THE RISK OF EXPOSURE TO AFLATOXINS THROUGH CONSUMPTION OF NSHIMA MADE FROM MAIZE MEAL IN SELECTED AREAS OF 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 Master of Science in Food Safety and Risk Analysis

The University of Zambia

School of Veterinary Medicine

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DECLARATION

I, **MUMBA MUSONDA,** do hereby declare that the contents of the dissertation being submitted herein are my original work and 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 MUMBA MUSONDA is approved as fulfilling the requirements for the award of Master of Science in Food Safety and Risk Analysis of the University of Zambia.

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	Signature	Date

DEDICATION

I dedicate this work to my late mother and my father, my siblings, my family (husband and children), and friends who believed in me and endlessly supported me throughout this master's journey.

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LIST OF ACRONYMS AND ABBREVIATIONS

AF	Aflatoxin
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M2
BMDL	Benchmark Dose Limit
BW	Body Weight
CSO	Central Statistics Office
DAD	Diode Array Detector
DE	Dietary Exposure
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EFSA	European Food Safety Authority
ERES	Excellence in Research Ethics and Science Converge
FAO	Food and Agriculture Organization
FPIA	Fluorescence Polarization Polarization Immuno Assays
FD	Fluorescence Detector
GAP	Good Agricultural Practices
GMP	Good Manufacturing Practices
HCC	Hepatocellular Carcinoma
HIV	Human Immunodeficiency Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IITA	International Institute of Tropical Agriculture
JECFA	Joint Expert Food Additives
L.C.	Liquid Chromatography

LFD	Lateral-Flow Devices
MOE	Margin of Exposure
MS	Mass Spectrometry
SADC	Southern Africa Development Committee
TFA	Trifluoroacetic Acid
TLC	Thin-Layer Chromatography
USAID	United States Agency for International Development
WHO	World Health Organization

ABSTRACT

Maize and its products, such as maize meal, are susceptible to aflatoxin contamination. Maize is the staple crop of Zambia and is widely consumed as thick porridge, commonly called *nshima*. Previous studies have reported high levels of AFs in maize produced in Zambia. However, no study has determined the risk of exposure to AFs through the consumption of maize nshima. This cross-sectional study was designed to determine the risk of exposure to aflatoxins through the consumption of maize nshima in households in the Lusaka District. A questionnaire was administered to households to investigate dietary consumption patterns and revealed that most Zambians consumed nshima twice a day, prepared from either breakfast or roller maize meal. It was also noted that males aged 18 years and above consumed more nshima per day (360 – 980g) than children and female adults. The Zambia Bureau of Standards Laboratory recorded aflatoxin concentrations ranging from 0.2 µg/kg to 150 µg/kg in breakfast (flour from polished maize grain) and roller (flour from whole maize grain) meal samples from 2019-2022. This was used as secondary data for determining the risk of exposure to aflatoxins during this study. The risk of exposure was estimated using the Model Risk Software® using the estimated daily intake of maize nshima and secondary aflatoxin data as input parameters. The maximum exposure of 80.2 to aflatoxins was highest for the males compared to other age groups. The risk was further characterized using the margin of exposure (MOE). The MOE was < 10000 for age categories, thus indicating potential adverse health effects, with the highest MOE of 0.005 recorded for the males. The high levels of AFs in maize meal and high exposures accentuate the need for preventive measures. Particular attention should be given to raising awareness of the impact of aflatoxin risk exposure and setting maximum allowable limits in maize meal considering maize product consumption patterns in Zambia.

CHAPTER ONE

INTRODUCTION

1.1.Background

Maize is the national staple crop in Zambia, attributed to more than 65% of farmed crops; on average, 105 kilograms of maize are consumed mainly as nshima prepared from breakfast or roller meal (Manda et al. 2018). The Zambian Standard for Maize Meal states that maize breakfast meal is the product obtained from cleaned milling grades of maize at a maximum of 70% extraction rate, while maize roller meal is obtained from cleaned milling grades of maize at a maximum of 85% extraction rate (ZS 189: 2014). Maize and maize products are susceptible to contamination by toxigenic fungi, especially during cultivation and storage, mainly resulting from *Fusarium* and *Aspergillus* species. These fungi produce mycotoxins, fumonisins and aflatoxins that contaminate the maize (Okoth, 2016; Kortei et al., 2021). Aflatoxins are contaminants of various crops and are produced as secondary metabolites of the *Aspergillus flavus* and *Aspergillus parasiticus* (Wild and Gong, 2010). These aflatoxins are highly prevalent in hot and humid regions, including sub-Saharan Africa and Southeast Asia, where conditions favour fungal growth (Gong et al. 2016). Aflatoxin poisoning may result from ingesting aflatoxins and exposure through dermal (skin) and inhalation routes. There are two forms of aflatoxicosis: acute severe intoxication leading to liver damage and subsequent illness or death and chronic sub-symptomatic exposure (Okoth, 2016). When high-level exposure to aflatoxin occurs within a relatively short period is referred to as acute aflatoxicosis. The United States Food and Drug Administration (FDA) defined high aflatoxin levels in food or feed as 20 to 300 ppb (Gong et al. 2016). Besides acute aflatoxicosis occurring on a case-by-case basis, there have been reports of large outbreaks in some parts of Africa, such as in Eastern Kenya in 2004. A total of 317 individuals were diagnosed with acute liver failure, and 37% of the patients subsequently died due to acute aflatoxicosis (Wild and Gong, 2010). A case-control study was further carried out and revealed that the source of the outbreak in Kenya might have been aflatoxin-contaminated home-grown maize (Wild and Gong 2010). Breast milk is also reported to be a potential source of aflatoxin exposure for very young infants (Wild and Gong 2010). Aflatoxin M1, the hydroxylated metabolite of Aflatoxin B1, was detected in breast milk 12 to 24 hours after the lactating mothers consumed foods contaminated with Aflatoxin B1 (Gong et al. 2016).

Aflatoxins are prevalent in Zambia's main food crops, including maize and groundnuts. High aflatoxin levels were detected using lateral-flow immuno-chromatography in about 17% of the groundnuts and maize crops from the markets in 27 districts in Zambia. The aflatoxin concentrations of these crops were above the allowable level of 10 μg kg−1 set by the Zambia Bureau of Standards (Kachapulula et al. 2017).

Food processing methods do not eliminate aflatoxins in harvested crops as these aflatoxins are heat stable. A study indicated that only 23% of the aflatoxin levels had been reduced by the home preparation of maize porridge (Okoth, 2016). However, this aflatoxin reduction may not bring the contaminants down to acceptable levels (Okoth, 2016). Similarly, the canning of contaminated food only reduced aflatoxin levels by 15% (Scudamore, 2009). A study by the Bureau of Social Welfare and Public Health in 2018 in Tokyo showed that even after boiling the food, there was still about 50 to 80% of mycotoxins left in the food, while 10 to 15% remained in the water used for boiling (Bureau of Social Welfare and Public Health, 2018).

Due to the public health concerns that these aflatoxins raise and their association with genotoxic effects, intensive studies have been conducted since their discovery to elucidate the mechanisms of their carcinogenicity and other toxicities. However, few or no studies determined the risk of exposure to aflatoxins through consuming maize and maize products such as nshima.

1.2. Problem statement and study justification

Maize is the primary source of daily calories in central, southern, and eastern Zambia. It is the leading staple food consumed mainly in the form of maize nshima by 52% of the local population, including both children and adults (Manda et al. 2018). In the Lusaka District of Zambia, mealie meal is a staple diet enjoyed with varieties of relish. It is popularly consumed as nshima, prepared from breakfast or roller maize meal flour.

Many Zambians are chronically exposed to uncontrolled amounts of aflatoxin in their diet by consuming maize and maize products (Kachapula et al. 2017). Mostly, exposure is low and does not cause acute illness, but may pose adverse health effects related to chronic low-dose exposure. Nevertheless, animals and humans have reported large-scale acute illness and death due to aflatoxicosis (Awuor et al., 2017; Kamala et al., 2018). Accounts of large-scale aflatoxicosis in

humans are explained mainly by ingesting infected maize and maize products (Kachapula et al. 2017).

Besides aflatoxin contamination of maize in Zambia being a significant threat to public health and economic burden, risk assessments of exposure to aflatoxin through consumption of maize and maize products such as nshima have not been determined considering that most families depend on maize nshima as their source of calories. This study will therefore determine the risk of exposure to aflatoxins and health risks associated with aflatoxins through the consumption of maize nshima in households of Lusaka. Furthermore, the data collected from the maize nshima consumption patterns can evaluate the intake and exposure of the general population to aflatoxins and establish policies in agriculture, food production, trade and health related to aflatoxins in Zambia.

1.3. Research question

Is the population in the Lusaka district at risk of adverse health effects from dietary exposure to aflatoxins through the consumption of maize meal nshima?

1.4. Objectives of the study

1.4.1 General objective

This research aimed to assess the risk of exposure to aflatoxin through the consumption of nshima made from maize meal flour in selected households in the Lusaka District.

1.4.2 Specific objectives

i. To determine the consumption patterns of maize nshima by both children and adults in selected households of the Lusaka district; and,
ii. To estimate the risk of exposure to aflatoxins through the consumption of maize nshima in the study population.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background and Definition of Aflatoxins

Aflatoxin is a naturally occurring toxin produced by *Aspergillus* species commonly found in grain storage and soil. *Aspergillus* have an optimum growth temperature of 25 °C and a minimum water activity of 0.75. These fungi produce secondary metabolites at temperatures within 10-12 ºC, but the most toxic ones are produced at 25°C and have a water activity of 0.95 (Gimeno and Martins, 2003). The fungus is identified by its grey-green or yellow-green appearance growing on corn kernels (as shown in Figure 2.1) in the field or storage (Gimeno and Martins, 2003). Relative humidity and temperature are the most important factors attributing to aflatoxin production (Cotty and Garcia, 2008). The current climate change trend in Zambia causes an increase in drought and temperature (UNDP, 2010). This changing climate provides an optimum water activity and temperature favourable for the growth of *Aspergillus flavus* (Medina et al. 2014). Among the many *Aspergillus species*, *Aspergillus flavus* and *Aspergillus parasiticus* are the leading producers of Aflatoxins (Wu et al., 2013). The presence of *Aspergillus flavus* does not always indicate harmful levels of aflatoxins, but it means there is potential for aflatoxin production (USDA, 2012). There are about 20 types of aflatoxins reported. However, the naturally occurring and well-known ones are aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 (Wu et al., 2013). Aflatoxin B1 is the most prevalent and toxic aflatoxin responsible for acute and chronic toxicity, carcinogenicity, teratogenicity, genotoxicity and immunotoxicity (Wild and Gong 2016).

