OCCURRENCE AND RISKS OF AFLATOXINS AND FUMONISINS IN LOCALLY PROCESSED BABY PORRIDGE FLOUR IN MALAWI

MASTER OF SCIENCE (ENVIRONMENTAL SCIENCE) THESIS

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UNIVERSITY OF MALAWI

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MSc (ENVIRONMENTAL SCIENCE) THESIS

By

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DECLARATION

This thesis is my own original work and has not been previously submitted to any other institution for a similar purpose. Proper acknowledgments have been included where the work of others has been used. I take full responsibility for the content of this work.

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CERTIFICATE OF APPROVAL

The undersigned certify that this Thesis represents the student's own work and effort and is submitted with our approval.

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DEDICATION

I dedicate this work to my super-amazing father, Charles Lumanga for pushing me and believing in my potential and supporting me all the way. To my sweet mother, Jane Lumanga, I say thank you for being my constant encourager when my energy was running low.

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ABSTRACT

Infants, once introduced to supplementary feeding, are at a high risk of exposure to mycotoxins such as aflatoxins and fumonisins. The aim of this study was to examine the occurrence and risks associated with aflatoxins and fumonisins in local baby porridge flour in Malawi. A structured questionnaire was administered to 338 households from 13 districts in Malawi to gather child anthropometric data as well as knowledge, attitude and practices (KAP) of caregivers. From each household, porridge flour samples were collected and analyzed for aflatoxin and fumonisin. A supplementary consumption study involving sixty 6-24 months old children was conducted to estimate maize flour intake. The risk of exposure to aflatoxins and fumonisins was estimated using the probabilistic Monte Carlo approach. Subsequently, the risk of aflatoxin induced Hepatocellular Carcinoma (HCC) was estimated. The average knowledge score for the caregivers was 7.024±1.871 out of 30 points and there was no association between the score and caregivers' education attainment. The average practice score was at 4.18±0.12 out of a possible 5 points. About 22% and 27% of the children were moderately and severely stunted respectively. Except for child's gender, age and education level of the caregiver, stunting had a direct significant ($p < 0.05$) association with, mycotoxin exposure, socio-economic status as well as residence in the southern region. There were significant differences ($p < 0.05$) in the occurrence and levels of aflatoxins and fumonisins amongst the districts. Aflatoxins were detected in 59 % of the total samples with lower levels in Dedza (mean, $\bar{x}$ = 3.4µg/kg) and highest in Balaka ($\bar{x}$ = 43.8µg/kg). Fumonisins were detected in 96% of the samples with the lower levels found in Nsanje ($\bar{x}$ = 884.6µg/kg) and higher levels in Balaka ($\bar{x}$ = 2842.3µg/kg). Aflatoxin and fumonisin co-occurred in 57% of the samples and only about 2% of the total samples complied with the EU limits for both aflatoxins (4µgkg) and fumonisins (200µg/kg). About 90% of the study population was concurrently exposed to aflatoxin and fumonisin levels beyond reference toxicological values with a risk of dietary-aflatoxin-induced liver cancer estimated between 3.19 and 3.27 per 100,000 population.

Reducing aflatoxin and fumonisin contamination of maize and dietary diversification can prevent infants and the public, in general, from exposure to the toxins. It is recommended that integrated interventions towards the prevention and mitigation of dietary mycotoxins for improved food safety and nutrition in Malawi, be established.

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LIST OF ACRONYMS

A. Flavus	Aspergillus Flavus
A. Parasiticus	Aspergillus Parasiticus
AEZ	Agro-Ecological Zone
AF	Aflatoxin
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of Variance
CI	Confidence Interval
DF	Dilution Factor
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
ELEM	Equine Leukoencephalomalacia
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Extension Planning Area
EU	European Union
FAO	Food and Agriculture Organization
FB	Fumonisin B
GEMS	Global Environment Monitoring System
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ID	Identity
IITA	International Institute of Tropical Agriculture
IPSC	International Power Control Systems
IYCN	Infant and Young Child Nutrition
JECFA	Joint Expert Committee on Food Additives
KAP	Knowledge, Attitudes and Practices

LB	Lower Bound
LOD	Limit of Detection
MAPAC	Malawi Program for Aflatoxin Control
MBS	Malawi Bureau of Standards
MDHS	Malawi Demographic and Health Survey
MOE	Margins of Exposure
NGO	Non-Governmental Organization
NNPSP	National Nutrition Policy and Strategic Plan
PPE	Porcine Pulmonary Edema
SPSS	Statistical Package for Social Science
TDI	Tolerable Daily Intake
UB	Upper Bound
UNEP	United Nations Environment Program
UNFPA	United Nations Population Fund
UNICEF	United Nations Children's Fund
USDA	United States Department of Agriculture
USFDA	United States Food and Drug Administration
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Children rely solely on breast milk for their nutrient, water, and energy needs during the first six months of life. However, as their needs double at six months, it is recommended that supplementary feeding programs be introduced. These programs commonly use nutrient-rich mixed flours, such as maize, soybean, and groundnuts, or commercial infant cereals. These cereals may be processed at home or purchased under different commercial brands. Unfortunately, many of these crops are prone to pre-harvest and post-harvest contamination by environmental toxins, including mycotoxins. Mycotoxins are associated with mycotoxicosis, a major public health concern that can affect infants when these toxins pass from contaminated cereals into their food and then their biological system. Mycotoxin levels have been increasing due to various anthropogenic activities, which is a major public health concern (Motamu et al., 2016; Sherif et al., 2009).

Mycotoxins are toxic secondary fungal metabolites that can contaminate crops and pose serious health risks to humans and animals globally (IARC, 2012). Aflatoxins and fumonisins are among the most well-studied mycotoxins and are particularly concerning as they contaminate a variety of crops, including maize, oil seeds, cereals, groundnuts, sunflower seeds, and soybeans, which raises questions about food quality and safety. Aflatoxins and fumonisins, produced by Aspergillus flavus and Fusariums miniliforme,

respectively, are carcinogenic and hepatotoxic, and infants are particularly vulnerable to their effects due to their underdeveloped biological organs and systems (Pinar et al., 2008). Mycotoxins are believed to be one of the major causes of acute and chronic illnesses among children under the age of five (Kiarie et al., 2016; Okong & Ohingo, 2004). Although the association between mycotoxin exposure and impaired child growth is well-documented, the specific biological mechanisms by which they exert their effects are not yet fully understood (Gong, 2003; Turner et al., 2003; Gong et al., 2008).

A report from an international meeting held in Washington DC in 2012 on the association of mycotoxins and child growth impairment emphasized that the burden of mycotoxin exposure is particularly high in the developing world where the population's diet is limited to mainly a single staple food that is vulnerable to fungal infection, either in the field or post-harvest during processing or storage (IFPRI, 2012) The report highlighted that the consumption of aflatoxin contaminated food is highly associated with stunting in both stature and cognitive development and is likely associated with susceptibility to disease and reduced response to vaccinations. The key period of vulnerability to mycotoxins has not been clearly defined, but includes both the pre-natal and the first few years of life; however, the negative effects are believed to have life-long implications upon the health of the affected child. Different types of mycotoxins may have different mechanisms of action, and lifelong exposure is common in many areas.

According to the 2020 Global Nutrition report, 149 million children under the age of 5 were reported to be stunted (too short for their age), this calculates to about 21.9% of the global under five population where Africa and Asia were reported with the highest prevalence of stunting (at 54% and 40% respectively) (Mannar & Micha, 2020). Malawi is no exception, as it is ranked at 37.10% as one of the countries with the highest prevalence of stunting in the world. (UNICEF/WHO/World Bank Group Joint Malnutrition Estimates, 2020 edition; International Food Policy Research Institute, 2016). At national level, the 2015-2016 Malawi Demographic and Health Survey reported that 37% of children under age 5 are stunted. The report revealed that stunting is more common in Neno and Mangochi (45%) and less common in Likoma district (25%), (NSO, 2016). According to studies,

stunting is likely to reflect a multi-factorial process where nutrition, environment, genetics, infections, economics and maternal factors have been deemed as the major contributing factors. However, reports have revealed that only about 35% of stunting can be directly attributable to studied health and dietary factors, and the role of mycotoxins in the remaining 65% is currently unknown and thus teasing out the aspects related to any specific factor is difficult. Therefore, there is a need to investigate on the health impacts associated with mycotoxins. This study focused on the prevalence of aflatoxins and fumonisins in baby food products as they are the most studied groups of mycotoxins so far highly associated with child growth impairment (IFPRI, 2012).

Matumba et al. (2009), in a study on the natural occurrence of aflatoxin in maize in Malawi, reported that 45.3% of the maize samples collected and analyzed were detected with AFB1 and of these, 12.3% exceeded the FAO acceptable limit of 5 µg/kg. Several studies have also reported significantly high levels of aflatoxins and fumonosins in Malawi's crops, grain and cereal-based foods (Matumba et al., 2014; Monyo et al., 2009; Chiona et al., 2014). Aflatoxins have been a major concern as they have been discovered to cause a number of adverse health effects in infants. Due to their carcinogenic nature, they damage internal organs by altering the DNA and suppressing the immune system of the developing bodies of infants. Furthermore, aflatoxins are associated with childhood cancers, brain disorders, dysfunction of the central nervous systems, and stunting, a major concern of child health and development in Malawi (Hendrickse, 1984; Department of Nutrition, HIV and AIDS, 2009; UNICEF/WHO/World Bank Group Joint Child Malnutrition Estimates, 2011).

1.2 Problem Statement

About 15% of Malawi's population are children under 5years of age (Ndawala, 2019) . This age group is more susceptible to acute and chronic illnesses that cause lifetime suffering, impaired growth, disabilities and worse still premature deaths. Malawi has high infant and under-5 mortality rates of 42 and 63 deaths per 1,000 live births, respectively. Over half of these deaths are linked to under nutrition, particularly stunting, which remains prevalent in Malawi. The 2015-2016 Malawi Demographic and Health Survey reported the

prevalence of stunting at 37%, with the highest rates in Neno and Mangochi districts at 45% and the lowest in Likoma at 25% (National Statistics Office, 2016). For years, under nutrition has been blamed on poverty, HIV/AIDS, food insecurity, and poor healthcare, water, and sanitation services. However, global research suggests that aflatoxins and fumonisins are acutely and chronically toxic to children's health and strongly associated with growth impairment (stunting), although the biological mechanism of their action is not yet fully understood (Matumba et al., 2014a; Jiang, 2008; Njobeh et al., 2010; MAPAC, 2013; Okong & Ohingo, 2004; Mottaghianpour et al., 2016; IFPRI, 2012).

About 40% of infants in Malawi are introduced to supplementary feeding from the ages of 4-5 months, with fruits, vegetables, and cereal-based porridge as the primary food supplement (National Statistics Office, 2016). Most of the porridge products are made from raw materials that are prone to pre- and post-harvest aflatoxin and fumonisin contamination.

The prevalence of aflatoxin and fumonisin contamination in Malawi's maize and groundnut crops has led to the collapse of the country's groundnut trade, raising concerns about the public health implications of this situation (MAPAC, 2013). Researchers have been studying the incidence of these toxins in various cereals, grain-based crops and products, and other food staples to investigate the associated health implications and guide the development of viable interventions. Several studies, including those by Matumba et al., 2009, 2011, 2014a, 2014b; Chiona et al., 2014; Monyo et al., 2012; Mwalwayo & Thole, 2016 indicate that aflatoxin and fumonisin levels in Malawi's cereals, grains, staples, and grain-based food products often exceed the maximum acceptable limits set by FAO and WHO. This suggests that a considerable percentage of Malawian children are exposed to these toxins at a young age during the introduction of supplementary feeding programs.

This study aimed to evaluate the levels of aflatoxin and fumonisin contamination in various baby porridge products provided to children during supplementary feeding programs in Malawi, with focus on the different agro ecological zones in Malawi. The study sought to generate data on the incidence of these toxins in locally processed household-level porridge

products made from different cereals and grain raw materials. The study serves as an empirical assessment of child health and development regarding aflatoxin and fumonisin contamination in Malawi, and its results should guide the general public and decision-makers in taking appropriate action to combat these toxins and protect infants and children from their harmful effects. (MAPAC, 2013)

1.3 Justification and Significance of the Study

The high prevalence of aflatoxins in Malawi's agricultural cash crops particularly maize and groundnuts has played a central role in the collapse of Malawian groundnut trade. This raises an alarm for researchers and scientists to conduct further studies on the public health implications of this situation in Malawi (MAPAC, 2013). Researchers are being urged to examine the frequency of aflatoxins and fumonisins in various cereal and grain-based crops, investigate their associated health effects, and establish effective interventions to address the aflatoxin and fumonisin issue. Specifically, this study generates data on the presence of aflatoxins and fumonisins in both local and commercial baby porridge products available in Malawi. The focus will be on districts with high, moderate, and low stunting rates according to the 2015-2016 Malawi Demographic and Health Survey. The study quantified the levels of aflatoxins and fumonisins in different baby porridge products made from various cereals and grains. The study empirically assessed the impact of aflatoxins and fumonisins on child health and development in Malawi. The findings will assist the general public and decision makers in taking appropriate action to combat these toxins and protect infants and children (MAPAC, 2013).

1.4 Objectives of the Study

1.4.1 Main Objective

The main objective of the study was to examine the occurrence and risks associated with aflatoxins and fumonisins in locally processed baby porridge flour in Malawi.

1.4.2 Specific Objectives

The specific objectives were to:

i. Assess the caregiver's state of knowledge, attitudes and practices (KAP) regarding aflatoxins and fumonisins in Malawi
ii. Analyze the concentration and distribution of aflatoxin and fumonisin in different Agro Ecological zones in Malawi
iii. Determine the association between mycotoxin exposure, demographic and socioeconomic factors and stunting.
iv. Determine the risk associated with aflatoxin and fumonisin dietary exposure amongst 6–24-month-olds in Malawi

1.5 Research Questions

The study was guided by the following Research Questions:

i. What is the level of knowledge, attitudes, and practices (KAP) regarding aflatoxins and fumonisins among caregivers in Malawi?
ii. What is the distribution and concentration of aflatoxin and fumonisin in different Agro Ecological zones in Malawi?
iii. Is there an association between mycotoxin exposure, demographic and socioeconomic factors, and stunting?
iv. What is the risk associated with aflatoxin and fumonisin dietary exposure among 6-24 month old infants and children in Malawi?

1.6 Research Hypotheses

i. Caregivers in Malawi have limited knowledge of aflatoxins and fumonisins, which could contribute to the high prevalence of exposure to these toxins among infants and children.
ii. The concentration and distribution of aflatoxin and fumonisin in different Agro Ecological zones in Malawi may vary due to differences in agricultural practices and climate
iii. Exposure to aflatoxin and fumonisin is associated with an increased risk of stunting among 6-24 month old infants and children in Malawi, and this relationship may be influenced by demographic and socioeconomic factors.
iv. Infants and young children in Malawi are at high risk of exposure to aflatoxin and fumonisin through contaminated food, and this exposure is associated with adverse health outcomes.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This literature review focuses on the global studies conducted to understand aflatoxins and fumonisins, with a special emphasis on Africa and Malawi. The purpose is to present the history and research findings related to the human and animal health impacts, as well as the socio-economic impacts associated with these mycotoxins since their discovery in the 1960s and 1980s, respectively. The review also focuses on the impact of aflatoxins and fumonisins on child health and development. In addition, literature on the control measures established to combat these mycotoxins is also discussed.

Aflatoxins and fumonisins remain a significant focus for researchers, as they are found abundantly in the environment and contaminate a wide range of food and feed products. Their hepatotoxic and carcinogenic nature raises important questions about food security, trade, animal, and human health. These fungal infections of agricultural commodities are inevitable, and no region in the world is free from the problem of aflatoxins and fumonisins. (Partnership for Aflatoxin Control in Africa, 2016).

Developed countries are better equipped to identify and isolate fungal-contaminated crops from their food chain, thus reducing the overall aflatoxin burden. Strict feed and food quality control programs play a significant role in this reduction. However, developing nations are still hard hit by aflatoxins and fumonisins, mainly due to hot and humid favorable climatic conditions for fungal

Synthesis. In addition, the lack of suitable regulation against mycotoxins is another contributing factor to the high prevalence of aflatoxin and fumonisin contamination in these nations. (Makun et al., 2011).

Fungal infections in agricultural crops are a widespread issue in many developing countries, where they make up a significant part of the daily diets of numerous people, including children in both urban and rural settlements. This problem is further compounded by food shortages, forcing many individuals to consume grains contaminated with molds that produce aflatoxins due to inadequate pre-harvest and post-harvest practices. Although virtually all agriculturally-based food and feed products are susceptible to fungal infections, the extent of aflatoxigenic fungal contamination will vary depending on whether the product contains aflatoxin or not. As a result, it is crucial to quantify these suspected toxins in various foods and feed products, especially in developing countries such as Nigeria, India, Pakistan, Bangladesh, and Kenya, where the problem is particularly prevalent. Addressing mycotoxins is critical in ensuring food safety and protecting public health in these countries. (Bankole & Adebanjo, 2003).

2.2 Knowledge, Attitudes and Practices on Aflatoxins and Fumonisins

Aflatoxins and fumonisins are mycotoxins produced by fungi that commonly contaminate food crops, especially maize, peanuts, and other nuts. Exposure to these mycotoxins can have serious health consequences, including liver cancer, immune suppression, and developmental delays in children.

Knowledge, Attitudes and Practices (KAP) on Aflatoxins and Fumonisins

The knowledge, attitudes, and practices (KAP) of individuals regarding aflatoxins and fumonisins can have a significant impact on their exposure to these toxins.

Several studies have examined the KAP of individuals regarding aflatoxins and fumonisins in developing countries, where mycotoxin contamination is a significant public health concern. A study by Mensah et al. (2018) in Ghana found that although the majority of participants were aware of the presence of fungi in food, only a small proportion knew

about the specific mycotoxins that can be produced by these fungi. Additionally, while many participants recognized the potential health risks of mycotoxin exposure, they did not take adequate measures to prevent contamination, such as drying crops before storage. A similar study by Muzhingi et al. (2014) in Zimbabwe found that while participants were aware of the risk of mycotoxin contamination, they lacked knowledge of specific prevention methods. The study also found that participants had poor practices regarding food storage and handling, which could contribute to mycotoxin contamination.

In contrast, a study by Sylla et al. (2018) in Mali found that while many participants were not aware of the health risks associated with mycotoxin exposure, they had good knowledge of prevention methods, such as drying crops before storage. However, the study also found that participants faced significant barriers to implementing these prevention methods, such as lack of resources and cultural beliefs regarding food storage.

In a study conducted by Kamala et al. (2020) in Malawi, the researchers aimed to assess the level of knowledge, attitudes, and practices of caregivers regarding mycotoxins. The study was conducted in the rural districts of Balaka and Machinga in Southern Malawi, and involved 401 caregivers of children aged 6-23 months. The study used a structured questionnaire to collect data on caregivers' knowledge, attitudes, and practices regarding mycotoxins.

The findings of the study showed that only 30% of caregivers had heard of mycotoxins, and only 9% were aware of the health effects of mycotoxin exposure. Additionally, only 21% of caregivers knew that mycotoxin contamination can occur during storage and handling of food, and only 6% knew that mycotoxins can be harmful to young children. The study also found that a majority of caregivers (84%) stored their baby porridge flour in non-airtight containers, and only 27% stored the flour in a dry place.

Another study conducted in Malawi by Makoka et al. (2016) aimed to assess the level of knowledge and practices of caregivers regarding mycotoxins and aflatoxin contamination in maize grain. The study was conducted in the rural district of Dedza in Central Malawi,

and involved 300 caregivers of children aged 6-59 months. The study used a structured questionnaire to collect data on caregivers' knowledge and practices regarding mycotoxins. The findings of the study showed that only 29% of caregivers were aware of aflatoxin contamination in maize grain, and only 9% knew that aflatoxin can be harmful to young children. Additionally, only 28% of caregivers knew how to identify aflatoxin-contaminated maize, and only 26% knew how to prevent aflatoxin contamination during storage. The study also found that a majority of caregivers (60%) stored their maize in non-airtight containers, and only 17% stored the maize in a dry place.

Similar findings were reported in a study conducted by Mwale et al. (2019) in Malawi. The study included 300 caregivers of children aged 6-23 months, and found that only 24% of caregivers were aware of mycotoxins, and only 3% knew about the health effects of mycotoxin exposure. The study also found that the majority of caregivers did not take any measures to prevent mycotoxin contamination, such as drying maize before storage or discarding moldy food.

