Building an Aflatoxin Safe East African Community

Technical Policy Paper 1

Aflatoxin and Human Health

Knowledge Platform 2015

Situational Analysis for East Africa Region

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EAC JUMUIYA YA AFRIKA MASHARIKI

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Cover: Inspection of maize. *IITA*

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Foreword

The control of aflatoxin throughout the value chain is an important element of the larger food security and food safety profile of a region, a nation, and a community. There are a number of critical points—as commodities are harvested, stored, traded, and processed into foods—at which aflatoxin contamination also has a significant potential to erode the economic vitality of the agriculture sector. Ultimately however, the greatest cost is the negative impact of aflatoxin on human health. This begins in the earliest stages of life, and is reflected by low birth weight and stunting in young children, both of which are correlated to aflatoxin exposure. While this is not the case across Africa, in some countries, a special category of downward adjusted permissible levels of aflatoxins for foods for infants and young child foods has been set.

Throughout childhood, aflatoxin ingestion can cause inflammation of the gut, contributing to enteropathy and the malabsorbtion of essential nutrients. In adulthood, aflatoxin has a negatively synergistic effect on persons suffering from hepatitis, accelerating the progression to hepatocellular carcinoma (HCC). Early studies have also indicated a correlation between aflatoxin and a more rapid progression from HIV to AIDS, in tandem with worsening co-infections. Mortality from acute aflatoxin poisoning—aflatoxicosis—is reported on a consistent basis throughout the region. At the same time, stunting rates remain some of the highest in the world for countries of East Africa, with less than desirable improvements achieved over the past decade. As the relationships between dietary aflatoxin exposure and human health outcomes are better understood, and as the pervasively high levels through the staple food supply of the East Africa region persist, the current situation could be justifiably described as a public health emergency.

To address this, the International Institute of Tropical Agriculture (IITA), the East African Community (EAC), and the United States Agency for International Development (USAID) have formed a partnership under the “Aflatoxin Policy and Programs for the East Africa Region” (APPEAR) projec to tackle one of the most urgent food safety issues currently affecting the African continent. Today, aflatoxin contamination of foodstuffs impacts hundreds of millions of men, women, and children across the region on a daily basis, regardless of socioeconomic status, occupation, age, and gender.

The control of aflatoxin is a complex undertaking, requiring the sharing of information, the advent and application of new technologies and best practices, appropriate communications, and relationship-building throughout a multi-sectoral network reaching across health, agriculture, trade, and the environment. As a first step to addressing aflatoxin issues, we seek to establish a science-based knowledge platform and the building of an enabling policy framework. Through a deeper understanding of the relationships between aflatoxin contamination and human health, we are better equipped to then formulate effective program strategies to realize an aflatoxin safe community for all.

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Executive Summary

Aflatoxins are fungal toxins with widespread exposure in tropical latitudes, especially in developing countries, where contamination of staple foods such as maize, milk, and groundnuts occurs in the field and during storage. Aflatoxins can cause fatal liver toxicity at high doses during acute outbreaks of exposure, and chronic exposure is associated with a range of health effects including liver cancer, child stunting, low birth weight, and immune suppression. Immune suppression may increase susceptibility to infections, particularly in children, affecting general health, nutrient uptake, and growth. Aflatoxin can also cause damage to intestinal epithelial cells, and this effect may contribute to environmental enteropathy by causing leaky gut and reducing the uptake of nutrients. Child stunting that is associated with aflatoxin exposure in utero and in the first two years of life may be due to these mechanisms or other effects, such as disruption of growth factor pathways.

The suppression of the immune system by aflatoxin has led to the hypothesis that aflatoxin exposure may be exacerbating infectious diseases, such as hepatitis B virus (HBV) (which acts together with aflatoxin to cause liver cancer), tuberculosis (TB), and human immunodeficiency virus (HIV). HBV vaccination programs are helping to reduce its high occurrence and the joint effect HBV and aflatoxin have on promoting liver cancer. It is not known to what extent aflatoxin promotes infection with HBV. Evidence is accumulating that associates aflatoxin exposure with increased rates of infection with HIV and enhanced progression to acquired immunodeficiency syndrome (AIDS). As secondary infections are the main cause of death in patients with AIDS, this may also be exacerbated by aflatoxin-induced suppression of the immune system. In all these effects, there may also be interactions with other mycotoxins that co-contaminate crops, especially maize.

Aflatoxin exposure can be estimated by combining the amount of consumed food with levels of contamination in food or by measuring biomarkers in blood or urine. Biomarkers of aflatoxin exposure are widely used for studies aimed at understanding the health effects for aflatoxin exposure. Such biomarkers have shown widespread exposure in adults and children across a range of countries, including high levels in populations in East and West Africa.