Figure 2.1 Fungal contamination of maize (*Source of Image: https://www.monitor.co.ug/uganda/magazines/healthy-living/the-real-threat-of-aflatoxins-in-your-food-3331202)

2.2 Epidemiology

Mycotoxigenic moulds are widespread worldwide; hence mycotoxins, including aflatoxin, contaminate various crops and foods, especially those grown in tropical regions. Although aflatoxin occurrence is widespread and affects many food crops, certain crops are reportedly more susceptible to aflatoxins than others (Reddy et al., 2010). The susceptibility of a crop to fungus invasion and toxin production is determined by a combination of environmental and crop intrinsic factors, including nutritional content, moisture content, and pH (Smith et al., 2016). To a large extent, the contamination of crops will also determine the levels of human exposure to aflatoxin (Smith et al., 2016).

2.3 Prevalence of aflatoxins in maize

A study was carried out to assess levels of contamination of aflatoxins in maize produced, stored and consumed in rural households in Malawi (Mwalwayo and Thole 2016). A total of 9 districts were selected, including three from each of Malawi's Northern, Central and Southern regions. Households

were randomly sampled in each district, and ten maize samples were also collected for laboratory analysis. The detection limit for aflatoxins was 2 μg/kg and a quantitation range of 2–150 μg/kg. It was reported that samples in the Southern region were the most contaminated, especially those in the Chikhwawa district. At the same time, the Northern region recorded the least contaminated maize. The maximum detected aflatoxins level was 140 μg/kg. About 20% of maize samples were above Malawi's maximum tolerable limit for aflatoxins (Mwalwayo and Thole 2016). Aflatoxin contamination in maize was also reported in Ghana, leading to substantial health and economic burden on the population (Agbetiameh et al., 2018) . A total of 326 maize samples were tested to determine the distribution and aflatoxin-producing potential of Aspergillus species associated with maize, and more than 15% had detectable levels of aflatoxins exceeding the aflatoxin threshold limits set by the Ghana Standards Authority of 15 and 20 ppb, respectively (Agbetiameh et al. 2018). A related study was conducted at the farm level and from domestic markets revealed that 11% of the maize samples were contaminated with aflatoxin levels ranging from 12.7 ppb to 123.5 ppb, which was above the 10 ppb acceptable limit for East African countries (Ankwasa et al. 2021). About 74% of the samples taken from households were found to be contaminated with aflatoxins, with the highest recorded levels of 268 ppb and average contamination levels of 22.2 ppb (Ankwasa et al., 2021).

2.4 Impact of aflatoxins on human health

Humans are mainly exposed to aflatoxins by consuming aflatoxin-contaminated foods or ingesting foods produced by animals previously exposed to aflatoxins (Leong et al., 2012). This chronic dietary exposure to aflatoxins poses adverse health problems in humans and animals (Williams et al., 2004).

Figure 2.2 below shows some of the health risks associated with aflatoxin exposure in humans.

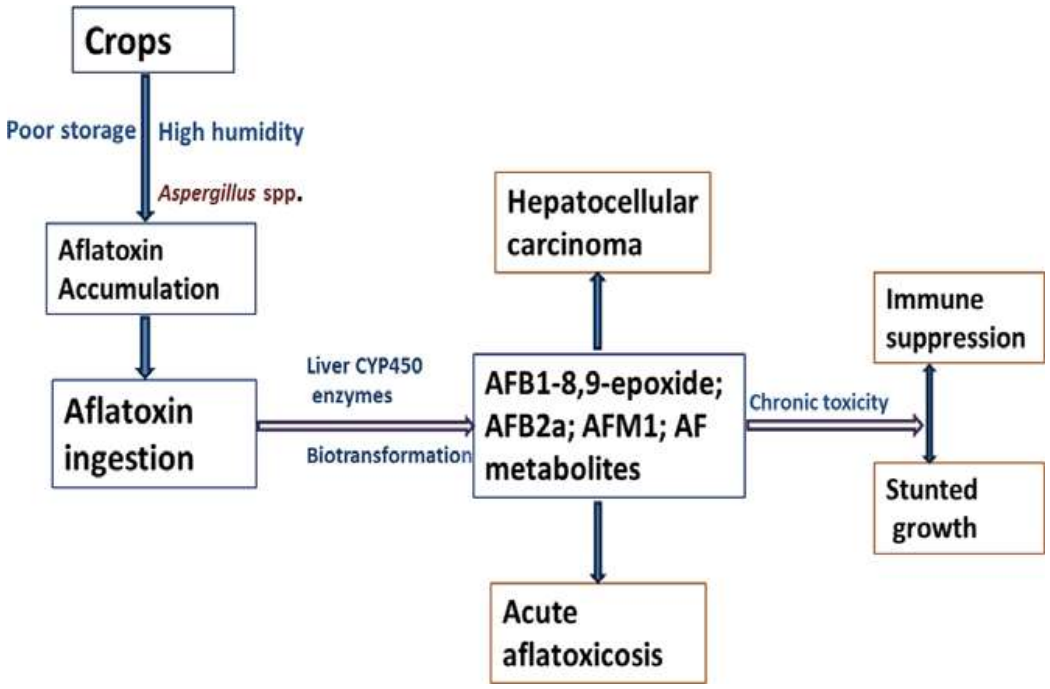

Figure 2.2 The effects of aflatoxins on human health (*Source of Image: https://www.researchgate.net/figure/The-effects-of-aflatoxin-on-human-health_fig4_319252568).

The International Agency for Research on Cancer (IARC) has classified Aflatoxin B1 as a group 1 carcinogen due to its genotoxic effect on humans and animals (IARC, 2017). Aflatoxin B1, as a potent carcinogen, may affect organs like the liver and kidneys (Alvarez et al., 2020; Li et al., 2018). Long-term exposure to aflatoxin has also been reported to be associated with congenital disabilities and stunting in children (Smith et al., 2015). Additionally, acute aflatoxin exposure can be life-threatening and cause aflatoxicosis (Williams et al., 2004). Recent reports of multiple acute aflatoxin exposure outbreaks, particularly from regions with tropical climates, such as Kenya and Tanzania (Awuor et al., 2017; Kamala et al., 2018).

A few epidemiological studies have indicated the relationship between Aflatoxin M1 in breast milk samples and impaired child growth. In Egypt, for instance, a cross-sectional study involving 46 children aged between 1 month to 4.5 years and 46 mothers revealed that 36.96% of the children's, and also 36.96% of mothers' serum samples had aflatoxin-albumin detected in them, that is, 51.61 (30.57−62.80) ppm in children and 50.0 (35.59−84.93) ppm in mothers (Shouman et al. 2012). Similarly, a cross-sectional study in Kenya involving 199 children aged 6 to 17 years with no record of the prevalence of malnutrition. Children with higher aflatoxin-albumin levels (>198.5 pg/mg)

were observed to be shorter in height compared to children with lower levels (<74.5 pg/mg) after adjusting for age, sex, school, disease state and infection status ($P < 0.001$) (Castelino et al., 2015). Children of weaning age in most developing countries are considered a high-risk population group for aflatoxin exposure, especially in sub-Saharan Africa (Gong et al. 2016). A 2013 and 2014 Zambian demographic and health survey revealed that 40% of the children under five were stunted, 6% were wasted, and 15% were underweight (Central Statistical Office,2014). Similarly, the 2018 survey reported that 35% of children under age five were stunted (short for their age), 4% were wasted (thin for their height), 12% were underweight (thin for their age), and 5% were overweight (heavy for their height) (Zambia Demographic and Health Survey, 2018).

Other studies have reported that aflatoxins increase the rate of progression from HIV infection to AIDS (Jolly et al., 2013; Jolly et al., 2022). The heavy dietary reliance on maize in Africa puts the population at high risk of exposure to aflatoxins (Jiang et al., 2008; Jolly et al., 2022). There was a consistently strong association between high aflatoxin-albumin levels and high HIV viral loads determined in cross-sectional studies carried out in Ghana; it was evident that aflatoxin exposure may contribute to high viral loads and faster progression to AIDS (Jiang et al., 2008; Jolly et al., 2022). A prospective study was conducted among HIV-positive asymptomatic Ghanaians to examine the association of aflatoxin-albumin levels in blood changes in CD4 cell count and uptake of ART over five years (Jolly et al., 2022). Aflatoxin-albumin levels in the study participants ranged from 0.20–109.87 pg/mg, and this indicated an immunological effect that contributed to a decrease in CD4 and that the effect of aflatoxin occurred early in HIV infection, and this remained consistent over time besides the initiation of ART (Jolly et al., 2022).

2.5 Economic impacts of aflatoxins

The contamination of agricultural products by aflatoxins goes beyond public health issues as it equally affects trade and economic ramifications for both developed and developing countries (Wu, 2015). Aflatoxin contamination can cause crop loss due to low yields, reduce the market value as the uses for a contaminated crop decrease, and aflatoxin-contaminated feed may also lead to stunted growth in farm animals and as well as lower yields of by-products such as milk and eggs (Strokes et al. 2017). Healthcare costs from aflatoxin exposure become a burden for the smallholder farmer families that are most likely to consume contaminated crops (Strokes et al. 2017). Other potential implications of aflatoxins are deterioration of food and nutritional value of agricultural products, accompanied by a reduction in sensory characteristics, e.g., taste, odour, texture and colour, etc.

