SOURCES AND LEVELS OF HUMAN EXPOSURE TO AFLATOXINS IN MAKUENI COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I would like to dedicate this thesis to my late Father Wilson Otok Wanyang', late mother Leah Were Otok who passed on during this project, to my husband Dr Tom Sangoro and to my children Dr Mike Sangoro, Susan Sangoro, Leah Sangoro and George Sangoro.

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ABSTRACT

Afllatoxicosis is a disease of great socioeconomic and public health importance. Several studies have been conducted in Kenya to establish the aflatoxin levels in food commodities especially during outbreaks where lives have been lost. Makueni County has had several of these outbreaks; however, no study has been done to establish the level of aflatoxin exposure in humans in this County. Most of the focus has been on the outbreaks ignoring the danger posed by chronic exposure.

A cross sectional study was carried out in Makueni County to: 1) establish the level of human exposure to aflatoxins in Makueni County. 2) to assess the associated pre- and post- harvest practices that pre-dispose cereals to aflatoxin contamination in the County. This study was stratified into three locations of Nguumo, Ukia and Wote in, Makindu, Kaiti and Wote divisions, respectively. These study sites were selected on the basis of dairy cattle population, maize growing and presence of children less than five years. Three- hundred- and-ten households (310) were proportionately selected in the three locations. A questionnaire was administered to the household head in the sampled households to establish household knowledge, attitudes and practices about aflatoxins. Samples consisting of maize, sorghum and millets, cow milk, goat milk and breast milk were collected for total aflatoxin analysis using an ELISA test. Urine from each index child under the age of 5 years in each household was collected twice six months apart and tested for aflatoxin using the ELISA test; anthropometric measurements (weight, and height) of the children were taken during these two visits.

The mean household size was six persons (5.9) across the three divisions. The main source of earnings was from sale of maize and fruits. The level of education was averagely primary.

Anthropometric measures showed overall stunting at 28%. Overall underweight children were 20.2% of the sampled 258 children. However as there were no controls, the stunting and the underweight seen could not totally be attributed to aflatoxins.

Ninety- two- point- eight percent (92.8%) of the households knew how to identify spoilt maize. Of the 278 households, 61.1% identified spoilt maize by change in colour of the grains, 14.3% by insect damage, and 5.7% by seeing rotten grains, 5% by mouldy grains, and 2.5% by smell. Concerning risks posed by spoilt maize, 78.6% were aware of some danger with 38.9% quoting stomach problems, 16.8% aflatoxins, 10% disease, 9.6% death and others cancer and liver problems. On whether mouldy feeds fed to dairy cows was safe, 53.6% knew it was not safe, 19.6% thought it was safe and 26.8% did not know. Of the 597 cereal samples collected, 83.4% had aflatoxins, 55.1% had levels up to 10ppb and 28.3% had levels above 10ppb. A high proportion (81.7%) of the maize samples were contaminated with aflatoxin with 27% of the samples having values ranging from 10ppb to 288.7ppb.The sorghum and millet samples had similar levels (86.5%) of aflatoxin contamination with 29.8% having values above 10ppb. The per capita consumption of maize in the study area was 0.30Kg/person/day, while the average aflatoxin exposure evel from maize was 6.94μg/person/day. The aflatoxin exposure level in Makindu was significantly (p<0.05) higher than in the other two divisions. From the 98 human breast milk samples collected, 86.9% were positive for aflatoxin M_1 with levels ranging from 0.215ppt to 47.5ppt. Of the 209 cow milk samples collected 88% were positive for aflatoxin (range 0.002ppt to 273.8ppt) and 93% of the 54 goat milk samples were positive for aflatoxin (range 0.84ppt to35.8ppt).

Per capita consumption for milk was 329.2ml/person/day while the exposure level through milk was 0.006ppt/person/day; exposure to children through breast milk was 5.5μg/child/day. A high proportion (79.2%) of the 250 urine samples collected on the first visit, were positive

for Aflatoxin M_1 (range 0.302ppt to 10415.1ppt). On the second visit, 126 samples were collected and 63.2% were positive (range 79.2ppt to2193ppt).

None of the potential risk factors for aflatoxin contamination considered were significant at 5% level of significance. However division appeared to be associated with aflatoxin contamination; Makindu Division had a significantly (p<0.05) higher exposure levels of aflatoxin (OR=1.4) relative to Kaiti and Wote Divisions.

It is recommended that extension messages targeting harvesting, drying, disposal of spoilt grains and shelling methods be availed to Makueni County and disseminated through women groups, churches and school farmers' clubs in order to address chronic exposure occasioned by consumption of aflatoxin contaminated foods in the daily diets.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Mycotoxins are secondary metabolites produced by fungi that are capable of causing disease and death in humans and other animals. Because of their pharmacological activity, some mycotoxins or mycotoxin derivatives have found use as antibiotics, growth promotants, and other kinds of drugs; still others have been implicated as chemical warfare agents (Bennet and Klich, 2003). These metabolites are known as mycotoxins and are especially produced by saprophytic moulds growing on poorly stored foodstuffs and animal feeds (CAST, 2003).

The world heavily relies on various cereals which differ from region to region. In many developed countries, storage of cereals and feed is more organized and properly done. In these countries, mycotoxin control measures have been implemented for agricultural commodities entering international trade and are strong in countries with centralized or large scale buying and distribution systems. However, in developing countries, where local food for subsistence is practiced by as much as 70% of the population, such measures are difficult to implement. Another challenge in developing countries is the poor storage of food and feed which contributes a great deal to production of natural toxic metabolites of fungi under favourable conditions, (Cast, 2003).

Mycotoxins have been a hazard to man and domestic animals but until the past 30 years, their effects had been largely overlooked. Moulds generally have been considered to cause aesthetic spoilage, without being dangerous to health. Between 1960 and 1970, it was established that some fungal metabolites were responsible for animal diseases and death (UNDP/FAO, 1989). In the decade following 1970 it became clear that mycotoxins had been the cause of human morbidity and mortality and had been responsible for major epidemics in

man and animals. They produce a wide range of adverse and toxic effects in animals in addition to being food borne hazards to humans (CAST, 2003).

A number of diseases are now known to have been caused by growth of specific moulds which produce one or more potent toxins, usually in one specific kind of food or feed. These diseases include: Ergotism which killed thousands of people in Europe in the last 1000 years; alimentary toxic aleukia (ATA) which was responsible for the death of many thousands of people in the USSR in the 1940s; stachybotryotoxicosis, which killed tens of thousands of horses and cattle in the USSR in the 1930s; and aflatoxicosis which killed 100,000 young turkeys in England in 1960 (UNDP/FAO, 1989).

Globally, the five most important mycotoxin-producing fungi are: *Aspergillus flavus, Aspergillus ochraceus, Penicillium verrucosum, Fusarium graminearum and Fusarium verticillioides*, (Miller, 2002). The five most important mycotoxins that occur naturally in agricultural products are: aflatoxin produced by *Aspergillus flavus*; ochratoxin produced by *Aspergillus ochraceus* and *Penicillium verrucosum;* deoxynivalenol and zearalenone produced by *Fusarium graminearum,* and fumonisin produced by *Fusarium verticillioides,* (Miller, 1995). Human diseases that have been associated with two of these mycotoxins in foods are: acute toxic hepatitis and liver cancer caused by aflatoxin; and esophageal cancer and neural tube defects caused by fumonisin. Deoxynevalenol in pig feed is known to reduce feed intake and cause occasional vomiting while zearalenone impaires growth and reproductive efficiency; and ochratoxin causes necrosis of the kidney tissue (Diekman and Green, 1992).

Mycotoxins may be detrimental to the health of humans and/or animals and may be produced on a wide range of agricultural commodities under a diverse range of conditions. Some of the mycotoxins, such as aflatoxin are among the most potent mutagenic and carcinogenic substances known (CAST, 2003). Mycotoxins are associated with many chronic health risks, including the induction of cancer, immune suppression, and digestive, blood and nerve defects (CAST, 2003; Shephard, 2006). They affect several agricultural products including cereals, oilseeds, pulses, root crops, dried fruits and coffee beans, which form the agricultural economic backbone of most developing countries in Africa (CAB International, 2008).

The current study was aimed at estimating the level of human exposure to aflatoxins from dietary sources and assessing the pre- and post- harvest practices that predispose Makueni County residents to this exposure.

1.2 Objectives

The overall objective of the study was to estimate the level of human exposure from dietary aflatoxins and to assess pre- and post-harvest practices that predispose them to this exposure.

The specific objectives were:

1. To establish the level of human exposure to aflatoxins in Makueni County.

2. To assess the associated pre- and post- harvest practices that predispose cereals to aflatoxin contamination in the County.

3. To determine occurrence and level of aflatoxins in cow milk, goat milk, human breast milk, maize, sorghum, millet and urine of children upto 5 years old in Makueni County.

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1.3 Justification and hypotheses

There have been many aflatoxicosis outbreaks in Makueni County and attention to aflatoxin poisoning has been drawn at these times without paying attention to what is consumed in the daily diets of the Makueni people. This study therefore set out to establish the levels of the daily dietary aflatoxins which could lead to chronic exposure.

Hypotheses

There is no human chronic exposure to aflatoxins in Makueni County; and there are no pre- and post-harvest practices that predispose cereals to aflatoxin contamination in Makueni County.

CHAPTER TWO: LITERATURE REVIEW

2.1. Mycotoxins and their effects

Mycotoxins are by-products of mould deterioration of foods and feeds. They are low molecular weight natural products produced as secondary metabolites by filamentous fungi. Mycotoxins should be distinguished from bacterial toxins which are proteins and produce characteristic symptoms in only a few hours, as the human body recognizes them and produce antibody mediated reactions to them. Fungal toxins, on the other hand, are low molecular weight chemical compounds which are not detected by the body immune cells and hence produce no obvious symptoms (Cast, 2003). They are generally of concern in human health, food safety, and trade because of their acute and chronic effects on humans and domestic animals.

Mycotoxins produce diverse effects depending on the particular mycotoxin involved. *Claviceps purpurea* produces ergotamine- ergocristine alkaloids which cause the gangrenous form of ergotism due to their vasoconstrictive activity, (King, 1979). There is also a convulsive form of ergotism related to intoxication with Clavine alkaloids from *Claviceps fusiformis* which is characterized by gastrointestinal symptoms followed by effects on the central nervous system, (Tulpule and Bhat, 1978, Krishnamachari and Bhat, 1976).

Aflatoxins are produced by *Aspergillus flavus*, *Aspergillus parasiticuss*, *Aspergillus niger* and *Aspergillus nomius* (Bennet, 1987). They are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic, (IARC, 1987).

Ochratoxin A has been shown to be nephrotoxic, immunosuppressive, carcinogenic and teratogenic, (WHO, 1990).

Trichothecenes produced mainly by members of the *Fusarium* genus are known to depress immune responses and cause nausea, (Saenz de Rodriguez, 1984)

Zearalenone produced mainly by *Fusarium graminearum* and related species produces estrogenic effects in various animal species,(Saenz de Rodriguez, 1984).

Fumonisins produced by *Fusarium moniliforme* and related species causes abdominal pain, borborygmus and diarrhea, (Bhat *et al.,* 1997).

These metabolites constitute toxigenically and chemically heterogeneous assemblages that are grouped together only because the members can cause disease and death in humans and other vertebrates (Bennett, 1987). They may also be produced before food harvest and passed through the food chain thereby contaminating food commodities that are not moulded, for example, aflatoxin M_1 in milk. Contamination of agricultural products occurs as a result of infection by toxigenic fungi under favourable environmental conditions in the field (CAB International, 2008) and may occur at various stages in the food chain, e.g. pre-harvest, drying, storage and or processing. In Kenya, The National Cereals and Produce Board (KNCPB) recommends a moisture content of 13.5% in maize to prevent fungal growth and aflatoxin production as agreed in the harmonized East African states standards, (http://www.newvision.co.ug/news/637036-eac-states-agree-on grainsandcerealsstandards.html, 2012). Harvesting early and artificial drying helps reduce the incidence of mycotoxins as well as preventing kernel breakage and stored grain insect infestations (Bruns, 2003).

Insect damage to seeds or nuts creates wounds and a microclimate that encourages fungal colonization, and the insects serve as vectors of fungal spores (Tuite, 1984; Munkvold and Hellmich, 1999; Waliyar *et al.,* 2003). Maize grains destroyed by insects predispose the grains to aflatoxin contamination and causes them lose their white colour and turn yellowish

as shown in (Plate 2.1). A survey done by Hell *et al*. (2000) found out that maize with no insect damage had no aflatoxin contamination, but maize which had 70% of the cobs damaged by insects had 30.3% of the cobs contaminated with aflatoxin.

Discoloured maize grain damaged by insects

Plate 2.1: Discoloured maize grains destroyed by insects (Source: Farm in Makindu Division)

2.2 Fungi growth and toxin production

Whether fungi will grow and produce toxins depends on the environmental conditions and the specific temperature and water activity requirements of the particular fungus and whether that particular fungus has the gene coding for toxin production (Marin *et al.,* 2001, 2004). In general, fungal growth during storage is at between 13%-18% moisture content while saprophytic fungi will grow at between 22%-25% moisture content. Humidity levels of more than 80% are ideal for fungal growth and production of mycotoxins. At 12°C, fungi will grow slowly and produce very little mycotoxins.

2.3 Economic impacts of mycotoxins

Huge quantities of food are wasted every year because they are invaded by toxic fungi or contaminated by fungal metabolic products. Such waste occurs most prominently in hotter countries where food shortages may already be a problem (Marin *et al.,* 2004). One estimate

(CAST, 2003) is that mycotoxins affect a quarter of the world's food crops, including many basic foodstuffs and animal feed, as well as crops, e.g., coffee, with high economic value. Mycotoxins have significant economic impacts in numerous crops, especially wheat, maize, peanuts and other nut crops, cottonseed, and coffee. The FAO (2007) estimated that 25% of the world's crops are affected by mycotoxins each year, with annual losses of around 1 billion metric tons of foods and food products (Smith *et al.,* 1994). Economic losses occur because of: 1) yield loss due to diseases induced by toxigenic fungi; 2) reduced crop value resulting from mycotoxin contamination; 3) losses in animal productivity from mycotoxin-related health problems; and 4) human health costs. Additional costs associated with mycotoxins include the cost of management at all levels– prevention, sampling, mitigation, litigation, and research costs. These economic impacts are felt all along the food and feed supply chains (crop producers, animal producers, grain handlers and distributors, processors, consumers, and society as a whole (due to health care impacts and productivity losses). The economic consequences of mycotoxin contamination are profound, as crops contaminated with high levels of mycotoxin are often destroyed. In the United States the mean economic annual costs of crop losses from mycotoxins are estimated to be $932 million, (Betran and Isakeit, 2003).

Diets in many developing countries are based on crops susceptible to mycotoxins, leading to high levels of chronic health problems in tropical, developing countries. A further concern is that the absence of visible mold does not guarantee the grain is free from mycotoxin, and cooking or processing the food product does not necessarily rid it off mycotoxin contamination. However, cooking of maize has been reported to reduce the level of aflatoxins by 46.6%, 28-72% and 80-93% in maize containing 10.7- 270ng/g of aflatoxin levels in Kenya (Mutungi *et al.,* 2008). These findings indicate that exposure to acute aflatoxin levels

is minimized during food processing. Toxigenic moulds have been isolated in processed food products such as bread, macaroni, cooked meat, cheese, and maize flour (Guerzoni, 1999).

Since the discovery of aflatoxins in the early 1960s (Blount, 1961) researchers worldwide have made significant progress identifying and understanding the major classes of mycotoxins and the fungi that produce them. Regulations exist worldwide against the sale of contaminated commodities, and the economic effect of these regulations is great. One study conducted in West Africa estimated crop losses (corn, wheat and peanuts) from mycotoxin contamination at $932 million annually, in addition to losses averaging $466 million annually from regulatory enforcement, testing, and other quality control measures (CAST, 2003). Wilson and Otsuki (2001) estimated that, for a group of 46 countries including the United States, the adoption of a uniform aflatoxin standard based on international Codex Alimentarius Commission (Codex) guidelines would increase trade of cereals (grains) and nuts by more than $6 billion, or more than 50 percent, compared with the divergent standards in effect during 1998.

Consumers in the developed world are well aware of the carcinogenic effect of aflatoxins and will thus stay away from a product that has aflatoxin beyond the acceptance level. Exports of agricultural products particularly groundnuts and other oilseeds from developing countries have dropped considerably in recent years resulting in major economic losses to producing countries as a result of these restrictions (Bhat and Visconti, 1999; Otsuki *et al.,* 2001).

According to the World Bank estimate, the policy change by the European Union will reduce by 64%, imports of cereals, dried fruits, oil seeds and nuts from nine African countries (Chad, Egypt, Gambia, Mali, Nigeria, Senegal, South Africa, Sudan and Zimbabwe) and this will cost African countries about US $670 million in trade per year (Kellerhal, 2000). Aflatoxins

impact,in maize in the U.S. has been estimated as a $225 million/yr not including mitigation costs ($20-30 million/yr just for testing) (Wilson and Otsuki, 2001). Estimates of the costs of mycotoxins in the United States vary and reduced crop value is a significant component of the losses caused by mycotoxins. This affects crops entered into local trade as well as crops intended for export. In aflatoxin-outbreak years in the U.S., many producers are turned away by grain elevators and other buyers because their crop has aflatoxin exceeding the 20 ppb limit. They are forced to accept a lower price on the local feed market, or even to dispose of their crop. Internationally, a standard limit of 4 ppb (adopting the European Union limit) for aflatoxins in peanuts would be estimated to cost about $450 million annually in lost exports, (Wilson and Otsuki, 2001).

 In developing countries, few estimates are available, but based on the elevated levels of aflatoxins regularly found in the developing world it is likely that losses consistently exceed those occurring in the United States. The impact of export losses is worsened by the situation in which developing countries, whose populations are most at risk for aflatoxin exposure, may be forced to export their highest quality maize and retain the poorer grain for domestic use. In 2010, 2.3 million bags of maize grown in eastern and the coastal regions of Kenya were declared by the Ministry of Public Health and Sanitation as being unfit for human consumption due to high levels of aflatoxins.This translates to about Ksh 3.2 billion.

In spite of four decades of research worldwide establishing the extent of mycotoxin contamination, recurrent episodes of chronic and acute toxicity following mycotoxin contamination, and the extensive economic costs associated with this, mycotoxins have continued to be a global problem to human and animal health since the earliest times. This threat will only increase as the demand on the available food supply increases in response to

the growth of the world population (Marasas and Nelson, 1987). If the food supply is limited, the mycotoxin hazard is exacerbated in at least two ways. First, more fungus-damaged, potentially mycotoxin-containing foodstuffs are consumed rather than discarded, and second, malnutrition enhances the susceptibility to lower levels of food-borne mycotoxins exposure and thus mycotoxin contamination is not considered an epidemic, (CAB, 2008).