Biomarkers have also been effective at assessing the outcomes of interventions aimed at reducing exposure. Such interventions have included the use of additives in the diet to lower absorption of the aflatoxin or inhibit its metabolism after ingestion, and changes to postharvest storage to reduce contamination of the stored crops. Low-cost, sustainable, community-based interventions to reduce exposure have potential for economic application in rural settings. Preharvest interventions can reduce contamination of crops.

Human health effects of aflatoxins in the EAC region were reported as early as the 1960s. In the EAC, aflatoxin contamination in staple foods is common and poor storage practices

and low resources for monitoring and regulating contamination, together with the high intake of contaminated staple crops, often leads to high exposure levels. As a result, aflatoxin exposure in East Africa is a major problem. The use of biomarkers of exposure has enabled more studies of aflatoxin exposure in the region, revealing high exposure in Tanzania, Kenya, and Uganda. A recent study in Tanzania found a significant association between aflatoxin M1 exposure and impaired growth in infants under six months old. The burden of aflatoxin exposure in the EAC is also reflected in the frequent outbreaks of acute aflatoxicosis, especially in Kenya. Human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/AIDS) is a major public health problem in East Africa, and women and children, and people living with HIV/AIDS, may be particularly susceptible to the effects of aflatoxin in their diet.

EAC countries have created a policy framework to aid formulation and implementation of intervention programs to address the human health threat of aflatoxin contamination. Several interventions are now being proposed within this policy framework to combat aflatoxin contamination and target nutritional problems of public health significance.

Although eradication of aflatoxin in staple foods in East Africa is impractical, there are a number of changes that could have a significant impact on exposure levels. Dietary diversification, along with good agricultural practices (GAPs) for aflatoxin-susceptible staple crops at the preharvest and postharvest stages, hold great promise.

There is a need for improved education about aflatoxins at all levels—community, farmers, and decision makers—as well as for a concerted effort to reduce exposure through policies aimed to introduce community-level interventions. These efforts would be boosted by East African countries cooperating in the fight against aflatoxin.

- Aflatoxin is highly toxic to humans and animals.
- Aflatoxin B_1 is the most frequent and potent of this family of toxins.
- Aflatoxin is a known human carcinogen.
- Aflatoxin causes human liver cancer synergistically with HBV infection.
- Aflatoxin has immune suppression effects, with potential to increase chances of infection in vulnerable groups.
- Aflatoxin is associated with slowed growth in children.
- Environmental enteropathy is prevalent in the developing world and may interact with aflatoxin.

Introduction

What is Aflatoxin?

Aflatoxin is the general name for a group of mycotoxins that are produced as secondary metabolites by certain species of the genus *Aspergillus* section *Flavi* (Horn 2003). There are four main types of aflatoxin: aflatoxin B_1 (AFB1), aflatoxin B_2 (AFB2), aflatoxin G_1 (AFG1), and aflatoxin G_2 (AFG2). Each has a different structure and fluorescent properties. Aflatoxin B_1 is the most potent toxin and is the most prevalent, accounting for around 70 percent of the total aflatoxin content in food, although this may vary. Aflatoxin M_1 (AFM_1) is a metabolite of aflatoxin B_1 that is also toxic, and can be found in milk of lactating mothers, and in the milk and meat of animals exposed to aflatoxin.

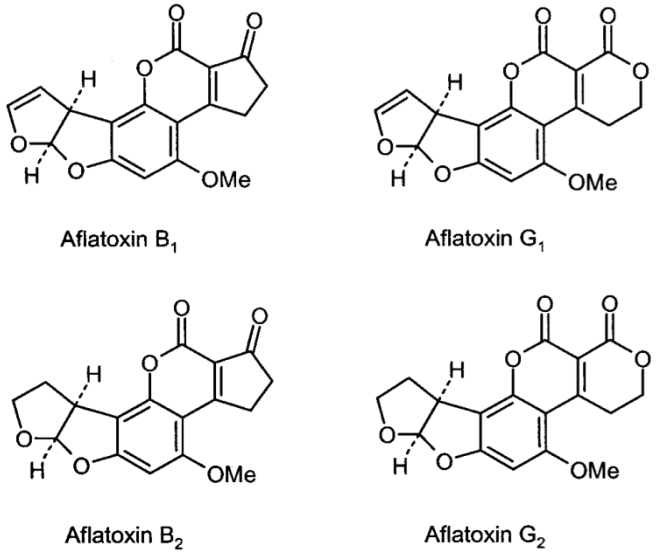


Figure 1: The molecular structure of aflatoxins.

