week						
6-8	0.00270963	-	62739.19	0.00249984	-	68004.35
9-11	0.00232029	-	73266.70	0.00213192	-	79740.33
12-23	0.00289653	-	58690.92	0.00270963	-	62739.19
24-59	0.00195342	-	87026.85	0.001884614	-	90204.15

Table 5.4b: DE of AFG2 (min) and Risk characterization based on MOE and HCC risk approach

AFG_2 (0.000006 min)						
	Female			Male		
Age	D.E	HCC	MOE	D.E	HCC	MOE
FFC = 1/day						

6-8	0.000019355	-	8783260.14	0.000017857	-	9520076.16
9-11	0.000016575	-	10256410.26	0.000015228	-	111634.59
12- 23	0.00002069	-	8216529.73	0.000019355	-	8783260.14
24-59	0.000013953	-	12183759.76	0.000013462	-	12628138.46
FFC = 2/ days						
6-8	0.00003871	-	4391630.07	0.00035714	-	4760038.08
9-11	0.00003315	-	5128205.13	0.000030456	-	558122.96
12-23	0.00004138	-	4108264.862	0.00003871	-	4391630.07
24-59	0.000027906		6091879.88	0.000026924	-	6314069232
FFC = 3/days						
6-8	0.0000058065	-	2927753.38	0.000053571	-	3173358.72
9-11	0.000049725	-	3418803.42	0.000045684	-	2927753.38
12-23	0.00006207	-	2738843.24	0.000058065	-	2927753.38
24-59	0.000041859	-	4061253.26	0.000040386	-	4209379.49
FFC = < 7 times/ week						
6-8	0.00011613	-	1463876.69	0.000107142	-	1586679.36
9-11	0.00009945	-	1709401.71	0.000091368		1860607.65
12-23	0.00012414	-	1369421.62	0.00011613	-	1463876.69
24-59	0.000083718	-	2030626.63	0.000080772	-	2104689.744
FFC = 14 times/ week						
6-8	0.00027097	-	627375.72	0.000249998	-	680005.44
9-11	0.00023205	-	732600.733	0.000213192	-	797403.28
12-23	0.00028966	-	586894.98	0.00027097	-	627375.72
24-59	0.000195342	-	870268.55	0.000188468	-	902009.89
FFC = 20 times/ week						
6-8	0.0003871	-	439163.01	0.00035714	-	476003.81
9-11	0.0003315	-	512820.51	0.00030456	-	558182.29
12-23	0.0004138	-	410826.49.	0.0003871	-	439163.01
24-59	0.00027906		609187.99	0.00026924	-	631406.92
FFFC = 21 times / week						
6-8	0.000406455	-	418250.48	0.000374997	-	194286.38
9-11	0.000348075	-	488400.49	0.000319788	-	531602.19
12-23	0.00043449	-	391263.32	0.000406455	-	418250.48
24-59	0.000293013	-	580179.04	0.000282702	-	601339.93

Table 5.5a: DE of AF (max) and Risk characterization based on MOE and HCC risk approach

AF (0.04867 Max)						
	Female			Male		
Age	D.E	HCC	MOE	D.E	HCC	MOE
FFC = 1/day						
6-8	0.157	0.0038465	1082.80	0.1448	0.0035476	1174.03
9-11	0.13447	0.0032945	1264.22	0.12352	0.00302624	1376.29
12- 23	0.16782	0.0041116	1012.99	0.157	0.0038465	1082.80
24-59	0.113186	0.0027731	1501.95	0.109195	0.00267527	1556.85
FFC = 2/ days						