Major classes of mycotoxins include, *Aspergillus, Claviceps, Fusarium, Penicillium*, *Neotypholium* and *pithomyces*, (www.mycotoxins.info Accessed on 15/9/2014).

Diagnosis of mycotoxicoses in animals is difficult as the symptoms induced may be similar to other diseases due to other causes. Moreover, the effects of mycotoxins often are synergistic (CAST, 2003).

2.4 Aflatoxins and fungi that produce them

Aflatoxins are difuranocoumarin derivatives produced by a polyketide pathway by many strains of *Aspergillus flavus* and *Aspergillus parasiticus*; in particular, *Aspergillus flavus* is a common contaminant in agriculture. *Aspergillus bombycis*, *Aspergillus ochraceoroseus*, *Aspergillus nomius*, and *Aspergillus pseudotamari* are also aflatoxin-producing species, but they are encountered less frequently (Goto *et al.,* 1996; Peterson *et al.,* 2001; Klich, 2002). From the mycological perspective, there are great qualitative and quantitative differences in the toxigenic abilities displayed by different strains within each aflatoxigenic species. For example, only about half of *Aspergillus flavus* strains produce aflatoxins (Klitch *et al.,* 1995), those that do may produce over 106 µg/kg (Cotty *et al.,* 1994). *Apergillus flavus* only produces the B toxins (Klitch and Pitt, 1988) while *Aspergillus nomius* and *Aspergillus parasiticus* produce both B and G toxins (Vaamonde *et al.,* 2003).

Some strains of *Aspergillus flavus* are regarded as the S strains based on the size of the sclerotia they produce. These strains are known to produce more toxin than toxigenic A. flavus which are L strains, (Cotty and Cadwell, 1999). The common aflatoxins are aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2. Their molecular weights are 312.3g/mol for aflatoxin B_1, 314.3g/mol for aflatoxin B_2, 328.3g/mol for aflatoxin G_I and 330.3g/mol for aflatoxin G_2. Aflatoxin M_1 and M_2 which are metabolites of aflatoxins were first isolated from milk of lactating animals that were fed on aflatoxin preparations (Bahout and El-Abbassy, 2004).

Aflatoxin B_1 is the most toxic of the aflatoxin types (Olaru *et al.,* 2008). During the outbreak that killed 125 people in Eastern Province in Kenya in 2004, aflatoxin B1 was detected from the samples collected (Azziz-Baumgartner *et al.,* 2005).

The Food and Agriculture Organization/World Health Organization (FAO/WHO, 1997) Joint Experts Committee on Food Additives has established guidelines for maximum food aflatoxin levels to reduce the amount of contaminated food that reaches consumers and animals as shown in Table 2.1.

Table 2.1: Recommended maximum levels of aflatoxin in food and feed (Source: FAO)

Product or animal	Total aflatoxin action level (µg/kg)
Human Food	20
Milk	0.5
Beef Cattle	300
Swine Over 100 Lbs	200
Breeding Beef Cattle, Swine, or Mature Poultry	100
Immature Animals	20
Dairy Animals	20

 Kenya has adopted the FAO /WHO (2005) limit of 10 parts per billion (ppb) total aflatoxins in cereals for human consumption.

2.5 Conditions for production of aflatoxins

Aflatoxins are produced in cereals and forages under ideal conditions of moisture, temperature and humidity. They often occur in crops in the field before harvest and are usually associated with drought stress (Diener *et al.,* 1987; Klitch, 1987). Common crops for fungal colonization and aflatoxin production include maize, groundnuts, wheat, rice and sorghum (Lisker *et al.,* 1993).

Post-harvest contamination can also occur if drying of crops is delayed and if water is allowed to exceed critical values (10 – 13%) for mould growth in stored crop (John, 2007). Even more problematic is the fate of crops stored under conditions that favour mould growth. Usually the most important variables are the moisture content of the substrate and the relative humidity of the surroundings (Detroy *et al.,* 1971; Wilson and Payne, 1994). Temperatures

between 20 to 35°C and relative humidity of more than 85% are optimal for Aspergillus species growth and aflatoxin production (Diener *et al*., 1987).

Kenya largely depends on agriculture for her economic development. The main staple food is maize, which accounts for more than 75% of the total area grown cereal and more than 60% of the total cereal marketed (Wilkister, 2008). These crops are produced under diverse weather conditions and harvested and stored in as diverse ways. Poor methods of harvesting and storage lead to mycotoxin contamination. Kenya National Produce Board recommends a moisture content of 12-15% in maize to prevent fungal growth and eventual aflatoxin production (Heather *et al.,* 2006).

2.6 Toxicity mechanism of aflatoxins

The human gastrointestinal tract quickly absorbs aflatoxins after consumption of contaminated feeds, and the circulatory system transports the toxins to the liver (Fung and Clark, 2004). From 1% to 3% of ingested aflatoxins irreversibly bind to protein and DNA bases to form adducts such as aflatoxin B1-lysine in albumin (Skipper and Tannenbaum, 1990). Disruption of proteins and DNA bases in hepatocytes causes liver toxicity (Tandon *et al.,* 1978). Cytochrome P450 enzymes convert aflatoxins to the reactive 8, 9-epoxide form (also referred to as aflatoxin-2, 3 epoxide), which is capable of binding to both DNA and proteins (Eaton and Groopman, 1994). Mechanically, it is known that the reactive aflatoxin epoxide binds to the N7 position of guanines. Moreover, aflatoxin B1-DNA adducts can result in GC to TA transversions in the P53, a DNA-repair, tumor suppressor gene, at codon 249. Inactivation of the P53 tumor suppressor gene leads to the development of primary liver cancer (Bressac et al., 1991; Hsu *et al.,* 1991).

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Aflatoxin contamination also has a reactive glutathione S-transferase system found in the cytosol and microsomes which catalyze the conjugation of activated aflatoxins with reduced glutathione, leading to the excretion of aflatoxin (Raj *et al.,* 1986). Variations in the level of the glutathione transferase system as well as variations in the cytochrome P450 system are thought to contribute to the differences observed in interspecies aflatoxin susceptibility (Eaton and Ramsdel, 1992; Eaton and Groopman, 1994).

2.7 Prevalence of aflatoxin in foods

Several prevalence studies of aflatoxin have been conducted in various countries.

In the United States, less than 50% of corn samples collected between 1978 and 1993 were aflatoxin contaminated with level of contamination ranging from 0.1 to 80 µg/Kg, (IARC, 1993).

In India, a study done in Magalore, Karnatake 6% of all samples gathered had aflatoxins.

In Jordan, toxin producing *Aspergillus flavus* and *Aspergillus parasiticus* were found in maize samples at prevalence rates of 18% and 22%, respectively (Nisreen *et al.,* 2012).

In Africa, the toxins have been found in foods from Botswana, Ethiopia, Lesotho, Malawi, South Africa, Swaziland, Tanzania, Uganda, Zambia, Zimbabwe and Kenya (CAB Intern., 2008). In Congo, the incidence in peanuts and levels of aflatoxin vary with season. Highly contaminated samples are obtained during the rainy seasons (90% frequency) than in the dry seasons (53.2%) frequency), (Kamika and Tokoy, 2011). The highest contamination noted in peanuts in Malawi (ICRISAT, 2010) was in districts that were prone to late season rains which enhance favourable conditions for post-harvest contamination. Lower aflatoxin

contamination levels were observed in drier North African countries than amounts reported in South African countries such as Uganda, Nigeria, Ghana and Malawi, (ICRASAT, 2010).

Kang'ethe *et al.* (2007) reported the prevalence of aflatoxin M_1 to be 45.5% in milk and 98.6% in feeds from urban smallholder dairy production in Dagoretti Division, Nairobi, Kenya. In another study Kang'ethe and Lang'at (2009) reported B_1 and M_1 contamination of animal feeds and milk from urban centres in Kenya.

During an acute aflatoxicosis outbreak in Makueni District, Kenya, in 2004, samples collected showed that 35.5% of the locally grown maize had levels of aflatoxins in excess of the recommended minimum of 20ppb by FAO/WHO, (2003). Of these, 20.2% had levels exceeding 100ppb while 10.6% had levels above 1000ppb. In Busia and Homabay districts in Kenya, peanuts were found to be contaminated with aflatoxins with levels ranging from 0-7525ppb (Mutegi *et al*., 2010).

In a study conducted in Nairobi, Kenya to determine maize flour contamination, Aflatoxin B_1 and B_2 were found at levels ranging between 0.4- 20ppb, this level was high taking into account a consumption level of 0.4Kg/person /day (Muriuki and Siboe, 1995). A study conducted in Nairobi, Kenya between the years 2006-2009 concluded that more than 83% of maize flour samples were contaminated with levels exceeding the recommended levels of 10ppb, with the highest levels reaching 4593.93ppb (Okoth and Kola, 2012).

2.8 Aflatoxicosis

Aflatoxins are associated with both toxicity and carcinogenicity in human and animal populations (Newberne and Butler, 1969; Shank *et al.,* 1972; Peers and Linsell, 1973; Eaton,

Groopman, 1994). The diseases caused by aflatoxin consumption are loosely called aflatoxicoses. Acute aflatoxicosis results in death while chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions (Hsieh, 1988). The liver is the primary target organ, with liver damage occurring in poultry, fish, rodents, and nonhuman primates fed aflatoxin B_1. There are substantial differences in species susceptibility. Even within a given species, the magnitude of the response is influenced by age, sex, weight, diet, exposure to infectious agents, and the presence of other mycotoxins and pharmacologically active substances (Hsieh, 1988).

Due to the differences in aflatoxin susceptibility in test animals, it has been difficult to extrapolate the possible effects of aflatoxin to humans, although acute toxicity of aflatoxins in Homo sapiens is rarely observed. It is believed that a 1974 Indian outbreak of hepatitis B in which 100 people died may have been due to the consumption of maize that was heavily contaminated with aflatoxin. Indeed some adults may have eaten 2 to 6 mg of aflatoxin in a single day (Chrishnamachari et al., 1975). Low dose consumption of aflatoxin contaminated food- stuff causes chronic aflatoxicosis. Subsequently, it was calculated that the acute lethal dose for adults is approximately 10 to 20 mg of aflatoxins (Pitt, 2000). One anecdotal report refutes this estimate. A woman who had ingested over 40 mg of purified aflatoxin in a suicide attempt was still alive 14 years later. Multiple laboratory tests of her urine and blood, and X-ray, ultrasound, and computerized axial tomography analyses of her abdomen, liver, and spleen all gave normal results (Willis et al., 1980).

It has been hypothesized that kwashiorkor, a severe malnutrition disease, may be a form of pediatric aflatoxicosis (Hendrickse, 1977). This is because children are exposed to aflatoxins from an early age through breast milk and weaning food. Early speculations that aflatoxin

might be involved in Reye's syndrome, an encephalopathy, and fatty degeneration of the viscera in children and adolescents (Hayes, 1980) have not been substantiated. Nevertheless, aflatoxins have achieved some notoriety as a poison. The plot of the Human Factor, a spy thriller by Graham Greene (Greene, 1979), revolves around the murder of a central figure whose whiskey was laced with aflatoxin (a toxicologically improbable way to kill someone). Nevertheless, aflatoxin's reputation as a potent poison may explain why it has been adopted for use in bioterrorism. There is substantial evidence that Iraq stockpiled aflatoxin to be delivered in missiles (Bennet and Klich, 2004).

Exposure to aflatoxins in the diet is considered an important risk factor for the development of primary hepatocellular carcinoma, particularly in individuals already exposed to hepatitis B. In classical epidemiology, several studies have linked liver cancer incidence to estimated aflatoxin consumption in the diet (Peers and Linsell, 1973; Van Rensburg *et al.,* 1985; Li *et al.,* 2001). The results of these studies have not been entirely consistent, and quantification of lifetime individual exposure to aflatoxin is extremely difficult. The incidence of liver cancer varies widely from country to country as shown in Table 2.2, but it is one of the most common cancers in China, the Philippines, Thailand, and many African countries.

Table 2.2: Estimated hepatocellular carcinoma incidence attributable to aflatoxin.

WHO region/country	Aflatoxin exposure(ng/kg body wt/day)	Estimated annual HCC/100000 HBsAgnegative	HBsAgpositive	References
Democratic Republic of Congo	0.07-27	0.0007-0.27	0.02-8.10	Manjula *et al*., 2009b
Ethiopia	1.4-36	0.01-0.36	0.42-10.8	Ayalew *et al*., 2006b
Nigeria	139-227	1.39-2.27	41.7- 68.1	Bandyopadhyay *et al*., 2007, Bankole and Mabeloje, 2004b
South Africa	0-17	0-0.17	0-5.10	Hall and Wild,1994; Shephard, 2003
Kenya	3.5-33	0.04-1.33	1.05-39.9	Hall and Wild,1994; Shephard, 2008
Tanzania	0.02-50	0.0002-0.50	0.06-15.0	Manjula *et al*., 2009b
Egypt	7-57	0.07-0.57	2.1-17.1	Anwar *et al.,* 2008b
Sudan	19-186	0.19-1.86	5.70-55.8	Omer *et al*.,2008b
Canada	0.2-0.4d	0.0020.004	0.06-0.12	Kuiper Goodman,1995
USA	0.26	0.003	0.008	IPCS/WHO 1998
Brazil	0.23-50	0.002-0.50	0.07-15.0	IARC,2002,Medio et al.,2001; Oliveira *et al.,* 2009; Vargas *et al*.,2001b
India	4-100	0.004-1.00	1.20-30.0	Vasanti, 1998
China	17-37	0.17-0.37	5.10-11.1	Li et al., 2001; Qiang *et al.,* 1994, Wang and Liu, 2007, Wang' *et al.,*2001b

Source: Environ Health Perspect (2010)

The presence of hepatitis B virus infection, an important risk factor for primary liver cancer, complicates many of the epidemiological studies. In one case-control study involving more than 18,000 urine samples collected over 3.5 years in Shanghai, China, aflatoxin exposure alone yielded a relative risk of about 2; hepatitis B virus antigen alone yielded a relative risk of about 5; combined exposure to aflatoxin and hepatitis B yielded a relative risk of about 60 (Ross *et al.,*1992). Vaccination against hepatitis B virus is recommended as a more realistic and cost-effective strategy for lowering liver cancer incidence than removing aflatoxin from the diet (Henry et al., 1999; Henry *et al.,* 2002).

In developed countries, sufficient amounts of food combined with regulations that monitor aflatoxin levels in these foods protect human populations from significant aflatoxin ingestion. However, in countries where populations are facing starvation or where regulations are either not enforced or nonexistent, routine ingestion of aflatoxin may occur ((Bhat *et al.,* 1997). Worldwide, liver cancer incidence rates are 2 to 10 times higher in developing countries than in developed countries (Henry *et al.,* 1999). Unfortunately, strict limitation of aflatoxin-contaminated food is not always an option. A joint Food and Agriculture Organization/World Health Organization/United Nations Environment Programme Conference report stated that "in developing countries, where food supplies are already limited, drastic legal measure may lead to lack of food and to excessive prices. It must be remembered that people living in these countries cannot exercise the option of starving to death today in order to live a better life tomorrow (Henry *et al.,* 1999).

2.9 Aflatoxins and their effects

These compounds are potent carcinogens found in crops such as maize, groundnuts, legumes and other grains that form the staple diet in many developing countries (Cardwell, 2001).

Among the aflatoxin chemotypes (B_1, B_2, G_1 and G_2), B_1 is the most common and toxic form (Park *et al.,* 2002, Olaru *et al.,* 2008). Aflatoxin B_1 is the most potent natural carcinogen known (Squire, 1981) and is usually the major aflatoxin. *Aspergillus flavus* produces only aflatoxins B_1 and B_2 whereas Aspergillus parasiticus produces aflatoxin B_1, B_2, G_1 and G_2 (CAB International, 2008). They are classified on the basis of the fluorescence colour they exhibit on thin layer chromatography; B for blue and G for Green fluorescence (Bennett and Klitch, 2003). Aflatoxin M_1 is a hydroxylated metabolite of aflatoxin B_1 found in the milk and urine of humans or other mammals that consume a diet contaminated with aflatoxin B_1.

The toxicological effects of aflatoxin are dose dependent. At high doses they are lethal if consumed, causing liver, myocardial and kidney tissue damage. At sub-lethal doses they cause chronic toxicity, e.g. liver cirrhosis, and at low doses they are potent human hepatocellular carcinogens (Wild and Turner, 2002). They are also mutagenic and teratogenic and can depress cell-mediated immunity (IARC, 1993, Neal *et al.,* 1998, Williams *et al.,* 2004). Chronic exposure of humans to low levels of aflatoxins occurs more commonly than acute toxic exposure. This exposure is associated with the development of hepatocellular carcinoma in humans (Bosch and Munoz, 1988; Ozturk, 1991; IARC, 1996), especially in those infected with hepatitis B virus (Ross *et al.,* 1992; Qian *et al.,* 1994; Wang *et al.*, 1996, 2001). Thus, chronic aflatoxin exposure is a major and significant public health problem.

Aflatoxins are less well known for their role in immune suppression, although numerous studies have been published since the late 1960s (Gallikeev *et al.*, 1968; Pier and Heddleston, 1970; Savel *et al.,* 1970) on the effects of aflatoxin on the immune system of animals (in vivo studies) and on animal and human immune cells in vitro. These studies have been reviewed extensively (Pier, 1986; Richard, 1991; Bondy and Pestka, 2000; Oswald *et al.,* 2005).

However, data on the immunotoxic effects of aflatoxins in humans are limited and only two reports have been made in recent years on the effects of aflatoxins on humans who are chronically exposed to it in the diet (Turner *et al.,* 2003; Jiang *et al.,* 2005). These reports indicated that chronic exposure to aflatoxins in childhood may have a critical influence on disease outcomes in later life. Animals can retain residues of aflatoxins or their metabolites in their tissues (Trucksess *et al*., 1982; Cook *et al.,* 1986; Fernandez *et al.,* 1994).

2.10 Effects of aflatoxins on humans

Human health impacts of mycotoxins are the most difficult to quantify. It is clear that mycotoxins affect human health, especially aflatoxins in developing countries. These effects are due to acute (single exposure) toxicoses and immunosuppression by mycotoxins, as well as chronic (repeated exposure) effects. Diseases modulated by mycotoxins accounted for 40% of lost disability-adjusted life years (DALYs) in a World Bank report, (1993) on human health. Of the reported $900 million impact of aflatoxins in Southeast Asia, $500 million of the costs were related to human health effects (World Bank, 2005). A report from the National Academy of Sciences (1996) concluded that mycotoxins probably contribute to human cancer rates, even in the United States. On a global scale, human health is the most significant impact of mycotoxins, with significant losses in monetary terms (through health care costs and productivity loss) and in human lives lost.