There might also be a loss of income from livestock resulting from contaminated feedstuffs, e.g., higher mortality rates and lower feed-to-weight conversion ratios for chickens, ducks, egg-layers, and pigs. The loss of the export market and other related economic losses due to regulations restricting international trade of aflatoxin-contaminated grain may also occur (Lubulwa and Davis, 1994).

2.6 Diagnostic methods for aflatoxins

Aflatoxins can be detected using different analytical methods categorized into two groups: classical analytical technologies and emerging technologies for aflatoxin analyses.

2.6.1. Classical analytical technologies for aflatoxin analysis

This group encompasses different diagnostic methods for aflatoxins as follows.

2.6.1.1 Thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) is a simple and cost-effective aflatoxin analytical method commonly used in developing countries for screening purposes, multi-mycotoxin analysis, and when low detection limits are not required (Pascale and Visconti, 2008). However, TLC cannot be used for sensitive or precise measurements unless densitometric analyses are performed (Pascale and Visconti, 2008)

2.6.1.2 High-performance liquid chromatography (HPLC)

HPLC is a highly sensitive, selective, accurate and repeatable technique most widely used for identifying the significant mycotoxins in food commodities (Giniani et al., 2011; Pascale and Visconti, 2008). HPLC has been adopted as an official or standard method by the International Association of Official Analytical Chemists or the European Standardization Committee. It can be used to identify aflatoxins B1, B2, G1 and G2 in both maize, raw peanuts and peanut butter (Pascale and Visconti, 2008; Fu et al., 2008). Figure 2.3 below shows the High-Performance Liquid Chromatography Method.

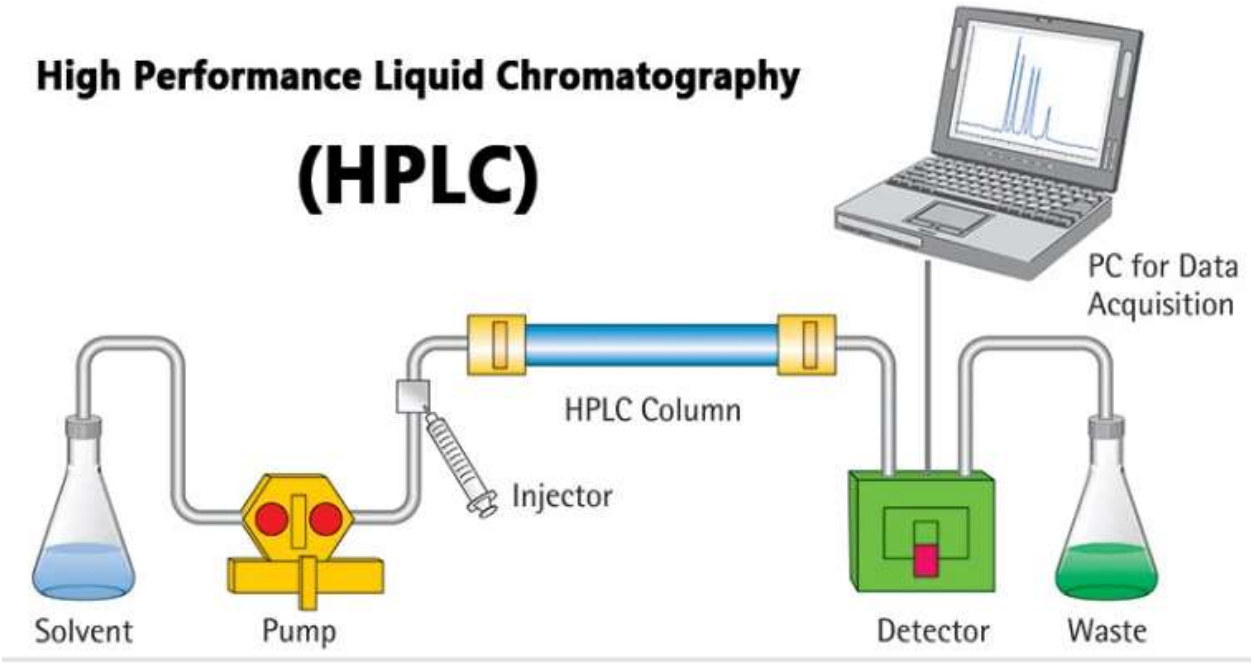

Figure 2.3 High Performance Liquid Chromatography Method (*Source of Image: https://www.sartorius.com/en/pr/cannabis/ultrapure-water-for-hplc-analysis)

2.6.1.3 Enzyme-linked immunosorbent assays (ELISAs)

The ELISAs are fast and inexpensive screening assays mainly used for qualitative, semi-quantitative and quantitative analysis of aflatoxins and other mycotoxins in several food matrices (Li et al., 2009). However, they are inaccurate, have very low concentrations and are limited in the range of matrices examined (Li et al., 2009).

2.6.2 Emerging technologies for aflatoxins analyses

This group includes a variety of emerging methods based on novel technologies that have been proposed for AFs analyses

2.6.2.1 Lateral flow devices (LFDs)

LFDs are user-friendly format, cost-effective and provide a rapid response, often used to determine AFB1 in maize and peanuts and AFM1 in milk and other mycotoxins (Tang et al., 2009; Van der Spiegel et al., 2013).

2.6.2.2 Capillary electrophoresis

Capillary electrophoresis is comparable in sensitivity, precision and accuracy to HPLC methods that allow good separation of mycotoxins from potential interfering species present in the extract based on electrical charge (Pascale and Visconti, 2008; Yiyang,1999).

2.6.2.4 Biosensors

A biosensor is an accurate and precise analytical device incorporating a specific biological element, e.g., an antibody that detects aflatoxins at deficient levels(Van der Gaag et al., 2003). Immunochemical biosensors that use surface plasmon resonance, quartz crystal microbalance and screen-printed carbon electrodes have been described for detecting mycotoxins (Van der Gaag et al., 2003).

2.7 Control and prevention to minimize exposure to aflatoxins

There are several mitigation and control measures employed to prevent or minimize exposure of humans and animals to aflatoxin, such as regulation enactment, implementation of quality control of agricultural products during pre- and postharvest, contaminated products can be degraded, or decontaminated to reduce the aflatoxins to acceptable levels, (FAO, 2004). Postharvest handling practices coupled with Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) have proven to be effective in minimizing aflatoxin contamination of agricultural products (Hell & Mutegi, 2011). The temperature and humidity in silos and other storage buildings are critical when preventing the increased risk of aflatoxin contamination of harvested crops and should therefore be controlled (Magan and Aldred, 2007) . Furthermore, insect activities in warehouses must be prevented as they tend to increase the temperature and level of humidity in the production, which could promote fungal growth and subsequently lead to the production of mycotoxins. Using silo bags to help guard against the infiltration of insects and moisture is a short-term control measure. However, for long-term storage, impermeability may result in fungal growth (Magan and Aldred, 2007).

Scientific advances allow biological, chemical, and physical measures to prevent and decontaminate contaminated agricultural products (Lizárraga-Paulín et al., 2013). Biological strategies that are environmentally friendly and natural are used instead of traditional chemical pesticides. These strategies include beneficial insects and plant extracts (Reddy et al., 2009). Adding non-toxigenic strains of *Aspergillus flavus* on crops to create competition with toxic ones can significantly reduce or eliminate the toxigenic aflatoxin-producing strains (Probst et al., 2010).

2.8 Aflatoxin studies in Zambia

Different studies have indicated the prevalence of aflatoxins in maize and maize products in Zambia. A survey carried out in the Lusaka Province of Zambia showed levels of aflatoxin and fumonisins

present in maize and various maize products (Mukanga et al., 2019). Sixty-six maize samples comprising grain, samp, maize flour, and popcorn were sampled from farms, markets, street vendors and hammer mills. A quantitative enzyme-linked immunosorbent assay for aflatoxin and fumonisins recorded positive results for all the maize products, of which 54.6% were above the Zambian regulatory standard of 10 μg/kg for total aflatoxin. There was a coexistence of aflatoxin and fumonisins found in all the tested maize products (Mukanga et al., 2019). However, no further studies were conducted to assess the extent to which the urban and peri-urban populations in Lusaka province were exposed to high levels of both aflatoxin and fumonisins and the health risks associated with exposure to these mycotoxins.

A related study in Monze and Chipata districts of Zambia was conducted on the nutritional and aflatoxin contents of complementary foods, i.e. maize nshima and maize porridge consumed by infants and young children. (Alamu et al., 2018). Data on breastfeeding and complementary feeding practices were collected twice at 3- month intervals using a structured questionnaire for 400mother–child pairs. The maize porridge samples from Chipata recorded the highest mean aflatoxin content of 5.8 ± 15.93 mg/100 g, while maize nshima was the most contaminated complementary food from Monze districts with a mean aflatoxin level of 3.8 ± 6.41 mg/100g. The study also indicated significant ($p < .05$) positive correlations between fat and aflatoxin contents for Chipata samples ($r = .12409$) and for Monze samples ($r = .13666$). It was observed that the traditional, complementary foods studied were low in fat and protein and high in aflatoxin contamination. This calls for implementing best practices and interventions to reduce the possible adverse health implications of the consumption of such complementary foods by children under five years (Alamu et al., 2018). This study established that maize porridge and maize nshima were prone to aflatoxin contamination but did not determine the extent to which children risked being exposed to the high doses of aflatoxins found in the complementary foods, i.e. maize porridge and maize nshima or the health risks associated with the aflatoxins.