A study by Muñoz et al. (2021) investigated the KAP of aflatoxins and fumonisins among maize traders in Malawi. The study found that traders had limited knowledge about mycotoxins and their associated health risks, but generally had good practices in place to prevent contamination, such as drying maize before storage. The study suggests that targeted education and training programs could be effective in improving trader knowledge and practices.

A study by Wambura et al. (2019) assessed the KAP of aflatoxins among smallholder farmers in central Malawi. The study found that farmers had good knowledge of aflatoxins and their associated health risks, but lacked knowledge of prevention methods. The study suggests that education and training programs that focus on prevention methods, such as crop rotation and drying, could be effective in reducing mycotoxin contamination.

These findings highlight the need for education and awareness-raising campaigns to improve caregiver knowledge and practices regarding mycotoxins. These campaigns

should focus on the sources of mycotoxin contamination in food, how to prevent it, and the health effects of mycotoxin exposure. These campaigns can be conducted through community health workers, radio programs, and other forms of mass media.

Furthermore, a study conducted by Banda et al. (2018) in Malawi found that involving caregivers in the production of safe and nutritious porridge flour can improve their knowledge and practices regarding mycotoxin contamination. The study involved training caregivers on how to produce porridge flour using safe and nutritious methods, and found that caregivers who participated in the training had improved knowledge and practices regarding mycotoxin contamination.

The limited knowledge and poor practices of caregivers regarding mycotoxin contamination in Malawi highlight the need for education and awareness-raising campaigns. These campaigns should focus on improving knowledge and practices regarding mycotoxin contamination in food, the health effects of mycotoxin exposure, and safe and nutritious food production methods. Additionally, involving caregivers in the production of safe and nutritious food can also improve their knowledge and practices regarding mycotoxin contamination.

These studies highlight the importance of educating individuals about the specific mycotoxins that can contaminate food, as well as providing information on effective prevention methods. Education campaigns should also address cultural beliefs and practical barriers that may prevent individuals from adopting safe food handling practices. In addition to education campaigns, interventions to improve food safety can also be effective. For example, a study by Onyango et al. (2018) in Kenya found that using a mycotoxin-binding product in animal feed reduced the levels of aflatoxin in milk, suggesting that interventions at the production level can also help to reduce mycotoxin exposure.

In conclusion, the KAP of individuals regarding aflatoxins and fumonisins can have a significant impact on their exposure to these toxins. Education campaigns and interventions

to improve food safety can be effective in reducing mycotoxin contamination and protecting public health.

2.3 Concentrations and Distribution of Aflatoxins and Fumonisins

Aflatoxins and fumonisins are two of the most important mycotoxins that pose a significant risk to human and animal health. These toxins are produced by fungi, including Aspergillus and Fusarium species, respectively. Aflatoxins and fumonisins are commonly found in food and feed crops, especially in areas with warm and humid climates, where fungal growth is favored (Devegowda et al., 1998). The consumption of aflatoxins and fumonisins contaminated food and feed has been associated with a range of health problems, including liver cancer, growth retardation, immune system suppression, and neural tube defects. Therefore, the concentration and distribution of these toxins are critical factors in assessing the risk to public health. In this literature review, we will examine the current knowledge on the concentration and distribution of aflatoxins and fumonisins in various food and feed commodities, highlighting the major sources of contamination.

Several studies have been conducted to determine the occurrence and distribution of aflatoxins and fumonisins in different food and feed commodities. For instance, studies in Nigeria have reported high levels of aflatoxins in peanuts, maize, and cassava (Oyelami et al., 2021; Ogbonna et al., 2019). Similarly, in India, high levels of aflatoxins have been detected in maize and groundnut samples (Bhat and Rai, 2019). In China, studies have revealed the occurrence of fumonisins in corn-based food products (Chen et al., 2019).
Several factors contribute to the contamination of food and feed with aflatoxins and fumonisins. These include the presence of the fungi in the soil, the climatic conditions, and the agricultural practices used (Bhat and Rai, 2019). Furthermore, improper storage and processing of the crops can lead to fungal growth and toxin accumulation (Devegowda et al., 1998).

The concentration of aflatoxins in food and feed varies widely depending on the geographic location, climate, storage conditions, and agricultural practices. Studies have shown that the concentration of aflatoxins in crops is highest in warm and humid climates, such as

sub-Saharan Africa, Southeast Asia, and parts of South America (Pittet, Wild, Montet, & Hsieh, 2021). Many studies have been conducted to investigate the concentration and distribution of aflatoxins and fumonisins in different regions and crops.

One such study was conducted by Muhimbula et al. (2020) in Tanzania to assess the levels of aflatoxins and fumonisins in maize-based foods. The study analyzed 119 maize samples from three regions in Tanzania and found that all samples were contaminated with aflatoxins and fumonisins. The mean levels of aflatoxins and fumonisins were 5.69 µg/kg and 1.58 mg/kg, respectively. The study highlights the need for effective control measures to reduce the contamination of maize with these mycotoxins in Tanzania.

Another study conducted by Kimanya et al. (2016) in Tanzania analyzed 300 maize samples from seven regions and found that all samples were contaminated with aflatoxins, with mean levels of 29.0 µg/kg. The study also found that 97.7% of the samples were contaminated with fumonisins, with mean levels of 1.12 mg/kg. The authors suggest that the high levels of mycotoxins in maize in Tanzania are due to poor post

Amare et al. (2020) conducted a study in Ethiopia to assess the contamination levels of aflatoxins and fumonisins in maize and groundnut samples. The samples were collected from different regions of the country and analyzed using high-performance liquid chromatography (HPLC). The study found that 60% of the maize samples and 35% of the groundnut samples were contaminated with aflatoxins, with concentrations ranging from 0.6 to 442.4 µg/kg. Fumonisins were detected in all maize samples, with concentrations ranging from 0.2 to 10.5 mg/kg, while only 20% of the groundnut samples were contaminated, with concentrations ranging from 0.1 to 1.5 mg/kg. The study highlights the need for proper crop management practices to reduce the risk of mycotoxin contamination in Ethiopia.

Another study by Akande et al. (2019) was conducted in Nigeria to determine the occurrence and levels of aflatoxins and fumonisins in maize and soybean samples. The samples were collected from six different states in the country and analyzed using enzyme-

linked immunosorbent assay (ELISA). The study found that all the maize and soybean samples were contaminated with aflatoxins, with concentrations ranging from 0.15 to 216 µg/kg and 0.08 to 177 µg/kg, respectively. Fumonisins were detected in 92% of the maize samples, with concentrations ranging from 0.3 to 4.4 mg/kg, and in 73% of the soybean samples, with concentrations ranging from 0.1 to 1.9 mg/kg. The study highlights the need for improved storage facilities and proper crop management practices to reduce mycotoxin contamination in Nigeria.

In a study conducted by Elzupir et al. (2020) in Sudan, the concentration and distribution of aflatoxins and fumonisins in sorghum and maize samples were investigated. The samples were collected from different states in the country and analyzed using HPLC. The study found that all the sorghum samples were contaminated with aflatoxins, with concentrations ranging from 1.4 to 53.9 µg/kg, while 60% of the maize samples were contaminated, with concentrations ranging from 2.2 to 28.7 µg/kg. Fumonisins were detected in 80% of the maize samples, with concentrations ranging from 0.1 to 1.7 mg/kg, while only 20% of the sorghum samples were contaminated, with concentrations ranging from 0.1 to 0.2 mg/kg. The study highlights the need for improved crop management practices and post-harvest handling to reduce mycotoxin contamination in Sudan.

One such study was conducted in Egypt in 2019 by Ahmed et al., who investigated the levels of aflatoxins and fumonisins in maize grains harvested from different regions. The researchers used high-performance liquid chromatography (HPLC) to analyze the samples and found that all the samples tested positive for both toxins, with concentrations ranging from 1.3 to 164.3 µg/kg for aflatoxins and 7.6 to 239.9 µg/kg for fumonisins. The study was done to provide information on the prevalence and distribution of these toxins in maize grains in Egypt and to raise awareness about the potential health risks associated with their consumption.

In another study, conducted in Mexico in 2018, Cortés-Ramírez et al. analyzed the presence of aflatoxins and fumonisins in different agricultural products, including maize, chili, peanuts, and sesame seeds, using HPLC. The researchers found that all the samples tested

positive for both toxins, with the highest concentrations found in peanuts and sesame seeds. The study aimed to provide updated information on the occurrence of these toxins in agricultural products in Mexico and to identify potential sources of exposure.

The studies discussed in this literature review demonstrate the widespread occurrence of aflatoxins and fumonisins in various crops in different regions of the world. The findings emphasize the need for proper crop management practices, improved storage facilities, and post-harvest handling to reduce the risk of mycotoxin contamination and ensure food safety.

Aflatoxins and fumonisins are still considered to be significant food safety concerns, particularly in developing countries where food storage and processing technologies may be inadequate (Gnonlonfin et al., 2013). In a study conducted in Nigeria, over 90% of maize and groundnut samples were found to be contaminated with both aflatoxins and fumonisins (Ezekiel et al., 2013). Similarly, in Kenya, high levels of contamination with both toxins were found in maize samples collected from various regions (Wakhisi et al., 2017).

Despite efforts to reduce mycotoxin contamination in food and feed, including the implementation of Good Agricultural Practices and Good Manufacturing Practices, aflatoxins and fumonisins continue to pose a significant threat to human and animal health (Bankole and Adebanjo, 2003). Aflatoxin contamination has been linked to increased risk of liver cancer, immune suppression, and stunted growth in children, while fumonisin contamination has been associated with neural tube defects and esophageal cancer (Wu et al., 2017).

Research has shown that climate change may exacerbate mycotoxin contamination in crops, as drought and extreme temperatures can promote fungal growth and toxin production (Magan and Medina, 2016). In addition, changes in agricultural practices and the increased use of genetically modified crops may have unintended consequences on mycotoxin contamination levels (Alberts et al., 2020).

Overall, continued efforts to monitor and control mycotoxin contamination in food and feed are necessary to protect public health and ensure food security. Improved storage and processing technologies, as well as the development of resistant crop varieties, may be key strategies in reducing mycotoxin contamination in the future.

Grains and cereals contribute significantly to the total energy intake in Africa, particularly in rural areas, where they are the main sources of nutrition (International Development Research Centre, 2016). However, this overdependence on grains and cereals has resulted in high levels of aflatoxin and fumonisin contamination in these commodities. Environmental factors, such as high temperature and humidity, promote the growth of molds that produce these toxins in agricultural crops, including maize, groundnuts, tree nuts, rice, millet, wheat, sorghum, sunflower seeds, and cottonseed (Mukanga, Derera, & Tongoona, 2015). Climate change is also believed to contribute to the incidence of mycotoxin contamination globally (Paterson & Lima, 2010).

Soil type is another factor that influences the occurrence of aflatoxins and fumonisins in crops. For example, groundnuts grown in sandy soils have been found to support rapid synthesis of these toxins due to the warm and dry characteristics of the soil, while those cultivated in heavier soils experience less contamination due to the soil's high water holding capacity, which inhibits fungal growth (Codex Alimentarius Commission, 2004).

Direct contamination occurs when products are infected with aflatoxigenic and fusarium fungi, resulting in toxin synthesis, while indirect contamination occurs when aflatoxin and fumonisin remain in the final food or feed product even after fungi have been removed or killed during processing. This indirect contamination is the most common point of entry of mycotoxins in the human and animal diet through cereals and oilseeds consumed worldwide (Smith & Moss, 1985; Wu, Duan, & Zhang, 2020).

2.3.1 Aflatoxins

For many years, *Aspergillus Flavus* and *Parasiticus* were considered the only species capable of producing aflatoxin until 1987 when Kurtzman et al. reported that *Aspergillus*

Nomius, a fungus closely related to *A. Flavus*, was also aflatoxigenic (Kurtzman et al., 1987). Since then, several more aflatoxin-producing fungi, such as *A. Parvisclerotigenus, A. Rambellii, A. Bombycis, A. Ochraceoroseus, A. Pseudotamari,* and *A. Tamarii*, have been discovered (Ito et al., 2001; Peterson et al., 2001). However, as compared to *A. Flavus* and *A. Parasiticus*, most of these other species are less copious in nature and rarely encountered in agriculture. Therefore, it is generally concluded that most of the aflatoxin that contaminates foods and feed products largely originate from *Aspergillus Flavus* and *Aspergillus Parasiticus* (Atehnkeng et al., 2008; Essono et al., 2009; Njobeh et al., 2010; Makun et al., 2012).

There are at least 14 different types of aflatoxins produced in nature; however, the main types are aflatoxin B1, B2, G1, G2, M1, and M2. Aflatoxin B1 is the major toxin produced by *Aspergillus Flavus* and *Aspergillus Parasiticus* that contaminates a variety of grain-based food products and animal feeds. It is also considered the most toxic type of aflatoxin as it is two times more carcinogenic than an equitoxic dose of X-rays (Galvano et al., 2005). Aflatoxin B1 is highly implicated in hepatocellular carcinoma in humans and has proved in many studies to be mutagenic, teratogenic, and genotoxic to both humans and animals (Matumba et al., 2014). Aflatoxin B2 is also produced by *A. Flavus* and *A. Parasiticus*. Aflatoxin G1 and G2 are exclusively produced by *A. Parasiticus*, while aflatoxins M1 and M2 are hydroxylated forms of AFB1 and AFB2, respectively, that are excreted in the tissues and fluids of animals and humans as metabolites after the consumption of aflatoxin-contaminated food and feedstuff (Geiser et al., 2000). Food contaminated with AF is imperatively considered unsafe for human and animal consumption as no animal species is immune to the toxic health hazards of aflatoxins. The increasing awareness of risks associated with aflatoxin exposure to humans and agricultural animals that has been ongoing over the years since their discovery in 1960, has opened a new avenue for researchers to conduct surveys on the prevalence and levels of aflatoxins in various food and feedstuffs that are generally consumed worldwide. Most studies have proved that aflatoxins occur all over the world in commodities used for production of food and agricultural animal feeds such as maize, peanuts, cottonseed and coconut cake groundnut cake, maize, cottonseed cake and other grains. Although A. Flavus occurs worldwide, the

warm and humid regions of Africa have been marked as areas with greater incidence of aflatoxin contamination of foods and feedstuff for human and animal consumption respectively. (Devegowda et al., 1998).

The results from the monitoring program on mycotoxin contamination that was conducted by FAO, WHO and UNEP in 1976-1983 suggested that most of the grains that were sampled for analysis, contained aflatoxins above 20-50 ppb which is higher than the acceptable limits in feeds in Most Countries. (Jelinek et al., 1989). Multitudes of studies have been conducted by researchers worldwide at both national and regional level to further investigate the occurrence of aflatoxins in food and feed products so as to better understand their effects on the biophysical and socio-economic environment. These studies have described a variety of general adverse human health effects associated with Aflatoxicosis such as increased risks of liver cancer in people with hepatitis B, kidney failure, impaired digestion, reduced sperm count and infertility, stunted growth and delayed development in children and immune suppression which increases the viral load in HIV positive patients (Jolly, et al., 2013). Worse still, acute Aflatoxicosis may result into premature deaths. (Ogobo & Ugbogu, 2016).

Several studies have been conducted to assess the impact of aflatoxin contamination on the economic development of countries that mainly rely on the export of agricultural commodities. Matumba et al. (2014b) found that groundnuts on the Malawi local market had distinctly high levels of aflatoxins, affecting the quality of this export commodity as a source of revenue for the nation (Matumba et al., 2014b). In another study on natural occurrence of aflatoxin B1, Matumba et al. (2009) reported a general widespread of aflatoxin contamination in Malawi, where 45.3% of the maize samples analyzed contained aflatoxin B1, of which 12.3% exceeded the FAO acceptable limit for human consumption (5 µg/kg). (Matumba et al., 2009). The study also revealed that traditional methods of maize flour preparation contributed to the reduction of aflatoxin B1 content in the final flour product for consumption as Nsima or Porridge. Matumba et al. (2011; 2014a) found that the final traditional beer products locally produced in Malawi exposed consumers to AFB1 and AFB2 ranging from 29-174 µg/kg body weight per day, which is above the

permissible limit set by FAO and WHO, marking opaque beer to pose high risks of aflatoxin exposure in Malawi.

Several researchers have quantitatively determined exceedingly high levels of aflatoxins in peanut butter, grain, and cereal-based products focusing on both local and imported goods. Matumba et al. (2014a, 2014b), Ndun'gu et al. (2013), Ngoroge et al. (2016, 2017), and Monyo et al. (2012) reported alarming high levels of aflatoxins present in groundnuts, maize, peanut butter, and cereals locally available in Malawi, Kenya, and Zambia, and those imported around Sub Saharan Africa. Chiona et al. (2014) conducted a study on the occurrence of aflatoxin in processed cassava in Malawi and Zambia as some regions of these countries depend entirely on it as a staple food. The study suggested that the levels of aflatoxin in Malawi's and Zambia's cassava are quite low, implying that consumers are safe from exposure. However, the researchers pointed out the need to conduct further intensive studies on the occurrence of other mycotoxins in cassava.

Razzazi-Fazeli et al. (2004) conducted a survey on the occurrence of aflatoxin in baby food, peanuts, and corn products sold at retail in Indonesia. The results indicated that aflatoxin contamination in most of these food products is serious, with peanut butter as the most consistently contaminated. Startlingly, out of all the baby food products sampled and analyzed in this survey, none were contaminated with aflatoxins. In Iran, a similar study was conducted by Mottaghianpour et al. (2016), where 48 commercial baby food products were analyzed. The results proved that the levels of aflatoxin in the cereal baby foods available in Iran are too high, ranging from 25% to 100% for different cereal-based products. The survey recommended that appropriate action must be taken in Iran.

Many other similar studies have been conducted in different parts of the world to study the occurrence of aflatoxins in various food products consumed globally. The general picture portrayed by most of these research findings points out that aflatoxin contamination is a widespread problem that sees no boundaries.

2.3.2 Fumonisins

Fumonisin was first discovered in South Africa in 1988. It was found after a predominant fungus, *Fusarium verticillioides (F. moniliforme),* was isolated from moldy corn that had been implicated in a field outbreak of equine leukoencephalomalacia (ELEM) back in 1970. This same fungus was also found to be widespread in moldy homegrown corn that was being consumed by people in high-incidence areas of esophageal cancer in South Africa's Transkei region (Walter, 2001).

Fumonisins are mycotoxins produced by various species of *Fusarium* fungi, including *Fusarium verticillioides* (also known as *F. moniliforme*) and *F. proliferatum*. They are found worldwide in corn and other cereals, posing a threat to animal and human health, though they are particularly associated with corn and corn-based foods. Fumonisins B1 (FB1), B2 (FB2), and B3 (FB3) are the most common, with FB1 being the most abundant, accounting for around 70% of total fumonisin contamination (JECFA, 2012).

Fumonisins cause equine leucoencephalomalacia (ELEM), a deadly disease in horses and related species, and porcine pulmonary edema (PPE) in pigs. However, ruminants appear to be relatively resistant to fumonisins, and no diseases have been reported in these animals. The occurrence of fumonisins in maize has been associated with human esophageal cancer in South Africa and China. Codex has set limits of 2-4 mg/kg fumonisins in maize for human consumption and 1-50 mg/kg for various livestock species (FAO; WHO, 2015).

In a study by Prelusky et al. (1996), there was no indication of significant carryover of fumonisin B1 (FB1) or its metabolites in animal products such as milk, meat, and eggs. Toxicokinetic studies in cows also revealed rapid metabolism of FB1 and no residue of the toxin in tissues. Consequently, the Scientific Committee on Food for the European Commission did not find it necessary to monitor fumonisin residues in meat (European Commission, 2000).

2.3.3 Aflatoxin and Fumonisin Regulations

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) collaborate to establish the science and develop risk assessments to determine safe toxin exposure levels. Based on these risk assessments, maximum tolerable limits for aflatoxins and fumonisins in different foods are recommended, forming the basis for national regulations to limit contamination (FAO, 2018).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has conducted toxicological evaluations and dietary exposure assessments of aflatoxins and fumonisins since their discovery in the 1960s and 1980s, respectively (FAO, 2018). These evaluations inform the Codex Alimentarius Commission, which has developed harmonized international food safety standards since 1963, protecting the health of consumers and ensuring fair trade practices (FAO, 2018).

Codex standards set the maximum acceptable limits for contaminants and natural toxins such as aflatoxins and fumonisins in food, serving as a reference for international trade in food and ensuring that consumers worldwide can be confident in the safety and quality of the food they purchase, regardless of its origin. To prevent and reduce the risk of aflatoxins and fumonisins in food and feed, Codex has developed codes of practice outlining preventive measures (FAO, 2018).