Outbreaks of aflatoxicosis in Kenya have led to hundreds of fatalities, even during the past decade. These Outbreaks that occur every year since the major outbreak in 2004 (CDC, 2004; Muture and Ogana, 2005, Azziz-Baumgartner *et al.,* 2005) indicate that the population is exposed to aflatoxins in their diet. Chronic exposure may be a more serious problem than the outbreaks of aflatoxicosis that attract attention at the time they occur (Sheila and Kola, 2012).

2.11 Aflatoxin effects in animals

Contaminated crops are sometimes diverted to animal feed which can reduce growth rates, lead to illness of animals consuming contaminated feeds and result in meat and milk containing toxic residues or biotransformation products. Aflatoxins in feed are known to be associated with liver damage in animals, reduced milk and egg production, poor weight gain, and recurrent infections due to immune suppression. The young of any particular species are most vulnerable, but the degree of susceptibility varies by species (Meissonnier *et al.,* 2008). Mycotoxins can be detected in meat, milk, and eggs from animals that have consumed feed ingredients containing mycotoxins, and many countries have tolerance standards for mycotoxin residues in these products. A study to determine natural occurrence of aflatoxin residues in fresh and sun dried meat in Nigeria in 2010 noted 100% prevalence in Oyoo state (Olufunmilayo and Akeeb, 2010). Another study to determine contamination and prevalence of aflatoxin and some antibiotic residues in eggs in Libya in 2008 found the prevalence to be 14% (Salem *et al.,* 2009). Another concern related to the consumption of mycotoxin-contaminated feed by livestock is the potential for economic losses from animal health and reduced productivity.

2.12 Signs of aflatoxin poisoning and effects of exposure

Consuming food products that contain high levels of certain mycotoxins can cause the rapid onset of mycotoxicosis, a severe illness characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and in rare cases, death (Groopman *et al.,* 1988). Although lethal cases are uncommon, acute illnesses from mycotoxins, particularly aflatoxins (aflatoxicosis), have been reported from many parts of the world; usually in developing countries, (Groopman *et al.,* 1988).

Studies in Kenya, Mozambique, Swaziland and South Africa have found that aflatoxin levels in the diet and the incidence of primary liver cancer are correlated (Groopman *et al.,* 1988). Aflatoxin consumption has also been implicated in some infant diseases such as Kwashiorkor, a form of protein malnutrition (Hendrickse *et al.,* 1982). Recently, these toxins have been implicated in stunted children in Benin and Togo, where growth was reduced in children eating foods highly contaminated with aflatoxins introduced at weaning (Gong *et al.,* 2002). Okoth and Ohingo, (2004) reported a significant correlation between wasting and aflatoxin exposure in children under 3 years of age in Kisumu, Kenya.

2.13 Aflatoxicosis outbreaks

Levels exceeding 100ng/g are common in foods in some parts of Africa (Van Egmond, 2004). Some notable outbreaks include the deaths of 3 Taiwanese in 1967, and the deaths of more than 100 people in Northwest India in 1974. Both outbreaks were attributed to aflatoxin contamination of rice in Taiwan and corn in India (Fuzhi, 2009). In Kenya there have been continuous incidences of acute poisoning due to mycotoxins especially through the staple food maize as shown in table 2.3.

Table 2.3: Aflatoxicosis outbreaks in Kenya

Year	Those affected	Numbers affected	Localities (Location/District)	Sorces of the toxin	Observed complications/effects	References
1960	Ducklings	16000	White settler farmer in rift valley	Aflatoxin contaminated groundnut feed	Death	Peers and Linsell, 1973.
1977	Dogs/poultry	Large numbers	Nairobi, Mombasa, Eldoret	Contaminated products due to poor storage	Death	FAO/WHO/UNEP, 1977.
1981	Humans	12	Machakos	Contaminated maize	Death	Ngindu *et al.,* 1982
1984/85	Poultry	Large numbers	Poultry farms	Contaminated imported maize	Death	Ngindu *et al.*, 1982
1988	Humans	3	Meru north	Contaminated maize	Death and acute effects	Autrup *et al.,*1987
2001	Humans	3	Meru north	Mouldy maize	Death, (16 deaths)	Probst et al., 2007
2002	Poultry/Dogs	Large numbers	Coast	Contaminated feeds	Death	Njapau et al, 2007
2003	Humans	6	Thika	Mouldy maize	Death	Onsongo, 2004
2004	Humans	331	Eastern, Central, Makueni, Kitui	Aflatoxin contaminated grains	Acute poisoning, (125 deaths)	Lewis *et al.,* 2005
2005	Humans	75	Makueni, Machakos, Kitui	Contaminated maize	Acute poisoning (75 cases with 32 deaths)	Eduardo Azziz-Baumgartner *et al.,* 2005

Year	Those affected	Numbers affected	Localities (Location/District)	Sorces of the toxin	Observed complications/effects	References
2006	Humans	20	Makueni, Kitui, Machakos	Contaminated maize	Acute poisoning, (10 deaths)	Muture and Ogana, 2005
2007	Humans	4	Kibwezi, Makueni	Contaminated maize	2 deaths	Wagacha and Muthomi, 2008
year	Those affected	Numbers affected	Localities (Location/District)	Sorces of the toxin	Observed complications/effects	References
2008	Humans	5	Kibwezi, Kajiado, Mutomo	Contaminated maize	3 hospitalized, 2 deaths	Muthomi *et al.*,2009
2010	Humans		29 districts in Eastern Kenya	Suspected contaminated maize	Price spiraldown and trade breakdown, unconfirmed dog cases	Muthomi *et al.,* 2010

Source: FAO Report, 2012

2.14 Methods of decreasing exposure to aflatoxins

The following methods are recommended for the control of Aflatoxins (WHO, 2005):

Plant breeding to select for fungi resistant varieties; in Kenya there are efforts to promote less vulnerable maize varieties.

Promotion of rapid and effective drying methods of grains after harvesting;

Evaluation and adaptation of storage technologies to local conditions, for example, grain warehouses with dryers and rapid testers of grain moisture. Promotion of good traditional practices such as sorting, sieving, steeping, density segregation and use of natural preservative agents;

Evaluation and promotion of packaging technologies that reduce fungal contamination;

Education of the general public, farmers and consumers on the risks associated with consumption of aflatoxin-contaminated food and promotion of good agricultural practices;

Biocontrol- this can be done by use of atoxigenic strains to out-compete the toxigenic strains and thereby reduce their population (UNIDO, 2010). An endophytic bacterium and a fungus are being tested as pre- and post-harvest biocontrols of mycotoxin accumulation, respectively. The bacterium, a strain from a subgroup of *Bacillus subtilis,* operates under the principle of competitive exclusion, reducing the fumonisin and aflatoxin concentration in plants. The fungus *Trichoderma* species is being tested for postharvest control of fumonisin and aflatoxin production in kernels during storage (Bacon *et al.,* 2001). Efforts of biocontrol are ongoing in Kenya (Ranajit, *et al.,* 2011).

2.15 Food detoxification methods

There are various methods that have been tried for detoxification but none provides optimal results for all commodities. Such methods include:

Hydrogen peroxide treatment (Sreenivasamurthy *et al., 1967)*. This is a promising laboratory method that has not been applied in the field.

Electronic and hand sorting (Dickens and Whitaker, 1975), this method works well for peanuts and other nuts in West Africa.

Ammonium treatment (Masri *et al.,* 1969), this method is receiving a lot of attention in the USA where some farms are using it on a continuous basis for cotton seed.

Formaldehyde and calcium hydroxide treatment (Codifer *et al.,* 1976) still require additional research. Sodium hypochlorite treatmen (Natarajan *et al.,* 1975b) which is still at the experimental stage.

2.16 Tests for aflatoxin

Methods for aflatoxin assay have been available for more than a decade. The technology is based on the ability of a specific antibody to distinguish the three-dimensional structure of a specific aflatoxin.

2.16.1 Enzyme Linked Immunosorbent Assay

The direct competitive Enzyme Linked Immunosorbent Assay is commonly used in mycotoxin analysis (Chu, 1996). A conventional microtitre plate ELISA requires equilibrium of the antibody–antigen reaction that would require an incubation time of approximately 1–2 hours. Currently, most of the commercially available ELISA test kits for mycotoxins are working in the kinetics phase of antibody–antigen binding, which reduces the incubation time to minutes. Although reduction of incubation time may lead to some loss of assay sensitivity, the test kit can provide accurate and reproducible results.

A typical principle of direct competitive ELISA is shown in Figure 2.1. After a mycotoxin is extracted from a ground sample with a solvent, a portion of the sample extract and a conjugate of an enzyme coupled mycotoxin are mixed and then added to the antibody-coated microtitre wells. Any mycotoxin in the sample extract or control standards is allowed to compete with the enzyme conjugated mycotoxin for the antibody binding sites. After washing, an enzyme substrate is added and blue color develops. The intensity of the color is inversely proportional to the concentration of mycotoxin in the sample or standard. A sulphuric acid is then added to stop the enzyme reaction. The intensity of the solution color in the microtitre wells is measured optically using an ELISA reader with an absorbance filter of 450 nm. The optical densities (OD) of the samples are compared to the ODs of the standards and an interpretative result is determined. High optical density means aflatoxins are low while a low optical density means aflatoxin levels are high.

Enzyme Linked Immunosorbent Assay test kits are favored as high throuput assays with low sample volume requirements and often less sample extract clean-up procedures compared to conventional methods such as Thin Liquid Chromatography and High Performance Liquid Chromatography (HPLC). The methods can be fully quantitative. They are rapid, simple, specific, sensitive and portable for use in the field (Trucksess, 2001).

Although the antibodies have the advantage of high specificity and sensitivity, the target compounds are mycotoxins but not the antigens, and therefore compounds with similar chemical groups can also interact with the antibodies. This so-called matrix effect or matrix interference commonly occurs resulting in underestimates or overestimates in mycotoxin concentrations in commodity samples (Trucksess and Koeltzow, 1995). Additionally, insufficient validation of ELISA methods causes the methods to be limited to those matrices

for which they were validated (Gilbert and Anklam, 2002). Therefore, an extensive study on the accuracy and precision of an ELISA method over a wide range of commodities is needed and a full validation for an ELISA method is essential and critical (Zheng *et al.,* 2005).
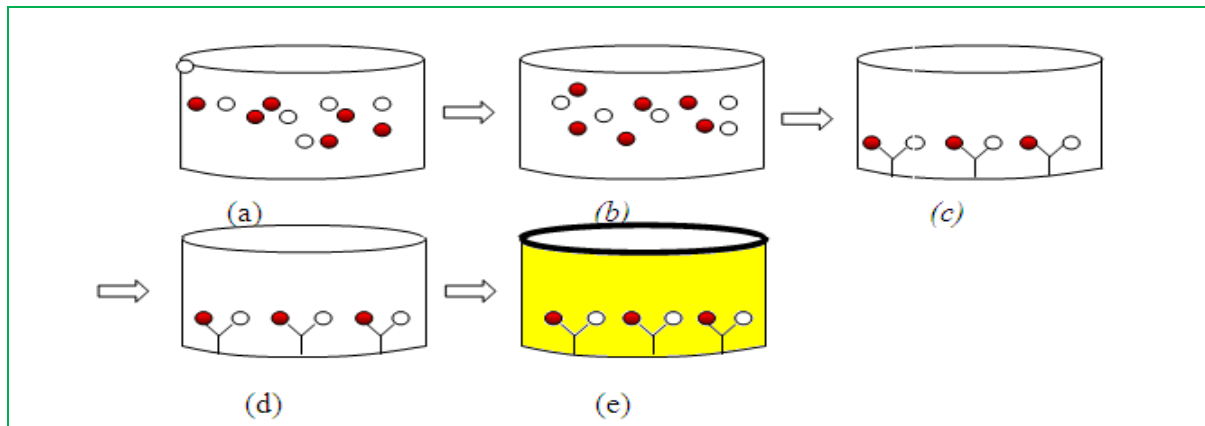
Key =   ● Mycotoxin

          Y Anti –mycotoxin antibody

          S Substrate

          O Conjugate

Figure 2.1: Principle of competitive ELISA for mycotoxin analysis. (Source:

Mycopathologia (2006) 161: 261–273)

(a) Sample mixed with conjugate; (b) mixed content added to antibody coated well; (c) mycotoxin binds to antibody in the first incubation; (d) unbound materials are washed away in the washing step; (e) substrate is added to develop colour and then stop solution is added to stop the reaction.

2.16.2 High Performance Liquid Chromatography

This method is used to separate the different aflatoxins and allows for calculation of content of individual aflatoxins in the sample (Wikipedia, 2013).

The sample mixture to be separated and analyzed is introduced, in a discrete small volume (typically microliters), into the stream of mobile phase percolating through the column. The

components of the sample move through the column at different velocities, which are function of specific physical interactions with the sorbent (also called stationary phase). The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called its retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte. Many different types of columns are available, filled with sorbents varying in particle size, and in the nature of their surface ("surface chemistry"). The use of smaller particle size packing materials requires the use of higher operational pressure ("backpressure") and typically improves chromatographic resolution (i.e. the degree of separation between consecutive analytes emerging from the column).

 In terms of surface chemistry, sorbent particles may be hydrophobic or polar in nature. Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol). Some HPLC techniques use water-free mobile phases .The aqueous component of the mobile phase may contain acids (such as formic, phosphoric or trifluoroacetic acid) or salts to assist in the separation of the sample components. The composition of the mobile phase may be kept constant ("isocratic elution mode") or varied ("gradient elution mode") during the chromatographic analysis. Isocratic elution is typically effective in the separation of sample components that are not very dissimilar in their affinity for the stationary phase.

In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength. The eluting strength of the mobile phase is reflected by analyte retention times with high eluting strength producing fast elution (short retention times). A typical

gradient profile in reversed phase chromatography might start at 5% acetonitrile (in water or aqueous buffer) and progress linearly to 95% acetonitrile over 5–25 minutes. Periods of constant mobile phase composition may be part of any gradient profile. For example, the mobile phase composition may be kept constant at 5% acetonitrile for 1–3 min, followed by a linear change up to 95% acetonitrile.

2.16.3 The Immunoaffinity Column

The immunoaffinity column (IAC) has been used widely for sample clean-up in the mycotoxin analysis (Zheng *et al.,* 2005). The IAC contains anti-mycotoxin antibody that is immobilized onto a solid support such as agarose gel in phosphate buffer, all of which is contained in a small plastic cartridge (Figure 2.2). The sample extract is applied to an IAC containing specific antibodies to a certain mycotoxin. The mycotoxin binds to the antibody and water is passed through the column to remove any impurities. Then by passing a solvent such as methanol through the column, the captured mycotoxin is removed from the antibody and thus eluted from the column. The mycotoxin in the methanol elute is then further developed by addition of a chemical substance to either enhance the fluorescence or render the mycotoxin fluorescent before measuring in a fluorometer. Prior to adding a fluorescent enhancing chemical, the methanol solution can be used for HPLC analysis as well. With IAC clean-up, the mycotoxin can be concentrated in the column, thereby increasing the fluorometric assay sensitivity or decrease its limit of detection. However, IACs have a limited loading capacity and the sample clean-up procedures are more complicated compared to others in the rapid methods for mycotoxins.

Solid-phase extraction (SPE) column clean-up using solid phase extraction columns for purifications are rapid and economical (Figure 2.3). The most commonly used packing

materials in the SPE columns or cartridges are silica gel, C18 bonded to silica gel, florisil or ion exchange resins. Conventional SPE column retain the analytes on the adsorbent, the non-mycotoxin materials are eluted and then the mycotoxins are eluted. A one-step SPE cleanup column has been developed for rapid clean-up of mycotoxin for the application in a fluorometric method (Malone *et al.,* 1998; Malone *et al.,* 2000). The packing material of the one step SPE column is packed with a porous frit at the top of the column packing, in a funnel-shaped, durable plastic tube with plastic caps at both ends (Figure 2.3). A sample extract is added to the sample reservoir and a rubber syringe plunger, or a similar device, is used to push the sample extract through the one-step SPE column. The purified extract collected at the lower end of the tube contains the mycotoxin, which can immediately be derivatized and placed in a fluorometer for analysis.
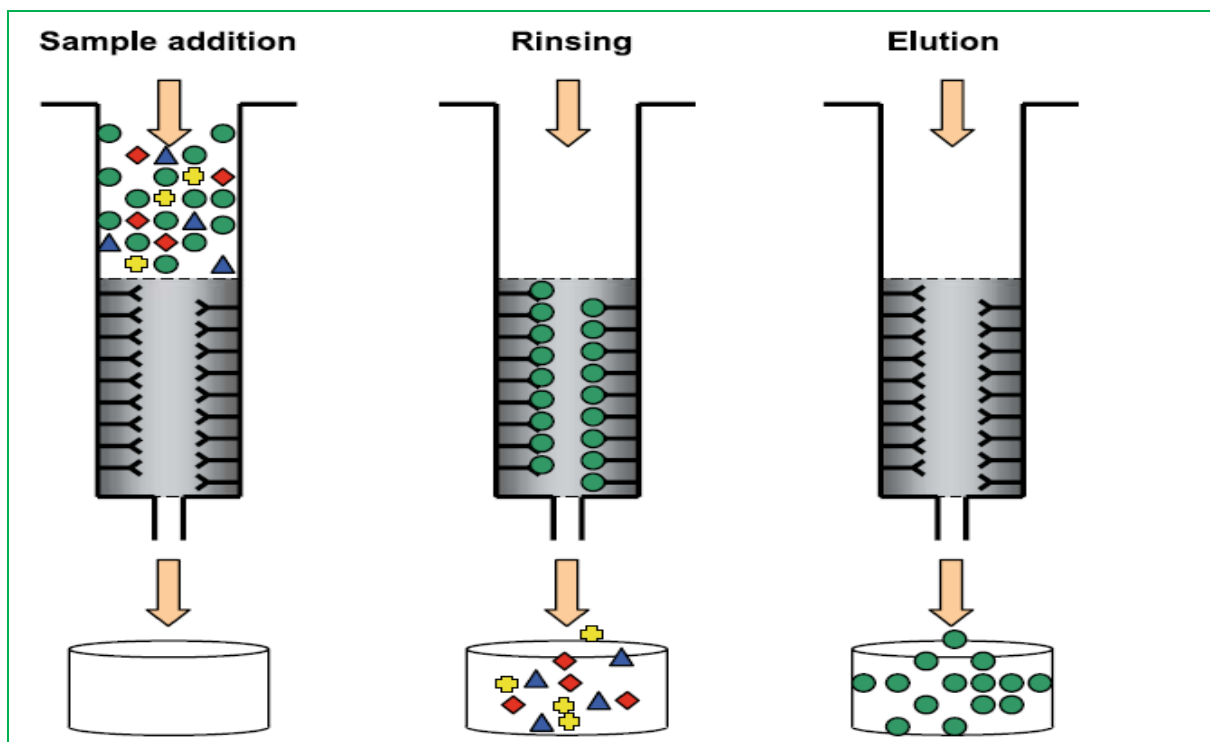
Figure 2.2 - Principle of immunoaffinity columns (Mycotoxin ● ● Impurities ◇▲ ⬡)