The United States Agency for International Development (USAID) Feed the Future aflatoxin mitigation project, the International Institute of Tropical Agriculture (IITA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), in partnership with other stakeholders, also carried out research on the prevalence of aflatoxins in maize and groundnuts in Eastern Province, Zambia (Ismail, 2013). The preliminary aflatoxin results ranged from 0–11ppb in

the maize at harvest, and 99% of maize samples had aflatoxin levels below the acceptable international standard of 4ppb. However, maize stored for up to three months recorded very high aflatoxin levels, depending on the types, storage conditions and facilities (Ismail, 2013). Nevertheless, this low-dose exposure was chronic because most Zambians consume maize(121–140g maize/per person/day (Ismail, 2013). This study by IITA and ICRISAT was based on aflatoxin concentrations in maize and groundnuts at harvest and postharvest, bordering more on the effects of storages conditions and facilities on the increase of aflatoxin levels in the crops. An exposure assessment to aflatoxins through consumption of maize and groundnut was not carried out even though the levels of aflatoxin detected in the samples after storage were very high, and this exposure to high aflatoxins levels could lead to serious health problems.

2.9 Risk Assessment of aflatoxins in maize

Food is a basic necessity for life as it is a source of essential nutrients and energy for optimal health (Nauta et al., 2018). Nevertheless, food may also be associated with hazards such as natural toxins, hazardous chemical substances or pathogenic microorganisms that can affect health negatively (Nauta et al., 2018).

Therefore, this calls for studies to risk assessment to determine the extent to which humans are exposed to such potential risks through their diet.

A Codex Alimentarius Commission has defined risk assessment as a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment and (iv) risk characterization (Codex Alimentarius Commission, 1999).

(i) Hazard Identification

Hazard identification is predominately a qualitative process, and hazards can be identified from relevant data sources such as scientific literature, databases such as those in the food industry, government agencies, and relevant international organizations and through the solicitation of opinions of experts (Codex Alimentarius Commission, 1999)

(ii) Hazard Characterization

This step provides a qualitative or quantitative description of the severity and duration of adverse effects that may result from ingesting toxins in food (Codex Alimentarius Commission, 1999). A dose-response assessment should be performed if the data is available, while in the absence of a known dose-response relationship, expert elicitations could be used as the source of information (Codex Alimentarius Commission, 1999).

(iii) Exposure Assessment

The exposure assessment is based on estimating the possible dietary intake of contaminants and the risk characterization provided (WHO IPCS, 2009).

Exposure assessment considers the portion size consumed by the study population and the frequency of contamination and level of a contaminant in a particular food over time (Codex Alimentarius Commission, 1999). This also includes investigating socio-economic and cultural backgrounds, ethnicity, seasonality, age differences (population demographics), regional differences, and consumer preferences and behaviour (Codex Alimentarius Commission, 1999).

(iv) Risk Characterization

Risk Characterization is an integration of Hazard Identification, Hazard Characterization, and Exposure Assessment determinations, thus providing a qualitative or quantitative estimate of the probability of occurrence of health outcomes in a population under defined conditions of exposure, including a description of the uncertainties associated with these estimates (Codex Alimentarius Commission, 1999).

AFB1 has been reported as the most harmful type of aflatoxin because of its association with hepatocellular carcinoma (HCC), leading to liver cell genetic changes (Wild and Gong, 2016). Worldwide, liver cancer has been ranked as the sixth most frequent type of cancer, and it has been reported that aflatoxins contributed to 4.6%–28.2% of all cases of HCC (Liu and Wu, 2010). HCC is more frequent in individuals with chronic hepatitis B virus (HBV) infections (Wild and Gong, 2016). The risk characterization can be determined using the margin of exposure (MOE) approach and the quantitative liver cancer risk approach (Gong et al. 2016). These MOE values for AfB1 exposure are determined using the mean estimated dietary intake (EDI) values on consumption data. A safe margin has been set as MOE values below 10000 have been set (EFSA, 2020). Therefore MOE values below 1,000 indicate that immediate action should be taken to manage the risk (Gong et al. 2016).

CHAPTER THREE

METHODOLOGY

3.1 Study area

Lusaka district (Figure 3.1) was selected as the study area because it is the capital of Zambia and a centre of both commerce and government in Zambia. The city was also cosmopolitan, with different nationalities and tribes enrolled; hence included respondents with diverse socio-economic characteristics. Lusaka Province has an estimated population of 2,191,225 (CSO, 2012). However, about 80% of this population (1,747,152) and major commercial activities are concentrated in Lusaka District (CSO, 2012).

Figure 3.1: Location of Lusaka District (*Source of Image: https://www.semanticscholar.org/paper/Users'-experience-of-primary-healthcare-services-of-Nyirenda/266c221a182f0f062144761421dfd6e2d0383371/figure/1)

3.2 Study design and data sources

A cross-sectional study design was carried out using a descriptive household-based survey involving maize nshima consumption patterns, and the second part involved a risk assessment based on the Codex Alimentarius commission framework.

***Primary data*:** a survey was undertaken, and a questionnaire (Appendix 2) as a data collection tool was designed and administered to the person in charge of preparing or planning meals for the household. Thus, in households where the one in charge of deciding and planning meals was different from the household head, the former became the primary respondent while the household head was consulted on matters if this person did not understand.

Secondary data: data was collected on the aflatoxin concentrations in the two commonly used types of maize meal, i.e., breakfast maize meal and roller maize meal, tested at the Zambia Bureau Standards Laboratory from 2019 to 2022, analyzed using high-performance liquid chromatography (HPLC).

3.3 Sampling size and frame

A total of 753 households were randomly sampled from three different strata, including low-cost, medium cost and high-cost residential areas, to ensure respondents with diverse socio-economic characteristics were included.

The epitools program (https://epitools.ausvet.com.au/samplesize) was used to calculate the sample size using the population size of housing units in the Lusaka district as an input for the calculation at 95% confidence interval and precision of 0.01.

The Central Statistical Office (2021) recorded a total of 358871 housing units in the Lusaka District.

Sample size was calculated using: $n = (Z2 \times P \times (1 - P))/e2$

Where:

- Z = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI)
- P is the expected true proportion
- e is desired precision (half desired CI width)

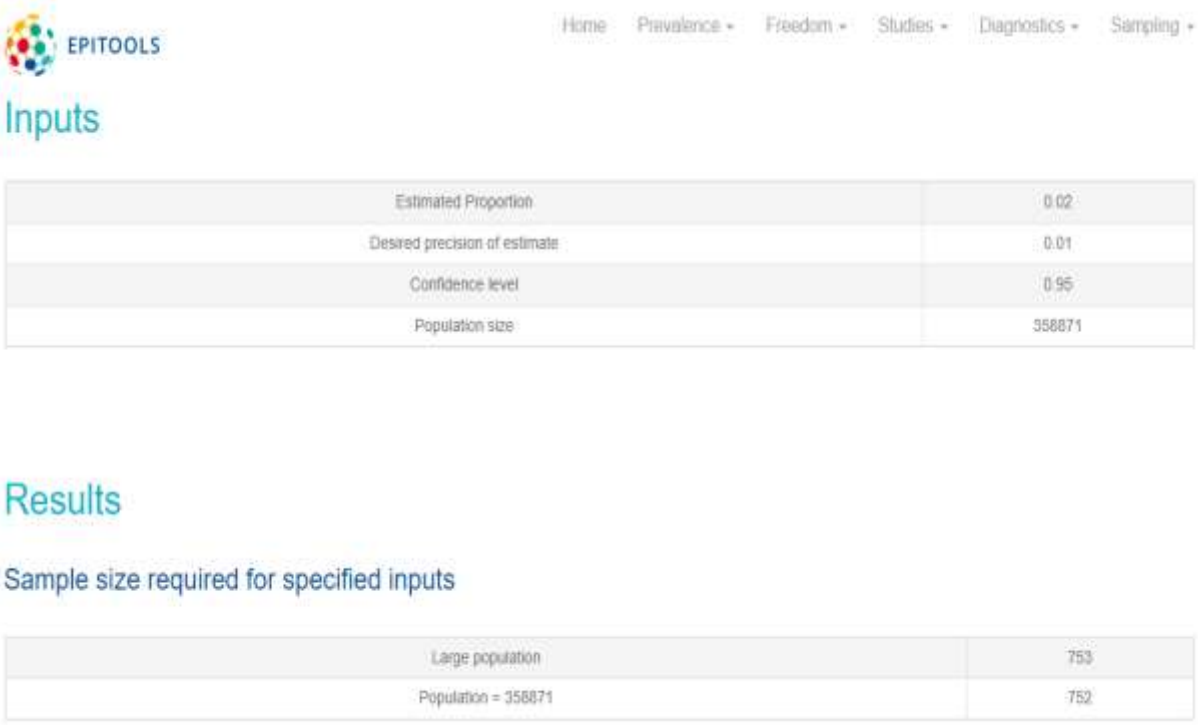

Figure 3.2: Epitools sample calculation (*Source of Image: https://epitools.ausvet.com.au/samplesize):

3.3.1 Stratification

The Stratification used in this study was based on one of the Central Statistical Office used in the Living Conditions Monitoring Survey for Urban Areas (Central Statistical Office, 2006).

Low-cost areas were characterized by having little access to amenities such as water and usually had a high population density, including areas such as Kanyama, Chawama and Chainda. The high-cost areas, on the other hand, had access to amenities and a low human population density, including areas such as Kabulonga, Roma and Olympia, while the medium-cost areas fall in between low and high-cost areas, including areas such as Kabwata, Emmasdale, Chelstone, and Libala (Central Statistical Office, 2006).

3.3.2. Sample allocation

Sample allocation to the three strata was done using square root allocation, with the number of households per Enumeration Area being the measure of size as follows.