WHO gathers food contamination data from nationally recognized institutions through the Food Contamination Monitoring and Assessment Program of the WHO Global Environment Monitoring System (GEMS/Food) to support these efforts. The GEMS/Food database updates governments, the Codex Alimentarius Commission, and other significant institutions, as well as the public, on levels and trends of contaminants in food (WHO, 2018).

Aflatoxin is considered the most intoxicating carcinogen known in mammals (Zinedine & Mañes, 2009), making it crucial to reduce food contamination to the lowest level possible, as no food or health organization has established an acceptable daily intake for humans

(tolerable daily intake, TDI) (Pietri et al., 2010). Exposure to hepatitis B virus infection and aflatoxin increases the risk of liver cancer by up to 30 times compared to the risk in people exposed to aflatoxin only (Udovicki et al., 2018). The risk of exposure to contaminated foods with varying levels of aflatoxins worldwide exists for more than 4.5 billion people (Assaf et al., 2019). Currently, about 100 countries have established levels of AFs in food and feed (Karunarathna et al., 2019). The EU maximum regulatory levels for aflatoxin in processed cereal foods are 0.10-2µg/kg (AFB1) and 4µg/kg (total aflatoxin) (Blankson et al., 2019; European Commission, 2006-2010; Quevedo-Garza et al., 2018).

2.4 Factors Affecting Mycotoxin Exposure

2.4.1 Type of Crop

Mycotoxin contamination can occur in a wide range of crops, but certain crops are particularly susceptible. For example, corn is one of the most commonly contaminated crops due to its susceptibility to fungal growth and mycotoxin production (Zain, 2011). A study by Wu et al. (2021) found that 95% of corn samples in China were contaminated with mycotoxins, with the most commonly detected mycotoxins being aflatoxins and fumonisins.

Peanuts are also highly susceptible to mycotoxin contamination, particularly from aflatoxins. This is due to their high oil content and tendency to grow in warm and humid conditions, which promote fungal growth (Iqbal et al., 2021). A study by Njumbe Ediage et al. (2017) found that 60% of peanut samples in Cameroon were contaminated with aflatoxins.

Wheat is another crop that is susceptible to mycotoxin contamination, with deoxynivalenol (DON) and zearalenone (ZEA) being the most commonly found mycotoxins (Mishra et al., 2019). The prevalence of mycotoxin contamination in wheat can vary depending on the geographic location, climate, and agricultural practices. (Chen et al., 2018).

Certain crops such as corn, peanuts, and wheat are highly susceptible to mycotoxin contamination due to various factors such as environmental conditions, storage, and

handling practices. It is, therefore, crucial to implement measures such as good agricultural practices and monitoring mycotoxin levels to reduce the risk of mycotoxin exposure.

Overall, it is important for farmers and food manufacturers to be aware of the susceptibility of certain crops to mycotoxin contamination and to take appropriate measures to prevent and manage contamination. These measures may include implementing good agricultural practices, monitoring crops for mycotoxin levels, and implementing appropriate storage and handling procedures.

2.4.2 Environmental Conditions

Environmental conditions play a significant role in the growth of fungi and the production of mycotoxins. Temperature, humidity, and rainfall are the primary factors that influence the growth of fungi and the production of mycotoxins in crops. Warm and humid conditions, in particular, create favorable conditions for fungal growth and mycotoxin production.

High temperatures and humidity levels increase the rate of fungal growth and enhance the production of mycotoxins in crops. For example, a study by Wu et al. (2021) found that the incidence of mycotoxin contamination in corn in China was higher in areas with high temperatures and high humidity levels. Similarly, a study by Ostry et al. (2017) showed that high temperatures and humidity levels in the Czech Republic contributed to the growth of Fusarium fungi and the production of mycotoxins in wheat crops.

Rainfall is another environmental factor that can influence mycotoxin contamination in crops. Excessive rainfall and moisture levels can increase the risk of fungal growth and mycotoxin production in crops. A study by Wacoo et al. (2018) found that heavy rainfall in Uganda contributed to the contamination of maize crops with aflatoxins.

Overall, the environmental conditions in which crops are grown are critical in determining the risk of mycotoxin contamination. To reduce the risk of mycotoxin contamination, farmers should take into account environmental factors when growing crops and implement

appropriate measures to manage the risks associated with high temperatures, humidity, and rainfall.

2.4.3 Geographical Location

The geographical location and climatic conditions of a region can have a significant impact on the occurrence and types of mycotoxins present in food. For example, in tropical and subtropical regions, such as Africa and Southeast Asia, high temperatures and humidity create optimal conditions for fungal growth and mycotoxin production in crops such as maize, peanuts, and cassava (Egal et al., 2005). As a result, these regions have higher incidences of mycotoxin contamination in food than regions with cooler and drier climates (Wu et al., 2019).

In sub-Saharan Africa, maize is a staple food crop, and it is often contaminated with aflatoxins and fumonisins (NSO, 2016). Aflatoxins are commonly found in peanuts, tree nuts, and other oilseeds, while fumonisins are often associated with maize and other cereal grains (Gong et al., 2020). In Asia, rice is a primary food crop, and it is often contaminated with mycotoxins such as aflatoxins and ochratoxins (Li et al., 2018).

Overall, the prevalence and types of mycotoxins in food can vary widely depending on the geographical location and the type of crop grown in that region. This highlights the need for region-specific monitoring and management strategies to minimize the risks of mycotoxin exposure in food.

2.4.3.1 Malawi Agro Ecological Zones

Malawi has three distinct agro-ecological zones (AEZs) based on differences in rainfall, temperature, and soil factors. The Lower Shire valley, Lakeshore plains and Upper Shire valley, mid-altitude plateau and highlands make up these zones. Each AEZ has its own unique climatic and environmental conditions, which influence agricultural production practices, crop yields, and the incidence of mycotoxins.

The highlands of Malawi are situated in the northern and central parts of the country and are characterized by high altitude, cool temperatures, and high rainfall. These conditions are suitable for growing crops such as maize, beans, and potatoes. However, high humidity and rainfall in this region also create favorable conditions for fungal growth and mycotoxin contamination. Studies have shown that the highlands of Malawi are at high risk of aflatoxin contamination, particularly in maize (Jolly et al., 2016).

The central region of Malawi is located in the lower altitude areas of the country and has a sub-tropical climate. This region experiences moderate rainfall and is ideal for growing crops such as maize, tobacco, and legumes. The incidence of mycotoxin contamination in this region is lower than in the highlands, although contamination levels can still be high under certain climatic conditions (Chilaka et al., 2017).

The southern region of Malawi is situated in the low-lying areas of the country and experiences hot and dry conditions for most of the year. This region is ideal for growing crops such as maize, sorghum, and groundnuts. The incidence of mycotoxin contamination in this region is also relatively low, although contamination levels can still be high under certain climatic conditions (Chilaka et al., 2017).

In summary, mycotoxin contamination levels in Malawi are influenced by various factors, including agro-ecological zones, climate, and crop production practices. While the incidence of mycotoxin contamination may be lower in certain regions of the country, such as the southern region, there is still a need to monitor and control mycotoxin contamination in all regions to protect human health and promote food safety.

2.4.4 Planting and Pre-Harvest Practices

2.4.4.1 Early Planting

Early planting has been shown to reduce the occurrence of aflatoxins and fumonisins in harvested maize crops compared to late planting. In their respective studies, Abbas et al. (2007) and Jones et al. (1981) found that maize planted in April contained lower levels of aflatoxin and fumonisin compared to that planted in May. Similarly, Lillehoj et al. (1978)

found that maize planted in June had higher aflatoxin levels than that planted in April and May. Parsons and Munkvold (2012) and Madege et al. (2019) also reported that early planting reduces aflatoxin and fumonisin contamination.

In North Carolina, research showed lower levels of aflatoxin B1 contamination in maize produced by April plantings compared to May plantings (Jones et al., 1981). Lillehoj (1978) conducted experiments in Florida and Georgia, reporting high levels of aflatoxin B1 in maize planted in June compared to that planted in April and May.

2.4.4.2 Timely Harvesting

Harvest is one crucial stage in the food production sector where moisture content must be observed. It is recommended that crops must not be harvested too late or too early so as to prevent the moist pathogen synthesis conditions (López-García et al., 1999). Optimal harvest time is a necessary factor as it ensures that crops are not overexposed to unfriendly environmental conditions. In general, it is recommended that maize grains must be harvested after they have attained physiological maturity and then artificially dried to a moisture content of below 13% for safe storage. Bankole et al. (2003) also discovered that early harvesting reduces fungal infections of crops in the field and their subsequent contamination during storage as opposed to late harvesting. (Zuza et al., 2016).

2.4.4.3 Crop Rotation

Atukwase et al. (2016) found that crop rotation is suggestively related to the synthesis of aflatoxins in maize, such that it facilitates the growth of mycotoxigenic molds, and care must be taken to avoid rotating crops that can promote contamination. Alakonya et al. (2008) also reported on the association between crop rotation and aflatoxin levels in maize. The USDA Multi-Crop Aflatoxin Working Group conducted an experiment in South Texas to study the impact of crop rotation on aflatoxin contamination. This study concluded that crop rotation generally influences the magnitude and composition of A. flavus communities in different ways. On average, the results revealed that A. flavus colony-forming units were higher in fields where the previous crop was corn as compared to where the previous crop was either cotton or sorghum (Cotty et al., 2006).

2.4.4.4 Biological Control

One of the most promising and potential strategies to mitigate mycotoxin contamination is biological control. A biological control technique greatly reduces aflatoxins in all susceptible crops in a cost-effective manner. This method is by far one of the most successful interventions in mitigating the aflatoxin crisis at the global level. Biocontrol uses native strains of A. flavus that do not produce aflatoxins to competitively eliminate aflatoxin-producing strains from the crop environment. This has been successfully implemented in Southern US, Nigeria, Northern Mexico, and West Africa under the commercial names AF36, Aflasafe, and AflaGuard (Donner et al., 2010; Atehnkeng et al., 2016).

2.4.4.5 Use of Resistant Varieties

Abbas et al. (2013) conducted a study which concluded that certain yellow maize hybrids with high yields provide resistance to Aspergillus and aflatoxin. However, commercial hybrid crops are not readily available to most local communities, especially in Africa.

2.4.5 Post-Harvest Practices

Post-harvest practices refer to the activities that take place after crops are harvested, such as milling. Good practices include rapid crop drying on surfaces that avoid contact with soil, proper shelling methods to prevent contamination, dehulling of maize before milling, hand sorting to separate molded grains, use of clean and aerated storage structures, preventing insect damage, employing good means of transportation, and avoiding long storage periods (Fandohan et al., 2003). All of these practices help to reduce aflatoxin and fumonisin contamination in agricultural crops to varying extents.

Harvesting methods greatly influence the incidence of mycotoxin contamination in agricultural crops. The general recommendation for controlling aflatoxin and fumonisin occurrence in maize is to harvest at a moisture content ranging from 25% to 20% and then artificially dry it to 15.5% for storage (FDA, 2021). Setamou et al. (1997) reported that fungal growth and mycotoxin production can occur in just a matter of days in improperly dried and cooled maize grain before storage. During transportation, it is also recommended

that grains must be at a safe moisture and temperature, especially if in transit for days. In most rural parts of Africa, grain-drying facilities are rare or almost non-existent, so many farmers rely on field drying to obtain a grain moisture level that is safe enough for handling and storage. The time required to achieve the safe moisture content of 15.5% grain also depends on the weather but will generally take from 14 to 28 days after grain physiological maturity (Bruns & Abbas, 2001). Vincelli and Parker (2001) cautioned that farmers must also avoid over-drying their maize grains, as overly dry grains are subject to kernel breakage during harvesting. Broken grains are subject to fungal infection, as the surface of the kernel's germ becomes more exposed. Research has shown that damage to the germ greatly increases the development of molds in maize grains during storage (Seitz et al., 1982; Tuite et al., 1985).

2.5 Risks Associated with Aflatoxin and Fumonisin Dietary Exposure

Aflatoxins alone can cause up to 25% damage to the world's food crops, leading to economic losses in developed countries and serious health implications in underdeveloped nations (WHO Food Safety Digest, 2018). Humans and animals are exposed to these toxins when contaminated agricultural commodities are consumed, and farmers, agricultural, and industrial workers may inhale the dust generated during the processing of contaminated crops (Flamatico & Pina, 2008). Aflatoxins are amongst the most carcinogenic substances in nature, classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC) (IARC, 2009). Children are particularly vulnerable to aflatoxin exposure, resulting in serious conditions collectively referred to as aflatoxicosis (Abbas, 2005; Hudler, 2000), leading to cancer, stunted growth, immune suppression, and other extreme conditions. Farmers in the poultry and animal husbandry businesses suffer significant losses from aflatoxin exposure, including the death of livestock, reduced growth rates, immune system suppression, and loss of feed efficiency (AU, 2016).

Fumonisin disrupts sphingolipid metabolism and inhibits folate transport, leading to fetal neural tube defects and potential links to esophageal cancer (Walter et al., 2004; Norred & Voss, 1994; Rheeder et al., 1992). Fumonisin exposure in animals can cause fatal diseases, such as Equine Leukoencephalomalacia (ELEM) and Porcine Pulmonary Edema (PPE)

(Norred & Voss, 1994). Therefore, it is crucial to monitor and control aflatoxin and fumonisin contamination in agricultural products, particularly in infant and baby porridge, to reduce the risk of adverse health effects in humans and animals.

The most vulnerable population to aflatoxin exposure is infants and young children, particularly those in low-income countries. The World Health Organization (WHO) estimates that up to 155,000 deaths occur annually due to aflatoxin exposure in Africa alone (WHO Food Safety Digest, 2018). In addition, long-term exposure to aflatoxins has been linked to liver cancer, immune suppression, and stunted growth in children (Gong et al., 2020; Mofid et al., 2020).

In recent years, several studies have been conducted to investigate the occurrence of aflatoxins and fumonisins in baby porridge. For instance, a study in Kenya found that 39% of baby porridge samples collected from retail outlets were contaminated with aflatoxins, with levels ranging from 0.5 to 51.8 µg/kg (Ngure et al., 2020). Similarly, a study in Nigeria reported that 48.6% of baby porridge samples tested positive for aflatoxins, with levels ranging from 0.10 to 1.35 µg/kg (Oladipo et al., 2018).

To mitigate the risks associated with aflatoxin and fumonisin exposure, several interventions have been proposed. These include the use of biocontrol agents, such as Aspergillus flavus strains, to prevent aflatoxin contamination in crops (Mehta et al., 2020). Post-harvest interventions such as sorting and cleaning of grains, drying and storage have also been recommended to reduce the levels of aflatoxins and fumonisins in food and feed (Wild et al., 2013).

In conclusion, aflatoxin and fumonisin exposure pose a significant threat to both human health and the economy. The occurrence of these toxins in baby porridge is particularly concerning due to the vulnerability of infants and young children. More research and interventions are needed to mitigate the risks associated with these toxins and ensure food safety for all.

2.5.1 Dietary Exposure Assessment

Probabilistic models have become a popular tool for estimating human exposure to potentially harmful chemicals and toxins via food consumption. These models attempt to calculate intake as accurately as possible by addressing variation in consumption patterns between individuals. The Monte Carlo approach, a widely used probabilistic model, is used to assess dietary exposure to aflatoxin and fumonisin.

In this approach, person-days are randomly selected from a food consumption study database, and the consumption amount of each relevant single food item is multiplied by a randomly selected concentration of aflatoxin or fumonisin available for that food item from a concentration database (Jolly et al., 2014). The resulting exposures per food are added together to find the overall intake for that particular person-day, which is then divided by the individual's body weight to obtain the exposure per kg body weight (Boon & Voet, 2015).

This procedure is repeated many times to generate a frequency distribution that reveals all possible combinations of daily consumptions and concentrations. The procedure is repeated until a stable frequency distribution is obtained, allowing for a more accurate estimation of human exposure to aflatoxin and fumonisin through food consumption (Codex Alimentarius Commission, 2019).

Overall, the use of probabilistic models for estimating human exposure to toxins in food is a valuable tool for ensuring food safety and public health.

2.6 Supplementary Feeding and Mycotoxins

The Malawi government has implemented various interventions to promote adequate nutrition for its populace. One of the interventions is intensive supplementary feeding, which is being promoted by many stakeholders. Traditional supplementary food products, such as porridge made from maize flour and soybean flour, are readily available throughout the country. However, nutrition advisors caution against serving plain porridge to children as it lacks sufficient energy and nutrients for proper growth and development. Instead,

parents and guardians are encouraged to add energy-rich foods, such as oil seeds, peanut butter, margarine, groundnuts, legume flours, cereals, and other grains, to enhance the nutritional value of the porridge. The addition of flours from germinated or fermented cereals, such as rice, is also encouraged to reduce the bulkiness of the porridge. Nutrition and health advisors recommend that parents and guardians mix these ingredients in proportions as advised (USAID, 2011).

Data collected during the 2015-16 Malawi Demographic and Health Survey on infant and young child feeding (IYCF) practices indicate that only 61% of infants under six months of age in Malawi are exclusively breastfed, which is contrary to the recommended practice. Instead, 9% of infants consume plain water, 3% are fed on non-milk liquids, 2% consume other milk, and 18% are fed on complementary foods plus breast milk. Shockingly, 7% of infants under the age of six months are not breastfed at all. As children grow older, the percentage of those who are breastfed declines sharply, from 18% of infants aged 0-1 month to 69% of those aged 2-3 months, and further to 34% of infants aged 4-5 months. By the time most infants reach six months of age, they have already become accustomed to foods other than breast milk. These findings suggest that a larger percentage of infants and children in Malawi are introduced to supplementary feeding too early in life (NSO, 2016).

Maize is the primary ingredient in the diets of children in Malawi, as highlighted by USAID (2011). However, studies have indicated that the agricultural commodities recommended for supplementation, such as maize and groundnuts, are potential substrates for fungal growth and the synthesis of aflatoxin and fumonisin (MAPAC, 2013). This suggests that while addressing malnutrition through supplementary feeding programs, children may be exposed to mycotoxins that have negative health implications. Children, particularly those in their first few years of life, are vulnerable to mycotoxins, as their immature detoxifying capacity, rapid growth, and higher food intake relative to their body mass make them more susceptible (Erkekoglu et al., 2008). Exposure to aflatoxin and fumonisin can have long-term implications on a child's health, including impaired development, immunosuppression, outbreaks of acute toxicity, and reduced response to vaccinations, as

discussed by Sherif et al. (2009). Studies on dietary exposure to aflatoxin and fumonisin among Tanzanian children have revealed that about 82% of children aged 12-22 months are chronically exposed to these mycotoxins through the consumption of contaminated supplementary foods, with maize being the primary ingredient (Candida et al., 2013; NSO, 2016).

Aflatoxins have been a significant concern for several years, and the number of global studies on aflatoxins continues to increase. While thousands of research articles exist on this topic, researchers and scientists are still exploring solutions to the aflatoxin crisis. This review has only presented a fraction of the literature currently available on aflatoxin occurrence and implications globally, but it aimed to provide a comprehensive overview of the current knowledge and proven facts (MAPAC, 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

To achieve the objectives of the study on the occurrence and risks of aflatoxins and fumonisins in locally processed baby porridge flour in Malawi, the research team employed a rigorous methodology. This chapter provides a detailed account of the study design, including the sampling strategy and the sampling size calculation. The sampling design aimed to ensure that the study sample was representative of the population of baby porridge flour produced and consumed in Malawi.

The chapter also describes the sample collection process, including the criteria for selecting the samples, sample collection location, and sample preservation and storage procedures. The sample collection procedure was designed to minimize the risk of contamination and ensure the accuracy of the results.

Furthermore, the chapter outlines the laboratory analysis methods used for the detection and quantification of aflatoxins and fumonisins in the samples. This includes the procedures for sample extraction, clean-up, and detection, as well as the equipment and materials used in the laboratory.

The data analysis section of the chapter describes the statistical methods used to analyze the data obtained from the laboratory analysis. The aim was to identify any patterns or trends in the occurrence of aflatoxins and fumonisins in the locally processed baby porridge flour samples, as well as to assess the associated risks to human health

The comprehensive description of the study methodology provided in this chapter will enable other researchers in the field to replicate the study and build on its findings. The transparency and replicability of the study are crucial in ensuring the reliability and validity of the results obtained, and in contributing to the body of knowledge on the occurrence and risks of aflatoxins and fumonisins in locally processed baby porridge flour in Malawi.