Source: Mycopathologia (2006).
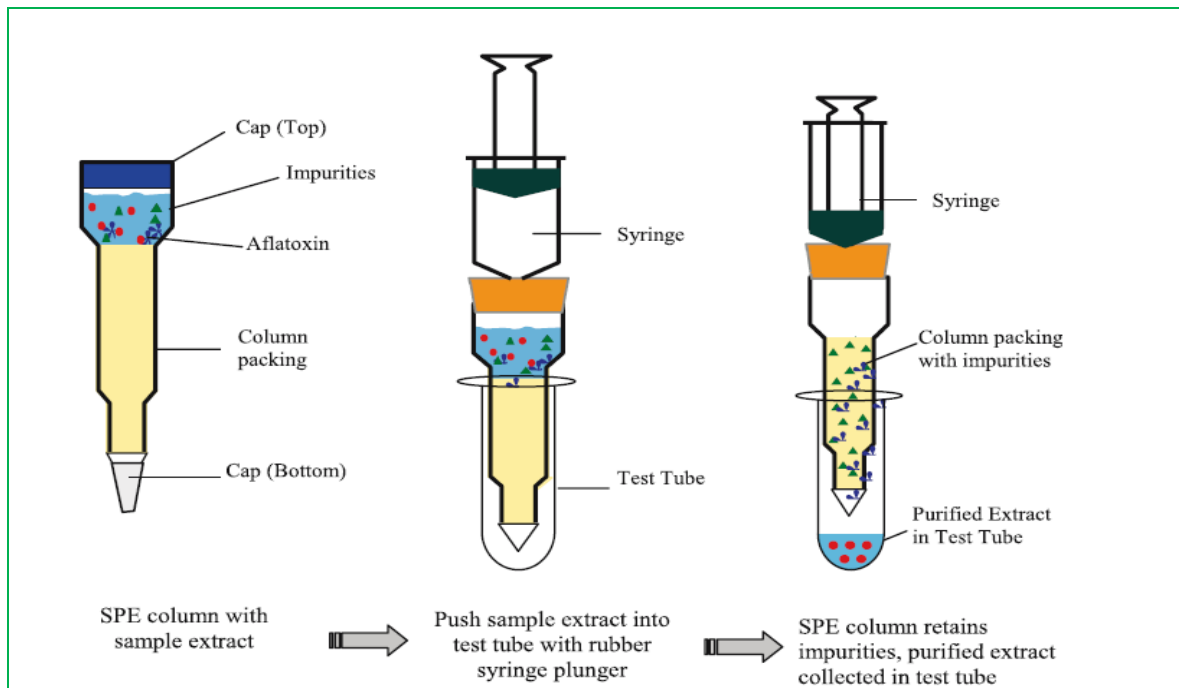
Figure 2.3: Solid -phase extraction column clean-up for mycotoxins

 Source: Mycopathologia (2006)

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was done in Makueni County in Eastern Province. It was purposively selected for the study due to the frequent aflatoxin outbreaks in the past (Mwihia et al., 2008). The County borders Kajiado County to the West, Taita Taveta to the South, Kitui to the East and Machakos County to the North (Fig.3.1). The area is predominantly inhabited by the Kamba people and covers approximately 20,000 km2. The estimated livestock population comprises 84,465 cattle, 192,329 goats 23111 sheep, 3 camels and 14,326 donkeys (Census, 2009). The County has a human population of 884,527 people (2009, Census) and is mainly arid, with an annual rainfall of between 800-1200 mm. Temperatures range from 20.2oC to 24.6oC. The area consists of 15 administrative divisions, namely, Kaiti, Kalawa, Kasikeu, Kathonzweni, Kibwezi, Kilome, Kilungu, Kisau, Makindu, Matiliku, Mbitini, Mbooni, Mtito Andei, Tulima and Wote (Fig.3.2). It falls under ecological zone 5. Agriculture is currently the economic backbone in Makueni County where a large percentage of the population undertakes subsistence farming with a little of commercial farming in some areas. In Makueni County, 34% of urban and 67% of rural population live on less than one dollar a day.

Commercial activities in the lower parts of the county are undertaken mainly in cotton farming. Fruit farming is another of the commercial undertakings where oranges, mangoes, pawpaws, bananas, water melons and lemons are grown. In addition, farmers of the hilly areas of Mbooni and Kilome grow coffee, sugarcane, and arrow roots. In terms of subsistence farming, the main crops are maize, beans, peas, millet, sorghum, sweet potatoes and cassava. Dairy farming is also undertaken both for subsistence and commercial purposes. The main livestock in the area are cows, goats, sheep and donkeys. Poultry is also reared.
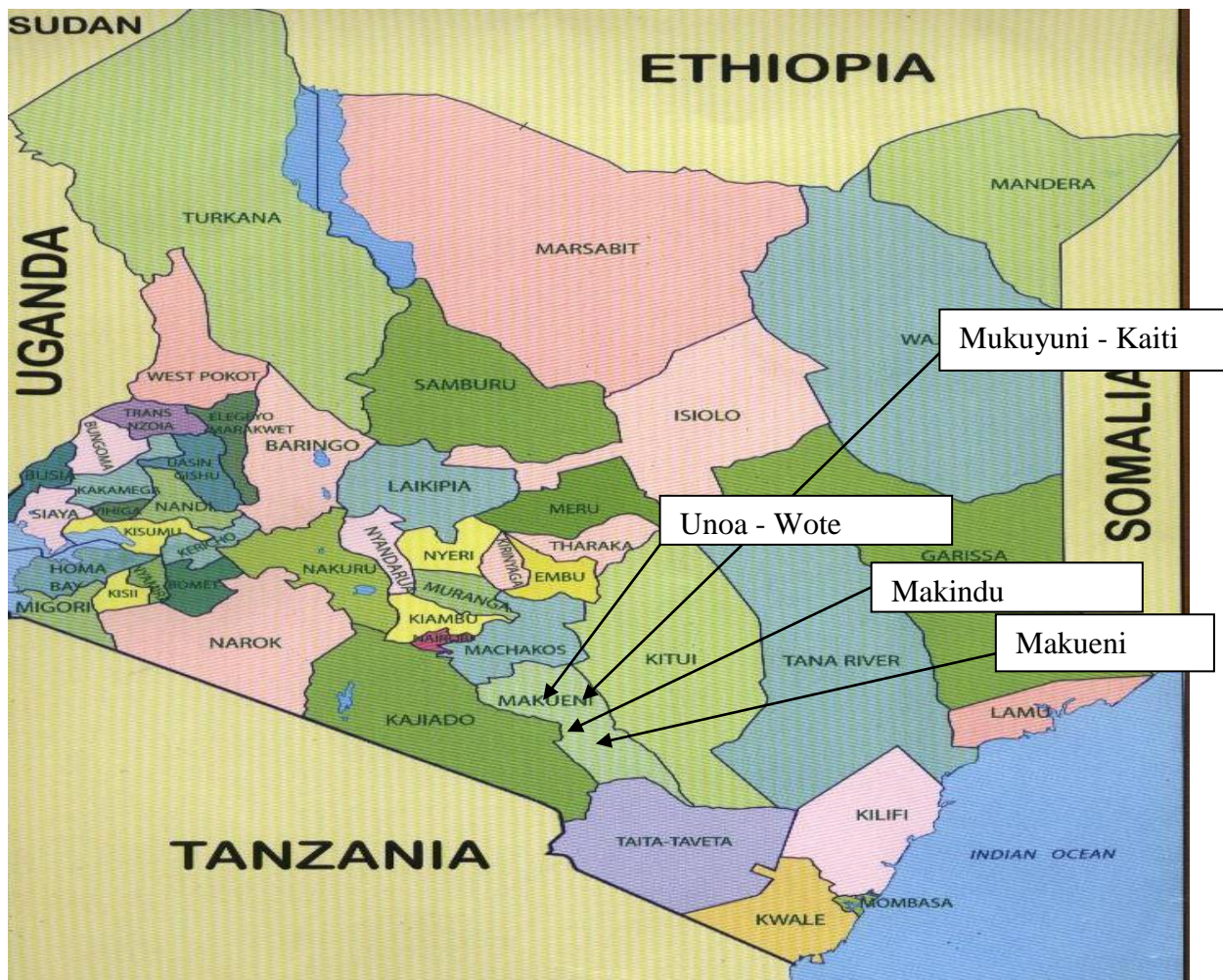
Figure 3.1: Map of Kenya showing the location of Makueni County (Source: Census 2009)
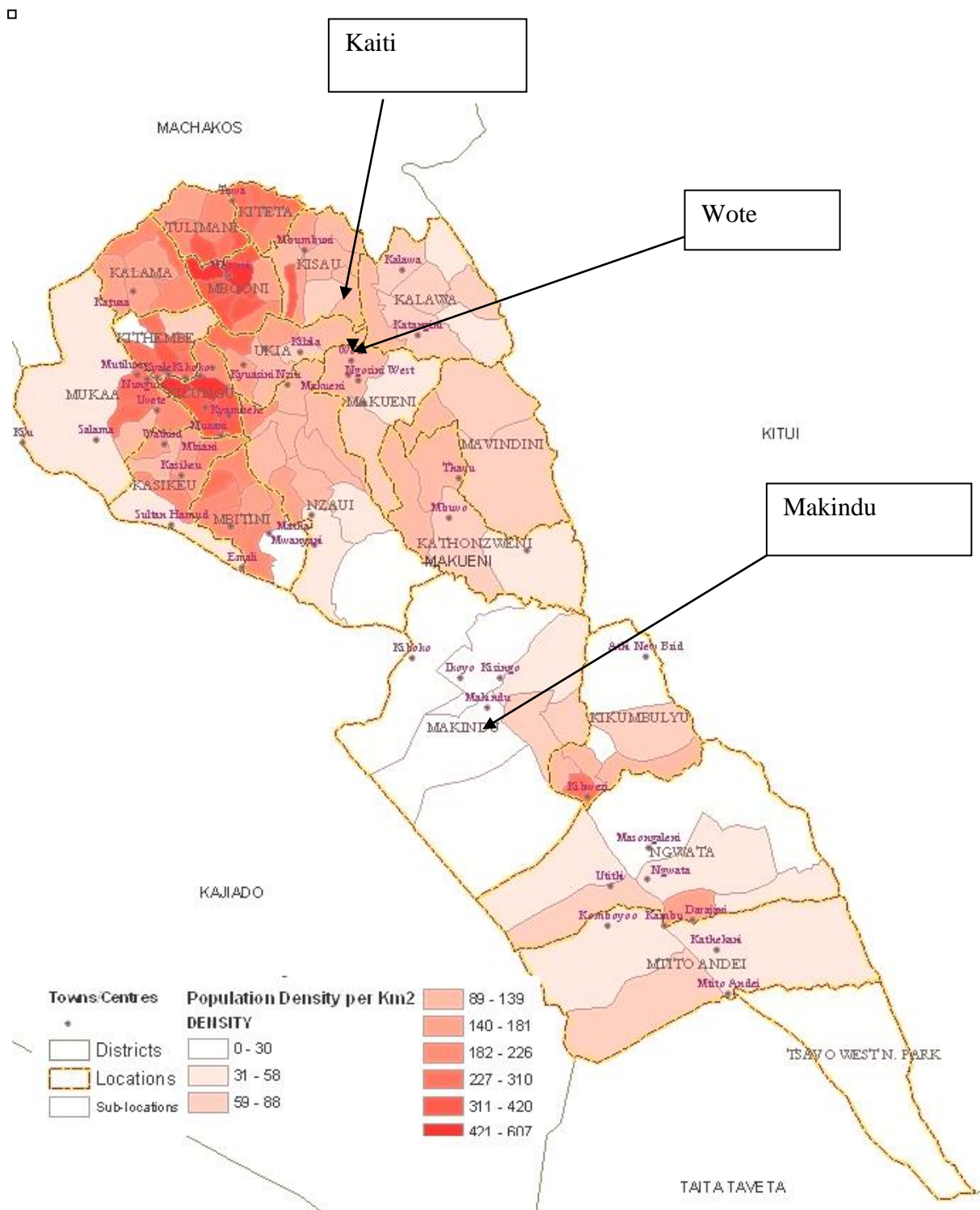
Figure 3.2: Map of Makueni county showing the administrative divisions - (Source: Makueni

District Plan Strategic Plan-2005-2010)

3.2 Selection of study sites and sample size determination

Selection of study divisions was purposive based on: At least 10 heads of dairy cattle and goats, growing of maize, sorghum and millet. Of the 15 administrative divisions, three met the selection criteria including Kaiti, Makindu and Wote. Within each of the three selected divisions, one sub-location was randomly selected for the study from a list provided by the provincial administration based on the same criteria as for the divisions. The selection of the households for inclusion in the study was based on the presence of a child whose age was less than five years of age, in addition to having livestock and growing maize, millet and sorghum. Through the assistance of the local chiefs and their assistants, a list of all households which fitted the criteria in the selected three sub-locations was provided. The presence of a pregnant woman was an added advantage but the selection criteria were followed. A total of 1400 households met the selection criteria as shown in Table 3.1.

The sample size of households to be selected for the study was determined using the formula in Dohoo et al. (2003): $n = \frac{Z^2 PE}{L^2}$

Where n is the sample size required,

Z is the value required to provide 95% confidence interval (1.96),

 L is the desired level of precision,

 p is a prior estimate of proportion of the attribute in the population and

 e is 1-p.

Using the proportion of households that met the selection criteria over the total number of households listed (Table 3.1) an estimate of p of 28% (1400/4922) was made. Using an allowable error (L) of 5%, the number of households to be sampled was:

$$n = \frac{1.96^2 * 0.28 * 0.72}{0.05^2} = 310$$

The number of households to be visited in each sub-location was proportionate to the total number of qualifying households in each division; 33% (96) for Kaunguni, 42% (124) for Mukuyuni, and 23% (73) for Unoa (Table 3.0). Before the household visits, meetings were held with the community leaders where the qualifying households were; and later with the households to explain the study objectives. Informed consent was also obtained from the household heads to participate in the study during the sampling.

Table: 3.1: Distribution of households by study division and sub-location in Makueni County.

Division	sub-location	Number of house holds	Human population size	House holds meeting selection criteria
Kaiti	Kaunguni	1722	9551	458
Makindu	Mukuyuni	1200	12000	593
Wote	Unoa	2000	12000	349
Total		4922	33551	1400

3.3 Data and Sample collection

3.3.1 Household sample collection

From each of the 278 households, maize, sorghum, millet, cow milk, goat milk, human milk and urine from children upto 5 years of age were collected.

3.3.1.1 Cereal sample collection

Cereal samples (maize, millet, sorghum and their flour) were collected in brown paper bags from the storage bags by obtaining small sub- samples from the bottom, the middle and the top of the bag. Where there were up to ten bags small subsamples were randomly picked

from all the ten bags and if bags were above ten, the number sampled was ten plus the square root of those above ten. In each case about 1kg of sample was taken. The subsamples were then mixed and treated as the sample. The same types of samples were also bought from posho mills and markets in the study area. These were then transported to the laboratory and stored in a dry place until analyzed within three months.

3.3.1.2 Milk sample collection

Milk samples were obtained from all milking cattle and goats in clean plastic bottles which had been left with the farmers the previous evening. These were then frozen, transported to the laboratory for analysis.

Breast feeding mothers were requested to use the sterile pumps to extract breast milk but most of them preferred to squeeze milk from their breasts into plastic bottles. These were also frozen, transported to the laboratory for analysis.

3.3.1.3 Urine sample collection and anthropometric measurements

Urine samples were collected from each index child (the youngest child) under five years of age in each household into plastic bottles. The samples were stored under refrigeration until analysis in the laboratory. Urine samples were collected a second time after 6 months from the same children sampled during the first visit in 50% of the households.

The height and weight of these children were also taken. Weighing was done using a hanging weighing scale and weight was rounded to the nearest one gramme while height was rounded to the nearest one centimeter. Weight for Age Z-score (WAZ), Height for Age Z-score (HAZ) and Weight for Height Z-scores (BAZ) were calculated according to the median value of the international reference population recommended by National Centre for Health

Statistics (NCHS), (WHO, 1986). Children with a Z-score of less than negative two standard deviations (-2SD) for HAZ are considered short for their age (stunted) and those with Z-scores less than -3 standard deviations (-3SD) are considered severely stunted. Those children with less than negative two standard deviation scores for BAZ are considered thin while those with less than minus three standard deviations are considered wasted. Children with WAZ scores less than minus two standard deviations are considered underweight and those with less than negative three standard deviations are considered extremely underweight.

3.4 Data collection on risk factors for aflatoxin contamination

Data on potential risk factors for aflatoxin contamination were collected using a standard questionnaire that was administered via personal interviews to 278 household heads. This was because 61 households either refused to continue to participate in the study or had moved away. Twenty nine (29) replacements were done for some of these households from the list of the qualifying households selected. Information on knowledge, attitudes, food consumption, income sources, asset index, household farm practices, household's perception about spoilt maize, were sought (Appendix 1). To ascertain individual food consumption, photographs of weighed cooked food were shown to the female spouse of the household and requested to identify the amount consumed by each member of the household. She was also asked about the amount of milk used to make tea and the number of cups (300 ml) of tea and porridge consumed by household members.

3.5 Geo-referencing sampling points

A hand held Global Positioning System (GPS) device (Garmin German made) was used to record the coordinates of the households from which sampling of cereals, cow milk, goat

milk, breast milk and anthropometric measures on children was done and questionnaires administered.

3.6 Laboratory analysis

3.6.1 Aflatoxin tests

Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) was used to determine total aflatoxin in the samples.

3.6.2 Screening for aflatoxin M1 in milk using competitive Enzyme Linked Immunosorbent Assay (Ridascreen test Kit)

The milk was warmed and 5 mls centrifuged for 15 minutes at 3500rpm at 10oC. The upper cream layer was removed by Pasteur pipette and this was put into a test tube. Five millilitres of ethyl acetate (88.11g/mol; melting point -83.6^{o}C-Sigma) was then added to the remaining whey part of the sample and vortexed for one minute. The mixture was centrifuged at 3500rpm for 15 minutes at room temperature. Three milliliters of ethylacetate layer was taken in a clean tube, put in a water bath at 38oC and evaporated to dryness under a stream of nitrogen. The dried sample was diluted with 250µl of the sample dilution buffer and 30µl of 70% methanol water. The tube was vortexed and the sample used in the proceeding steps.