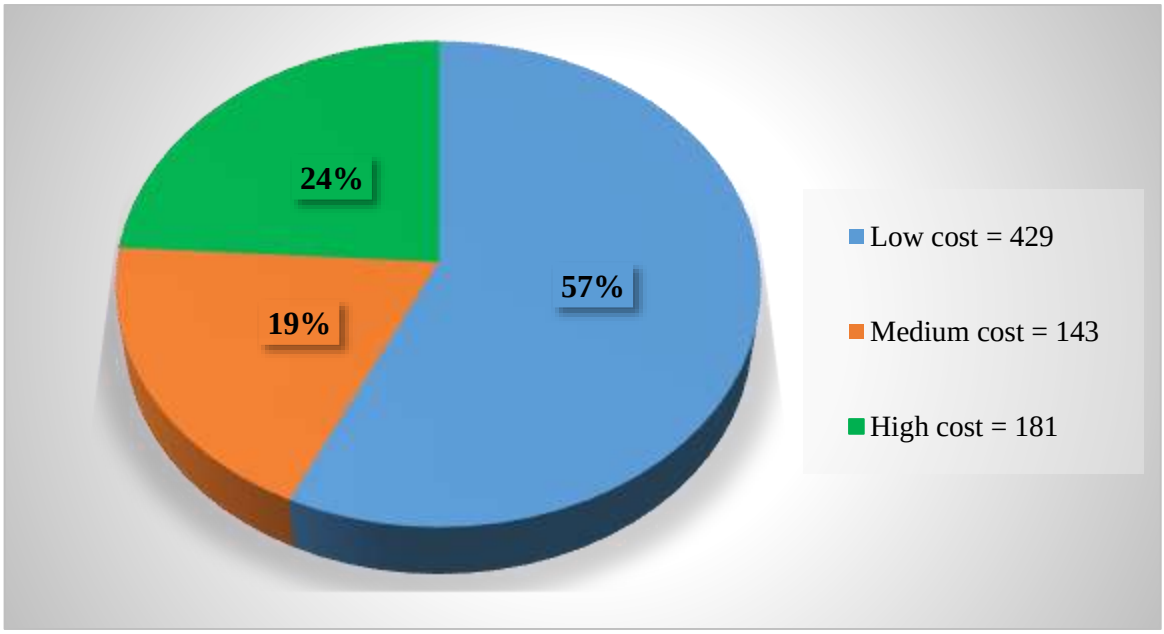

Figure 3.3 Sample allocation per stratum

3.4 Risk Assessment process

A Quantitative risk assessment of exposure to aflatoxins was carried out based on the Codex Alimentarius commission framework consisting of the following steps: (i) hazard identification; (ii) hazard characterization; (iii) exposure assessment; and (iv) risk characterization.
Figure 3.4 below shows the exposure scenario tree of aflatoxins in maize meal.

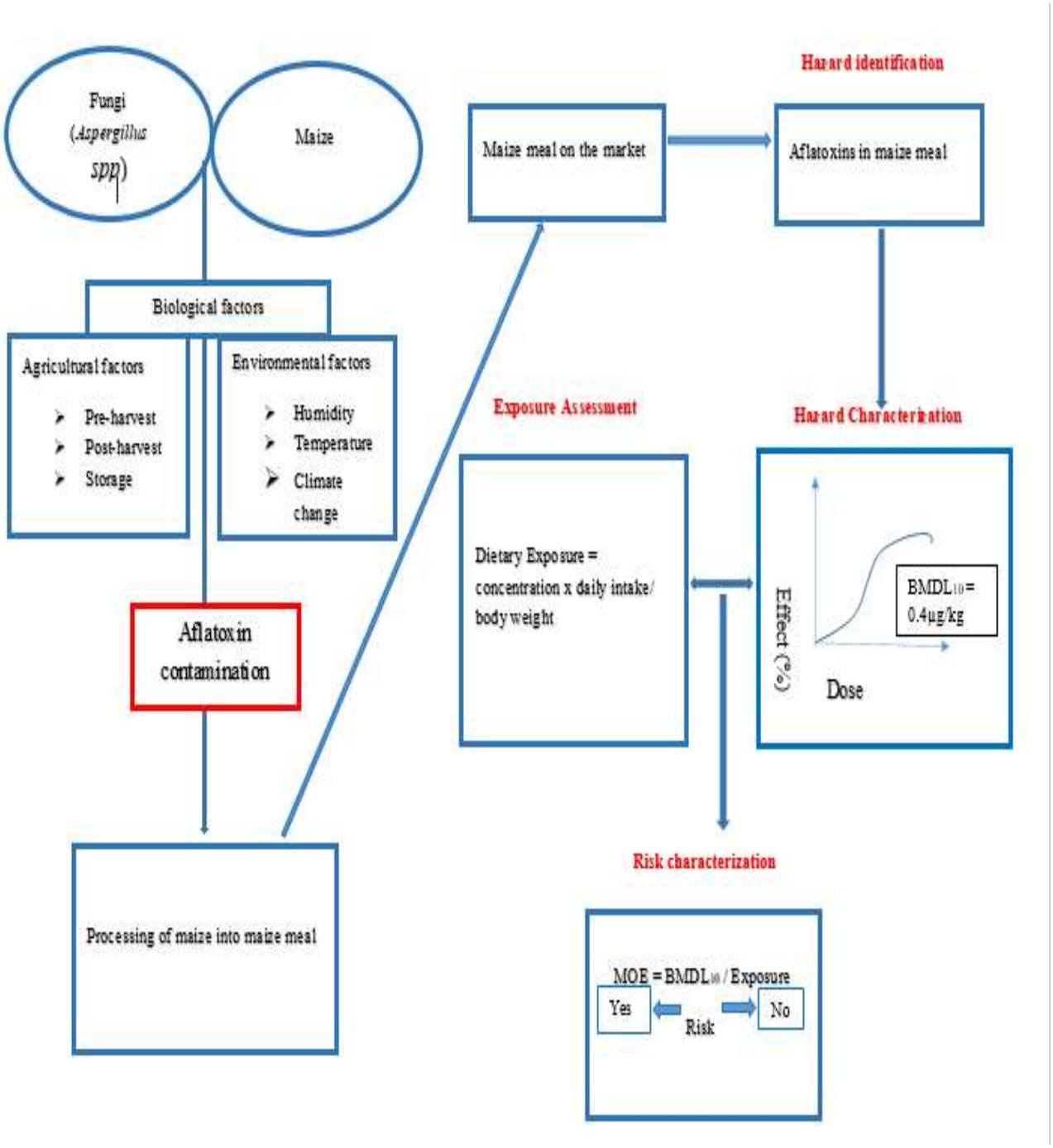

Figure 3.4: Exposure scenario tree of aflatoxins in maize meal

3.4.1 Hazard identification

The hazard was identified as aflatoxins in maize meal samples tested by the Zambia Bureau of Standards Laboratory.

3.4.2 Hazard characterization

The hazard could not be characterized due to the lack of parameters to determine the dose-response relationship.

3.4.3 Exposure Assessment

The likelihood of exposure to aflatoxins through consumption of maize nshima was assessed using the Model Risk® software. The pert distribution and Monte Carlo simulation were run for 5000 iterations. Table 3.1 below shows the input parameters used to determine exposure to aflatoxins in model risk.

Table 3.1 Input parameters for exposure assessment

Age Group	Parameter(s)	Lower value	Expected value	Upper value
6mnts - 2yrs	Quantity of nshima eaten per day (kg)	0.06	0.12	0.18
	AF Potential Value (µg/kg)	0.2	2	150
2 -5 yrs	Quantity of nshima eaten per day (kg)	0.12	0.24	0.36
	AF Potential Value (µg/kg)	0.2	2	150
5-10 yrs	Quantity of nshima eaten per day (kg)	0.12	0.24	0.36
	AF Potential Value (µg/kg)	0.2	2	150
10-13 yrs	Quantity of nshima eaten per day (kg)	0.12	0.24	0.36
	AF Potential Value (µg/kg)	0.2	2	150
13-18yrs	Quantity of nshima eaten per day (kg)	0.24	0.36	0.48
	AF Potential Value (µg/kg)	0.2	2	150

	Quantity of nshima eaten per day (kg)	0.36	0.48	0.98
18 yrs and above (Males)	AF Potential Value (µg/kg)	0.2	2	150
	Quantity of nshima eaten per day (kg)	0.24	0.36	0.48
18 yrs and above (Females)	AF Potential Value (µg/kg)	0.2	2	150

3.4.4 Risk Characterization

The carcinogenic potency of aflatoxins and the cancer risk from exposure to Aflatoxins in the diet were estimated using the margin of exposure (MOE).

The MOE has been defined as the ratio between a toxicological reference, usually, the benchmark dose level that causes 10% cancer incidence in rats ($BMDL_{10}$) and the total intake (European Food Safety Authority (EFSA), 2020). A $BMDL_{10}$ of 10% of 0.4 µg/kg BW/day was estimated by EFSA (2020).

The larger the MOE, the smaller the risk, and a value lower than 10 000 may indicate a human health concern (EFSA, 2020).

3.4.5 Assumptions

The following assumptions were considered when carrying out this risk assessment;

i. The aflatoxin levels in maize meal did not reduce after cooking nshima.
ii. Aflatoxins are heat-stable (Okoth, 2016).
iii. The total aflatoxin levels tested in the maize meal samples comprised AFB1
iv. Aflatoxin B1 is responsible for acute and chronic toxicity (Wild and Gong 2016)

3.5 Data analysis and management

The data collected from the survey on consumption patterns were summarized and analyzed using Excel® 2013 and Stata® version 13.0 software. Then the Model Risk® software was used for risk modelling and calculations for the exposure.

3.6 Ethical considerations

Clearance to conduct the study was obtained from the School of Veterinary at the University of Zambia and the National Health Research Authority (NHRA), while ethical clearance was obtained from Excellence in Research Ethics and Science Converge (ERES). Written or thumb-printed voluntary informed consent was sought from participants (Appendix 1). The participants' privacy and confidentiality were ensured, with no names or national identities used in the questionnaire.

CHAPTER FOUR

RESULTS

4.1 Respondents' demographics

A total of 753 households were successfully interviewed. The selection of the households stratified to necessitate a balance of socio-economic classes; these were high, medium or middle and low classes; 57% were from low-class areas, 24% were from high class, and 19% were from the medium or middle class. Females, with a proportion of 76 % (95% CI; 72.78 -78.89), were the largest group of respondents, while males accounted for 24% (95% CI; 21.11- 27.23). Appendix 3 provides a statistical summary of the socio-demographic characteristics.