3.2 Study Site

The study site for this research was in Malawi, a country in southeastern Africa that relies heavily on agriculture. To understand the different types of agricultural practices and crops that can be grown in different regions, Malawi's districts are classified into agro-ecological zones based on altitude, temperature, and rainfall patterns, as defined by the Ministry of Agriculture and Food Security (2016). For this study, the focus was on the three agro-ecological zones: the Lower Shire valley, Lakeshore plains and Upper Shire valley, and the mid-altitude plateau and highlands. Each zone has its own unique climatic and environmental conditions, which influence agricultural production practices, crop yields, and the incidence of mycotoxins.

The study was conducted in thirteen districts within these zones, selected for their location and known history of mycotoxin contamination in maize. The Lower Shire valley is characterized by high temperatures and humidity, while the Lakeshore plains and Upper Shire Valley have moderate temperatures and moderate to high rainfall, and the mid-altitude plateau and highlands have moderate temperatures and low to medium rainfall. Most of these districts are predominantly rural areas with a high proportion of smallholder farmers practicing subsistence farming, and the primary crops grown are maize, beans, groundnuts, vegetables, and legumes.

The thirteen selected districts were Nsanje in the Lower Shire valley, Mwanza, Balaka, Mangochi, Blantyre, Machinga, and Neno in the Lakeshore plains and Upper Shire valley, and Mchinji, Dedza, Ntcheu, Lilongwe, Zomba, and Phalombe in the mid-altitude plateau and highlands. **Table 1** and **Figure 1** present the details of their Extension Planning Areas (EPA's). The selection of these districts aimed to provide a representative sample of the different agricultural practices, crop yields, and incidence of mycotoxins within the agro-ecological zones of interest. The data collected from these districts will inform the analysis and discussion of the study findings and contribute to the development of appropriate mycotoxin management strategies in Malawi.

Table 1. Malawi Districts as Classified by their Different Agro-Ecological Zones (AEZ's) and Extension Planning Areas (Ministry of Agriculture and Irrigation, 2005)

Agro Ecological Zone	District	EPA
Lower Shire Valley	Nsanje	Nyachilenda
		Zunde
Lakeshore and Upper Shire Valley	Mwanza	Mwanza
		Thambani
	Balaka	Mpilisi
		Ulongwe
	Mangochi	Mthiramanja
		Ntiya
	Blantyre	Lirangwe
		Ntonda
	Machinga	Nanyumbu
		Nyambi
	Neno	Neno
		Lisungwi
Mid-altitude Plateau and highlands	Mchinji	Mlonyeni
		Mkanda
	Dedza	Chafumbwa
		Kabwazi
	Ntcheu	Sharpevalley
		Bilira
	Lilongwe	Malingunde
		Mlomba
	Zomba	Malosa
		Thondwe
	Phalombe	Naminjiwa
		Kasongo

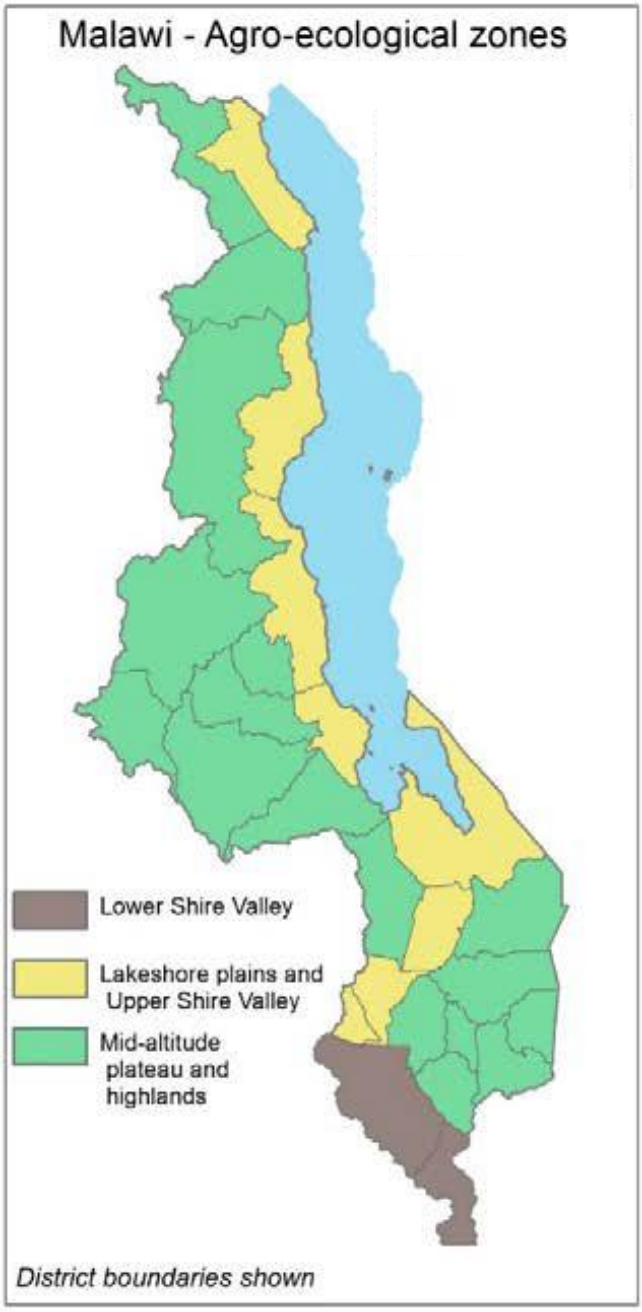

Figure 1 Agricultural Zonation Scheme for Malawi (Benson et al., 2016)

3.3 Sample Size and Sampling Techniques

3.3.1 Sample Size Dertermination

On overall the household sample size of the study was determined by this formula

$$n = \frac{(Z\alpha/2)^2(1-p)p}{c^2}$$ (Taherdoost, 2017)

Where: $Z\alpha/2$ is the critical value of the normal distribution at $\alpha/2$ (with the confidence level set at 95%, α = 0.05 and the critical value = 1.96), **p** is the sample proportion expressed as a decimal (15% expressed as 0.15. This 15% is a representation of the population of under five children in Malawi as reported by the 2018 UNFPA Malawi Population Matters (Ndawala, 2019) **c** is the margin of error expressed as a decimal (set at ±5%, 0.05) and **n** is the Population sample size. Substituting the values in the formula gives a sample size of 196 households from which the survey questionnaire was to be administered and local porridge samples collected. However, the sample size was adjusted to n=338 covering the 13 districts.

3.3.2 Sampling Techniques

The sampling technique used for the KAP study and porridge flour analysis in the laboratory was a multi-stage design that aimed to ensure the selection of representative samples from the study area. The first stage involved the selection of agro-ecological zones. The study area was divided into three zones, each with distinct characteristics in terms of climate, soil type, and vegetation. This was done to ensure that the sample was representative of the study area as a whole.

The second stage involved the selection of districts. 13 districts were systematically drawn with probability proportional to size in each agro-ecological zone. This was done to ensure that the sample included a range of districts that were representative of the study area.

The third stage involved the selection of Extension Planning Areas (EPAs) from each district. Two EPAs were randomly selected from each of the 13 districts. This was done to ensure that the sample included EPAs that were representative of each district. The EPAs are administrative units with similar characteristics in terms of climate, soil, and vegetation.

The fourth stage involved the selection of households. From each of the selected EPAs, 13 households targeting only those with under-five children were randomly selected for the KAP study and the collection of porridge flour samples. The selection of households was carried out with the help of local leaders and community members. This was done to ensure that the sample included households that were representative of each EPA.

The final stage involved the administering of a KAP study and collection of porridge flour samples. From each of the selected households, a KAP study questionnaire was administered and a 500-gram raw porridge flour sample was collected for aflatoxin and fumonisin analysis in the laboratory. The samples were collected using clean, dry, and sealed polythene bags to avoid contamination. This was done to ensure the accuracy of the laboratory results.

In summary, the multi-stage design ensured that the sample was representative of the study area, with districts, EPAs, and households randomly selected to minimize bias in the sample selection process. The collection of porridge flour samples using standard procedures further ensured the accuracy of the laboratory results.

3.3.2.1 KAP Study and Sample Collection

On overall, 338 households from 26 EPA's and 13 districts comprised the study sample size. A questionnaire (Appendix 1) was administered at household level to gather data on the demographic and anthropometric information of under-five children and caregiver, to learn more about the supplementary feeding practices as well as the knowledge, attitudes and practices on aflatoxins and fumonisins. The questionnaire comprised of four main sections covering general household location details, demographics and anthropometric data, supplementary feeding practices and knowledge, attitudes and practices on mycotoxins.

3.3.2.2 Mycotoxin Knowledge, Attitudes and Practices Scores

Mycotoxin knowledge was composed of seven questions, which ranged from basic knowledge on molds to specific knowledge for molds and mycotoxins. Questions included those on occurrence of molds, health implications associated with consumption of moldy grains, molds and child nutrition, agricultural practices to control and reduce molds. Knowledge questions had 30 possible scores such that each respondent would have a maximum score of 30 points and a minimum of zero. The mycotoxin attitudes section was composed of four questions with a maximum of four possible scores and a minimum of zero scores for each respondent. The attitude questions were tailored in relation to attitudes toward molds, mycotoxins and child health. Mycotoxin practices included questions on supplementary feeding practices in relation to molds, mycotoxins and child health. Practices such as sorting of grains, separating moldy fraction and well as food preparation methods. This section was composed of 5questions with a maximum of five possible scores and a minimum of zero scores for each respondent with. A total of 10 points were allocated to nutritional practice. All the KAP questions were scored using a two-point scale (zero = wrong and don't know responses, 1 = correct response) because of multiple correct responses, some questions had more than 1 score.

3.3.2.3 Under 5 Year Olds Anthropometric Measurements

For under 5-year-olds, the crown-heel length was taken using a portable length/height measuring board for children with centimeters as units of measure attached on the board. The weights of the children were measured using a portable digital weighing scale in kilograms. These allowed the measuring of a child's weight while an adult was holding it by use of tare function, (WHO, 2019).

3.3.2.4 Sample Collection for Mycotoxin Analysis

During each household visit, samples of locally processed porridge flour were collected in their raw powder state, with a quantity of 500 grams obtained from each household. The samples once collected were stored in properly sealed polythene sterile bags for not more than 48hrs before aflatoxin and fumonisin analysis in the laboratory. Samples were transported in cool and dry boxes to inhibit further fungi growth which might have

contributed towards the biasness of final results. All laboratory work and proceedings were conducted at the IITA Research Center Microbiology laboratory situated in Lilongwe at Chitedze Research Center along Mchinji road.

3.4 Research Tools and Instruments

Interviews were conducted at household level using a semi-structured questionnaire to gather demographic and anthropometric data of children as well as the socio-economic and knowledge, attitudes and practices of caregivers in relation to aflatoxins and fumunosins**.** The quantification of aflatoxins and fumunosins in the collected porridge flour samples was done using a REVEAL Q+ FOR AFLATOXIN and FUMUNOSIN test kits respectively (NeogenAccuscan Gold Reader).

3.4.1 Assay Principles

Reveal Q+ for aflatoxin and fumonisin are both single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. In the test, the extract is passed through the reagent zone, which contains antibodies specific for toxin conjugated to colloidal gold particles (gold complex). If toxin (aflatoxin or fumonisin) is present, the gold complex will capture it. The gold complex, along with any free gold-complex, is then passed onto a membrane, which contains a zone of aflatoxin or fumonisin conjugated to a protein carrier. This zone captures any unbound gold complex, allowing the particles to concentrate and form a visible line. As the level of toxin in a sample increases, free toxin will bind with the gold complex allowing less gold complex to be captured in the test zones. Therefore, as the concentration of mycotoxin in the sample increases, the test line density decreases.

Algorithms programmed into the readers convert the line intensities into a quantitative result displayed in parts per billion (ppb) for aflatoxin and (ppm) for fumonisin. The membrane also contains a control zone where an immune complex present in the reagent zone is captured by an antibody, forming a visible line. The control line will always form regardless of the presence of aflatoxin, ensuring the strip is functioning properly (Sarver, et al., 2020).

3.5 Materials

3.5.1 Test Kit and Apparatus

To minimize potential sample contamination, gloves and other protective wear were utilized throughout the procedures. The quantification of aflatoxins and fumonisins was conducted using a Neogen AccuScan Gold Reader and the REVEAL Q+ test kits for aflatoxin and fumonisin, respectively (Neogen, 2021a,b). Each kit comprised of test strips for aflatoxin and fumonisin, as well as red and clear sample dilution cups and sample diluents. (Neogen, n.d).

Other apparatus used included; 100µL and 500µL pipettors, sterile 100µL and 200-1000µL pipetting tips, dispensing pumps, graduated cylinders, funnels, filter papers, an Agri-Grind grinder to ensure that at least 95% of the ground material passed through the filter, digital timers for reaction time accuracy, laboratory scales for measuring samples and ethanol for preparation of extraction solvent (Neogen, 2021a,b)

3.6 Analytical Procedures

3.6.1 Sample Extraction

For each sample, 20 g (± 0.2) of ground flour mixture was transferred into a clean disinfected blender, to it, 100mL of the extraction solvent (65% ethanol) was added. The mixture was then carefully blended at low speed for 5 minutes ensuring that the blending container was tightly closed so as not to spill the mixture. Once fully blended and mixed, the mixture was then filtered through a funnel with filter paper into a clean sample collection flask. From this flask 5ml of filtrate was directly transferred by pouring into clean and sterile sample cells labeled with the specific sample codes. Afterwards, the extract was tested for both aflatoxin and fumonisin.

3.6.2 Sample Dilution

The REVEAL Q+ for aflatoxin and fumonisin readers both have a detection limit of 2-100 ppb and 3-6ppm respectively. All samples with concentrations above these upper limits were diluted and tests were re-run. Using pipettors and pipetting tips, 0.1ml of sample filtrate was placed into a sterile sample cell. To the filtrate, 9.9ml of 65% ethanol was added

and mixed thoroughly by pippeting up and down 5 times. Then the diluted sample extracts were labeled as diluents of corresponding sample codes and ready for aflatoxin and or fumonisin testing on the Reveal Q+ AccuScan Gold Reader.

For all diluted samples, the results displayed on the reader were finally multiplied by the dilution factor (DF) = 100, calculations below;

$DF = \frac{Vf}{Vi}$ where Vf= Final Volume and Vi= Initial Volume

Vf = aliquot volume + diluent Volume = (0.1 + 9.9) ml = 10ml

Vi = aliquot volume = 0.1ml

$DF = \frac{10ml}{0.1ml} = 100$

Therefore, the Dilution Factor (DF) is 100

3.6.3 Aflatoxin and Fumonisin Determination

An appropriate number of red sample dilution cups and clear sample cups for each sample were placed into a sample cup rack, well-labeled with sample codes. In each red cup, 100 µL of sample extract was thoroughly mixed with 500 µL of sample diluent by pipetting up and down five times, using appropriate and sterile pipetting tips for each sample (Neogen, 2021a). Then, 100 µL of each sample extract and diluent mixture was transferred into new clear sample cups for testing. A new Reveal Q+ test strip for aflatoxin was placed into each clear cup with the sample end pad directly in contact with the liquid for exactly six minutes to develop (Neogen, 2021a). After the reaction period, the test strip was removed from the sample cup for result reading under a Neogen's RevealQ+ AccuScan Gold Reader, which had an upper detection limit of 100 ppb (Neogen, n.d.). The readings were then recorded against the sample ID in a spreadsheet. Whenever the sample readings exceeded the machine's detection limit, the sample was diluted, and the entire test procedure was repeated.

Reading was done between 6 and 7 minutes. Results read after 7 minutes, were considered inaccurate due to over development of the device and were not be reported. Immediately

after the reaction time elapsed, the test strips were read (within 30 seconds) under a Neogen's AccuScan Gold Reader for analysis where results were displayed and stored in the reader. The Reveal Q+ test strip was fully inserted into the device's black cartridge adapter with the sample end first and results facing out. The cartridge was then inserted with test strip facing upwards into the Neogen's AccuScan Reader. The reader automatically analyzed the strip in the cartridge, giving readings in ppb. All the readings were systematically recorded with corresponding sample codes. Fumonisin test procedure was similar to aflatoxin analysis except the specific fumonisin test kits were used.

3.7 Determination of Maize Intake through Complementary Porridge

Maize intake (amount of maize flour consumed) by the children in form of complementary porridge was calculated based on consumption data obtained from a supplementary experiment involving sixty 6-24 months olds from an underprivileged community living in a rural setting of Lilongwe District (Traditional Authority Maliri). In this supplementary experiment, the 60 children were randomly divided into three groups and were fed plain maize porridge ad libitum on two separate days and the volume consumed was determined by subtracting the left over from the supplied volume. Local women were engaged to prepare the porridge to ensure acceptable consistency of the porridges. The amount of maize intake on dry matter basis was determined through moisture determination subsamples of the porridges. The maize intake did not correlate with children age (Month = 0.0079*Maize mass + 15.32; $R^2 = 0.0057$) and therefore the children were not segregated into infants and toddlers based on consumption amount.

3.7.1 Mycotoxin Exposure Analysis

Mycotoxin exposure assessment was performed, probabilistically using @Risk Trial version 8.1 by combining the mycotoxin contamination data of 338 samples from the survey with maize consumption data obtained from the supplementary experiment outlined above (Palisade Corporation, 2021). The study utilized a Monte Carlo simulation model to estimate mycotoxin exposure, using @Risk Trial version 8.1 (Palisade Corp., USA). The model combined mycotoxin contamination data from 338 flour samples collected during the survey with maize consumption data obtained from the supplementary experiment. To

estimate mycotoxin intake, the mycotoxin level in the flour (ng/kg for aflatoxin B1 and µg/kg for fumonisins) was multiplied by the amount of maize flour consumed (g/day) and then divided by an average child's body weight of 9.95 kg. The average body weight was based on median weight values for a 15-month-old girl child (9.3kg) and a 15-month-old boy child (10.3kg) (De Onis, 2006). As the Neogen assay used in the study only provided total aflatoxins, the values were divided by 2 to estimate AFB1 based on the occurrence pattern of AFBs and AFGs in Malawi (Matumba et al., 2015a). The study considered two mycotoxin exposure scenarios: mycotoxin contamination values below the limit of detection (LOD) were replaced with LOD to obtain upper bound (UB) and with zero to obtain lower bound (LB) means. To ensure the accuracy of mycotoxin intake estimation, three Monte Carlo simulations were performed with 50,000 iterations for each estimation (Lambe, 2002).

3.7.2 Mycotoxin Exposure Risk Characterization

The exposure estimates for fumonisin were compared with a tolerable daily intake (TDI) of 1µg/kg bw/day (as set for FB1 and group fumonisins (FB_1, FB_2, FB_3 and FB_4), as set by European Food Safety Authority (EFSA) (Knutsen et al., 2018). Since aflatoxin is a genotoxic carcinogen, no authority, let alone JECFA can set a tolerable daily intake (TDI) for it (JECFA, 1999). Therefore, risk characterization for this toxin was based on margins of exposure (MOE) (Benford et al., 2010), which according to according to EFSA is based on cutoff point considered to be of low public health concern of exposure of 0.017 ng/kg bw/day (obtained by dividing the benchmark dose lower limit ($BMDL_{10}$) of 170 ng/kg bw/day by 10,000) (EFSA, 2007).

3.7.3 Estimation of Population Risk for Aflatoxin-Induced Liver Cancer

The risk of developing Hepatocellular Carcinoma (HCC) due to aflatoxin exposure in the population was estimated using the method outlined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 1998, 2017). JECFA considers the synergistic effect of AFB1 and hepatitis B virus (HBV) infection in causing liver cancer and specifies HCC potency factors of 0.30 cases per 100,000 persons per year per ng of

aflatoxin/kg bw/day for individuals who are positive for hepatitis B surface antigen (HBsAg+), and 0.01 corresponding cases for HBsAg negative individuals (HBsAg-).

In Malawi, the prevalence of HBV positive individuals is 12.22% (Schweitzer et al., 2015), and based on this, the population risk for aflatoxin-induced HCC was estimated as follows: Population HCC risk = AFB1 Exposure (ng/kg bw/day) × Average HCC potency (P).

Where;

Average HCC potency = $0.3 \times P + 0.01 \times (1 - P) = 0.3 \times 12.22\% + 0.01 \times 87.78\% =$ 0.045438 cancers per year per 100,000 population per nanogram AFB1 per kg body weight per day

3.8 Data Analysis

Data entries from the questionnaire as well as contaminants concentrations obtained from laboratory analyses were entered and processed using IBM SPSS Statistics software v 22. Significance of the Aflatoxin and fumonisin data was tested using t-test statistics at 95% confidence interval. Aflatoxin and fumonisin data were log-transformed (log x+1) for statistical analysis due to normality assumption violation. The resultant transformed data had equal variance and were normally distributed. This was established through Levene's test and normal probability plots, respectively (Kuehl, 2000). The difference between means of Aflatoxins and Fumonisins was assessed by analysis of variance (ANOVA) and Post Hoc Turkey's test.