The milk samples were run in duplicates in the microtitre plates which were coated with antibodies to Aflatoxin M$_1$. Six standards with known aflatoxin levels ranging from 0 to 40ppt (Ridascreen Aflatoxin M1 30/150 ELISA, R-Biopharm AGD-Darmstadt, Germany), were always included in every plate.

A hundred microlitres of the positive and negative controls and the test sample solutions were added to separate duplicate wells. Mixing was done by shaking the plate using a machine shaker. The plates were then incubated for 30 minutes at room temperature in the dark. A

well washer (THERMO SCIENTIFIC) machine (Plate 3.1) was used to wash out the liquid using about 250 µl washing buffer and suck the wells completely dry. Washing and sucking was repeated three times using the machine. A hundred microlitres of the diluted (1:60) enzyme conjugate (Horse radish peroxidase) was added, mixed gently by shaking the plate manually and incubated for 15 minutes at room temperature in the dark. Washing and sucking was repeated three times using the machine. One hundred µl of the substrate (Tetramethylbenzidine) was added to each well, mixed gently and incubated for 15 minutes at room temperature in the dark.

 A hundred microlitres of the stop solution (1 mol/L Sulphuric acid) was added to each well, mixed gently and the absorbance was measured at 450nm against an air blank using an ELISA reader (Multiskan Plus, Labsystems, Helsinki, Finland).

3.6.3 Assay procedure for determination of aflatoxin M1 in urine using Enzyme Linked Immunosorbent Assay

Five millilitres of urine were aliquoted into centrifuge tubes and centrifuged at 3000rpm for 10 minutes. Nine hundred and fifty microlitres of distilled water was pippeted into skartron (Manufactured by SKATRON AS LIER, Norway. CAT. No 7071) tubes and 50µl of standards and the supernatant-urine were added into the 950µl of distilled water in the skartron tubes respectively to make-up a total of 1000µl. They were mixed by priming pipette at least 5 times. Two hundred microlitres of the assay-buffer was added into the mixing well/plate using a multi-channel pipette. Then a 100µl of the diluted standards ranging from 0 to 40ppt and the urine samples were added into the mixing well/plate, each sample per well to make-up a total of 300µl. These were then mixed using the micro-shaker (DYNATECH) for 2 minutes. A hundred microlitres of the mixture were transferred using a multi-channel

pipette into the Reaction-Assay plate (Aflatoxin M1 assay for urine, (RIDASCREEN®) Helica biosystems Inc, 231E imperial Hwy, suite 250 Fullerton) which was coated with antibodies for aflatoxin in duplicate. Mixing was then done using the micro-shaker (DYNATECH) for 1 minute and incubation was then done at room temperature in the dark for 1hr. Phosphate Buffer Saline-Tween packet was reconstituted by washing out the contents with distilled water into 1liter. This was stored refrigerated when not in use. The plate was washed using the well wash (THERMO SCIETIFIC) machine.

A hundred microlitres of conjugate was then added into each well, mixed gently by tapping and incubated at room temperature for 15 minutes in the dark. The plate (Aflafoxin M1 assay for urine kit) was then washed using the well wash.

A hundred microlitres of substrate reagent (Tetramethylbenzidine) was then added into each well, mixed gently by tapping and incubated at room temperature for 15 minutes in the dark.

The well-wash machine

WASHING BUFFER BOTTLE

ELISA PLATE, READY FOR

WASTE BOTTLE
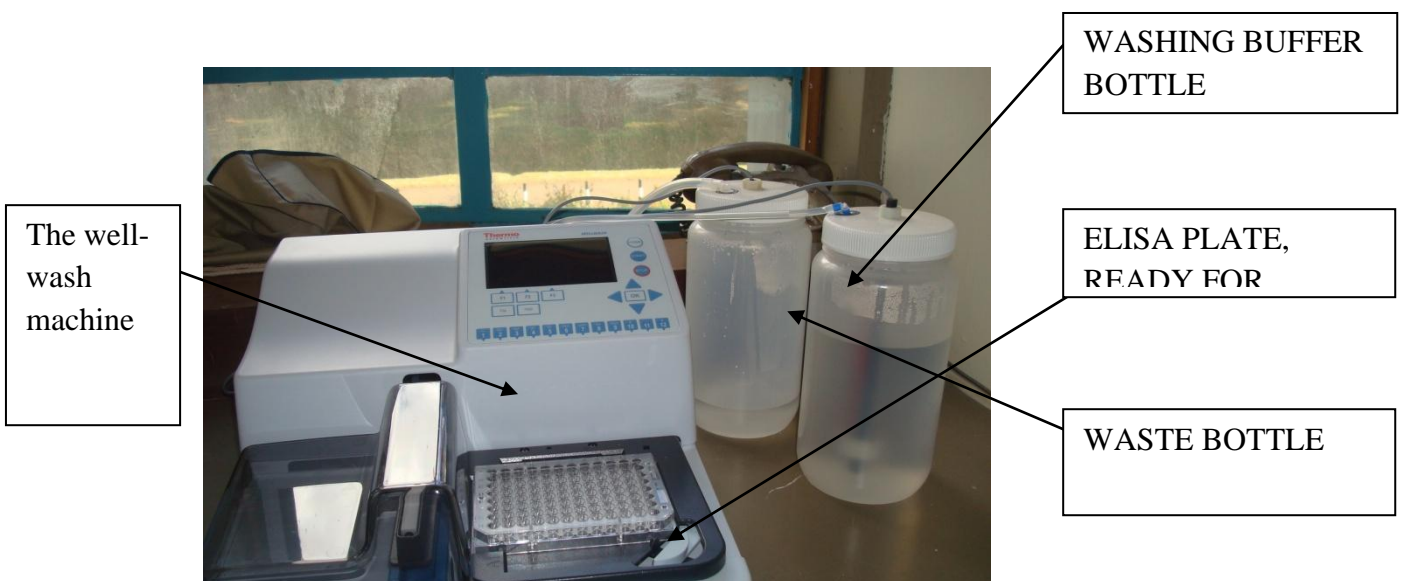
Plate 3.1: The Well-wash Machine

A hundred microlitres of stop solution was then added into each of the wells using a multi-channel pipette. The optical density (OD) was read at 450nm within 15 minutes of adding the stop solution.

3.7. Determination of total Aflatoxin B1, B2, G1 and G2 in cereals

3.7.1. Grain sample preparation

Portions of sorghum, maize, and millet samples obtained from the collected samples were ground using a grinder (Grindomix® GM200 knife mill, Retsch GmbH, Germany) at 8000 rpm for 40 seconds to obtain a fine sample.

3.7.2 Grain sample extraction and determination of aflatoxin content by c-ELISA

All the reagents were brought to room temperature before use. Two grams of the representative ground sample was weighed and put in a screw top glass vial. To the sample, 25mls of methanol/distilled water (70/30; v/v) was added and mixed thoroughly for 10 min at room temperature using a shaker. The extract was filtered through Whatman No. 4 filter paper and diluted 1:6 in phosphate buffered saline containing 500µl/l Tween-20 (PBS-Tween) provided by the manufacturer and analyzed for aflatoxin with a competitive ELISA. Prepared antibody pre -coated plates with 96 microtitre wells were used and fifty micro-liters per well of each sample and 6 standards was added in duplicates and 50µl/well of conjugate solution was also added and the plates incubated at room temperature in the dark for 1-2 hours. After washing (using the machine washer) to remove the unbound aflatoxins and other impurities, substrate solution was added at 100µl/well and the plate incubated for 10-20 minutes for colour development. The reaction was stopped by adding 100µl/well of 1 mol/L H2SO4 and the absorbance read at 450 nm in an ELISA reader. High reading shows samples

with no aflatoxin while low readings show samples positive for aflatoxins. Total aflatoxin was then calculated using a computer delivered program.

3.8 Estimation of aflatoxin exposure levels

3.8.1 Aflatoxin exposure from maize consumption

The consumption for maize was determined by showing photographs of weighed ugali and muthokoi to the female spouse of the household who then identified the amount consumed by each member of her household. The totals for each household were then computed. The amount of flour in the ugali and muthokoi was determined by using the amount used to cook the starndad portions on the pictures: 1Kg of ugali or muthokoi = 1.046 Kg of maize flour (Flour was weighed when ugali was made). From this the total consumption per household was obtained and the consumption per person was then derived by dividing this quantity by the average household size. To calculate the aflatoxin consumed per person, the mean aflatoxin quantities in the division was multiplied by the amount of flour consumed per person.

3.8.2 Aflatoxin Exposure from milk consumption

The milk consumption was obtained by asking the female spouse to state how many cups of milk (300ml) she gives to each household member each day or how many cups of milk she uses to make tea per day. The total for the household was then computed and average per household member determined. Aflatoxin consumption per person was then determined by multiplying the average aflatoxin content for the division by the individual milk consumption for the particular division.

3.9 Data analysis

Data were entered into Microsoft excel 2007, cleaned and coded for analysis. This was then exported into IBM SPSS version 20 for statistical analysis. Descriptive statistics were generated using the same package. Analysis of Variance (ANOVA) was done to compare the means of aflatoxin levels among the study sites for different sample types and χ^2 statistic was used to assess associations between household practices and aflatoxin contamination of cereals. Logistic Regression was done to determine how pre- and post-harvest household practices influence presence of aflatoxins in cereals. The dependent outcome variable was the level of aflatoxin in cereals collected from households. The independent/explanatory variables included the study divisions; preservation methods; method of harvesting; mode of handling; drying methods; methods for shelling; and storage methods. A backward elimination procedure was used to fit the model and a p-value =0.1 was used for retaining the variables. A p-value =0.05 was used for the final model.

CHAPTER FOUR: RESULTS

4.1 Response rate

Out of the 310 households targeted for questionnaire administration, 278 agreed to participate in the study for a response rate of 90%. The location of the households within the study area is shown in Fig. 4.1.
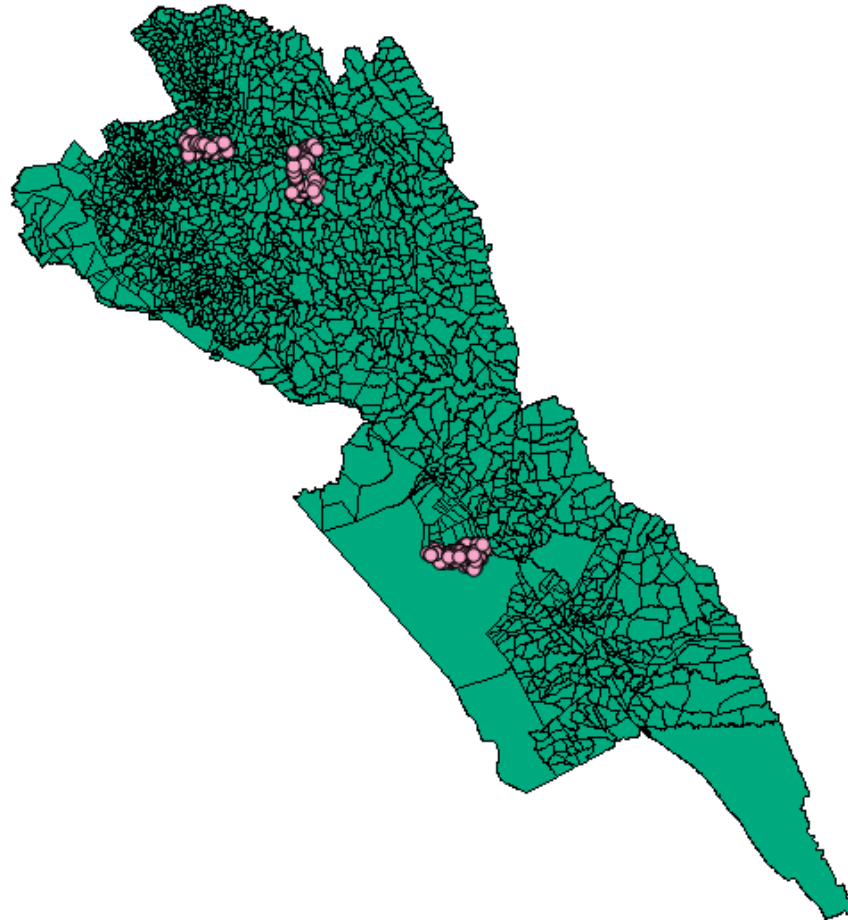
Figure 4.1: Locations of households as captured by the hand held global positioning system (pink dots). Black lines indicate location of other households.

4.2 Household characteristics

4.2.1 Household composition

The average number of people in the 278 study households was 5.9 consisting of 51.5% males and 48.5% females. The average age was 20.0 years for males and 19.6 years for females. Children five years and less constituted 12.7% of the study population.

4.2.2 Level of education

The majority (55.7%) of the respondents had attained primary level of education, and only one (0.36%) was a university graduate. The level of education in the three divisions was averagely primary level of education, however there was a significant ($p<0.05$) difference between the divisions. Makindu had the most people with primary level education (349/925), followed by Kaiti (313/925) and Wote (263/925), respectively. Those with secondary education were most in Wote (106/216), followed by Kaiti (62/216) and Makindu (48/216), respectively. For tertiary level of education, Kaiti had the most people (13/28), followed by Wote (12/28) and Makindu (3/28), respectively. At the University level, Kaiti and Wote both had the same number (1/2) while Makindu had none. The individual divisional percentages are shown in Fig. 4.2.
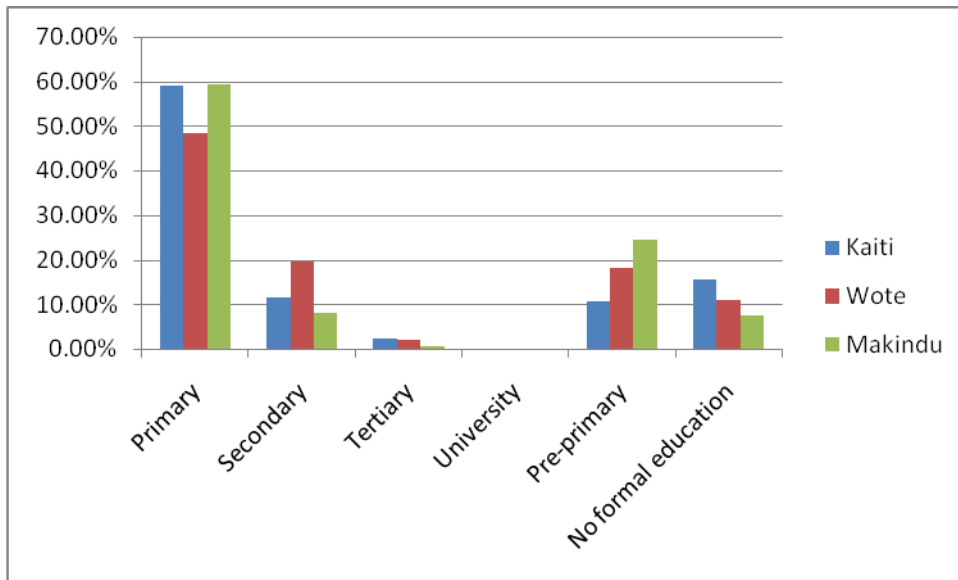
Figure 4.2: Distribution of study participants by highest level of education by division in Makueni County.

4.2.3. Household income and total assets

The major sources of income for the respondents are displayed in Figure 4.3. The three leading sources were sale of crops (grains and seed) (16.1%), paid employment (15.7%) and fruits and vegetable sales (14.79%). The highest income came from paid employment followed by self-employment, livestock/milk sales, fruits and vegetable sales, earnings from other sources and lastly remittances from children and other relatives. Average income per day was Ksh295.80 in Kaiti, Ksh373.10 in Wote and Ksh330.60 in Makindu while average total assets per household were Ksh 1,028,800 in Kaiti, Ksh 1,086,909.474 in Wote and Ksh 430,723.1579 in Makindu. Mean for the District was Ksh 845,596.79.