6-8	0.314	0.007693	541.40	0.2896	0.0070952	587.02
9-11	0.26894	0.0065890	587.42	0.24704	0.00605248	688.15
12-23	0.33564	0.00822318	506.49	0.314	0.007693	541.40
24-59	0.226372	0.005546	750.98	0.21839	0.00535055	778.42
FFC = 3/days						
6-8	0.471	0.0115395	360.93	0.4344	0.0106428	391.34
9-11	0.40341	0.0098835	421.41	0.37056	0.00907872	458.77
12-23	0.50346	0.01233477	337.66	0.471	0.0115395	360.93
24-59	0.339558	0.008319171	500.65	0.327585	0.00802583	518.95
FFC = < 7 times/ week						
6-8	0.942	0.023079	180.47	0.8688	0.0212856	195.67
9-11	0.80682	0.01976709	210.70	0.74112	0.01815744	229.38
12-23	1.00692	0.02466954	168.83	0.942	0.023078	180.47
24-59	0.679116	0.0166383	250.33	0.65517	0.01605167	250.33
FFC = 14 times/ week						
6-8	2.198	0.053851	77.34	2.0272	0.0496664	83.86
9-11	1.88258	0.04612321	90.30	1.72928	0.04236736	98.31
12-23	2.34948	0.05756226	72.36	2.198	0.053851	77.34
24-59	1.584604	0.0388228	107.28	1.52873	0.037453885	111.20
FFC = 20 times/ week						
6-8	3.14	0.07693	54.14	2.896	0.070952	58.70
9-11	2.6894	0.0658903	63.21	2.4704	0.0605248	68.81
12-23	3.3564	0.0822318	50.67	3.14	0.07693	54.14
24-59	2.26372	0.05546144	75.09	2.1839	0.05350555	77.84
FFFC = 21 times / week						
6-8	3.297	0.0807765	51.56	3.0408	0.0744996	55.91
9-11	2.82387	0.069184815	60.20	2.59392	0.06355104	65.54
12-23	3.52422	0.08634339	48.24	3.297	0.0807765	51.56
24-59	2.376906	0.058234197	71.42	2.293095	0.056180828	74.14

Table 5.5b: DE of AFB1 (min) and Risk characterization based on MOE and HCC risk approach

AF (0.000014 min)						
	Female			Male		
Age	D.E	HCC	MOE	D.E	HCC	MOE
FFC = 1/day						
6-8	0.000045161	0.000001106	3764309.91	0.000041667	0.000001021	4079967.36
9-11	0.000038674	0.000000948	4395718.05	0.000035533	0.000000871	4784285.08
12- 23	0.000048276	0.000001183	3521418.51	0.000045161	0.000001106	3764309.91
24-59	0.000032558	0.000000798	5221450.95	0.00003141	0.00000077	5412289.08
FFC = 2/ days						
6-8	0.000090322	0.000002213	1882154.96	0.000083334	0.000002042	2039983.89
9-11	0.000077348	0.000001895	2197859.03	0.000071066	0.000001741	2392142.52
12-23	0.000096556	0.000002366	1760709.26	0.000090322	0.000002213	1882154.96
24-59	0.000065116	0.00000159	2610725.48	0.00006282	0.000001539	2706144.54
FFC = 3/days						

6-8	0.000135483	0.000003319	1254769.97	0.000125001	0.000003063	1359989.12
9-11	0.000116022	0.000002843	1465239.35	0.000106599	0.000002612	1594761.68
12-23	0.000144828	0.000003548	1173806.17	0.000135483	0.000003319	125476.97
24-59	0.000097674	0.000002393	1740483.65	0.00009423	0.000002309	1804096.36
FFC < 7 times/ week						
6-8	0.000270966	0.000006639	627384.99	0.000250002	0.000006125	679994.56
9-11	0.000232044	0.000005685	732619.68	0.000213198	0.000005223	797380.84
12-23	0.000289656	0.000007097	586908.09	0.000270966	0.000006639	627384.99
24-59	0.000195348	0.000004786	870241.82	0.00018846	0.000004617	902048.18
FFC = 14 times/ week						
6-8	0.000632254	0.00001549	268879.28	0.000583338	0.000014414	203998.37
9-11	0.000541436	0.000013265	313979.86	0.000497462	0.000012186	239214.25
12-23	0.00675864	0.000165587	25152.99	0.000632254	0.00001549	268879.28
24-59	0.000455812	0.000011167	372960.78	0.00043974	0.000010774	270614.45
FFC = 20 times/ week						
6-8	0.00090322	0.000038387	188215.49	0.00083334	0.000020417	203998.37
9-11	0.00077348	0.00001895	219785.90	0.00071066	0.000017411	239214.25
12-23	0.00096552	0.000023655	176070.93	0.00090322	0.000038387	188215.49
24-59	0.00065116	0.000015953	261072.55	0.0006282	0.000015953	270614.45
FFC= 21 times / week						
6-8	0.000948381	0.000023235	179252.85	0.000875007	0.000021438	194284.16
9-11	0.000812154	0.000019898	209319.91	0.000746193	0.000018282	227823.09
12-23	0.001013796	0.000024838	167686.59	0.000948381	0.000023235	179252.85
24-59	0.000683718	0.000016836	248640.52	0.00065961	0.00001616	257728.05