WHO AnthroPlus software was used to produce weight for height, height for age and weight for age Z-scores to assess wasting, stunting and underweight, respectively. After entering the 338 observations for this study in the software, it was discovered that 24 observations had flagged values. These observations were removed in order to have reliable data such that for this particular analysis the total number of entries were 314.

The likelihood of a child to be stunted, based on gender, age, location, household assets value, education level of guardian, exposure to aflatoxins , exposure to fumonisins, and

overall exposure risk to mycotoxins, were determined using binary logistic regression analysis as previously described by Gama et al., (2018. The logistic model used is represented as follows:

$$\mathrm{P}[Y_i = 1|X] = \frac{1}{1+e^{-(\beta_0+\sum_{i=1}^{n}\beta_i X_i)}} \qquad \text{(Gama et al., 2018) (2)}$$

The binary dependent response (Y_i) for child *i* takes a value of 1 if the child is stunted. If not, Y_i takes the value of 0. Vectors of the exploratory variables are represented by X_i while the intercept and variable coefficients are represented by β_0 and β_i (i = 1, 2, …n), respectively. In the model, gender was categorized as male and female, age was categorized as infant (≤ 12 months) and toddler (> 12 months), location as Centre and South, household assets value as low (<MK68500) and high (≥ MK68500), education level of guardian as utmost primary education and at least secondary education, and exposure to aflatoxin, fumonisins, and to both mycotoxins as low and high, respectively.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Demographics

4.1.1.1 Gender, Marital Status, Age and Level of Education

Out of the 338 respondents, 87.9% were married and 91.4% were above 20 years of age (Table 4.1). About 27% of the population had attended school up to secondary level and 63% up to primary level.

Table 2: Gender, Marital Status, Age and Level of Education of the Respondents

Variable			Level of Education			
		N	None	Primary	Secondary	Tertiary
Gender of respondent	Male	1	0	0	1	0
	Female	337	34	217	85	1
Marital status	Married	297	30	190	76	1
	Single	13	0	6	7	0
	Widowed	4	1	2	1	0
	Divorced	24	3	19	2	0
Age categories of respondent	Under 20	29	1	25	3	0
	20-29	183	16	117	50	0
	30-39	106	14	63	28	1
	40 & Above	20	3	12	5	0

N = 338

4.1.1.2 Source of Livelihood

The majority of the respondents from all the districts were farmers with Dedza, Lilongwe, Mangochi, Mchinji, Ntcheu and Phalombe having farmer proportion roughly greater than 60%. However, Blantyre had the greatest number of business dependent households (38.5%) while those depending on piecework were high in Balaka, Mwanza and Neno all at 34.6% each.

The study further looked at cross-agro-ecological differences whereby three AEZ were identified as; Lower Shire Valley, Lakeshore Plains and Upper Shire Valley, and Mid-altitude Plateau and Highlands. In the same way as district approach, the AEZ were dominated by farmers despite Lakeshore Plains and Upper Shire having relatively low number of farmers (44.2%) and a relatively higher number of business dependent households (19.2%) than all other zones.

Table 3: Age and Source of Livelihood by District and AEZ

		N	Mean Age	% Farmer	% Piece work	% Business	% Fully employed
District	Balaka	26	26	34.6	34.6	26.9	3.8
	Blantyre	26	29	34.6	19.2	38.5	11.5
	Dedza	26	27	65.4	26.9	7.7	0.0
	Lilongwe	26	28	69.2	19.2	15.4	0.0
	Machinga	26	28	42.3	26.9	30.8	0.0
	Mangochi	26	25	61.5	26.9	3.8	7.7
	Mchinji	26	29	57.7	11.5	26.9	0.0
	Mwanza	26	26	42.3	34.6	19.2	0.0
	Neno	26	33	38.5	34.6	26.9	7.7
	Nsanje	26	28	65.4	23.1	15.4	0.0
	Ntcheu	26	27	61.5	23.1	15.4	11.5
	Phalombe	26	27	61.5	26.9	7.7	3.8
	Zomba	26	27	42.3	30.8	23.1	3.8
Agro Ecological Zone	Lower shire Valley	26	28	65.4	23.1	15.4	0.0
	Lakeshore plains and upper shire valley	104	28	44.2	32.7	19.2	4.8
	Mid-altitude plateu and highlands	208	28	54.3	23.1	20.7	3.8

N = 338

4.1.1.3 Household Monetary Value of Assets

Despite households having different main sources of income, the study further found that the highest mean monetary value of assets was in Nsanje and Neno and lowest in Mwanza and Mangochi. However, at 95% confidence interval, there was a higher variability of income among households in Nsanje and Neno and the variation was lowest in Dedza. Despite that being the case, the majority of the households had assets of less than MK200, 000.

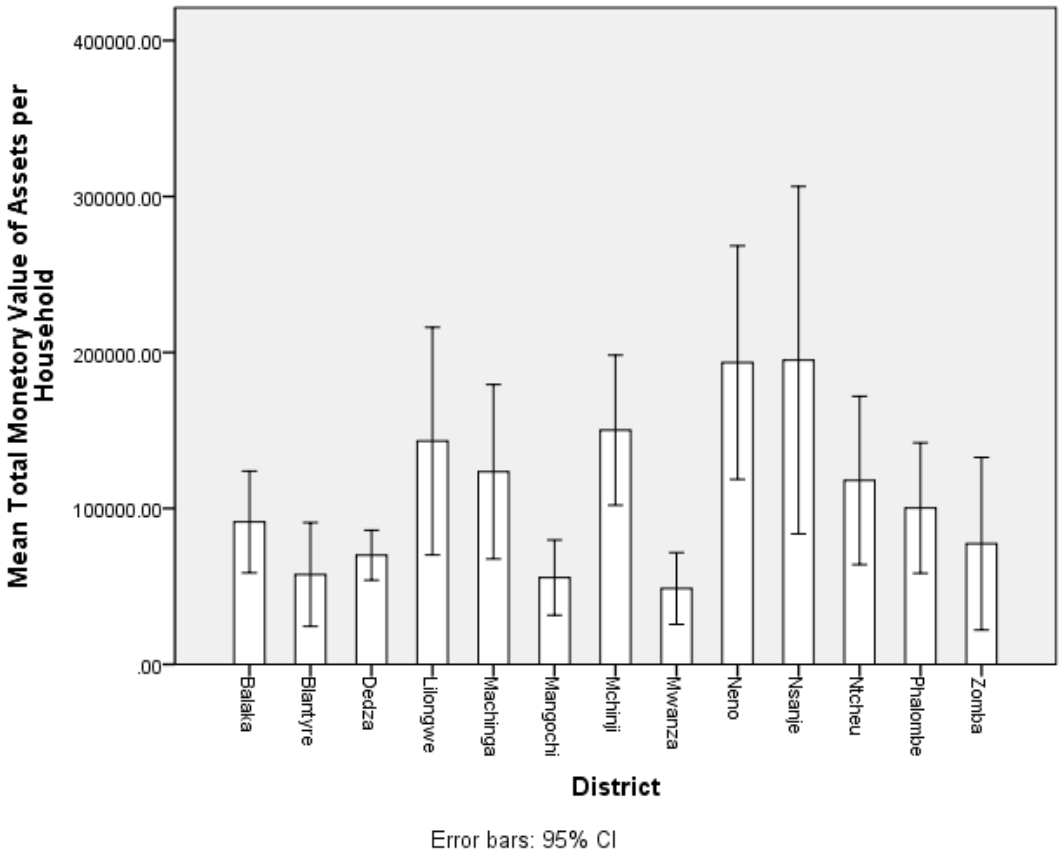

Figure 2: Household Monetary Value of Assets

4.1.2 Knowledge, Altitudes and Practices Related To Mycotoxins in Food

4.1.2.1 Knowledge of Aflatoxins and Fumonisins

The average KAP score for knowledge of aflatoxins and fumonisins among the different categories of respondents was 7.024±1.871 out of a possible 30points, which indicates that knowledge about aflatoxins and fumonisins is very low across the sample population (Table 4). There was no significant difference in variation of knowledge level among people of different age groups, agro-ecological zones and level of education. However, the study found significant ($p < 0.05$) differences in the variation of knowledge levels of aflatoxins and fumonisins across the districts. A Tukeys post-hoc test shows that across the districts, respondents from Nsanje District had significantly higher knowledge scores ($p<0.05$) than respondents from the rest of the districts (Appendix 2).

Table 4: Differences in Knowledge of Aflatoxin and Fumonisin

Variable		Knowledge Score			Chi-Square Tests (α = 0.05)	
		Frequency	Mean	Standard Deviation	Value	Sig. (2-sided)
Gender						
	Male	1				
	Female	337	6.89	1.98		
Age categories						
	Under 20	29	6.62	1.76	34.742	0.528
	20-29	183	6.94	2.01		
	30-39	106	6.91	2.05		
	40 & Above	20	6.85	1.69		
District						
	Balaka	26	6.27	1.64	174.049	0.045*
	Blantyre	26	7.23	1.82		
	Dedza	26	5.96	1.87		
	Lilongwe	26	5.81	2.40		
	Machinga	26	7.27	2.07		
	Mangochi	26	7.04	2.14		
	Mchinji	26	6.27	1.89		
	Mwanza	26	7.23	1.58		
	Neno	26	6.85	1.59		
	Nsanje	26	8.04	1.40		
	Ntcheu	26	6.58	1.98		
	Phalombe	26	7.58	1.81		
	Zomba	26	7.54	2.32		
Agro Ecological Zone						
	Lower shire Valley	26	8.04	1.40	22.289	0.562
	Lakeshore plains and upper shire valley	104	6.85	1.77		
	Mid-altitude plateau and highlands	208	6.78	2.11		
Education						
	None	34	6.24	1.776	29.176	0.783
	Primary	217	6.88	1.921		
	Secondary	86	7.17	2.165		
	Tertiary	1	9	.		
KAP		338	7.070	1.882		

*Significant at 95% confidence interval

4.1.2.2 Attitudes

The KAP study showed that the average score for attitude towards aflatoxins and fumonisins among the respondents was 1.87±0.45 out of a possible 3 points, indicating a relatively fair attitude towards these toxins across the study population (Table 5). The results also revealed that there was no significant difference in the level of attitude towards aflatoxin and fumonisin among respondents of different age categories ($p>0.05$) and education levels ($p>0.05$).

However, the study found a significant difference ($p<0.05$) in the level of attitude towards aflatoxins and fumonisins across districts and agro-ecological zones. Specifically, people from Dedza and Blantyre districts exhibited higher levels of attitude towards these toxins compared to respondents from other districts.

Table 5: Differences in Attitude of the Respondents to Aflatoxin and Fumonisin

Variable		Total Attitude Score out of 3Marks			Chi-Square Tests ($\alpha = 0.05$)	
		Frequency	Mean	Standard Deviation	Value	Sig. (2-sided)
Gender						
	Male	1				
	Female	337	1.87	0.51		
Age categories						
	Under 20	29	1.69	0.66	15.658	0.405
	20-29	183	1.89	0.54		
	30-39	106	1.87	0.44		
	40 & Above	20	1.95	0.39		
District						
	Balaka	26	1.42	0.81	81.622	0.033*
	Blantyre	26	2.00	0.00		
	Dedza	26	2.15	0.73		
	Lilongwe	26	1.92	0.27		
	Machinga	26	1.88	0.33		
	Mangochi	26	1.65	0.75		
	Mchinji	26	1.88	0.52		
	Mwanza	26	1.81	0.63		
	Neno	26	1.96	0.20		
	Nsanje	26	1.88	0.33		
	Ntcheu	26	1.96	0.20		
	Phalombe	26	1.96	0.20		
	Zomba	26	1.77	0.59		
Agro Ecological Zone						
	Lower shire Valley	26	1.88	0.33	19.472	0.035*
	Lakeshore plains and upper shire valley	104	1.71	0.66		
	Mid-altitude plateu and highlands	208	1.94	0.42		
Education						
	None	34	1.71	0.579	14.153	0.514
	Primary	217	1.86	0.509		
	Secondary	86	1.94	0.494		
	Tertiary	1	2			
KAP		388	1.87	0.45		

When asked whether it is necessary to sort moldy fractions of grains, most of the respondents (302) agreed that it was necessary to do so (Table 6). Only 36 thought it was not necessary. For those that thought it was not necessary to sort moldy grains, most of the (16) said there is no need because the bad ones are well mixed with the good ones after grinding, while some thought it reduces grain volume and some find the task time consuming.

On what they know about moldy grains promoting malnutrition, majority of the respondents (291) expressed having no idea while 45 thought there is no relationship between moldy grains and malnutrition. Only two respondents understood that moldy grains in a child's diet promote malnutrition among infants. Despite the majority not knowing whether or not moldy grains promotes malnutrition in infants, 234 of them rated the impact of moldy grains to a child's health as bearing detrimental impacts, 85 said the impacts are fatal while 17 thought the moldy grains are harmless.

Table 6: Responses Regarding Sorting and Consuming Moldy Grains and Reasons

Aspect (question)	Views (Answer)	Frequency
Do you think it is necessary to sort moldy fractions of grains	Yes	302
	No	36
IF NO. Why don't you think sorting is necessary	Grain sorting is time consuming	6
	No need to sort since the bad ones are well mixed with the good ones after grinding	16
	Roasting, cooking and grinding solves the bad grain problems	2
	Soring grains reduces volume	8
	Other	2
The use of moldy grains in a child's diet promotes malnutrition amongst infants	Yes	2
	No	45
	Don't Know	291
How would you rate the impact of mouldy grains to a child's health	Harmless	17
	Detrimental	234
	Fatal	85

4.1.2.3 Practices

The average KAP score for management practice of aflatoxins and fumonisins among the different categories of respondents was 4.18±0.12 out of a possible 5 (4 variables plus 1), which indicates that there is good aflatoxins and fumonisins management practices across the sample population (Table 7). The study did not find statistical difference in the level of knowledge of aflatoxin and fumonisin management across the districts, the agro-ecological zones as well as education levels ($p>0.005$). However, there was statistical difference in level of knowledge of practices to manage aflatoxins and fumonisins among different age group ($p<0.005$), where people of age group 30-39 were more knowledgeable than the rest.

Table 7: Differences in Practices of Aflatoxin and Fumonisin Handling/Management

Variable		Total Practice Score Out of 5 Marks			Chi-Square Tests (α = 0.05)	
		Frequency	Mean	Standard Deviation	Value	Sig. (2-sided)
Gender						
	Male	1	5.00			
	Female	337	4.13	0.95		
Age categories						
	Under 20	29	3.93	1.13	21.275	0.046*
	20-29	183	4.11	0.90		
	30-39	106	4.22	0.97		
	40 & Above	20	4.15	0.99		
District						
	Balaka	26	3.27	1.08	63.268	0.069
	Blantyre	26	4.38	0.80		
	Dedza	26	4.23	0.82		
	Lilongwe	26	4.00	0.94		
	Machinga	26	4.00	1.13		
	Mangochi	26	3.88	0.86		
	Mchinji	26	4.31	0.93		
	Mwanza	26	4.12	1.03		
	Neno	26	4.38	0.90		
	Nsanje	26	4.50	0.81		
	Ntcheu	26	4.42	0.58		
	Phalombe	26	4.08	0.69		
	Zomba	26	4.15	1.12		
Agro Ecological Zone						
	Lower shire Valley	26	4.50	0.81	13.117[a]	0.108
	Lakeshore plains and upper shire valley	104	3.91	1.04		
	Mid-altitude plateu and highlands	208	4.20	0.89		
Education						
	None	34	3.71	1.06	18.125	0.112
	Primary	217	4.12	0.93		
	Secondary	86	4.33	0.9		
	Tertiary	1	5	.		
KAP		388	4.19	0.93		

Table 7 presents the differences in practices of aflatoxin and fumonisin handling/management among different groups of participants in the study. The total practice score out of 5 marks is reported, along with the frequency, mean, and standard deviation for each variable. The chi-square tests are also reported, with the significance level ($\alpha = 0.05$).

The results show that females had a lower mean score (4.13) than males (5.00) for handling/management practices. In terms of age categories, those under 20 had the lowest mean score (3.93), while those aged 30-39 had the highest (4.22), but there was no significant difference among the groups.

There were differences among the districts, with Balaka having the lowest mean score (3.27) and Nsanje having the highest (4.50), although the chi-square test did not reach statistical significance.

The agro-ecological zone also showed differences in practices, with the Lower Shire Valley having the highest mean score (4.50) and the Lakeshore Plains and Upper Shire Valley having the lowest (3.91), but again, the chi-square test did not reach statistical significance.

In terms of education, those with tertiary education had the highest mean score (5.00), but there was only one participant in this category. Those with no education had the lowest mean score (3.71), but there was no significant difference among the groups.

When asked whether they actually sort grains to remove the moldy fractions before preparing a child's porridge flour, majority of them (301) said yes and only 27 said they do not. Majority of those that do sort the grains (285) said they throw away the bad/moldy grains while (12) of them give the grains to livestock and (4) use it as manure. Of all the respondents, only (100) have ever heard of aflatoxins. Of those that they heard, most of them (46) heard it from other different (informal) sources while 32 heard from radio/TV, 11 from IITA personnel and 9 from health personnel.

When asked whether a child ever gotten ill after consuming food prepared from unsorted grains, 48 said yes, while the majority (290) said no. For those that said yes, majority (23) admitted that the children sulfured “stunting” while 8 mentioned Kwashiorkor and 7 mentioned other illnesses.

Table 8: Responses Regarding Practices of Moldy Grains Management

Aspect (question)	Views (Answer)	Frequency
Do you sort grains to remove the moldy fractions before preparing your child's porridge flour	Yes	301
	No	37
If Yes, What do you do with the moldy ones	Give to livestock	12
	Throw them away	285
	Mix the good and bad grains	1
	Use as manure	4
	NA	36
	No	238
From which source, did you get the information on Aflatoxins	Radio and TV	32
	IITA	11
	Health workers	9
	Other	46
	N/A	240
Has your child ever gotten ill after consuming food prepared from unsorted grains	Yes	48
	No	290
IF YES. What was the illness	Kwashiorkor	8
	Stunting	23
	Marasmus	5
	Marasmic-Kwashiorkor	1
	Other	7
	N/A	294
Has your child ever been diagnosed with malnutrition	Yes	48
	No	290
IF YES. What condition was it	Kwashiorkor	8
	Stunting	23
	Marasmus	5
	Marasmic-Kwashiorkor	1
	Other	7
	N/A	294

4.1.3 Under Five Anthropometric Status

The anthropometric measurement of 314 children (51.59% males) are presented in Table 9. About 48% were stunted where 21.7% were moderate cases of stunting and 26.8% were severe cases.

Table 9: Prevalence of Stunting Based on Height-for-Age Z-Scores

	All n = 314	**Boys** n = 162	**Girls** n = 152
Prevalence of stunting (<-2 z-score)	(152) (42.9 - 53.9 95% C.I.)	(86) (45.4 - 60.6 95% C.I.)	(66) (35.8 - 51.4 95% C.I.)
Prevalence of moderate stunting (<-2 z-score and >=-3 z-score)	(68) (17.5 - 26.5 95% C.I.)	(45) (21.5 - 35.1 95% C.I.)	(23) (10.3 - 21.7 95% C.I.)
Prevalence of severe stunting (<-3 z-score)	(84) (22.2 - 31.9 95% C.I.)	(41) (19.2 - 32.5 95% C.I.)	(43) (21.7 - 35.9 95% C.I.)

Figure 3 shows the distribution of the children's stunting (height for age) status as compared to WHO standards. As illustrated in the graph, the height for age z scores were more skewed towards the negative side indicating that a greater proportion of the children were short for their age.