There was a significant ($p<0.05$) difference in the proportion of the population living below one dollar a day; the highest being in Kaiti (43.6%) and Makindu (40%) and the lowest in Wote division(22.3%).
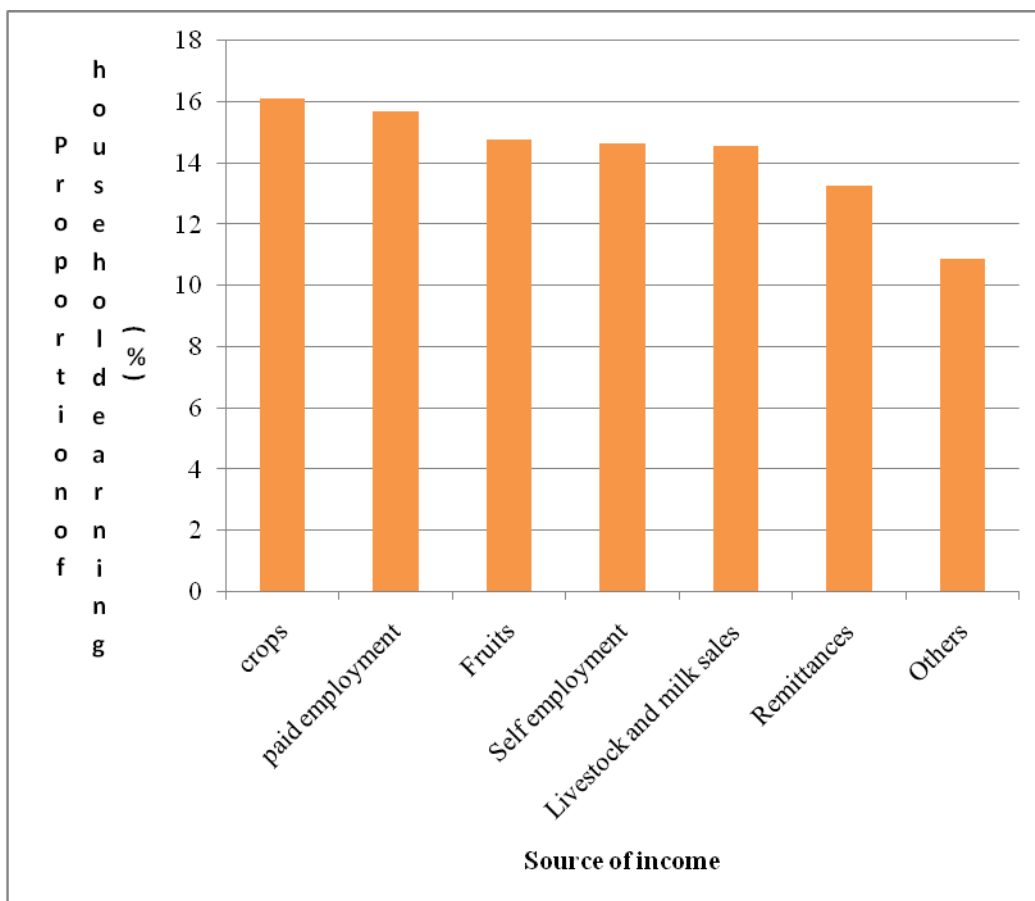
Figure 4.3: Sources of income in households sampled in Makueni County, Kenya, 2011,

(n=278)

KEY- % = proportion of households that had income from a particular source

4.3. Harvesting and handling practices of crops by households

Almost all (99%) of the households used hands to harvest crops while 1% used machines. There was no significant difference between divisions (p>0.05). The individual divisional percentages are shown in Figure 4.4.
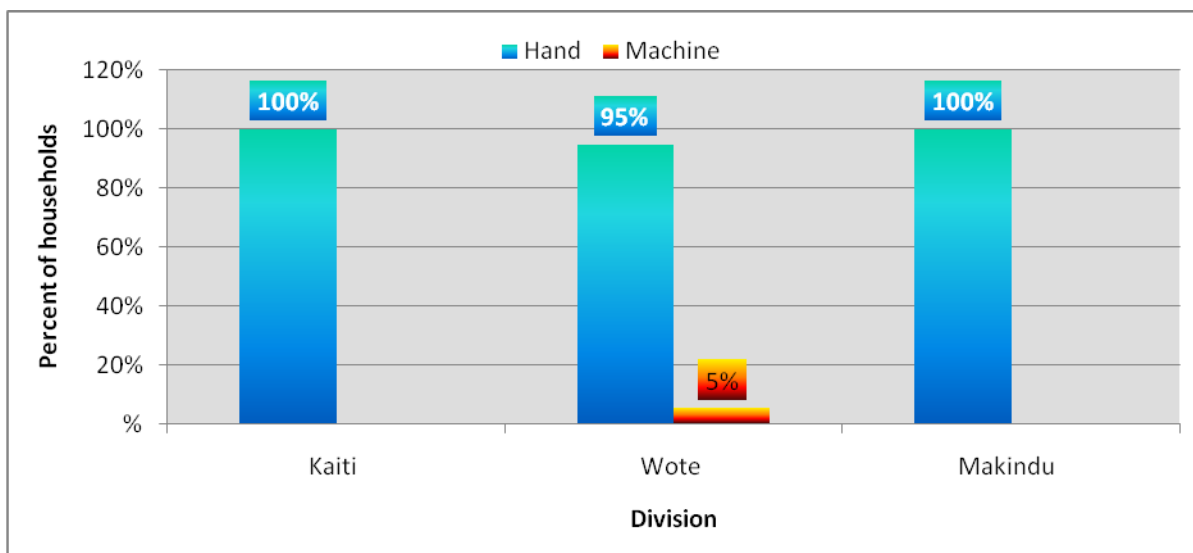
Figure 4.4: Modes of harvesting cereals by respondents in Makueni County in 2011

Over half (55%; 152/278) first cut the maize stovers and stack them in heaps before removing the maize cobs, 44%, (122/278) removed the maize cobs on standing stovers while 1.4% (4/278) used a combination of both methods Figure 4.5 shows the distribution of the study participants by harvesting method.
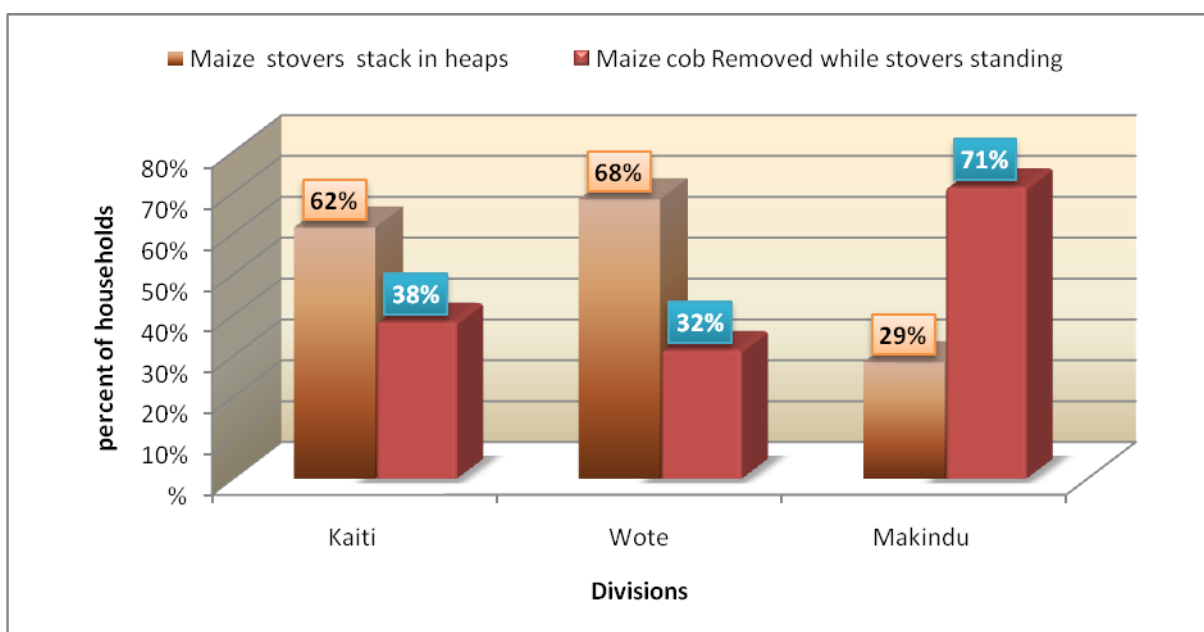
Figure 4.5: Modes of handling cereal stovers during harvest by respondents in Makueni County in 2011

Several methods were used to sun-dry the maize after harvesting. The most widely used (60% of respondents) was drying the maize on the ground without canvas. Other methods used are shown in Fig. 4.6. There was no significant difference in the drying methods used between divisions.
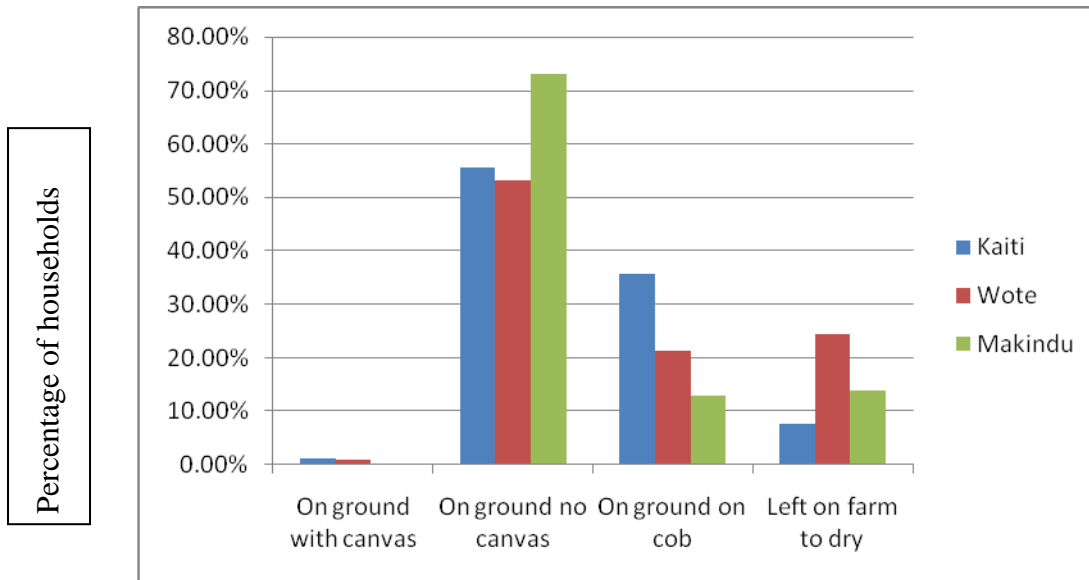
Figure 4.6: Methods used by households to dry cereals by division in Makueni County in 2011(n=278)

Key for drying: 1= on ground with canvas, 2= on ground no canvas, 3= on ground on cob, 4= on tarmac.

Shelling of the maize cobs to remove the grains was done mainly by using three methods. The most popular method used by 77% of the respondents was pounding the maize cobs in a sack manually using a piece of wood. Other methods included hand shelling and shelling by machine. There was no significant difference in shelling methods between divisions. Figure 4.7 illustrates the shelling methods by division.
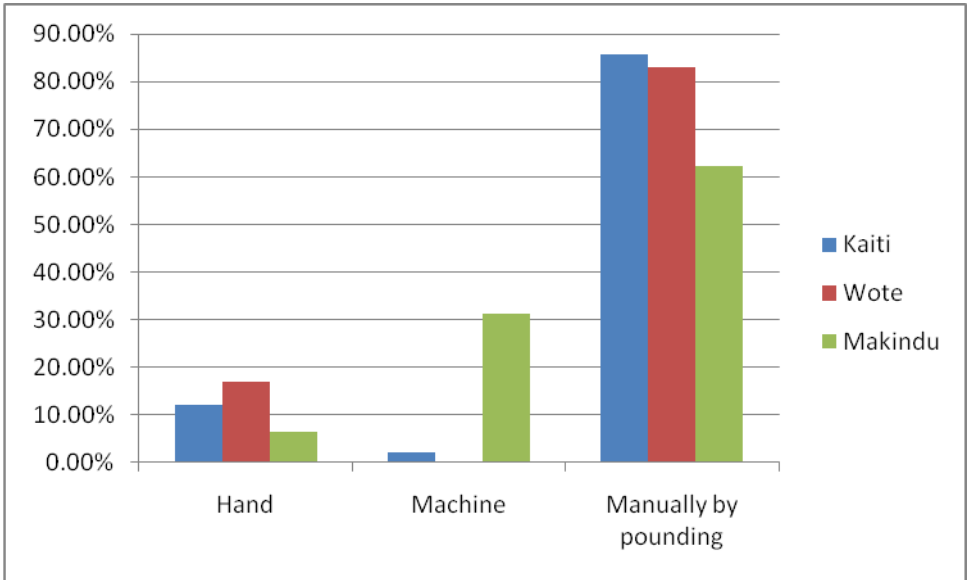
Figure 4.7: Shelling methods practiced by respondents in the study divisions, Makueni County

After shelling, the maize was stored using a variety of methods but the majority (51.8%) of the households stored their maize in nylon bags. Other methods used included iron sheet granary (21.8%), grass thatched granary (17.5%) and cribs (8.9%), (Figure 4.8). There was no significant difference between divisions on storage methods.

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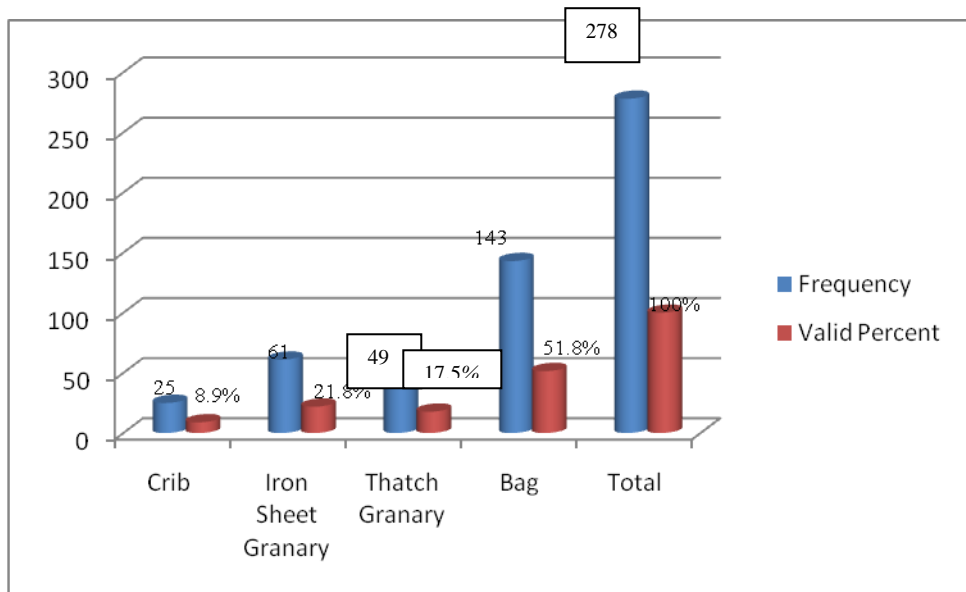
Figure 4.8: Storage methods used for cereals by households in Makueni County, 2011

The vast majority (80.2%) of the respondents preserved their stored maize by application of the pesticide Actellic®. The rest used application of wood ash to the cereals and others did not use any preservative for their stored cereals as illustrated in Figure 4.9. There was no significant difference between divisions on the methods used for preservation.
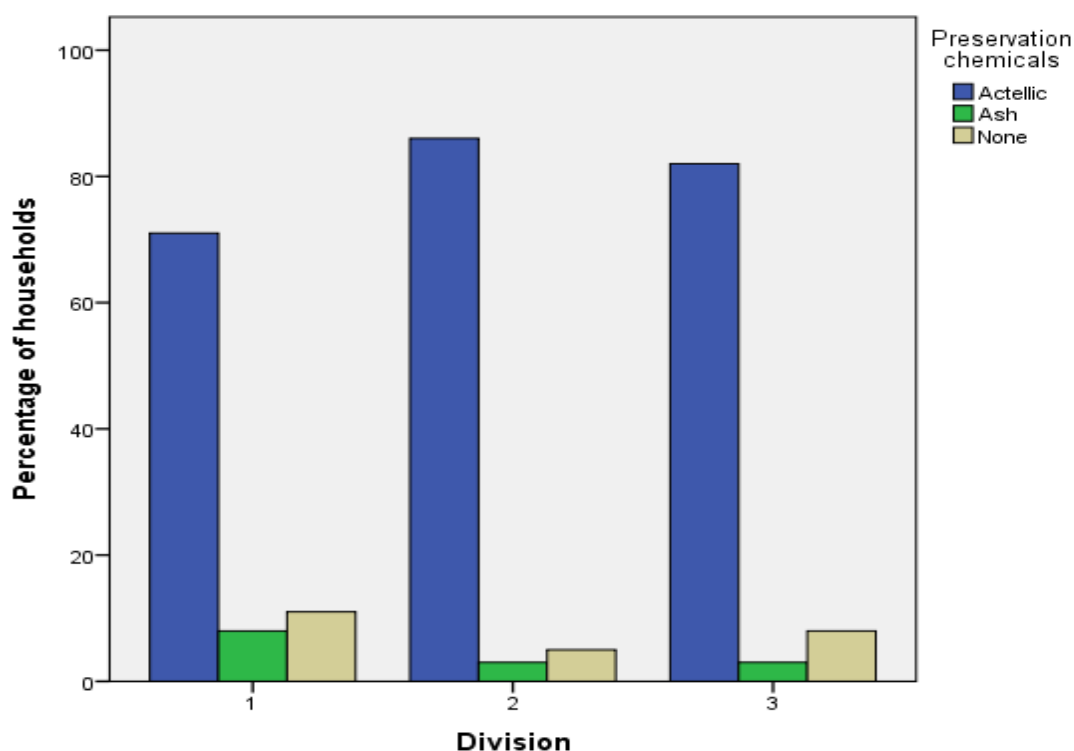
Figure 4.9: Preservation methods used by respondents in Makueni County for stored maize in 2011

4.4 Maize yields and spoilage

The reported usual yields in 90Kg bags in the study divisions and sub-locations are displayed in Table 4.1. Wote Division had a reportedly higher maize yield at an average of 10.5 bags per acre compared to Makindu Division at 8.2 bags per acre and Kaiti Division with a relatively lower yield of 6.1 bags per acre.

Table 4.1: Maize yields in 90Kg bags by division and sub-location in Makueni County, Kenya, 2011

Division	Number of households	Total No of 90Kg bags of maize harvested	Bags/household
Kaiti	91	555.1	6.1
Wote	95	997.5	10.5
Makindu	92	754	8.2
Total	278	2306.5	8.3

The occurrence of spoilt maize was common in the study area with 80% (223/278) of the survey households reporting having had spoilt bags of maize in the last harvest. The proportion of households reporting spoilt maize ranged from a high of 90% in Makindu Division to a low of 70% in Kaiti Division (Table 4.2). However, the mean number of bags of spoilt maize per household was highest in Kaitiision Div (1.8) and lowest in Wote Division (0.5) (Table 4.2). Of the 2306.5 bags of maize harvested (Table 4.1), 244 (10.6%) reportedly was spoilt maize (Table 4.2).

A variety of methods were employed in the disposal of spoilt maize as illustrated in Figure 4.10.

Table 4.2: Distribution of maize spoilage by division and sub-location in Makueni County, Kenya, 2011

Division	Number of households	Number of households with spoilt maize	Proportion of (%) households whose harvest had spoilt maize	Number of bags of spoilt maize	Mean spoiled bags per household/site
Kaiti	91	64	70	115	1.8
Wote	95	76	80	38	0.5
Makindu	92	83	90	91	1.1
Total	278	223	80	244	1.1
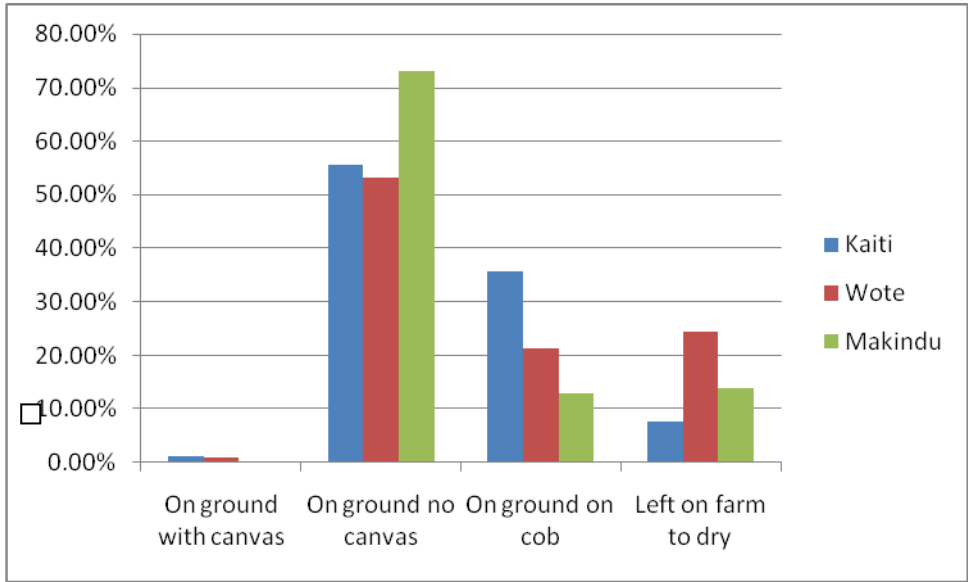
Figure 4.10: The different disposal methods for spoilt maize in Makueni County in 2011

Several indicators of spoilt maize were reported as shown in Table 4.3. The vast majority (61.1%) of the respondents reported colour change in the maize as the most significant sign of spoilage and 14.3% reported maize destruction by insects (Weevils) as another important indicator of spoilage. A total of 92.5% of the households could identify spoilt maize and had various ways of identifying it as shown in Table. 4.3. Concerning risks posed by spoilt maize, 78.6% were aware of some danger with 38.9% citing stomach problems, 16.8% aflatoxins, 10% disease, 9.6% death, 1.5% cancer and 1.8% liver problems.