4.2 Source, preparation and storage of maize meal

This study showed that most households bought or sourced their maize meal from different sources. Appendix 4 summarizes that 49.0%(95% CI; 45.44-52.58) bought maize meal from the supermarket, while 16.73% (95% CI; 14.23 - 19.58) used their maize grains which were milled into maize meal using a hammer mill *(Chigayo)* at the market, and 15.27% (95% CI; 12.87 - 18.03) sourced their maize from the open market mainly bought maize meal pre-packed into 2-5kg plastic bags popularly known as "*Pamela packs*". The majority (41.17% at 95% CI; 37.69 - 44.73) of the selected households indicated that they cooked their maize nshima for more than 20 minutes, while 17.80% (955 CI; 15.22 -20.70) cooked their nshima for less than 20 minutes. Storage of maize meal is critical when considering aflatoxin contamination. Most respondents [83.13% (95% CI; 80.24 - 85.65)] stored their maize meal in plastic-based containers.

4.3 Daily dietary intake of maize nshima (consumption patterns)

The study established that 97.08% of the selected households consumed maize meal nshima, most (66.8%; 95% CI: 63.34-70.08) of which used breakfast maize meal as shown in figure 6 compared to those that used roller meal as shown in figure 7 (18.33% at 95% CI; 15.71 -21.25).

The aflatoxin concentrations in maize meal obtained from the Zambia Bureau of Standards laboratory test results for 169 breakfast and 139 roller maize meal samples between 2019 and 2022 ranged between 0.2 µg/kg to 150 µg/kg. The roller meal samples had an average aflatoxin level of 13.53 µg/kg, while the breakfast meal had an average aflatoxin level of 10 µg/kg. No significant

difference was observed in the levels of AFs between roller and breakfast maize meal samples (p = 0.20) when compared using an unpaired two-sample t-test.

In most households, maize nshima was consumed twice daily, accounting for 53.1% (95% CI; 49.54 - 56.67) of the selected households. The study also showed that most households (34.4%; 95% CI: 31.08 - 37.87) consumed nshima with relish mixed with groundnut powder more than five times a month. A difference was observed in the amounts of nshima eaten between the male and female adults; the males consumed about 360g to 980g of maize nshima per day, while the females consumed 240g to 480g of maize nshima per day. Table 4.1 summarizes the amounts of maize nshima eaten by each age group compared to the recommended amounts described in the Zambia Food-Based Dietary Guidelines Technical Recommendation 2021. The data on the quantities of nshima eaten per day was also used as input parameters for the exposure calculations in Model Risk software®.

Table 4.1: Summary of the Food Frequency Consumption by Age

Age group	Frequency of consumption per day	Amount of nshima eaten (g)	Recommended* Amount (g)
6mnts - 2yrs	1-2 times	60 – 120	171
2yrs - 5yrs	1-2 times	240 – 360	171
5yrs -10yrs	1-2 times	240 – 360	358
10yrs -13yrs	1-2 times	240 – 360	358
13yrs - 18yrs	1-2 times	240 – 480	358
Above 18yrs (Males)	1-2 times	360 – 980	559
Above 18yrs (Females)	1-2 times	240 – 480	559

*Source: Zambia Food-Based Dietary Guidelines Technical Recommendation 2021

4.4 Aflatoxin awareness

Determining awareness of aflatoxin contamination of maize and its products, such as maize meal, among the respondents was important in this study. Only 13.68% (95% CI; 11.40 -16.33) of the

respondents were aware, while the majority (86.32 % at 95% CI; 83.67 -88.60) were unaware of the presence of aflatoxin in maize meal.

4.5 Exposure assessment and risk characterization

. The maximum exposure to aflatoxin based on the daily consumption for all the age groups varied; it was observed to range between 15.72 -33.32 for children below the age of 10 years, 28.58 - 44.64 for young adolescents and teenagers aged between 10 years to 18 years. The maximum exposure for adult males was 80.21, while for females, it was 54.25. Table 4.5 shows the minimum and maximum exposure values to aflatoxins.

Table 4.2 Exposure to aflatoxin min and max values from Model Risk Software®

Age group	Exposure value (min)	Exposure value (max)
6mnts - 2yrs	0.02	15.72
2yrs - 5yrs	0.06	32.21
5yrs -10yrs	0.05	33.32
10yrs -13yrs	0.08	28.58
13yrs - 18yrs	0.06	44.65
Above 18yrs (Males)	0.12	80.21
Above 18yrs (Females)	0.07	54.25

The margin of exposure (MOE) for each age group was determined using the exposure values in table 4.1 obtained from the Model Risk Software® and recommended $BMDL_{10}$ of 0.4 µg/kg BW/day.

The MOE results are summarized using a box plot below (figure 4.1).

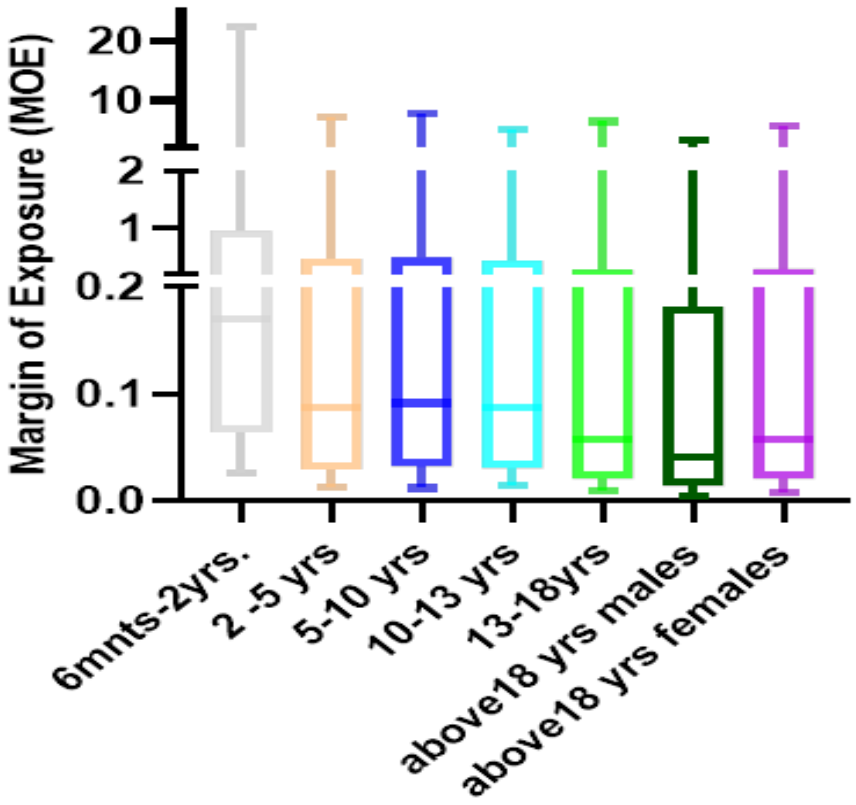

Figure 4.1: Margin of Exposure among different age groups

CHAPTER FIVE

DISCUSSION

This study aimed to determine the risk of exposure to aflatoxins in households of Lusaka District through the consumption of maize nshima.

It was observed that most of the respondents were females comprising 76 % (95% CI; 72.78 -78.89), compared to males, who accounted for 24% (95% CI; 21.11- 27.23). This could be attributed to the fact that most male members of the households are not actively involved in meal planning and preparation in Zambia. A Catholic Relief Services Rwanda report showed that a minority of men expressed more open attitudes to involving themselves in tasks such as caring for children or cooking (Catholic Relief Services Rwanda 2016). Most of the time, this is only acceptable when the wife is not available for such tasks. This cultural attitude is prevalent among most African countries, including Zambia, where men perceive the preparation of meals or cooking as a female activity (Catholic Relief Services Rwanda 2016).

The source of the maize meal is essential when considering aflatoxins contamination of the meal; most respondents accounting for 49.0% (95% CI; 45.44-52.58), bought their bought maize meal from the supermarket. Most supermarkets provide safe and quality commodities sourced directly from manufacturers who tend to implement quality and food safety practices. This is also in line with the finding in this study indicating that there has been a remarkable increase in the number of supermarkets involved in food retailing in Zambia (Jaffee 2002). One direct effect of this supermarket growth was a significant increase in higher quality products and a tremendous increase in various food products.

However, 16.73% (95% CI; 14.23 - 19.58) of the households milled maize grains into maize meal flour using a hammer mill (Chigayo) at the market. Lack of pre-cleaning of the hammer mills before use could lead to cross-contamination during the milling of maize into maize meal. An article in *Miller Magazine* published on 5 August 2013 reported that mould growth can be seen at uncontrolled points in some parts where the washing and annealing units inside the flour mill and at some points where vapour is intensified inside the closed systems. These moulds can cause health problems for

the consumer because of both themselves and the mycotoxins they produce by mixing with the flour in time (https://millermagazine.com/blog/food-safety-risks-in-flour-and-hygiene-and-sanitation-in-flour-mills-for-producing-safe-flour-1830).

The studies also revealed that 15.27% (95% CI; 12.87 - 18.03) sourced their maize from the open market, mainly bought maize meal pre-packed into 2-5kg plastic bags popularly known as "Pamela packs". Maize meal sold in open markets has a high risk of fungal contamination related to practices by most marketeers who display uncovered foodstuffs on their stalls. This can be compared with studies by (Omodele et al. 2020) indicating the presence of spoilage fungi species, including *Aspergillus spp* and *Candida spp*, respectively, in maize meal sampled from the open market or street vendors.

The majority (41.17% at 95% CI; 37.69 - 44.73) of the selected households indicated that they cooked their maize nshima for more than 20 minutes, while 17.80% (955 CI; 15.22 -20.70) cooked their nshima for less than 20 minutes. Besides aflatoxins being heat-stable, some studies have indicated that heat has little impact on destroying these metabolites (Okoth, 2016; Bureau of Social Welfare and Public Health, 2018).