It has been estimated that mycotoxins contaminate approximately a quarter of the world's food supply (CAST 2003), and that approximately 4.5 billion people are exposed to aflatoxin contamination worldwide (Williams, et al. 2004).

Aspergillus molds occur in soil across a wide geographic area and tend to contaminate certain agricultural crops under drought conditions (Pitt et al. 2012). Further growth of the fungus and production of aflatoxin is favored by high humidity postharvest storage conditions (Mutegi et al. 2013).

Aflatoxin contamination of crops has a range of effects on humans, including health effects (discussed below) and economic impact on the ability of farmers to sell their crops, especially on the international market.

Aflatoxicosis: Acute and Chronic

Aflatoxin is highly toxic to animals and humans (International Agency for Research on Cancer [IARC] 2002). Aflatoxin ingestion and absorption leads to metabolism of the toxin to reactive metabolites that react with cellular molecules such as DNA and protein, causing a range of toxic effects. The most severe of these effects occur in the liver. Acute exposure of humans to high levels of aflatoxin can lead to fatal liver damage. The symptoms of acute aflatoxin intake begin with low grade fever, anorexia, and malaise, which can develop into abdominal pain, vomiting, and acute liver failure leading to death (Ngindu et al. 1982). There have been a number of outbreaks of acute aflatoxicosis that have led to deaths in different countries (Krishnamachari et al. 1975; Ngindu et al. 1982; CDC 2004; Williams et al. 2004; Probst et al. 2007; Mohd-Redzwan et al. 2013). One of the most severe reported outbreaks of acute aflatoxicosis occurred in Kenya in 2004, with 125 deaths resulting from 317 cases of acute liver failure (CDC 2004; Azziz-Baumgartner et al. 2005; Probst et al. 2007). Samples of maize from the affected area were found to be contaminated with up to 4400 parts per billion (ppb) aflatoxin (the food limit in Kenya being 20 ppb). It has been estimated that fatal exposure levels would be between 29 and 117 μg/kg body weight, although the threshold probably varies depending on individual susceptibility.

Lower levels of exposure are associated with chronic aflatoxicosis. The most clearly established health outcome associated with chronic aflatoxin exposure is primary hepatocellular carcinoma (HCC), which is common in regions with high aflatoxin exposure and endemic HBV infection. The epidemiological evidence that aflatoxin is a human liver carcinogen is very strong, and aflatoxin has been classified as a known human carcinogen by IARC (IARC 2002). While aflatoxin is a carcinogen on its own, the interaction with HBV has been reported to produce a synergistic effect (IARC 2002). In addition, chronic aflatoxin exposure has been associated with effects on immune function and child growth impairment, as well as other effects such as hepatomegaly (Turner et al. 2003; Jiang et al. 2005; Gong et al. 2012). Chronic aflatoxin exposure is likely to be exacerbating the existing health problems associated with infection and malnutrition of children and adults living in the developing world. Suppression of the immune system could increase the likelihood and the severity of infectious disease, while reduction in nutrient uptake could impair growth in children and increase susceptibility to infection.

Knowledge Base

Animal Experiments: Immune System Effects, Growth, and Nutrient Uptake

There have been many animal studies reporting the carcinogenic effects of aflatoxins in various species (Wogan et al. 2012). Non-cancer effects of aflatoxin, including suppressed immune function, particularly cell-mediated immune responses, have been observed in animal models. B and T lymph cell activity decreased (Reddy et al. 1987) and macrophage activity was suppressed (Moon et al. 1999) in mice treated with aflatoxin. Cellular immune responses were reduced in piglets exposed to aflatoxin during gestation and lactation (Silvotti et al. 1997), and various species have been shown to be less resistant to infection after aflatoxin exposure (Edds et al. 1973; Wyatt et al. 1975; Jones et al. 1981; Venturini et al. 1996). Aflatoxin in the diet causes suppressed growth in pigs, with stronger effects in younger animals (Andretta et al. 2012; Dersjant-Li et al. 2003). Growth impairment is at least partly explained by reduced feed intake, reduced nutrient uptake due to intestinal epithelial damage, or altered energy metabolism (Grenier and Applegate 2013). Reduced growth in agricultural animals due to aflatoxin-contaminated feed results in major economic losses in affected areas (Desjardins et al. 2003).