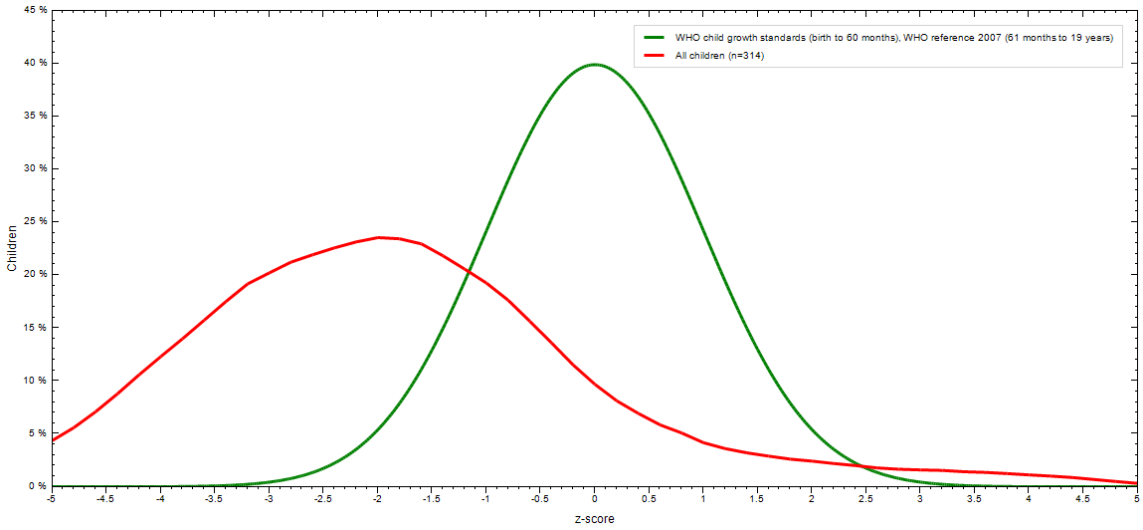

Figure 3 Height for Age

4.1.3 Aflatoxin and Fumonisin Distribution

4.1.3.1 Occurrence of Aflatoxins in Baby Porridge-Flour Samples

Table 10 reflects the contamination of baby porridge-flour samples collected from various districts in Malawi in the summer of 2018. Aflatoxin incidence ranged from 15.4% in Dedza to 96.2% in Balaka district with an average overall national incidence of 59.2%. There were significant mean differences in aflatoxin concentrations ($p < 0.05$) amongst the samples collected with Lowest levels observed in Dedza (3.35µg/kg) and highest in Balaka (43.80µg/kg)

Table 10: Aflatoxin occurrence in baby porridge-flour samples collected from different districts in Malawi in 2018 and ANOVA. Mean values with different subscript letters have significantly ($p < 0.05$) different means according to Post Hoc Tukey's test.

District	Number of Samples	Aflatoxin positive (%)	Aflatoxin Concentration (μg/kg)								
						Geo. mean ± *Std DEV*	% exceeding Limit				
			Mean	Min	Max		0.1	3	4	10	20
Balaka	26	96.2	43.80_{c}	1	350	13.95±87.10	100	76.9	69.2	61.5	38.5
Blantyre	26	50	12.41_{abc}	1	91	3.61±23.24	100	42.3	42.3	26.9	15.4
Dedza	26	15.4	3.35_{a}	1	38	1.46±7.63	100	**15.4**	**15.4**	11.5	**3.8**
Lilongwe	26	57.7	34.91_{abc}	1	500	4.52±104.90	100	50	42.3	26.9	19.2
Machinga	26	92.3	35.19_{c}	1	230	15.56±46.45	100	**80.8**	76.9	57.7	**53.8**
Mangochi	26	38.5	9.76_{ab}	1	60	3.01±5.39	100	34.6	34.6	26.9	19.2
Mchinji	26	38.5	18.56_{ab}	1	200	2.93±45.19	100	30.8	26.9	23.1	15.4
Mwanza	26	38.5	10.78_{ab}	1	70.60	2.91±18.24	100	34.6	30.8	26.9	23.1
Neno	26	80.8	21.57_{bc}	1	90.80	8.27±27.43	100	**80.8**	61.5	38.5	34.6
Nsanje	26	53.8	17.86_{abc}	1	130.60	4.71±29.76	100	46.2	42.3	38.5	30.8
Ntcheu	26	57.7	27.0_{abc}	1	144.20	6.24±40.17	100	57.7	50	38.5	34.6
Phalombe	26	69.2	25.84_{bc}	1	129.20	9.80±30.69	100	69.2	69.2	57.7	50
Zomba	26	80.8	23.70_{bc}	1	100.90	8.45±30.49	100	73.1	65.4	42.3	34.6
Total	338	59.2	21.90	1	500	5.31±47.76	100	53.3	48.2	36.7	28.7

According to WHO guidelines (IPCS/GEMS/Food 1995), whenever the number of non-detects was less than 60% of the samples analyzed, values below the LOD were replaced by half of the LOD and when the number of non-detected samples was more than 60% of the total analyzed, the value below the LOD was replaced by zero. In this study the number of non-detects for aflatoxins was less than 60% (40.8%) and the detection limit ranged from 2-100µg/kg. therefore, all the non-detects were replaced with 1µg/kg. This clearly indicates that our method of aflatoxin analysis (Reveal Q+ for Aflatoxin) was unable to detect anything below 2µg/kg hence the percent exceeding the EU limit of 0.1µg/kg was 100% for all the districts in this study. The regulatory limits displayed above are set for total aflatoxin. 0.1µg/kg is the EU regulatory limit in processed cereal-based foods and baby foods for infants and young children; 3µg/kg is the limit in maize for human consumption in Malawi (Malawi Standard Board, 1990, 1996); 4µg/kg is the limit set by the Joint Expert Committee on Food Additives (JECFA); 10µg/kg the median for total of aflatoxins limits used worldwide (FAO, 2004) and 20µg/kg, the total aflatoxin limit for human consumption enforced by U.S.FD

4.1.3.2 Occurrence of Fumonisin in Baby Porridge-Flour Samples in Malawi

Fumonisin incidence ranged from 88.5% in Mwanza to 100% in Balaka, Lilongwe, Mchinji, Ntcheu, Phalombe and Zomba districts with an average overall national incidence of 96.4%. There were significant mean differences in fumonisin concentrations ($p < 0.05$) amongst the flour samples from the different districts with Lowest levels observed in Nsanje (884.61µg/kg) and highest in Balaka (2842.30µg/kg). Ntcheu district recorded the highest fumonisin contamination levels where 100% of the samples exceeded both the 2µg/kg and 200µg/kg limits (European Commission, 2000), 88.5% exceeded the worldwide limit of 2000µg/kg by JECFA (FAO/WHO Expert Committee, 2018) and 19.2% percent exceeded the USFDA limit of 4000µg/kg (Table 11).

Table 11: Fumonisin occurrence in baby porridge-flour samples collected from different districts in Malawi in 2018 and ANOVA. Mean values with different subscript letters have significantly ($p < 0.05$) different means according to Post Hoc Tukey's test.

District	Number of Samples	Fumonisin positive (%)	Fumonisin Concentration (µg/kg)									
			Mean	Min	Max	Geo. mean ± *Std DEV*	% exceeding Limit					
							2	200	1000	2000	3000	4000
Balaka	26	100	2842.30_{bc}	300	17000	2093.34±3092.07	100	100	88.5	57.1	34.6	7.7
Blantyre	26	96.2	2178.84_{bc}	150	4700	1821.98±1095.09	100	96.2	88.5	46.2	23.1	3.8
Dedza	26	92.3	892.30_{a}	150	3700	694.76±733.03	100	92.3	**23.1**	3.8	3.8	0
Lilongwe	26	100	1815.38_{b}	400	4000	1489.36±1101.88	100	100	65.4	38.5	15.4	0
Machinga	26	92.3	1942.30_{b}	150	4000	1513.29±1099.79	100	92.3	73.1	42.3	19.2	0
Mangochi	26	96.2	1621.15_{ab}	150	4000	1343.27±899.57	100	96.2	73.1	30.8	7.7	0
Mchinji	26	100	1946.15_{b}	300	4300	1475.26±1319.16	100	100	69.2	42.3	23.1	11.5
Mwanza	26	88.5	894.23_{a}	150	2800	706.90±580.91	100	88.5	34.6	3.8	0	0
Neno	26	96.2	1736.53_{bc}	150	3600	1538.97±713.80	100	96.2	88.5	34.6	3.8	0
Nsanje	26	92.3	884.61_{a}	150	2900	695.19±664.19	100	93.2	30.8	7.7	0	0
Ntcheu	26	100	4003.84_{c}	300	20000	3083.98±3727.89	100	100	92.3	**88.5**	**57.7**	**19.2**
Phalombe	26	100	1750.0_{b}	300	5400	1384.36±1225.47	100	100	69.2	26.9	11.5	7.7
Zomba	26	100	1311.53_{ab}	300	3400	1125.67±754.89	100	100	57.7	19.2	3.8	0
Total	338	96.4	1832.24	150	20000	1335.61±1780.98	100	96.4	65.5	34	15.7	3.8

Whenever the number of non-detects was less than 60% of the samples analyzed, values below the LOD were replaced by half of the LOD and when the number of non-detected samples was more than 60% of the total analyzed, the value below the LOD was replaced by zero. In this survey the value of fumonisin non-detects was less than 60% (3.6%) and the range of the REVEAL Q+ for fumonisin detection method was 300-600µg/kg. Therefore all non-detects were replaced with 150µg/kg and all the samples that exceeded the maximum detection limit were diluted and retested, the reading was multiplied by the dilution factor to get the final sample contamination concentration. The regulatory limits displayed above are set for total fumonisin. 2µg/kg is the JECFA regulatory limit in cereals and foods for infants and young children; 200µg/kg is the EU fumonisin Regulatory limit in processed maize-based foods and baby foods; 1000-3000µg/kg is the fumonisin worldwide regulatory limit range and 4000µg/kg is the (European Commission, 2006). USFDA regulatory limit in food for human consumption in the USA (Petersen, 2018)

Table 12: Aflatoxin and fumonisin Occurrence in Baby Porridge-Flour Samples from Different Agro Ecologic Zones and ANOVA. Mean values with different subscript letters have significantly ($p < 0.05$) different means according to Post Hoc Tukey's test.

Agro Ecological Zone	Number of Samples	Aflatoxin positive (% exceeding Minimum Limit)	Fumonisin positive (% exceeding Minimum limit)	Concentration (µg/kg)									
				Aflatoxin					Fumonisin				
				Geo. mean ± *Std DEV*	Min	Max	Arithmetic Mean	% exceeding Max-Limit (20µg/kg)	Geo. mean ± *Std DEV*	Min	Max	Arithmetic Mean	% exceeding Max-Limit (4000µg/kg)
Lower Shire Valley	26	46.2	92.3	4.71±29.76	1	130.60	17.86_a	30.8	695.52±664.19	150	2900	884.61_a	0
Lakeshore Plains and Upper Shire Valley	104	56.7	95.2	5.63±48.49	1	350	21.48_a	28.8	1322.51±1792.29	150	17000	1773.55_b	1.9
Mid-Altitude Plateau and Highlands	208	52.4	97.6	5.23±49.33	1	500	22.62_a	28.4	1456.27±1837.32	150	20000	1980.04_b	5.3
Total	338	53.3	96.4	5.31±47.76	1	500	21.90	28.7	1335.61±1780.98	150	20000	1832.24	3.8

Table 12 presents the occurrence of aflatoxin and fumonisin in baby porridge-flour samples from different agro-ecological zones, along with ANOVA. The table includes the number of samples tested, the percentage of samples that were positive for aflatoxin and fumonisin, the concentration of aflatoxin and fumonisin in the samples, and the arithmetic mean.

The results showed that the percentage of samples that exceeded the maximum limit for aflatoxin (20µg/kg) and fumonisin (4000µg/kg) varied across the different agro-ecological zones. The highest percentage of samples exceeding the maximum limit for aflatoxin was observed in the Lakeshore Plains and Upper Shire Valley zone (56.7%), followed by the Mid-Altitude Plateau and Highlands zone (52.4%), and the lowest percentage was observed in the Lower Shire Valley zone (46.2%). For fumonisin, the highest percentage of samples exceeding the maximum limit was observed in the Lower Shire Valley zone (92.3%), followed by the Lakeshore Plains and Upper Shire Valley zone (95.2%), and the Mid-Altitude Plateau and Highlands zone (97.6%).

The concentration of aflatoxin and fumonisin also varied across the different agro-ecological zones. The highest concentration of aflatoxin was observed in the Mid-Altitude Plateau and Highlands zone (1980.04µg/kg), followed by the Lakeshore Plains and Upper Shire Valley zone (1773.55µg/kg), and the lowest concentration was observed in the Lower Shire Valley zone (884.61µg/kg). The highest concentration of fumonisin was observed in the Mid-Altitude Plateau and Highlands zone (1456.27µg/kg), followed by the Lakeshore Plains and Upper Shire Valley zone (1322.51µg/kg), and the lowest concentration was observed in the Lower Shire Valley zone (695.52µg/kg).

The ANOVA results indicated that there were significant differences in the means of aflatoxin and fumonisin concentrations across the different agro-ecological zones ($p<0.05$). Post-hoc Tukey's test revealed that the means of aflatoxin and fumonisin concentrations in the Lower Shire Valley zone were significantly lower than those in the other two zones. Additionally, the means of fumonisin concentration in the Mid-Altitude Plateau and Highlands zone were significantly higher than those in the other two zones.

4.1.4 Variation in Quantities of Aflatoxin and Fumonisn

4.1.4.1 Aflatoxin and Fumonisn by Agro-Ecological Zones (AEZ'S)

Despite significant mean differences ($p < 0.05$) amongst districts, there were no significant differences in the overall concentrations of aflatoxins amongst the 3 agro ecological zones. However, there were significant mean differences ($p < 0.05$) in fumonisin concentrations despite widespread fumonisin occurrence across the agro ecological zones (Table 12).

4.1.4.2 Co-occurrence of Aflatoxin and fumonisin in porridge flour samples

Co-occurrence of aflatoxins and fumonisins was found to be at 57.4%. However, there was no relationship (R^2=0.007) between the contamination levels of aflatoxin and fumonisin in a sample. Only 7 samples (2.1%) complied to both aflatoxin and fumonisin regulatory limits of 4µg//kg and 200µg/kg respectively, this implies that, 97.7% of the total samples were contaminated by either or both mycotoxins above the EU maximum levels for various population categories (Table 13). About 132 samples 39.1% violated the EU maximum levels for both aflatoxins (4µg/kg) and fumonisins (1000µg/kg) (European Commission., 2006-2010).

Table 13: Compliance of Samples to EU Aflatoxin and fumonisin Regulatory Limits.

Fumonisin EU Limits (µg//kg)	**Aflatoxin EU Limits (µg/kg)**					
	Less than 4µg//kg		**4µg/kg & Above**		**Total (fumonisins)**	
	N	**%**	**N**	**%**	**N**	**%**
200$^{\beta}$ µg/kg	7	**2.1**	5	1.5	12	3.6
200-1000$^{\Omega}$ µg/kg	55	16.3	32	9.5	87	25.7
1000-4000$^{\alpha}$ µg/kg$^{\alpha}$	90	26.6	132	**39.1**	222	65.7
4000 µg/kg & Above	6	1.8	11	3.3	17	5
Total (aflatoxins)	158	46.7	180	53.3	338	100

β = Maximum level of fumonisin for processed maize-based foods and baby foods for infants and young children

Ω = Maximum level of fumonisin for maize intended for direct human consumption, maize-based foods for direct human consumption

α = Maximum level of fumonisin for unprocessed maize, with the exception of unprocessed maize intended to be processed by wet milling

4.1.5 Effect of Demographic, Socioeconomic and Mycotoxin Exposure-Related Factors on Stunting

The results show that except for child's gender, age and education level of the guardian, the rest of the factors had significant associations with stunting ($p < 0.05$). Children from the southern region of Malawi were 1.38 times more likely to be stunted than those from the central region when all other factors were held constant. Likewise, children from households with low-value assets (resource poor), those highly exposed to aflatoxin, fumonisins or to both were 1.28, 2.66, 2.44, and 1.43 times more likely to be stunted than their counterparts. The likelihood of association is presented as odds ratio with 95% confidence interval (Table 14).

Table 14: Logistic regression estimates for demographic and socioeconomic, and risk variables affecting stunting

Predictor	Odds ratio	χ^2	*P* - value
Gender of child			
Male vs. Female	1.244	3.384	0.066
Age of child			
≤12 months vs. >12 months	1.175	1.716	0.190
Education level of guardian			
Utmost primary vs. At least secondary	1.023	0.032	0.858
Location of household			
South vs. Centre	1.380	6.140	**0.013**
Household assets value			
Low vs. High	1.282	4.429	**0.035**
Exposure to aflatoxin			
High vs Low	2.655	4.703	**0.030**
Exposure to fumonisins			
High vs Low	2.440	5.679	**0.017**
Exposure to both aflatoxin & fumonisins			
High vs Low	1.426	5.146	**0.023**

P-values in bold are significant at 5% significance level. CI = 95% confidence Interval.

4.1.5 Aflatoxin and Fumonisin Exposure Risk Analysis

A summary of the statistical distribution for simulated maize flour consumption, mycotoxin (aflatoxin B1 and fumonisin) contamination and associated dietary exposure is presented in Table 15. Graphical presentation of these data is provided in Figure 4. The lower bound mean aflatoxin B1 and fumonisin exposures were 70 ng/kg bw/day and 11.9 µg/kg bw/day, respectively. The upper bound mean aflatoxin B1 and fumonisin exposures were 72 ng/kg bw/day and 12.2 µg/kg bw/day, respectively. There was no significant difference ($p > 0.05$) between the mean mycotoxin exposures for lower and upper bounds. In fact, 1 percentile (P1, 99% of the population) for aflatoxin exposure was found to be exposed to 1.1 ng/kg bw/day thus exceeding the aflatoxin B1 public health cutoff point considered to be of low public health concern of exposure of 0.017 ng/kg bw/day by a factor of 65. Likewise, P10 (90% of the population) exceeded the TDI of 1µg/kg bw/day for fumonisins. Thus, about 90% of the study population was simultaneous exposed to aflatoxin and fumonisin levels beyond reference toxicological values.

Table 15: Best-Fit Distributions for Maize Intake and Upper and Lower Bound Scenario of Mycotoxin Concentration and Associated Dietary Exposures for 6 to 24 Month Olds

	‡Lower Bound (LB) Scenario	Upper Bound (UB) Scenario*									
					Percentile (%)						
Description	Mean	Distribution fit function	Mean	Std. Dev	P1	P5	P10	P50	P90	P99	Skewness
Maize flour consumption (g)	Same as UB	RiskPert(1.5777,13.349,332.64)	65	49	3	7	12	53	135	211	1.0
Maize flour intake (kg/kg BW per day)	Same as UB	RiskPert(0.00015856,0.0013416,0.033431)	0.0065	0.0049	0.0003	0.0007	0.0012	0.0053	0.0136	0.0212	1.0
Aflatoxin B1 Concentration (ng/kg)	10,716‡	RiskExpon(10157,RiskShift(969.95))	11,127	10,158	1,072	1,491	2,040	8,010	24,356	47,735	2.0
Fumonisin Concentration (µg/kg)	1,837β	RiskLognorm(1859.1,1749.6,RiskShift(17.783))	1,877	1,746	230	383	506	1,372	3,775	8,647	3.5
Aflatoxin B1 Exposure (ng/kg BW/day)	70	-	72.0	98.7	1.1	3.3	5.8	37.2	178.8	475.6	3.6
Fumonisin Exposure (µg/kg BW/day)	11.9	-	12.2	17.2	0.3	0.7	1.2	6.8	28.2	80.9	5.8

*Values below the limit of detection (LOD) were replaced by LOD; ‡Values below LOD were replaced by zeros (0) †RiskExpon(10749, RiskShift(-31.803)); βRiskPearson5(5.2742,12231,RiskShift(-1026))

The nationwide population risk for aflatoxin-induced liver cancer attributable to consumption of maize based complementary porridge was estimated to be between 3.19 and 3.27 per 100,000 population which translates to a total national aflatoxin induced HCC burden of 629 to 645 cases per year, based on the 2018 population estimate of 19.72 million in Malawi.

Table 16: Estimated population risk (cases per 100,000 persons per year) of aflatoxin-induced hepatocellular carcinoma associated with combined effect of aflatoxin exposure through maize based complementary porridge consumption and hepatitis B virus (HBV) prevalence in Malawi.