An earlier study demonstrated that home preparation of maize porridge reduced only 23% of aflatoxin (Okoth, 2016). However, even these reductions in aflatoxin levels may not bring the contaminants down to safe levels. Similarly, the Bureau of Social Welfare and Public Health in 2018 in Tokyo also reported that after boiling, 50 to 80% of mycotoxins remained in food, with around 10 to 15% present in the water used for boiling (Bureau of Social Welfare and Public Health, 2018). These studies showed that boiling destroys only a small number of potential mycotoxins in food.

Consequently, the storage conditions after milling and packaging are crucial for the quality of the mealie meal because they affect the shelf life and safety of the consumer (Ntuli et al. 2015). The most common (83.13% at 95% CI; 80.24 - 85.65) storage practice of maize meal in most households was in a plastic storage container. Most respondents transferred the maize meal from the polythene bag in which it was purchased into a plastic container with an air-tight lid or cover. This is likely to reduce exposure of the maize meal to high moisture and humidity. This finding corroborates the findings of a study carried out on the storage of flour or food powders, which indicated that when storing in a plastic container (LDPE) (up to 24 weeks), the moisture content increased from 8.30 to

12.04%, which still falls within the permissible range between 12 and 14% and as such attracts minimal mould and bacteria attack during storage (Daramola et al.2010).

Similarly, for high-density polyethene (HDPE) storage (up to 16 weeks of storage), the moisture contents of samples were also within acceptable levels (Daramola et al.2010). However, the moisture content of samples stored in LDPE, irrespective of storage temperature and relative humidity, was higher than that of samples stored in HDPE and plastic containers (Daramola et al., 2010). Hence, plastic containers give the best barrier protective ability under all storage conditions.

Most households consumed breakfast meal (66.8% at 95% CI; 63.34-70.08) compared to roller meal (18.33% at 95% CI; 15.71 -21.25). This preference can best be explained by the fact that breakfast meal is cleaned milling grades of maize more than roller meal. The unpaired two-sample t-test was used to compare the aflatoxin levels in breakfast and roller maize meals; the p-value was 0.20, more significant than the significance level of 0.05. Therefore, there was no significant difference in the aflatoxin concentrations between breakfast and roller maize meals. This finding is supported by a study in Kenya that showed that there was no significant difference in aflatoxin concentration in ppb among three different types of maize flours ($P>0.05$) (Nduti et al. 2017). Aflatoxins in the maize flour samples were slightly above the international upper limit of 5ppb; all the results were lower than the Kenya standard, whose upper limit is 10ppb (Nduti et al. 2017). Thus, the households that consumed breakfast maize meal had the same chance of exposure to aflatoxins as those that consumed roller maize meal.

Furthermore, most households consumed maize nshima twice daily for lunch and supper, accounting for 53.1% (95% CI; 49.54 - 56.67). This corroborates the earlier findings, which reported that in Zambia, traditional stiff maize porridge, called nshima, is extensively consumed (Chikowo, 2020) and contributes up to 50% of daily calorie intake (Kachapulula, 2017).

There was also a difference observed in the portion size of nshima eaten between the male and female adults; the males consumed about 3-5 lumps of maize nshima (360g to 980g) per day, while the females ate 2-4 lumps of nshima (240g to 480g) per day. This borders more on the socio-cultural and psychological factors as most women tend to eat more femininely, preferring to eat smaller portions of carbohydrate-rich foods, including maize nshima, to avoid body weight gain. Related studies by (Liu et al., 2021) found that the average carbohydrate intake was 267.4 (SD 112.0) g/day in males and 204.9 (SD 90.7) g/day. Furthermore, more than 80% of carbohydrates were derived

from refined grains. Another researcher showed that both sexes had different eating styles because women experienced more food-related conflict than men, especially high-calorie or fattening foods (Rolls et al. 1991). Women experienced more dissatisfaction with their body weight and shape than men (Rolls et al. 1991). These eating habits put men at higher risk of exposure to higher levels of aflatoxins.

The study revealed that 86.32 % (95% CI; 83.67 -88.60) of the respondents lacked awareness of the aflatoxin contamination of maize and its products and their adverse effects. This may be explained due to little or no information on aflatoxins disseminated to the general public or the lack of aflatoxin awareness campaigns by the public health departments in Zambia. Awareness campaigns are important in improving the knowledge on aflatoxins which could lead to change towards attitudes and practices of both agricultural and food production methods in the community, thus preventing or reducing aflatoxin contamination of maize and its product. This method proved effective during the aflatoxicosis outbreak in Kenya in 2005 (Strosnider et al., 2006).

In this study, the aflatoxin concentrations in maize meal were obtained from the Zambia Bureau of Standards Laboratory between 2019 and 2022 for 139 roller meal and 169 breakfast meal samples. The aflatoxin levels in the breakfast and roller meal samples ranged between 0.2 µg/kg to 150 µg/kg, indicating the presence of aflatoxin in maize meal.

Related studies have shown a prevalence of aflatoxins in maize and maize products. Mukanga (et al. 2019) reported that all the maize products tested positive for aflatoxin with 54.6%, above the Zambian regulatory standard of 10 µg/kg for total aflatoxin. Significantly higher amounts of aflatoxins were obtained from hammer mill samples, 11.28 µg/kg, while the least was in the maize products sold by street vendors, 2.62 µg/kg. Alamu (2018) indicated in a study on the nutrient and aflatoxin contents of complementary foods, i.e. maize porridge and maize nshima consumed by children (6–24 months) and infants, that the maize porridge samples from Chipata were the most contaminated with mean aflatoxin content of 5.8 ± 15.93 mg/100 g, while maize nshima was the more contaminated of the two complementary foods from Monze districts with mean aflatoxin level of 3.8 ± 6.41 mg/100g. These results show that aflatoxins were also present in various maize products, including raw products such as maize meal samples from the hammer mill and cooked products such as maize porridge and maize nshima samples.

The maximum exposure to aflatoxin ranged between 15.72 -32.31 for children below the age of 5 years who consumed 60-360 g of maize nshima per day, while for young adolescents aged between 5 years and ten years consumed 240-360 g of maize nshima per day recorded a maximum exposure of 33.32 and those between the age of 10 years and 18 years consumed 240-480g of maize nshima per day had a exposure maximum range of 28.58 - 44.64. However, for adult males, it was 80.21 and 54.25 for adult females, who consumed 360-980g and 240-480g of maize nshima per day, respectively. The risk of exposure was observed to increase with the increase in quantity or portion size of maize nshima consumed among the different age groups. In this case, male adults would be more at risk than other age groups.

Another study reported that children are more vulnerable to aflatoxin exposure from other sources, such as groundnuts, since peanuts powder is often added to maize porridge for infants because of its high protein, a typical constituent of weaning food (Gong et al. 2016). Hence this double exposure to aflatoxin levels relative to body weight is higher for children than for adults, thus indicating the impact of aflatoxin on child growth (Gong et al. 2016). Another source of aflatoxin dietary exposure for children, especially those below the age of two years, is breast milk. Breast milk was also a potential source of aflatoxin exposure for very young infants. Gong et al. (2016) detected Aflatoxin M1, the hydroxylated metabolite of Aflatoxin B1, in breast milk 12 to 24 hours following ingestion of foods contaminated with Aflatoxin B1.

This study also found that most households (34.4% at 95% CI; 31.08 - 37.87) consumed nshima with relish mixed with groundnut powder more than five times a month. This increased the risk of double exposure to aflatoxins as groundnuts are also prone to aflatoxin contamination (Bumbangi 2016). This is supported by the findings of a study done by Bumbangi (2016) on peanut samples from open markets and supermarkets in the Lusaka District, which were analyzed for concentration levels of aflatoxins and AFB1, the most carcinogenic type was 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 (0.0466ng/kg).

The MOE values for all the age categories in the study population were below 10,000. This indicated serious health concerns. The adult males had the highest MOE value of 0.005. Hence they were most likely to suffer the adverse health effects from aflatoxin exposure through the consumption of maize nshima.

This further shows that high dietary exposure results in a high hepatocellular carcinoma (HCC) risk (EFSA, 2005). For both genotoxic and carcinogenic substances, the EFSA Scientific Committee stated that a MOE of 10,000 or higher, if based on the BMDL10 from an animal carcinogenicity study, would be of low concern from a public health point of view (EFSA, 2005).

Related risk assessment studies have reported low MOE values below 10,000 associated with cancer risks. Federal District of Brazil (Andrade et.al. 2012) reported that the risk of exposure to aflatoxins through consumption of maize and rice was twice as high as that of the entire country. The MOEs ranged from 2833 to 10.4 for the entire country and 25 to 3.6 for the Federal District (Andrade et al. 2012). Similar studies in Africa (Shepherd, 2008) carried on the risk of exposure to aflatoxins by consuming various staple foods such as maize, yam, millet sorghum, rice and groundnuts in Kenya, Tanzania, Gambia, Botswana, Benin and Gambia. The MOE values were below 10,000 in the maize from Kenya, ranging between 2.5-6.5 and 241.7 in maize from Gambia. Thus, as for the cancer potency approach, this MOE approach clearly highlights the need for action by risk managers.

Actual liver cancer incidence rates reported in sub-Saharan Africa differ across countries, with high rates in Mozambique (incidence in males of 79.4 per 100,000) and Gambia (45.6 per 100,000 males) to low rates in Namibia (3.1 per 100,000 males) and Botswana (4.5 per 100,000 males) (IARC 2007).

CHAPTER SIX
LIMITATIONS CONCLUSION AND RECOMMENDATIONS

6.1 Limitations

a) Time constraints affected the sample size.
b) Limited funds to test for aflatoxins in maize nshima.
c) Carrying out the study in some low-cost areas notorious for crimes and violence was a challenge.
d) The current Zambian Standard for maize meal does not include acceptable levels of aflatoxins.

6.2 Conclusion

The results of this study indicate that the exposure risk to aflatoxin in households in Lusaka District could result in health risks. This is related to the presence of aflatoxins in maize meal used to prepare nshima. The overall exposure was highest in adult males who consumed 360 to 980g of nshima per day. There was little or no knowledge about aflatoxin among respondents besides the public health concerns associated with aflatoxins. This calls for awareness of exposure to aflatoxins through consuming aflatoxin-contaminated foods.