Table 4.3: Indicators of maize spoilage in a survey of 278 households in Makueni County, 2011

Indicators of maize spoilage	Frequency	Proportion (%)
Colour change	171	61.1
Insect destruction	40	14.3
Moist	2	0.7
Mouldy	14	5.0
Pests	2	0.7
Rotten	16	5.7
Rust	2	0.7
Smell	7	2.5
Sprouting	2	0.7
Bitter taste	1	0.4
No response	21	7.5
Total	278	100.0

Thirty-four point five percent (96/278) of the households were not aware of how aflatoxin contaminated cereals could be made safe, 11.9% (33/278) thought it can be done by thorough drying while 39.9% (111/278) thought it cannot be done. The others mentioned soaking before cooking, de-hulling, boiling, making *muthokoi* and making maize germ.

4.5 Cereal samples

4.5.1 Sources of cereals with the households

Figure 4.11 shows different sources of cereals across the three divisions. Most of the maize was from the respondents' farms in all the three divisions. However, Makindu Division relied mores on relief food (35%) compared to Kaiti (16%) and Wote (11%) divisions.
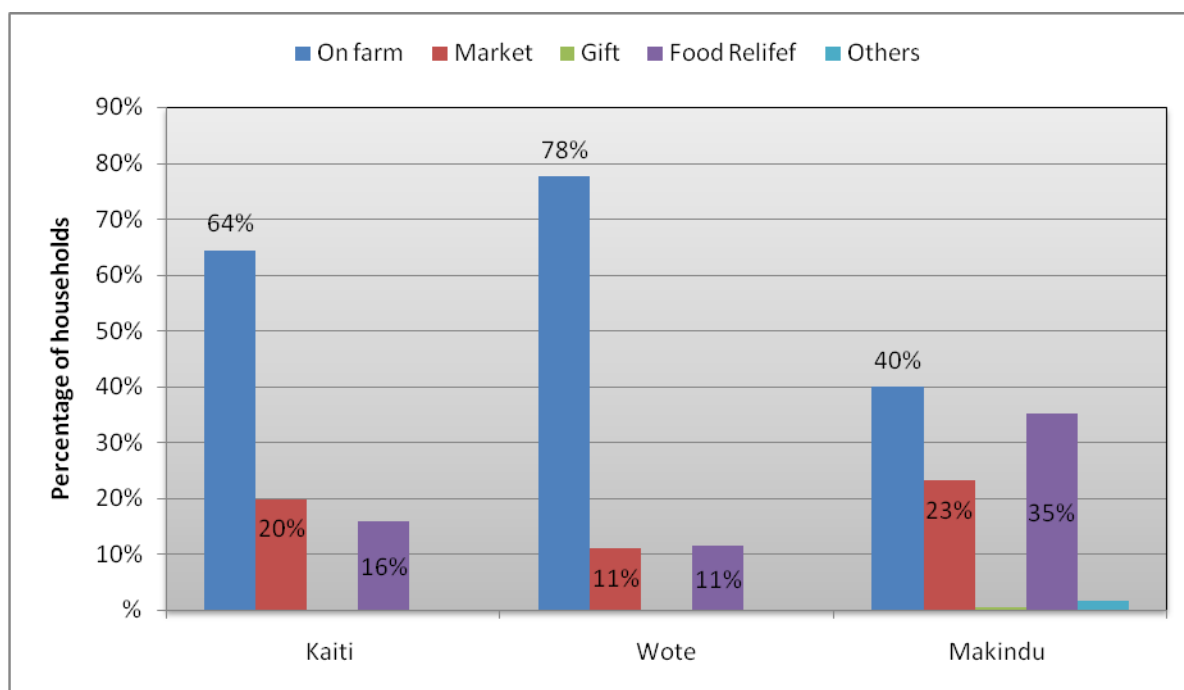
Figure 4.11: Sources of maize by division in Makueni County, 2011

4.5.2 Cntamination of household cereal samples with aflatoxin

A total of 597 cereal samples were collected, of these; 378 were maize and maize products, 215 were sorghum/millet, 2 were finger millet and 2 were mixtures meant for animal feed (Table 4.4). Of the total maize and maize products samples taken 81.7% (309/378), were contaminated with aflatoxin while 26.98% (102/378) had levels above the regulatory limit of 10ppb (FAO/WHO). Of the Sorghum and millet samples collected, 86.5% (186/215) were aflatoxin contaminated while 29.76% (64/215) were above the regulatory limit. Aflatoxin levels ranged from 0ppb to 288.7ppb in maize and maize products, 0ppm to 238.2ppb in sorghum and millet samples.

Table 4.4: Distribution of aflatoxin levels determined by Enzyme Linked Immunosorbent

Assay test by type of sample in Makueni County

Type of Sample	< 10ppb	>10<20ppb	>20ppb	Total
Finger millet	0	1	1	2
Animal feed	1	0	1	2
Maize for human consumption	276	22	80	378
Sorghurm and millet	151	32	32	215
TOTAL	428	55	114	597

Key= ppb – parts per billion

A total of 64 cereal samples were collected from market centres in the study area of which 58 (90.6%) tested positive for aflatoxin on ELISA test, (Table 4.5). Twenty seven (27) of the samples (42.2%) had aflatoxin levels above the regulatory limit of 10ppb.

Table 4.5: Types of cereal samples collected from markets in Makueni County and numbers

testing positive for aflatoxin on Enzyme Linked Immunosorbent Assay

Type of sample	Number of samples	Number positive	Proportion positive (%)_	Number and Proportion (%) > 10ppb
Maize	43	38	88.4	18 (42)
Maize flour	8	8	100	4 (50)
Sorghum	9	9	100	3(33)
Finger millet	2	2	100	1(50)
Animal Feed	2	1	50	1 (50)
Total	64	58	90.6	27(42.2)

The distribution of the cereal samples and the proportions (%) having aflatoxin levels above 10ppb according to the study divisions is shown in Table 4.6. Significantly (p<0.05) more samples from Makindu Division had aflatoxin levels above 10ppb (42.1%) than those from Kaiti (21.7%) and Wote (22.1%) divisions. There was significant difference (p<0.05) in the mean aflatoxin levels in cereal samples from the three division of Kaiti, Wote and Makindu.

Table 4.6: Distribution of cereal samples and proportions (%) with aflatoxin levels above 10 parts per billion by divisions

Division	Number of samples tested	Number of samples with aflatoxin levels >10ppb	Proportion (%) with aflatoxin levels >10ppb
Kaiti	212	46	21.7
Wote	195	43	22.1
Makindu	190	80	42.1
Total	597	169	28.3

4.6 Aflatoxin levels in milk and urine samples

4.6.1 Cattle milk

A total of 209 cow milk samples were collected from cattle in the three divisions and tested for the presence of aflatoxin M1 using the ELISA test. The proportion of the milk samples testing positive were similar across the divisions ranging from 88.1% for milk samples from Kaiti to 90.3% for samples from Makindu Division (Table 4.7). However, the mean aflatoxin M_1 in ppt attained for the 109 milk samples from Kaiti division (25.88ppt) was significantly (p= 0.004) higher than the means obtained for samples from both Makindu (13.68ppt) and Wote (10.43ppt) divisions (Table 4.7).

Table 4.7: Proportion of cattle milk samples testing positive for Aflatoxin M1 and its quantities in ppt in three divisions of Makueni County

Division	No. of milk samples tested	No. positive for Afatoxin M1	Proportion (%) positive for AflatoxinM1	Mean Aflatoxin M1 in ppt and SD	p-value
Kaiti	109	96	88.1	25.88 ± 41.87	
Wote	69	62	89.9	10.43 ± 9.74	0.004
Makindu	31	28	90.3	13.68 ± 10.52	
Totals	209	186	89.0	18.97 ± 31.8	

Key: No. =Number, Ppt = parts per trillion, SD = Starndard Deviation

4.6.2 Goat milk

Fifty- four goat milk samples were tested for aflatoxin M_1 from Kaiti (1), Wote (3) and Makindu (50) divisions. Fifty out of the fifty four (93%) samples tested positive. The mean Aflatoxin M_1 in ppt for the three samples from Wote and the 50 from Makindu were 11.9 and 12.4, respectively (Table 4.8); the difference was not statistically significant (p=0.733).

Table 4.8: Levels of aflatoxin M1 in goat milk in the study divisions of Makueni County.

Division	No. of samples tested	No. of samples positive for aflatoxin M_1	% of positive goat milk samples	Mean aflatoxin M_1 in ppt and SD	p-value
Kaiti	1	1	100	11.0	
Wote	3	2	66.7	18±1.41	
Makindu	50	47	94.0	13.1±9.15	0.733
Total	54	50	86.9	13.26±8.93	

Key: No. =Number, ppt = Parts per trillion, SD = Standard deviation

4.6.3 Human milk

A total of 98 human breast milk samples were tested for presence of aflatoxin M_1 out of which 87.8% (86/98) were positive (Table 4.9). The quantities of the toxin ranged from a mean of 3.45ppt in samples from Kaiti to 13.33ppt in samples from Makindu. The mean for Makindu was significantly different (p=0.000) from the means for samples from Kaiti and Wote divisions.

Table 4.9: Summary of aflatoxin M_1 in human milk in the study divisions of Makueni County

Division	No. of milk samples tested	No. positive for Aflatoxin M1	% of Positive human milk samples	Mean aflatoxin M1 in ppt and SD	p-value
Kaiti	40	31	77.5	3.45±5.01	
Wote	25	23	92.0	5.76±8.53	0.000
Makindu	33	32	94.1	13.33±12.53	
Total	98	86	87.87	7.36±9.95	

Key: No. =Number, Ppt = Parts per trillion, SD =Standard deviatio

4.6.4 Urine samples

Three- hundred- seventy- six (376) urine samples from children five years old and less were tested for Aflatoxin M_1 out of which 79.2% (298/376) were positive (Table 4.10). The proportions of the urine samples positive were similar across the study divisions. However there were statistically significant ($p<0.05$) differences in the mean quantities in ppt of the toxin by division; the mean for samples from Makindu (1354.67ppt) was statistically significant (p=0.003) compared to the means for samples from Kaiti (756.3ppt) and Wote (600.26ppt) divisions (Table 4.10).

Table 4.10: Proportion of human urine samples testing positive for aflatoxin M1 on Enzyme Linked Immunosorbent Assay and mean quantities (ppt) of the toxin in three divisions of Makueni County.

Division	No. of urine samples tested	No positive for AflatoxinM1	Proportion(%)positive for aflatoxin M1	Mean (range) Aflatoxin M1 in ppt	p-value
Kaiti	166	125	75.3	756.3±1834.83	
Wote	89	72	80.9	600.26±1299.13	0.003
Makindu	121	101	83.5	1354.67±1915.52	
Total	376	298	79.9	911.98±1774.41	

Key: No. = Number, ppt = parts per trillion, SD =Standard deviation

4.7 Nutritional status of children

A total of 258 children ≤ 5 years of age were evaluated for being underweight using the weight-for-age Z-scores during a first visit and a second visit 6 months later. At the time of collecting data, 20 children had gone beyond the required five years of age and therefore their Z- scores were not analyzed. Two urine collections were done to monitor continuous presence of aflatoxin M1 in the urine of these children. Kaiti Division had the highest proportion (31%) of underweight children while Wote Division had the lowest proportion (14.8%), (Table 4.11). Overall, 20.2 % of the children were underweight. There was a decrease in the proportion of underweight children between the first and the second visits in all the three divisions but the pattern remained the same with Kaiti Division having the highest proportion (21.4% and Wote division the lowest (7.4%). The probability of children with aflatoxin in their urine being underweight was 0.814 while that for those without aflatoxin in their urine was 0.186.

Of the 123 children evaluated for stunting using the height-for- age Z-scores in the first visit, 28 % were stunted. Kaiti Division had the highest proportion (35.71%) of stunted children compared to children from Wote (18.51%) and Makindu (25.93%) divisions (Table 4.12). There was a drop in the proportion of stunted children during the second visit in Kaiti and Makindu while the proportion of stunted children remained the same in Wote. Children from Kaiti Division were more stunted (28.57%) than children from the other two divisions. The most dramatic drop was recorded for children from Kaiti Division from 35.71% stunted children in the first visit to 28.57% in the second visit (Table 4.12). Those children with aflatoxin in their urine had a probability of 0.94 of being stunted while those without aflatoxin in their urine had a probability of 0.063 of being stunted.

One- hundred- and- twenty- three children were evaluated for wasting on the first visit- 7% of these children were thin. Wote Division had the highest proportion (7%) of children who were thin compared to Kaiti Division (5%) while Makindu Division had no wasted children. During the second visit, Wote Division still had the highest proportion (7%) followed by Makindu Division (4%) and Kaiti (2%), (Table 4.13). The probability of children with aflatoxin in their urine being wasted was 0.08 while that of those without aflatoxin in their urine was 0.27.

Table 4.11: Distribution of weight–for- age Z scores for children ≤ 5 years of age by division in Makueni County.

Visit	Division	Number of children	Mean Z score (SD)	% Below-2SD
1st visit	Kaiti	42	- 0.979	30.952
	Wote	27	-0.493	14.815
	Makindu	54	-1.175	24.074
2nd visit	Kaiti	54	-1.355	21.43
	Wote	27	-0.756	7.41
	Makindu	54	-1.059	14.81

Key: SD = Standard Deviation, % = Percent

Table 4.12: Distribution of height –for- age Z- scores for children ≤ 5 years of age by division in Makueni County.

Visits	Division	Number of children	Mean Z score (SD)	Proportion (%) Below-2SD
1st visit	Kaiti	42	-1.741	35.71
	Wote	27	-1.593	18.51
	Makindu	54	-2.009	25.93
2nd visit	Kaiti	42	-1.772	28.57
	Wote	27	-1.187	18.51
	Makindu	54	-1.664	20.37

Key: SD = Standard Deviation, % = Percent

Table 4.13: Distribution of weight –for- height Z- scores for children ≤ 5 years of age by division in Makueni County.

Visits	Division	Number of children	Mean Z-score (SD)	Proportion (%) below 2SD
1st Visit	Kaiti	42	0.132	5
	Wote	27	0.921	7
	Makindu	54	0.259	0
2nd Visit	Kaiti	42	-0.328	2
	Wote	27	0.034	7
	Makindu	54	0.031	4

Key:SD = Standard deviation, % = Percent

4.8 Maize and milk consumption and levels of human aflatoxin exposure

Maize consumption was highest in Kaiti Divison (0.35Kg/person/day) and lowest in Wote Division (0.26Kg/person/day), however aflatoxin exposure was highest in Makindu Division (11.34ppb/person/day) and lowest in Wote Division (3.57ppb/person/day) (Table 4.14).

Milk consumption was highest in Wote Division (469.3 ml/person/day) and lowest in Makindu Division (146.7 ml/person/day), while aflatoxin exposure was highest in Wote and lowest in Kaiti (0.011ppt/person/day), (Table 4.15).

Table 4.14: Maize consumption and estimated aflatoxin exposure levels by Division in Makueni County.

Division	Number of households	Average household size	Total maize consumption (kg)	Household maize consumption (Kg/hh/dy)	Per-Capita consumption	Average total aflatoxin per household in parts per billion	Mean household aflatoxin consumption (µg/Kg/day)	Exposure estimates (µg/person/day)
Kaiti	91	5.9	190.36	2.092	0.35	35.7	17.07	5.9
Wote	95	5.7	140.05	1.47	0.26	3.8	13.74	3.57
Makindu	92	6.2	179.22	1.907	0.30	11.5	37.81	11.34
Average	93	5.9	169.88	1.824	0.34	17	22.87	6.94

Key: Kg = Kilograms, Kg/hh/dy = Kilograms per household per day, kg/person/dy = Kilograms per person per day,   µg/kg/day = Micrograms per kilogram per day.

Milk consumption per household and per person was calculated (Table 4.15) and this was then used to calculate aflatoxin exposure per household and per person (Table 4.15). Makindu had the least aflatoxin exposure from milk, this is mainly because the percapita milk consumption is lowest in this division.

Table 4.15: Milk consumption and estimated Aflatoxin M1 exposure by division in Makueni County.

Division	Number of households	Average household size	Total milk consumption (ml)	Household milk consumption (ml/hh/dy)	Per capita milk consumption in ml.	Average total AF M1/hh (ppt)	Mean hh AF M1 consumption (ppt/dy)	Exposure estimates (ppt/person /day)
Kaiti	91	5.9	199510.71	2,192.4	371.6	65.8	0.03	.011
Wote	95	5.7	254122.66	2,675.0	469.3.	27.8	0.01	0.0046
Makindu	92	6.2	83679.955	909.6	146.7	12.6	0.014	0.002
Average	92.67	5.9	179104.44	1902.4	329.2	35.4	0.018	0.006

Key = ml =milliliters, ml/hh/dy = milliliters per household per day, µg/dy = micrograms per day, AF M1/hh= aflatoxin M1 per household

Assuming consumption of breast milk for children between 570-900ml per day at ages of 3months to 2years (Kelly Mom, 2012) and taking an average of 735ml/day, aflatoxin exposure through this route was 2.504ppt/child/day, 4.196ppt/child/day and 9.793ppt/child/day for Kaiti, Wote and Makindu, respectively. The average for the County was 5.5ppt/child/day.

From the total population of 1657 whose consumption of milk and maize were considered, 530 were from Kaiti, 536 were from wote and 591 were from Makindu.

4.9 Household practices associated with aflatoxin contamination

Rgression was done to determine the household practices that influenced aflatoxin contamination of cereals. Aflatoxin content of household cereal samples was treated as the outcome and the practices as the independent variables. Only two variables were retained in the final model (Table 4.16). The only independent variable that was significantly ($p < 0.005$) associated with the level of aflatoxin contamination was division; cereals from Makindu Division were 1.4 times more likely to have aflatoxins relative to cereals from both Kaiti and Wote Divisions (Table 4.16).