Description	Lower Bound Scenario Mean	Upper Bound Scenario*								
		Mean	Std. Dev	Percentile						Skewness
				P1	P5	P10	P50	P90	P99	
Cancers/year/100,000 for HBsAg- individuals	0.70	0.72	0.99	0.01	0.03	0.06	0.37	1.79	4.76	3.60
Cancers/year/100,000 for HBsAg+ individuals	21.04	21.60	29.61	0.34	0.99	1.74	11.16	53.63	142.69	3.60
Cancers/year/100,000 individuals	3.19	3.27	4.48	0.05	0.15	0.26	1.69	8.12	21.61	3.60

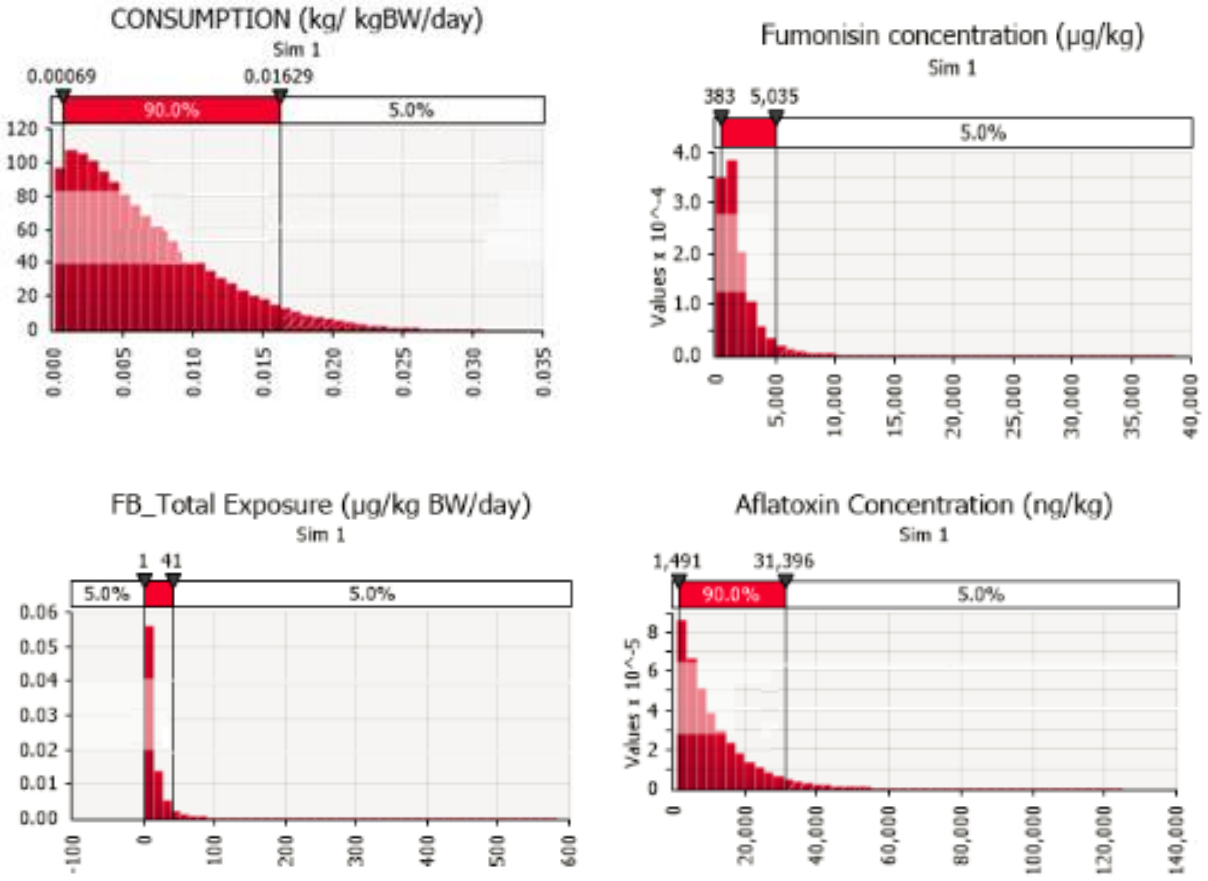

Figure 4 Monte Carlo Simulation Outputs for Maize Flour Consumption, Mycotoxin (Aflatoxin and Fumonisin) Contamination and Associated Dietary Exposure for 6 to 24 Month Olds in Malawi

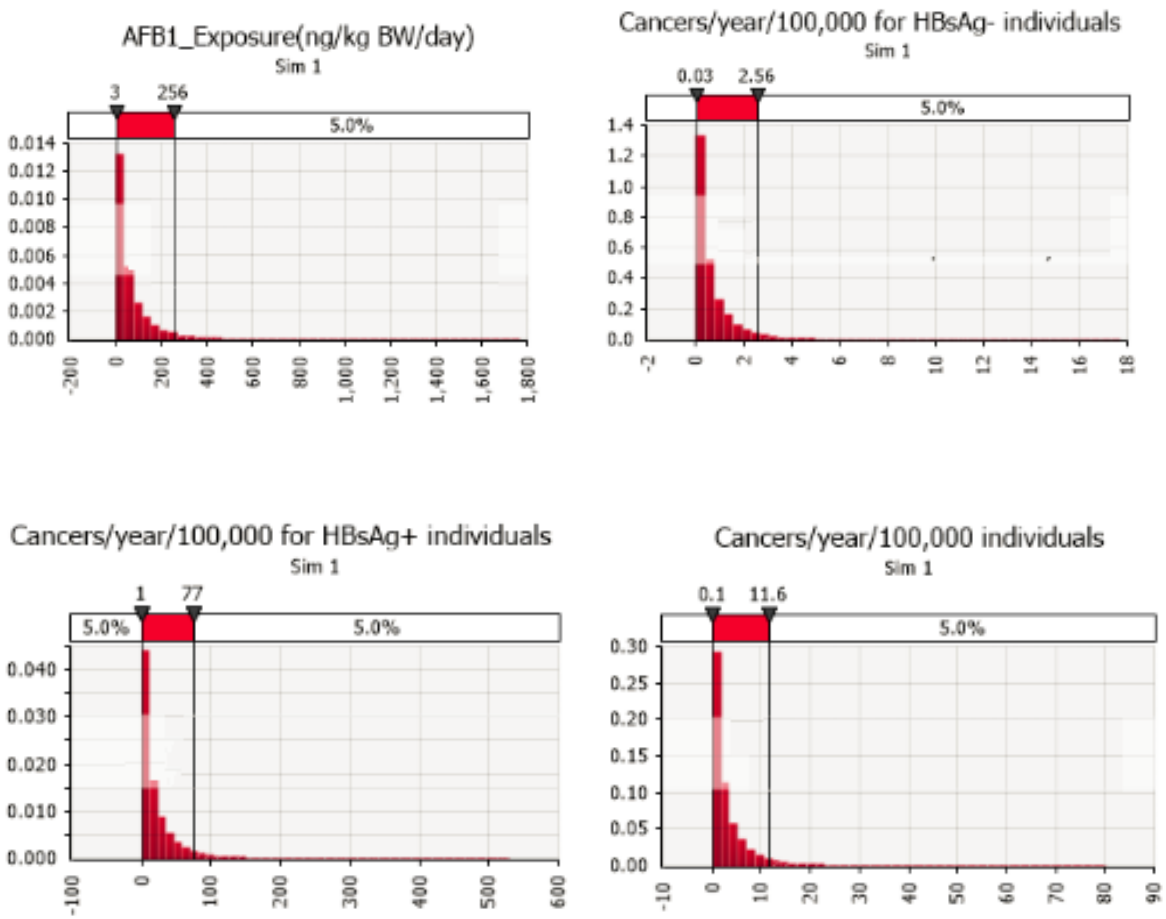

Figure 5 Monte Carlo Simulation Outputs for Maize Flour Consumption, Mycotoxin (Aflatoxin and Fumonisin) Contamination Associated Dietary Exposure for 6 to 24 Month Olds in Malawi

4.2 Discussion

4.2.1 Knowledge Attitudes and Practices

The knowledge, attitudes and practices (KAP) study findings from this survey reflect that all the 338 respondents were able to identify the condition of moldy grains with different local names used across districts. It was not surprising that *chuku* was well known across the districts because it is a chichewa name and is a national language. Furthermore, this could be associated with how the Malawi Program for Aflatoxin Control generally sends out radio messages through local stations as an initiative to raise awareness of aflatoxins. (MAPAC, 2013).

The survey results showed that although many participants were able to recognize moldy grains, a significant portion of them lacked knowledge about the health risks associated with aflatoxins and fumonisins, which are commonly found in moldy grains. This was not surprising, as previous KAP studies in Malawi had also found that many respondents were unaware of the health implications of moldy grains. The study also found that there was no significant correlation between a mother's education level and their knowledge about aflatoxins and fumonisins, likely due to the fact that these issues are not typically covered in the Malawian education curriculum. Instead, awareness about mycotoxins is often spread through mass media campaigns conducted by NGOs and government agencies. (Monyo et al., 2009).

It is not surprising that many of the respondents reported sorting their grains before food preparation, as this is a common practice among subsistence farmers in developing countries. However, the high levels of aflatoxins and fumonisins found in the samples collected in this study suggest that the hand sorting methods used may not have been fully effective in reducing contamination. Effective hand sorting involves systematically screening each individual grain and separating the good grains from the bad ones (Matumba et al., 2015).

Some participants expressed reluctance to sort their grains due to concerns that it is a time-consuming and labor-intensive process that could reduce the volume of their harvest. These

attitudes are often linked to poverty, as many people already struggle to harvest enough food to meet their needs. A study on this topic identified several barriers that prevent individuals from taking action to control molds and mycotoxins in food, including social, financial, and economic obstacles (Matumba et al., 2015a). The study found that many respondents cited food shortages as a reason for consuming moldy food, a lack of maize as a reason for purchasing moldy agricultural crops, and a reduction in volume as a reason for not sorting their grains. The overall higher practice score as compared to that of knowledge and attitudes towards molds and mycotoxins would be associated with good agricultural practices that are employed by farmers to improve crop yield rather than directly intended to control molds (Hell & Mutegi, 2011).

4.2.2 Aflatoxin and Fumonisin Distribution in Porridge Flour Samples

The results of this study indicate that the concentration of contamination in baby porridge-flour was significantly different among districts. Based on the Agro ecological zones, the risk of aflatoxin contamination appeared to rank in the decreasing order of: Lake Shore plain and upper Shire River valley > lower Shire > medium altitude plateau and highland. Aflatoxins are produced optimally at temperatures between 28 and 30 °C (Brian et al., 2007). These optimal temperatures are typical for low-lying agro-ecological zones of the lower Shire River valley and Lakeshore which are distinctly higher than average temperatures for the highlands. This partly explains the low prevalence of aflatoxins in the samples collected in the highlands. To the contrary, it was expected that samples from the lower Shire (Nsanje) would have had more aflatoxin contamination due to the climatic conditions as the temperatures in this area are extremely high. The low contamination could be a reflection of another study where vendor interviews revealed that 60% of maize samples (the main baby porridge component) collected in the lower Shire area were purchased from high and mid-altitude areas of Dedza and Lilongwe districts (Matumba et al., 2013). This suggests that the baby porridge samples collected from Nsanje district might not necessarily have been cultivated in the district but rather, would have been purchased from other districts.

Similarly, based on the agro ecological zones, the results revealed significant differences in fumonisin concentrations that were observed among the various districts and regions with the contamination risk ranking in the decreasing order of: Lake Shore and Upper Shire River valley > medium altitude plateau and highland > lower Shire River valley. This was somewhat an unexpected pattern as previous studies observed the highest concentration of fumonisin in the lower Shire due to warm moist conditions prevalent in the area that favor growth of the fusarium fungi (Mwalwayoa & Bernard Thole, 2016). However, this pattern could be as a result of delayed harvests as a major factor influencing fumonisin contamination of baby porridge raw materials particularly maize. Amongst other factors, long production periods expose maize to Fusarium infection and subsequent fumonisin contamination (Atukwase et al., 2009). In comparison with aflatoxin contamination, a greater proportion of the baby porridge-flour samples appear to have been contaminated with fumonisins.

The primary mycotoxins occurring in maize worldwide are aflatoxins and fumonisins (Abbott, 2013; Rodrigues & Naehrer, 2013; Giorni et al., 2016). The results show that both aflatoxins and fumonisins were detected in baby porridge samples from all the districts at different levels with concentrations significantly higher than Malawi's Limit (3µg/kg for total aflatoxins) (Malawi Standards Board, 1996) and the European Union (EU) limit for cereal foods (4µg/kg for total aflatoxin & 200µg/kg for total fumonisins) (European Commission, 2006). There are no limits set in Malawi for fumonisins. The sub-tropical climate of Malawi generally creates conducive conditions for the growth of both aflatoxin and fumonisin fungi Aspergillus flavus (Af) and Fusarium verticillioides (Fv) as both tend to flourish in hot and humid environments (Misihairabgwi et al., 2019).

4.2.3 Effect of Demographic, Socioeconomic and Mycotoxin Exposure-Related Factors on Stunting

Early childhood development is critical during the first 1000 days of an infant's life, after which stunting is irreversible (Ekholuenetale et al., 2020; de Onis & Branca, 2016). The current study aimed to identify factors associated with stunting among children under the age of five who were observed. The prevalence of stunting was 48.4%, and the difference

in prevalence between males and females was not significant. This finding contradicts other studies conducted worldwide that documented a higher prevalence of stunting in boys than in girls (Agho et al., 2009; Wamani et al., 2007; Cruz, 2017). The sex differences in stunting prevalence could be due to certain behavioral patterns of communities, such as favoritism towards the girl child (Chirande et al., 2015). Additionally, epidemiological evidence shows that boys are biologically more vulnerable to morbidity (Elsmén et al., 2007; Kilbride & Daily, 1998). This unusual pattern requires further investigation.

The lack of significant association between the mother's age and stunting is contrary to most findings of similar studies (Chirande et al., 2015; Darteh & Kumi-Kyereme, 2014). Typically, younger mothers tend to have poor knowledge and practices of good nutrition for young children. A study in India found that children born to teenage mothers are more likely to be malnourished than those born to adult mothers, highlighting that adolescence is a critical stage for the mother's physical and neuro-maturational changes, with maternal nutritional demands exerting extra pressure on the mother's body (Nguyen et al., 2019). The lack of association in this study could have been due to the few teenage mothers, resulting in under-powering the correlation analysis.

Previous studies have suggested that maternal education has a protective effect against malnutrition, including stunting, in children (Chirande, 2015; Fenske et al., 2013; Broeck, 2007; Rannan-Eliya et al., 2013). However, in this study, there was no observed association between maternal education and stunting. This finding is not unusual, as other studies conducted in Ethiopia and Pakistan have also found no significant association between stunting and maternal education (Fikadu et al., 2014; Khan et al., 2016). Therefore, the importance of maternal education may vary from one country to another, and the differences may be due to variations in study design and the different socioeconomic statuses of countries.

Over the years, studies have observed that populations living in areas with high poverty incidence are more susceptible to nutritional deficits due to issues such as food shortages and poor-quality nutritional diets, leading to inadequate nutrient intake (Menon et al., 2000;

Nzala et al., 2011). This study's results agree with this, as a significant ($p < 0.05$) association was observed between poverty and stunting. Furthermore, children from the southern region of Malawi were more likely to be stunted than those in the central region. The southern region of Malawi has a higher incidence of poverty than the central and northern regions, creating a food-insecure situation that affects the general nutritional status of children in the region (NSO, 2016-2017). Additionally, the Malawi Integrated Household survey report revealed that the proportion of households providing three or more meals to children under the age of 5 years in Malawi is lowest in the Southern Region due to localized dry spells and erratic rainfall resulting in below-average food production. These factors leave the region highly dependent on purchased maize as the main source of food, and only a few can afford enough for their families. However, it is difficult to isolate the effect of low socioeconomic status from the mycotoxin influence on stunting, as both are high in the southern region.

Regarding aflatoxins and fumonisins, the study shows that children exposed to high concentrations of either or both mycotoxins were more likely to be stunted than those exposed to lower concentrations. This finding supports other studies reporting associations between mycotoxin exposure and child growth impairment resulting in stunting and other types of malnutrition (Moe, 2018). Aflatoxins and fumonisins are highly associated with immunosuppression, outbreaks of acute toxicity, and reduced response to vaccinations in both stature and cognitive development amongst under 5-year-olds (Sherif et al., 2009). Children are more susceptible to the effects of mycotoxins than adults due to their immature detoxifying capacity, rapid growth, and higher food intake relative to their body mass. As such, exposures to aflatoxin and fumonisin could be particularly detrimental (Erkekoglu et al., 2008). Studies by Candida et al. on dietary exposure to aflatoxin and fumonisin among Tanzanian children revealed that about 82% of children aged 12-22 months are chronically exposed to the two mycotoxins through the consumption of contaminated supplementary foods, where maize is the major ingredient (Candida et al., 2013).

4.2.4 Aflatoxin and fumonisin Exposure Risk Analysis

The present study investigated the risks associated with exposure to mycotoxins from maize-based complementary porridge among children aged 6 to 24 months. The findings indicate that children in this age group are exposed to high levels of mycotoxins, which can contribute to various diseases, including the incidence of hepatocellular carcinoma (HCC). Mycotoxin exposure can occur through high consumption of moderately contaminated foods or consumption of foods with high contamination levels in moderate amounts.

The high exposure to aflatoxins and fumonisins observed in this study was attributed to both high contamination and high intake of maize. The estimated mean daily maize intake of 65 g per child found in the study is higher than the average reported for neighboring Tanzania (59 g) and more than 7 times higher than the average intake in adults in Europe as reported by WHO GEMS/Food Regional Diets (WHO, 1998; Kamala et al., 2017). Since the majority of the population relies on subsistence consumption, there is a need to promote mycotoxin exposure reduction strategies, including good pre- and post-harvest practices such as sorting of grains meant for complementary feeding (Matumba et al., 2015b, 2017). It is also crucial to shift from monotonous maize-based complementary diets to more diverse diets that include less susceptible commodities such as rice (Reddy et al., 2008). Additionally, it is necessary to develop complementary foods with high mycotoxin counteracting capacity (Galvano et al., 2002; Hamzawy et al., 2013).

The study's observation of simultaneous exposure to aflatoxin and fumonisin levels beyond reference toxicological values by >90% of the study population raises concern, particularly due to the synergistic effects of these mycotoxins (Gelderblom et al., 2002; Clarke et al., 2014; Qian et al., 2016). It is likely that the children were exposed to more than just these two toxins, considering that a previous study demonstrated that maize samples from Malawi could be co-contaminated with up to 41 different metabolites (Matumba et al., 2015c). Unfortunately, until now, there is no risk assessment approach that considers the co-occurrence of different mycotoxins; hence the current use of a single mycotoxin risk assessment methodology. The high rate of co-occurrence of aflatoxins and fumonisins

observed in this study reinforces the need for the development of risk assessments for cocktails of mycotoxins in food.

The nationwide population risk for aflatoxin-induced liver cancer attributable to the consumption of maize-based complementary porridge, ranging from 3.19 to 3.27 per 100,000, is consistent with the findings from a previous probabilistic assessment based on adult aflatoxin B1 exposure through groundnut and maize consumption (Matumba et al., 2019). However, these figures are higher than those reported by Msyamboza et al. (2012), who estimated new liver cancers in Malawi to be at 0.8 per 100,000 population for men and 0.4 per 100,000 for women. The discrepancy is likely due to differences in the methodology used. The latter estimates were largely based on cancer registries, which are likely to suffer from underreporting because liver cancer is rarely biopsied, and therefore, many of the cases may not be admitted to hospitals (Banda et al., 2001; Chasimpha et al., 2017).

Fumonisins are also implicated in esophageal cancer risk (Norred & Voss, 1994), and the risk of cancer is high in Malawi (Mlombe et al., 2015). Unfortunately, currently, there is no model for the estimation of esophageal cancer induced by fumonisin. Hence, in this study, only the estimated cancers induced by aflatoxins, the HCC (liver cancer), were considered. This suggests that the under-5-year-old population in Malawi might be subjected to even more health risks associated with mycotoxin exposure.

Nevertheless, the current risk analysis had its own flaws as it presumed porridge as the only source of food in a child's diet, yet in a day, a child is most likely to eat other foods such as legumes, milk, and other snacks, which are also prone to mycotoxins. Another weakness is that the current risk analysis was based on data that came from one season. Mycotoxins are believed to vary from one season to another (Kanwal et al., 2018); therefore, the risk analysis would have been more comprehensive if it had considered multi-year data. Furthermore, the data was not segregated by districts, yet the current results showed that mycotoxin occurrence varied from one district to another. All the same,

the risk analysis gives a better projection of the aflatoxin and fumonisin exposure amongst under-5-year-olds in Malawi.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

Molds and mycotoxins in food are a significant public health concern, particularly for vulnerable populations such as young children. In Malawi, where maize-based porridge is a staple food for young children, the risk of mycotoxin exposure is high. This study aimed to explore the knowledge, attitudes, and practices of caregivers of under 5-year-olds regarding molds and mycotoxins in food, as well as to assess the prevalence and distribution of aflatoxin and fumonisin in baby porridge flour in Malawi. The findings reveal a lack of knowledge among caregivers regarding the health risks associated with moldy food and highlight the high risk of mycotoxin exposure for young children. The study also demonstrates a significant association between levels of mycotoxins and their distribution based on region/agro-ecology. These findings highlight the need for targeted interventions to reduce mycotoxin exposure and mitigate the associated health risks for young children in Malawi.