6.3 Recommendations

a) The best approach is preventing fungal growth on maize in the field and during storage (Bruns, 2003). This calls for implementing good agricultural practices (GAPs) by commercial and small-scale farmers in Zambia.
b) There is a need for the Government to carry out public awareness of aflatoxins and their potential health effects.
c) The Zambia Bureau of Standards (ZABS) could recommend maximum limits for aflatoxins in maize meal as the current standard for maize meal (ZS 189: 2014) has not defined the maximum limits for aflatoxins.
d) There is a need to study exposure to aflatoxins in children below two years through break milk.

e) Research on the compounding effect of aflatoxin from maize and grounds used in baby porridge should be conducted.

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APPENDICES

APPENDIX 1: LETTER OF INTRODUCTION AND INFORMED CONSENT FORM

My name is Mumba Musonda, and I am a student at the University of Zambia. I amrequesting your participation in the study.

What is the project title: Exposure Assessment of Households in Lusaka District to Aflatoxins through Consumption of Maize Meal Nshima.

Brief description of the study

Maize is the sample crop of Zambia mainly consumed as nshima. However, maize and its products, such as maize meal nshima, are prone to aflatoxin contamination. Aflatoxins are metabolites of fungi belonging to the *Aspergillus spp,* which are grown on maize in fields and storage when not well managed.

This aims to estimate the risk of human exposure to aflatoxins by consuming maizemeal nshima. The study will also determine the consumption patterns of maize meal nshima by households in the Lusaka District.

The information gained will help design intervention and control measures against aflatoxins.

Who is running the study: The study is conducted by a master's student from the University of Zambia pursuing Food Safety and Risk Analysis.

Do I have to participate: It is not a must for you to participate in the study. You are free to make a voluntary decision about participation or not. There is no penalty for refusing to participate. In case you agree to participate, you are free to skip questions you may deem personal or otherwise and to withdraw from the study at any time without penalty.

What will happen to me if I participate in the study: If you agree to participate in the study, we will ask you a few questions about where you live, your educational background, the number of people living in your household, if your household consumes maize meal nshima, etc.

Are there any risks if I participate in the study: Asking of questions will take some of your time.

Are there any benefits from the study: There is no direct benefit to participation in the study, but it will lead to a better understanding of the level of exposure to aflatoxins when consuming maize meal nshima. This study's findings will help design intervention and control measures against aflatoxin contamination of maize meal nshima.

Will there be any compensation for being in the study: There is no compensation to volunteers for your participation.

How long does the study last: This study requires only the completion of a short questionnaire. There is no follow-up or further information needed. The questionnaire will take about 25 minutes.

Who will be able to see my information: Any information about you and your household will be kept very confidential. Only the people directly involved in the study will be able to see your information. Your name will not be used in any report resulting from this study. Any report from this study will refer to you only by a study identification number and not by a name.

Whom can I contact about the study or my rights as a volunteer in this research study: If, during the course of this study, you have questions concerning the nature of the research, you should contact:

Mumba Musonda
University of Zambia
Department of Disease Control
School of Veterinary Medicine
P. O. Box 32379

Email: musondamumba91@gmail.com
Phone: +26096327794

What if you have questions about your rights as a research participant: All research on human volunteers is reviewed by ERES Converge IRB, a committee that protects your rights and welfare. If you have questions or concerns about your rights as a research participant,you may contact the following:

The Chairperson

ERES Converge IRB

272 Meanwood Road, Meanwood Ibex

LUSAKA

Email: eresconverge@yahoo.co.uk

Telephone: +260211-230-581

Mobile phone: +260 955 155633 | +260 955 155634

as describe

IF THERE IS ANY PORTION OF THIS CONSENT AGREEMENT THAT YOU DONOT UNDERSTAND, PLEASE TALK TO SOMEONE FROM THE STUDY TEAM BEFORE SIGNING.

Principal Investigator: Mumba Musonda

Participant's Agreement:

The above document describing this study's benefits, risks and procedures has been read andexplained to me. I have been told that joining this study is voluntary and that I can withdraw anytime. I have been allowed to ask any questions about the study, and all my questions have been answered. I voluntarily agree for my household to participate in this research study

Name (print)

Signature or Thumbprint Date

I certify that the nature, purpose, potential benefits, and possible risks associated with participation in this study have been explained to the individual participant.

Signature of Research Team Member Obtaining ConsentDate

I was present throughout the entire informed consent process with the volunteer. All questions from the volunteer were answered, and the volunteer agreed to participate in the study.

Signature of Witness Date

Printed Name of Witness

*Note: Witness name, signature and date are required on this consent form only when the consenting volunteer is not able to read (illiterate).

APPENDIX 2: QUESTIONNAIRE
EXPOSURE TO AFLATOXINS THROUGH CONSUMPTION OF MAIZE MEAL NSHIMA IN LUSAKA DISTRICT

1. RESPONDENT'S GENERAL INFORMATION

Participant Identification ….....

Area of residence ….....

How long have you been living in this area? ….....

Sex of the respondent

	Male
	Female

Age of the respondent ….....

Marital status

	Married
	Single
	Cohabiting
	Divorced
	Widow

What is the highest level of education you completed?

	None (did not attend school)
	Primary (grade 1-7)
	Junior Secondary (grade 8-9)
	Senior Secondary (grades 10-12
	Tertiary (University/College)

What is your occupation/profession?

	Government employer
	Private employer
	Private business
	Student

What type of food(s) do you order at a restaurant?

……

	Unemployed
	Other (specify) ….....

Structure of your nuclear family unit in your household

	Number of persons living in the household
	Number of persons aged six months- 2 years
	Number of persons aged two years – 5 years
	Number of persons aged five years -10 years
	Number of persons aged ten years- 13 years
	Number of persons aged 13 years – 18 years
	Number of persons aged above 18 years

2. SOURCE(S) OF MAIZE MEAL USED AND PREPARATION OF NSHIMA

Which type of maize mealie meal do you use to prepare nshima?

	Roller meal
	Breakfast meal
	A mix of breakfast meal and roller meal
	Others (specify: ……)

Where do you buy or get your maize mealie meal from?

	Open market
	Supermarket
	Grocery shop
	Kiosk (*Kantemba)*
	Maize meal ground at home
	Maize meal ground at a market hammer mill *(Chigayo)*
	Others (specify :……)

How long do you boil the maize meal porridge before making the pulp (nshima)?

	<20mins
	>20mins
	Do not Know

Do you use any other flour for nshima apart from maize? Indicate the type

	Millet
	Cassava
	Sorghum

Which type of material is the container for the maize meal storage made of?

	Plant material (wood, calabash)

	Plastic
	Metal
	Polythene bag

3. MAIZE MEAL NSHIMA CONSUMPTION

Do you eat maize meal nshima in your household?

	Yes
	No

How many lumps of maize meal nshima does each person in your household consume per day? (Indicate the age of each person)

*One lump of nshima was equivalent to 120g.

…….

…….

……..

Do you eat other types of carbohydrates apart from nshima?
If yes, list them.

……...

……..

Do you eat meals with relish prepared with peanut flour (Ifisashi)?
If yes, how often to eat relish prepared with peanut flour (Ifisashi)?

……

……

…….……

Are you aware of the aflatoxin contamination of maize and maize products?

……..……

APPENDIX 3: Respondents Demographic Characteristics

Table 5: Respondents' demographic characteristics

Demographic variables		Types of Variables	n (753)	%	95% CI.
Gender	Male	Categorical	181	24	21.11- 27.23
	Female	Categorical	572	76	72.78 -78.89
	18-25	Continuous	126	17	14.23 - 19.58
	25-35	Continuous	344	45	42.21 - 49.27
	Above 35	Continuous	283	38	34.18 - 41.12
Marital status	Single	Categorical	244	32.4	29.15 - 35.84
	Married	Categorical	415	55.1	51.53 - 58.64
	Divorced	Categorical	33	4.4	3.13 - 6.11
	Widowed	Categorical	61	8.1	6.35 - 10.28
Residential area	Low class	Categorical	429	57	53.40 -60.48
	Medium class	Categorical	143	19	16.34 - 21.96
	High class	Categorical	181	24	21.11 - 27.23
Level of education	None	Categorical	24	3.2	2.14 - 4.72
	Primary	Categorical	92	12.2	10.06 -14.76
	Secondary	Categorical	358	47.5	43.99 - 51.12
	Tertiary	Categorical	279	37.1	33.67 - 40.57
Employment status	Employed	Categorical	429	57	53.40 - 60.48
	Unemployed	Categorical	74	9.8	7.90 - 12.17
	Business	Categorical	209	27.8	24.67 -31.07
	Student	Categorical	14	1.8	1.11 - 3.12
	Retired	Categorical	27	3.6	2.47 - 5.18

n = Number of Respondents; CI= Confidence interval

APPENDIX 4: Source, Preparation and Storage of Maize Meal

Table 6: Source, preparation and storage of maize meal

Variables		Types of Variables	n (753)	%	95% CI.
Source(s) of maize meal	Supermarket	Categorical	369	49	45.44-52.58
	Grocery store	Categorical	91	12.08	9.94 - 14.62
	Open market	Categorical	115	15.27	12.87 - 18.03
	Kiosk	Categorical	16	2.12	1.30 - 3.44
	Grounded at the market (chigayo)	Categorical	126	16.73	14.23 - 19.58
	Grounded at home	Categorical	8	1.06	0.53 - 2.11
Cooking time for nshima	Less than 20 minutes	Categorical	134	17.8	15.22 -20.70
	More than 20 minutes	Categorical	310	41.17	37.69 - 44.73
	Not sure	Categorical	284	37.72	34.31 -41. 24
Type of storage container	Plant material (wood, calabash)	Categorical	2	0.27	0.66 - 1.06
	Plastic	Categorical	626	83.13	80.24 - 85.65
	Metal	Categorical	14	1.9	1.10 - 3.11
	Polythene bag	Categorical	71	9.43	7.53 -11.7

n = Number of Respondents; CI= Confidence interval