Table 4.16: Household practices which influenced the presence of aflatoxins in cereals obtained from households in Makueni County of Kenya, 2011

Variable	Levels	Estimate	Std	lower	Upper ratio	Odds Ratio	95% LCL	95% UCL	Chi-square	P-value
Division	Kaiti	-0.354	0.0754	-0.507	-0.203	0.7	0.6	0.8	20.964	0.000
	Wote	-0.359	0.0736	-0.494	-0.198	0.7	0.6	0.8	20.996	0,000
	Makindu	0a.	.	.				.	.	
Shelling	Hand	-0.713	0.4244	-1.551	0.158	0.5	0.21	1.17	2.556	0.093
	Machine	-0.705	0.4489	-1.585	0.175	0.5	0.20	1.19	2.467	0.116
	Manual by pounding	-0.805.	0.435.	-1.657.	0.048	0.4	0.19	1.05.	3.425.	0.053
	Manual pounding + Hand	-0.765	0.449	-1.627	0.097	0.4	0.2	1.10	3.026	0.055
	Machine and Hand	0[a]								

Key – 0[a] = Reference point for the levels of the factor, LCL = Lower confidence level, UCL = Upper confidence level   .

CHAPTER FIVE: DISCUSSION

In this study, the mean household size was 5.9 persons, which was higher than the national average of 4 persons (KDHS, 2009). This implies that there is pressure on the scarce resources available in Makueni County and may be a contributor to the high poverty levels observed. The level of education across the three divisions was averagely primary level although there was a significant difference between the study divisions. In Makindu, there was a large number of household heads who dropped out of school at primary level. This group of people is at a high risk of aflatoxin exposure as their understanding is low and their access to information may be limited. This appeared to be substantiated by the high percentage of cereals from Makindu that were contaminated and the high levels of aflatoxin in the cereals. Indeed Wote Division which had the highest proportion of household heads with secondary education had reportedly the least number of bags of spoilt maize and had the least exposure levels compared to Kaiti and Makindu Divisions.

The results of this study concur with the report by Sabran *et al.* (2012) who demonstrated a link between education levels and exposure to aflatoxins; those with high education levels had significantly high knowledge on the occurrence of fungal infection in the diets compared to those with low levels education, the study also showed that in relation to aflatoxin contamination in foodstuffs, there was a tendency for those with primary or no formal education to be twice as likely to have cereals high in AFB_1 levels compared to those with secondary education or above. Another study by Jolly *et al.,* (2006), concluded that the educated individuals are more likely to seek information about aflatoxin and consequently develop an action plan to prevent them from being exposed to aflatoxin.

The prevalence of aflatoxin contamination in maize and maize products was 81.7%, which compares well with a study by Ayugi, (2013) who isolated Aspergillus flavus in over 70% of maize kernels collected from Makueni County. The prevalence of aflatoxin at levels above 20ppb in maize was 27.2%, which was lower than the 58.7% reported by Mwihia *et al.* (2008) in Makueni. Results from the current study show that maize and sorghum grown in Makueni were contaminated by aflatoxins and aflatoxin exposure in humans was widespread in the County. These results are consistent with results by Muthomi *et al.* (2009) who showed that samples collected from Eastern Province had aflatoxin B1 levels of up- to 136.4µg/kg in semi-processed maize, 77.4µg/kg in whole grain and 40.9µg/kg in flour. These aflatoxin levels were above the National tolerance levels of 10µg/kg (FAO, 2005).

The levels of aflatoxin in maize and maize products ranged from 0 to 288.7ppb. This was lower than estimates by Hell *et al.,* (2003) in Benin and Togo where the aflatoxin levels of up to 532ppb were recorded in maize samples. However, the presence of aflatoxin in all samples collected including maize, sorghum, millet, cow milk, goat milk and human milk, indicate that aflatoxin exposure is wide spread in Makueni County.

Although most of the populations across the study sites were able to identify spoilt maize (92.8%), about twenty-four percent (23.9%) still used spoilt maize as animal feeds and 12.1% of farmers reportedly left the spoilt maize in the farms without destroying it. This practice obviously led to re-infection of the soil and therefore continuous propagation of Aspergillus flavus and Aspergillus parasiticus. Indeed a study by Ayugi (2013) found that Aspergillus flavus incidence in Makueni County soils was 83%. The Aspergillus flavus acts by forming sclerotia which allows it to survive saprophytically in the soil, maize residues and cobs for extended periods of time (Schedegger and Payne, 2003; Jaime and Cotty, 2004; Wagacha and

Muthomi, 2008).The inoculums in the soil and crop debris then acts as the primary source of inoculums that infect maturing maize crop (Jaime and Cotty, 2004; Horn, 2007; Atehnkeng et *al.,* 2008). According to Olanya et al. (1997) and Strosnider et al. (2006), elimination of inoculums sources such as infected debris from the previous harvest, may prevent infection of the crop. Public education on the dangers of leaving spoilt cereals in the farm would go a long way in reducing soil contamination and therefore reduce mycotoxin levels in the cereals harvested from these farms.

Despite the fact that most households knew about the dangers of consuming spoilt grains (78.5%), only a few households associated them with liver problems (1.8%). This shows that many respondents were mainly aware of the acute aflatoxicosis that is frequent in the County but unaware that there could be any relationship between spoilt cereals and cancer of the liver.

It is worth noting that no household mentioned sorting of cereals to remove spoilt ones as a way of reducing aflatoxin exposure although it is a common practice among the rural folk in many parts of Kenya. This indicates that the relationship between aflatoxin contamination and cereals that are normally sorted out is not clear to the people of Makueni County.

The way grains are handled from the time of harvesting to storage determines whether aflatoxin levels are high or low (Codex Alimentarius, 2003)). Most farmers harvested using hands and about half of the farmers stacked the maize stovers or left it on standing stovers to dry before harvesting. Although this practice was not significantly associated with aflatoxin contamination of maize the study may have lacked the power to detect any differences as there were no controls. It is possible that stovers stacked in heaps could increase the chances

of aflatoxin contamination of cereals. This is because stacked cereals leads to increased temperatures and this coupled with the fact that the moisture content at harvest is still high; could provide the ideal conditions for aflatoxin production. It has been shown that aflatoxin contamination can occur after maturation when the crop is exposed to high moisture either before harvest or in storage (Payne and Brown, 1992; Atehnkeng *et al.,* 2008). Indeed drying of maize to safe moisture levels of less than 13% is known to hinder mould growth and aflatoxin production. This is not well achieved when maize is stacked in heaps in the shamba or even when standing stovers are left to dry before harvesting as the cob is not exposed to direct sunlight and the cob coverings trap any moisture from even slight showers during this period. This makes drying difficult. Thirty- eight percent of the farmers dried the maize on the ground without canvas; this proportion was lower than77.6% reported by Mwihia et al. (2005). This practice could expose maize with high moisture content to fungal contamination. Although a high (43.2%) percentage of households stored their cereals in bags that were raised, the bags were of nylon material as opposed to sisal bags that minimizes moisture build up (Hell and Mutegi, 2011).

Farmers stored these either in their houses or in iron sheet granaries; this practice could lead to increased temperatures and thus providing favourable conditions for aflatoxin production. Results showed that shelling method used was marginally insignificant at 95% confidence level (p=0.053 for manual pounding and p= 0.055 for mixed hand removal from cob and manual pounding). The Odds ratios for these two predictors were 0.4 and 0.5, respectively. This finding is important as it shows that there is a protective association on grains from aflatoxin contamination when these methods are used. This could be due to the fact that for grain to be easily removed from cobs or bushels by pounding or hand removal, the cereals have to be very dry thus lowering the chances of aflatoxin contamination due to the low

moisture content; however during storage, depending on preservation methods and storage methods contamination levels can increase.

Children in Makueni are exposed to aflatoxin from an early age through breast milk, and although the flatoxin levels in breast milk (5.5ppt/child/day) are below the acceptable infant exposure level of 25ppt (WHO, 2005), this poses a health hazard through chronic exposure. Thereafter, they are exposed through consumption of weaning diet which is mainly composed of porridge made from maize, sorghum and millet either separately or mixed, they are also exposed through milk in the tea or fresh milk. Weights and heights of the children showed under performance as earlier shown by Okoth and Ohingo (2004) in Kisumu, Kenya. The daily exposure levels for maize of 5.97µg/person/day for Kaiti, 3.57µg/person/day for Wote, although below the acceptable levels of 10ppb, this level leads to chronic exposure which is known to potentiate cancer of the liver, compromise immune system and also aggravates illness in those infected by hepatitis B virus (Ross *et al.,* 1992). The daily exposure levels for maize in Makindu of 11.34ppb/person/day are higher than the acceptable limit of 10ppb (WHO, 2005). This exposure range is less than that found by Frank *et al*. (2006) (3.1 to 17.5µg) in a study carried out in Swaziland which found aflatoxin exposure to be a more important determinant of the variation in liver cancer incidence than the prevalence of hepatitis infection. Hall and Wild (1993) also reported lower exposure level range in Kenya (0.0035ppb and 0.0148ppb) and in other parts of the world (Swaziland- 0.0114ppb and 0.1586ppb, Mozambique- 0.0386ppb and 0.184ppb, South Africa- 0.0165ppb, Thailand- 0.006ppb and 0.053ppb, Gambia- 0.004ppb and 0.115ppb and U.S.A.- 0.0027ppb) . Sirma, (2013) also reported lower aflatoxin exposure levels in Nandi County ranging from 0.27ppb to 0.38ppb/person/day.

Aflatoxin M_1 detected in the urine of young children indicated that the children were recently exposed to aflatoxins in their diet. The underweight findings (20.2%) closely compare with studies done by Gong *et al.* (2004) of (29%) in Togo and Benin which showed that aflatoxin albumin levels in children rose from birth up to the age of 3years before flattening out. The results of this study also showed a similar percentage of stunting (28%) in children as that estimated by Gong *et al*. (2004) of (33%) which associated aflatoxin exposure with impaired growth. Wasting was lower (4%) than that recorded by Gong *et al.,* (2004) of (6%).

In this study there were significant differences in the aflatoxin content in urine with Wote having the least average. This was generally reflected in the percentage of children whose growth was affected. Results showed that the children whose urine had aflatoxins were more likely to be underweight. This agrees with what Gong *et al*. (2004) found in Togo and Benin. However no association was found between wasting and urine aflatoxin which is in agreement with the findings by Gong (2004) who did not find any association between wasting and aflatoxin albumin adducts levels in children in Togo.

The results of this study show that Makindu Division was significantly associated with aflatoxin contamination of cereals. Since the household practices were similar in all the three divisions, the fact that Makindu is the driest, poorest and with the highest proportion of household heads with only primary level of education; these three factors could be the contributing factors to the higher level of aflatoxin contamination of cereals seen in Makindu compared to Wote and Kaiti Divisions. Therefore, there is need for continued aflatoxin awareness creation among the farmers in Makueni County with special attention to Makindu Division. Awareness campaigns should use systems that are in place for disseminating

information to subsistence farmers (James, 2005). Such systems include information through local women groups, schools and other community based organizations.

From this study it is clear that focus should shift to close monitoring of aflatoxin levels in the common staple foods in order to avoid chronic exposure which begins at a very early age and avoid it being overshadowed by the acute incidences as observed by Nyikal *et al*. (2004).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions can be drawn from this study:

The level of human exposure to aflatoxin in makueni was 6.94µg/person/day from maize, and 0.006µg/person/day from milk and 5.5ppt/child/day from breast milk.

The human population was continuously exposed to aflatoxins from infancy as shown by M1 detected in the urine of children under the age of five (Range 0.302ppt to 10415ppt)

Cereals grown in Makueni County were highly contaminated with aflatoxins; a prevalence of 81.7% was estimated in maize and 86.5% in sorghum and millet.

Milk from Makueni County was contaminated with aflatoxins with a prevalence of 89.0% estimated in cow milk, 86.9% in goat milk and 86.9% in human breast milk.

Of the risk factors for aflatoxin contamination of cereals assessed, only division was significantly ($p<0.005$) associated with the contamination with Makindu Division having the highest risk (OR= 1.4).

6.2 Recommendations

Targeted extension messages should be developed to educate the Makueni farmers on pre- and post- handling of harvests to reduce aflatoxins in their food. Particular attention should be paid to the shelling methods used and manual pounding of cereals should be encouraged. The dangers of chronic exposure to aflatoxins should be made clear to these farmers.

Efforts to enhance use of more resistant varieties of cereals should be encouraged in Makueni County in order to reduce aflatoxin contamination.

Further studies should be conducted to establish what contribution stacking of stovers during harvest makes to contamination of cereals by aflatoxin.

CHAPTER SEVEN
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APPENDICES

APPENDIX 1: QUESTIONAIRE: HOUSEHOLD SURVEY ON AFLATOXINS IN MAKUENI COUNTY, 2011.

Household Head Name					
ENUMERATOR NAME:		HHID:		DATE:	/ /20 __

(A) Household location and GIS Positioning

District	Division	Location	Sublocation	Village	Logitude	Latitude	Elevation
1=Makueni 2=Makindu	1= Kaiti 2=Wote	1= Ukia 2=Malivani 3=Ngumo	1=Mukuyuni 2= Unoa 3=Kaunguni				

Members of household	Name	Age	Sex	Formal education
		(Years)	1=Male 2=Female	(Years) 1 = Primary 2 = Secondary 3 = Other post secondary 4 =University/tertiary 5 = Other

(B) Household ID and characteristics

© Foods consumption

Food item	Source(s)	Preparations for under fives	Quantity consumed	Frequency of consumption /day
1. 2. 3.	1 = Market 2 = Own farm 3= Food relief 4 = Gift 5 = Relief 6 = Others	1= Ugali 2= Uji 3= Muthokoi 4= Milk 5= Others(specify)	1= 2kg 2= 1.75 Kg 3= 1,5kg 4= 1.25kg 5= 1kg 6= 0.75kg 7= 0.5kg 8= 0.25kg cups(indicate number)	Record times mentioned

(D) Household income by sources

Category	Amount KShs/ month	Category	Amount KShs/month
Crops (grains/seeds) sales		Paid employment	
Fruits and vegetables sales		Self employed	
Livestock/fish sales		Remittances	
Petty trading		Other (specify)	

(E) Asset ownership.

Asset item	Units (or pairs)	Estimated value KShs	Asset item	Units (or pairs)	Estimated value KShs	Main House type	Number of units
Commercial motor vehicle			Tractor			Mud / thatch	
Private motor vehicle			Tractor trailer			Mud /iron sheets	
Motor cycle			Tractor plough			Timber /iron sheets	
Bicycle			Tractor harrow			Stone/Iron sheets	
Television			Bullock/donkeys/horse			Wood/thatch	
Radio			Bullock/ donkey plough				
Private well			Bullock/ donkey harrow				
Private borehole			Bullock/ donkey cart				
Water pump			Wheel barrow				
Cultivator			Mobile Phones				
Diesel pumps			Fixed phone				
Water tanks							
Generator			Other (specify)_____				

*To be obtained from market prevailing rates/ or ask community

(F) Farming practices

Crop/Commodity	Mode of harvesting	Mode of handling	Storage	Shelling	Drying	Preservation. chemicals	Yield Bags/Kg	Spoilt Bags/Kg	Disposal
1. Maize									
2. Sorghum									
3. Millet									
4. Wimbi									
	1.= Hand 2.=Machine	1 = stacked in heaps 2.=Harvested with stovers standing	1.= Crib 2.=Granary / thatch 3.= Granary/ iron sheet 4. = Bag	1= hand 2 = machine 3=Manually by pounding	1.= on ground with canvas 2. = on ground no canvas 3 = on ground on cob 4= left in farm to dry	1. = Actellic 2. = Ash 3=None			1. = Destroy 2. = Feed 3. = Sell 4. = Give away 5 = Consume 6 = Seed

(G) Household Perceptions ON SPOILT Maize

Food / Feed Grain	Criteria for "SPOILT"			How SPOILAGE is dealt with				Awareness of dangers posed by spoiled grain			Minimum level or type of spoilage that will still allow feeding?
	Criterion 1	Criterion 2	Criterion 3	Anim. feed	Throw away	Give away	Leave it in the field	Risk 1	Risk 2	Risk 3	
1.											
2.											
3.											

(H) Household Perceptions ON MOULDY maize

FOOD/FEED Grain	Total harvest	% MOULDY	Why specified proportion of grain is considered mouldy			Other uses of mouldy grain
						= Destroy = Animal feeds = Sell =Give away = Consume = Seed = Leave in shamba = Other (specify)

(I) Household Perceptions ON Human Health Risks Associated with Consumption of Mouldy Maize.

Mouldy Grain of	If consumption of mouldy grain can cause health problems, which ones?			If mouldy grain can be made safer, how?			Has anybody in this household suffered health problem?
	Risk 1	Risk 2	Risk 3	Action 1	Action 2	Action 3	
1.							
2.							
3.							

(J). Household Perceptions ON Animal Health Risks Associated with Feeding of Mouldy Maize

Mouldy Grain Feed of	If consumption of mouldy grain can cause Animal health problems, which ones?			If mouldy grain FEED can be made safer, how?			Is MILK of cows fed on mouldy grain safe?
	Risk 1	Risk 2	Risk 3	Action 1	Action 2	Action 3	
(K) Can eating mouldy grain or groundnuts cause any problems to health? Please circle							1=Yes 0=No

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What problems?	Has anyone in your hh had this problem	Is it possible to make mouldy grain safe?	How?
1= Poisoning 2= Cancer 3= Other (specify)	0= No 1 = Yes 2 = Not certain	0 = No 1= Yes	

Can mouldy feed or grain cause any problems to animal health? _____

Yes = 1 No = 2

If you feed mouldy grain or feed to dairy cows is the milk safe to drink? _____

Yes = 1 No = 0

APPENDIX 2: PREPARATION OF EXTRACTION SOLUTIONS AND SALINIZATION OF GLASSWARE.

1.1 Preparation of solvent mixtures

1.1.1 Extraction solution

AOC: 70% methanol/ water (v/v) was prepared for cereals extraction.

Easy extract: 60% acetonitrile /water (v/v) was prepared for feeds.

For dilution of extract, phosphate saline buffer was made from PBS tablets.

For silanization of glassware, 10% of dimethylchlorosilane in toluene was made.

1.2 Silanization of glassware

One milliliter of the silanization liquid was transferred into a test tube and blended in a test tube blender and the solution was then poured out of the test tube, the test tube was then kept at 60oc in an oven for 60 minutes. One milliliter of hexane was then transferred into the test tube and the test tube was blended in a test tube blender. The solution was then poured out and test tube was warmed in an oven.