5.2 Conclusion

Based on the findings of the study, it can be concluded that locally processed baby porridge flour in Malawi is highly contaminated with aflatoxins and fumonisins, and this poses a significant health risk to the under 5-year-old population. Caregivers of these children lack

Knowledge regarding the health risks associated with moldy food, regardless of their level of education, which further increases the risk of mycotoxin exposure. The distribution of mycotoxins is significantly dependent on the region/agro-ecology, emphasizing the need for targeted interventions. The study also establishes a direct association between mycotoxin levels and the severity of stunting, which is linked to low economic status and residence in the southern region. Porridge contributes significantly to aflatoxin exposure in children, which can induce a substantial number of hepatocellular carcinoma cases in Malawi, especially when synergistically interacting with Hepatitis B virus (Kamala et al., 2021). Therefore, there is an urgent need for interventions to reduce mycotoxin exposure in Malawi, particularly in the under 5-year-old population, and increase awareness among caregivers about the health risks associated with moldy food.

5.3 Recommendations

To reduce the potential health risks associated with mycotoxin exposure in under 5 year olds in Malawi, several recommendations have been put forward. First, nutritionists should develop and implement policies to raise awareness of the dangers of mycotoxicosis in supplementary feeding diets, including proper postharvest handling practices and processing of maize-based foods. Additionally, diversification of maize with other cereal-based foods that are less prone to mycotoxin contamination should be explored. This will help reduce the potential for exposure to mycotoxins in this vulnerable population.

Further studies should be conducted to investigate the prevalence of mycotoxin contamination in other food commodities, such as dairy products, nuts, and legumes. This information will help develop effective risk management strategies to reduce exposure to mycotoxins. By identifying the sources of mycotoxins in the food chain, appropriate interventions can be developed to prevent contamination and ensure safe food practices.

It is also recommended that education on food and mycotoxins be integrated into the Malawi primary education curriculum to raise awareness and promote safe food practices among the general population. The Malawi Bureau of Standards should also perform regular monitoring of food commodities and formulate mycotoxin regulatory limits in baby

foods for infants and young children to enforce safe levels of mycotoxin exposure and reduce the health risks associated with contaminated food.

Future research should explore the potential impact of interventions aimed at reducing mycotoxin exposure, such as dietary interventions or interventions targeting postharvest handling practices. This will help evaluate the effectiveness of these interventions in reducing mycotoxin contamination in the food chain and minimizing the health risks associated with exposure. Additionally, research should be conducted to determine the economic impact of mycotoxin contamination on the food industry in Malawi.

Finally, it is important to investigate the broader implications of mycotoxin contamination on food security and public health in Malawi. This will help inform policy decisions and resource allocation towards reducing the impact of mycotoxins on vulnerable populations. By understanding the broader implications of mycotoxin contamination, appropriate interventions can be developed to prevent contamination and ensure safe food practices. This will help to ensure food security and promote public health in Malawi.

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APPENDICES

Appendix 1: Household Survey Questionnaire

Household Survey Questionnaire

ASSESSMENT OF AFLATOXIN AND FUMONISIN CONTAMINATION IN LOCALLY PROCESSED BABY PORRIDGE PRODUCTS AT HOUSEHOLD LEVEL IN MALAWI

Greetings, my name is _____, I am working with the International Institute of Tropical Agriculture (IITA) Research Center and University of Malawi, Chancellor College on an important survey focusing on the occurrence of aflatoxins and fumonisins in locally processed baby porridge products in Malawi. This study is a partial fulfillment of Miss Freda Lumanga's studies for a Master of Science in Environmental Science at the University of Malawi. IITA is a non-profit institution that generates agricultural innovations to meet Africa's most pressing challenges of hunger, malnutrition, poverty and natural resource degradation. The institution is working with various partners across sub-Saharan Africa to improve livelihoods, enhance food and nutrition security, increase employment and preserve natural resource integrity.

This interview has been designed to gather data on various knowledge, altitudes and practices associated with supplementary feeding programs for children aged 0-5years. The survey aims to investigate more on the aflatoxin and fumonisin exposures of children through the food supplements they are introduced to during their early child development stages.

Do you have a child aged between 0-5years in your household?

A. Yes
B. No

IF NO. Thank you for your time

IF YES.

I will request about 30 minutes of your time, to ask you a few detailed questions, primarily about the child's biodata, health and supplementary feeding patterns. The overall information collected under this survey, will help enlighten the government on the status and interconnectedness of child health, nutrition and aflatoxin exposure for the implementation of appropriate measures if need be.

The survey is voluntary and confidential, this means that whatever we discuss in our interview will not be shared with others. I will not ask you for any embarrassing or personal information. Please feel free to express yourself, as there are no right or wrong answers, you may choose to refuse to respond to any question or stop at any point but your full participation in this exercise is IMPORTANT for us to gather accurate information about the under-five years' child community in Malawi.

May we proceed with the interview now.

A. GENERAL INFORMATION

A1 Questionnaire No._____

A2 Name of enumerator _____

A3 Name of parent/guardian _____Sex:

1=Male 2=Female

A4 Please tick whichever applies

A. Biological Mother

B. Biological Father

C. Guardian

A5 Marital Status.

A. Married

B. Single

C. Widowed

D. Divorced

A6 Age: _____ Height _____

A7 District _____

A8 Village _____

A9 Name of Section_____

A10 Name of EPA _____

A11 GPS

Coordinates_____

____

Date: _____ /_____ /_____ Time: _____

B. DEMOGRAPHIC AND ANTHROPOMETRIC DATA

B1 How many under five children do you have in your house?

A. 1

B. 2

C. 3

D. Other _____

	Child Biodata					
	Child's Name	**1.**	**2.**	**3.**	**4.**	**5.**
	Gender (M/F)					
	DOB	___/___/___	___/___/___	___/___/___	___/___/___	___/___/___
	Current Weight (Kg)					
	Height (Cm)					
	Head circumference (Cm)					

B8 What is your highest level of education?

A. None
B. Primary
C. Secondary
D. Tertiary
E. Other _____

B9 What do you do for a living?

A. Farming
B. Piece work
C. Business
D. Permanent employment
E. Other_____

	I will mention a list of items that you may or may not have in your household. Please say YES if you have an item and NO if you do not.	Yes (1)	No (0)

B10	Bed		
B11	Chair		
B12	TV		
B13	Mobile phone		
B14	Latrine		
B15	Solar Electricity		
B16	Bicycle		
B17	Motor cycle		

C. SUPPLEMENTARY FEEDING PRACTICES (3 Marks)

C1 When did you first decide how you would feed your child/children?

A. Before pregnancy
B. During pregnancy
C. After child's birth
D. Not sure

C2 Who or what helped you with the decision about feeding your child/children? (Do not read out, select all that apply)

A. No one
B. Mother/mother in-law
C. Any other relative/friend
D. Doctor/Midwife
E. Village Nutritional advisor
F. Husband
G. Radio/TV
H. Previous experience with another child

I. Other

C3 Have you received any information about your child's feeding program from a community health worker or any parenting program?

A. Yes

B. No

C4 Did you breastfeed or are you currently breastfeeding your child?

[Practice]

A. Yes [1]

B. No [0]

C5 At what age, did you introduce supplementary foods to your child's diet?

[Practice]

A. Less than 1 month old [0]

B. 1-2 months' old [0]

C. 3-5 months' old [0]

D. 6 months' old [1]

E. Over 7 months' old [0]

C6 Could you tell me why you decided to introduce supplementary foods to your child's diet at that age?

A. Doctor's health care advice

B. Relative or friend's advice

C. Child was hungry

D. Child weight was not going up

E. Mother was ill

F. Not enough milk

G. Previous experience with another child

H. Child fell ill

I. Child old enough for it

J. Cultural belief and family tradition

K. Other_____

C7 How old was your child when you completely stopped breastfeeding? -or if you are still breastfeeding, at what age do you intend to stop your child from breastfeeding?

Months _____

C8 Who advised you to stop breastfeeding your child at that age?

A. No one
B. Doctor/Nurse
C. Friend/relative
D. Husband
E. Community health advisor
F. Previous experience with another child

C9 What supplementary food do you constantly provide your child with? (select all that applies)

A. Porridge
B. Nsima
C. Fruits and vegetables
D. Formula milk
E. Juice
F. Junk food
G. Eggs and dairy milk
H. Other

_____
__

C10 IF A is part of the response. What raw materials do you use to prepare the porridge flour?

A. Maize
B. Soy
C. Maize and soy
D. Maize, soy and groundnuts
E. Maize, soy, groundnuts and beans
F. Maize, soy, groundnuts, beans and rice

G. Maize, soy, groundnuts and rice

H. Other_____

_____

C11 How often does your child consume this food?

A. Once a day

B. Twice a day

C. Three times a day

D. Other_____

C12 Are the grains straight from your recent harvest or from your storage piles **[Practice]**

A. Recent harvest [1]

B. 1 year storage [0]

C. 2 years storage [0]

D. Over 2 years storage [0]

E. Unknown because its bought [0]

C13 What flour preparation methods do you use?

A. Pounding raw grains with a mortar and pestle

B. Pounding raw grains at a milling station

C. Soaking then pounding grains with a mortar and pestle

D. Soaking then pounding grains at a milling station

E. Roasting then pounding grains

C14 IF C OR D. How long do you soak the raw materials?

_____

C15 How do you finally cook or prepare the porridge for your child?

_____

_____

_____

_____

C16 Do you think early supplementary feeding has any health and development implications in a child's life?

A. Yes

B. No

C. Don't know

C17 What are these possible implications?

A. Malnutrition

B. Diarrhea

C. Fever

D. Numerous diseases

E. Other

_____

D. KNOWLEDGE, ALTITUDES AND PRACTICES ON AFLATOXINS

KNOWLEDGE [Total=32 Marks]

D1 Have you ever experienced moldy grains/crops in your HH? (**flash card of moldy grains) [Knowledge 1 Mark]**

A. Yes [1]

B. No [0]

D2. What name is given to toxins produced by molds in agricultural crops?

A. Nkhungu

B. Chuku

C. Chivundi

D. Other_____

D3. At what stage of production do these moldy conditions develop in your crops? **[Knowledge 3 Marks]**

A. Field [1]

B. Pre-harvest, before storage [1]
C. Post-harvest, during storage [1]
D. Throughout the production chain [3]

D4. Which agricultural crops are more prone to this moldy condition? **[Knowledge 1 Mark]**

A. Maize [0]
B. Groundnuts [1]
C. Soy beans [0]
D. Beans [0]
E. All grains [0]

D5. What are the conditions that enhance the development of molds on agricultural crops? **[Knowledge 10 Marks]**

A. Temperature [1]
B. Humidity [1]
C. Insect damage [1]
D. Presence of fungi [1]
E. Poor storage [1]
F. Premature harvest [1]
G. Late harvest [1]
H. Too much rainfall [1]
I. Lack of Rainfall [1]
J. Weeds [1]

D6. How do you reduce the occurrence of moldy grains? **[Knowledge 10 Marks]**

A. Sun drying [1]
B. Use of aerated storage containers [1]
C. Use of insecticides and storage chemicals [1]
D. Timely harvest [1]
E. Overcooking/boiling [1]

F. Timely planting [1]
G. Burning farm residues [1]
H. Crop rotation [1]
I. Use biocontrol [1]
J. Timely weeding [1]

D7. Are you aware of any health implications associated with the consumption of moldy grains? **[Knowledge 1 Mark]**

A. Yes [1]
B. No [0]

D8. IF YES TO D7. What are the health implications associated with moldy grains or 'Nkhungu' (use local name as responded in D2)? **[Knowledge 7 Marks]**

A. Diarrhea [1]
B. cancer [1]
C. Heart disease [1]
D. Stunting [1]
E. Immune suppression [1]
F. Stomach aches [1]
G. Malnutrition [1]

PRACTICES

D9. Do you sort grains to remove the moldy fractions before preparing your child's porridge flour? **[Practice 1Mark]**

A. Yes [1]
B. No (skip to D7) [0]

D10. IF YES. What do you do with the moldy ones? **[Practice 1Mark]**

A. Give to livestock [0]
B. Throw them away /destroy them [1]
C. Rinse and Cook them [0]
D. Rinse and Roast them [0]
E. Rinse and sun-dry them [0]
F. Mix the good and bad grains [0]
G. Use as manure [0]

D11. Have you ever heard of Aflatoxins ?

A. Yes
B. No (skip to D14)

D12. From which source, did you get the information on Aflatoxins ?

A. Radio and TV
B. IITA
C. Health workers
D. Newspaper, publications and journals
E. Other

_____

_____

_____

D13. Has your child ever gotten ill after consuming food prepared from unsorted grains?

A. Yes
B. No
C. Don't know

D14. IF YES. What was the illness?

A. Diarrhea
B. Fever

C. Headache
D. Skin diseases
E. Stunting
F. Liver cancer
G. Heart problems
H. Diabetes
I. Other

_____

D15. Has your child ever been diagnosed with malnutrition?

A. Yes
B. No (skip to D22)

D16. IF YES. What condition was it?

A. Kwashiorkor
B. Stunting
C. Marasmus
D. Marasmic-kwashiorkor
E. Other

_____

ATTITUDES (3 Marks)

D17. Do you think it is necessary to sort moldy fractions of grains? **[Attitude 1Mark]**

A. Yes [1]
B. No [0]

D18. IF NO. Why don't you think sorting is necessary? [Attitude]

A. Grain sorting is time consuming

B. No need to sort since the bad ones are well mixed with the rest after grinding
C. Roasting, grinding and cooking solves the moldy grain problems
D. Sorting grains reduces volume
E. Other

_____

D19. The use of moldy grains in a child's diet promotes malnutrition amongst infants **[Attitude 1 Mark]**

A. Yes [1]
B. No [0]
C. Don't Know [0]

D20. How would you rate the impact of bad grains to a child's health? **[Attitude 2 Marks]**

A. Harmless [0]
B. Detrimental [1]
C. Fatal [1]
D. Other _____

GENERAL COMMENTS:

Do you have any additional comments from the issues we have discussed on our interview?

_____

_____

_____

_____

This marks the end of our interview. Thank you so much for your time and responses towards this survey.

Enjoy the rest of your day!

Raw Sample Checklist

Sample Code:

Sample Composition:

Appendix 2: Tukey's Test for District and Knowledge on Aflatoxins and Fumonisin

Dependent Variable: Aggregated Knowledge Score on Aflatoxins and fumonisins

Tukey HSD

(I) Name of District	(J) Name of District	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Balaka	Nsanje	-1.769*	.529	.049	-3.53	.00
Dedza	Nsanje	-2.077*	.529	.007	-3.84	-.31
Lilongwe	Nsanje	-2.231*	.529	.002	-4.00	-.47
	Phalombe	-1.769*	.529	.049	-3.53	.00
Mchinji	Nsanje	-1.769*	.529	.049	-3.53	.00
Nsanje	Balaka	1.769*	.529	.049	.00	3.53
	Lilongwe	2.231*	.529	.002	.47	4.00
Phalombe	Lilongwe	1.769*	.529	.049	.00	3.53

*. The mean difference is significant at the 0.05 level.

Appendix 3: Test Statistics_ Chi Square Tests for KAP and demographic categories

Age categories of respondent * Aggregated Knowledge Score on Aflatoxins and fumonisins out of 30 Marks)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	34.742[a]	36	.528
Likelihood Ratio	36.384	36	.451
Linear-by-Linear Association	.074	1	.786
N of Valid Cases	338		

a. 36 cells (69.2%) have expected count less than 5. The minimum expected count is .06.

Age categories of respondent * Total Practice Score Out of 5 Marks

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	21.275[a]	12	.046
Likelihood Ratio	22.818	12	.029
Linear-by-Linear Association	1.516	1	.218
N of Valid Cases	338		

a. 8 cells (40.0%) have expected count less than 5. The minimum expected count is .24.

Age categories of respondent * Total Attitude Score out of 3Marks

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.658[a]	15	.405
Likelihood Ratio	13.628	15	.554
Linear-by-Linear Association	1.596	1	.206
N of Valid Cases	338		

a. 17 cells (70.8%) have expected count less than 5. The minimum expected count is .06.

District * Aggregated Knowledge Score on Aflatoxins and fumonisins out of 30 Marks)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	174.049[a]	144	.045
Likelihood Ratio	163.410	144	.128
N of Valid Cases	338		

a. 143 cells (84.6%) have expected count less than 5. The minimum expected count is .08.

District * Total Practice Score Out of 5 Marks

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	63.268[a]	48	.069
Likelihood Ratio	62.854	48	.074
N of Valid Cases	338		

a. 39 cells (60.0%) have expected count less than 5. The minimum expected count is .31.

District * Total Attitude Score out of 3Marks

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	81.622[a]	60	.033
Likelihood Ratio	63.070	60	.368
N of Valid Cases	338		

a. 65 cells (83.3%) have expected count less than 5. The minimum expected count is .08.

Agro Ecological Zone * Aggregated Knowledge Score on Aflatoxins and fumonisins out of 30 Marks)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	22.289[a]	24	.562
Likelihood Ratio	25.750	24	.366
Linear-by-Linear Association	5.483	1	.019
N of Valid Cases	338		

a. 23 cells (59.0%) have expected count less than 5. The minimum expected count is .08.

Agro Ecological Zone * Total Practice Score Out of 5 Marks

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.117[a]	8	.108
Likelihood Ratio	12.990	8	.112
Linear-by-Linear Association	.116	1	.733
N of Valid Cases	338		

a. 5 cells (33.3%) have expected count less than 5. The minimum expected count is .31.

Agro Ecological Zone * Total Attitude Score out of 3Marks

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	19.472[a]	10	.035
Likelihood Ratio	19.358	10	.036
Linear-by-Linear Association	6.458	1	.011
N of Valid Cases	338		

a. 12 cells (66.7%) have expected count less than 5. The minimum expected count is .08.

What is your highest level of education * Aggregated Knowledge Score on Aflatoxins and fumonisins out of 30 Marks)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	29.176[a]	36	.783
Likelihood Ratio	26.792	36	.867
Linear-by-Linear Association	5.641	1	.018
N of Valid Cases	338		

a. 36 cells (69.2%) have expected count less than 5. The minimum expected count is .00.

What is your highest level of education * Total Practice Score Out of 5 Marks

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	18.125[a]	12	.112
Likelihood Ratio	16.909	12	.153
Linear-by-Linear Association	10.378	1	.001
N of Valid Cases	338		

a. 10 cells (50.0%) have expected count less than 5. The minimum expected count is .01.

What is your highest level of education * Total Attitude Score out of 3Marks

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	14.153[a]	15	.514
Likelihood Ratio	13.772	15	.543
Linear-by-Linear Association	4.876	1	.027
N of Valid Cases	338		

a. 18 cells (75.0%) have expected count less than 5. The minimum expected count is .00.

Appendix 4: ANOVA Tests

ANOVA

Sample Composition

		Sum of Squares	df	Mean Square	F	Sig.
Aflatoxin detected by dilution factor in ug/kg	Between Groups	87202.286	5	17440.457	8.495	.000
	Within Groups	681573.377	332	2052.932		
	Total	768775.663	337			
Fumonisin detected by dilution factor in ug/Kg	Between Groups	9216152.002	5	1843230.400	.544	.743
	Within Groups	1125492220.779	332	3390036.810		
	Total	1134708372.781	337			

ANOVA

District

		Sum of Squares	df	Mean Square	F	Sig.
Aflatoxin detected by dilution factor in ug/kg	Between Groups	41680.117	12	3473.343	1.553	.104
	Within Groups	727095.546	325	2237.217		
	Total	768775.663	337			
Fumonisin detected by dilution factor in ug/Kg	Between Groups	216729911.243	12	18060825.937	6.394	.000
	Within Groups	917978461.538	325	2824549.112		
	Total	1134708372.781	337			

ANOVA

Agro-Ecological Zone

		Sum of Squares	df	Mean Square	F	Sig.
Aflatoxin detected by dilution factor in ug/kg	Between Groups	549.331	2	274.665	.120	.887
	Within Groups	768226.332	335	2293.213		
	Total	768775.663	337			
Fumonisin detected by dilution factor in ug/Kg	Between Groups	27732928.070	2	13866464.035	4.196	.016
	Within Groups	1106975444.712	335	3304404.313		
	Total	1134708372.781	337			