Health Risks Associated With Aflatoxin Exposure

Hepatocellular Carcinoma and Hepatitis

Liver cancer causes more than 600,000 deaths per year worldwide, with the majority of cases occurring in China, Southeast Asia, and sub-Saharan Africa. It is known that chronic infections such as HBV and hepatitis C virus (HCV) are major risk factors, contributing to 59 percent and 33 percent, respectively, of liver cancer in developing countries (Parkin 2006).

Early studies in Kenya and Swaziland pointed to dietary aflatoxin exposure as an explanation for the high incidence of HCC in certain African countries (Wogan 1975). Aflatoxin acts synergistically with HBV to increase liver cancer risk (Qian et al. 1994). Aflatoxin B_1 is a genotoxic carcinogen with DNA adducts formed through the reaction of the aflatoxin 8,9-epoxide metabolite with guanine to form a mutagenic N7-guanine adduct (Groopman et al. 1981). Mutation in key genes leads to carcinogenesis. Liver tumors from high aflatoxin exposure regions contain a high incidence of G to T mutations at a very specific site in the p53 tumor suppressor gene, at codon 249 (Hsu et al. 1991, Ozturk 1991). Aflatoxin also promotes carcinogenesis, most likely as a result of induced cellular hyperplasia and toxicity in

the liver (Kew 2013). A more recent study in Taiwan suggests that the combined effect of aflatoxin and HBV is additive rather than multiplicative (Wu et al. 2009; 2012). Allen et al. (1992) observed higher levels of aflatoxin exposure using the aflatoxin albumin adduct (AF-alb) biomarker in Gambian children who were HBV+ compared to controls. Turner et al. (2000) found an association between high AF-alb levels and HBV viral load in children in The Gambia, and, more recently, the aflatoxin signature p53 codon 249 mutation has been shown to be associated with the presence of HBV antigens in liver cancer patients in The Gambia (Gouas et al. 2012). The inflammation associated with HBV or HCV promotes cell division that increases cancer risk (Neuveut et al. 2010). The mechanism by which aflatoxin acts with HBV to increase risk of HCC is not fully understood. Aflatoxin may be associated with a range of conditions related to chronic liver damage. A study in China found that high exposure was associated with biochemical changes suggesting damaged liver function (Tao et al. 2005). In The Gambia, one study has linked lifetime groundnut intake as a surrogate aflatoxin exposure measure, to increased risk of liver cirrhosis (Kuniholm et al. 2008). Aflatoxin exposure has also been associated with increased risk of hepatomegaly in school age children in Kenya (Gong et al. 2012).

Cassava can be a significant source of aflatoxin contamination in the diet, leading to the development of liver cancer (Bulatao-Jayme, J. et al. 1982). In a case-control dietary study of Filipino primary liver cancer risk from aflatoxin exposure, researchers compared the aflatoxin intake of 90 confirmed primary liver cancer cases against 90 age-sex matched controls. Dietary recall, obtained by the same nutritionist, was taken for each change of residence lasting one year or more. The frequency and amounts of food items consumed were calculated into units of aflatoxin load per day using a Philippine table of aflatoxin values for these items. Of the total subjects' aflatoxin load, 51.2 percent came from cassava, 20.3 percent from corn, 6.8 percent from groundnuts, and 5.8 percent from sweet potato. The mean aflatoxin load per day of the cases was found to be 440 percent that of the controls. Upon grouping dietary aflatoxin loads (and alcohol intakes) into *heavy* and *light* groups, and upon comparing cases versus controls, the relative risk (RR) of developing primary liver cancer was found to be statistically significant in the following order of rank: cassava, groundnuts, sweet potato, corn, and alcohol.

Aflatoxin and Enteropathy

Environmental enteropathy (EE), also called tropical enteropathy, is a chronic intestinal inflammation caused by repeated fecal-oral contamination due to poor sanitation and unhygienic living conditions (Korpe and Petri 2012). The chronic inflammation is accompanied by shortened intestinal villi, increased intestinal permeability (“leaky” gut), reduced intestinal absorption, and damaged intestinal immune function. Despite these characteristics,

EE can be asymptomatic, rarely having gastrointestinal symptoms like diarrhea (Korpe and Petri 2012). The mechanism of this pathogenic condition is poorly understood.

EE can cause malnutrition, which further impacts on Daily Adjusted Life Years (DALYs) in the tropical world (DeBoer et al. 2012; Prendergast et al. 2014). It is estimated that EE contributes to 43 percent of stunted growth, affecting one-fifth of children worldwide and one-third of children in developing countries (Guerrant et al. 2013). EE is also thought to contribute to the unsatisfactory growth improvement in many large nutritional intervention studies (Bhutta et al. 2013), as well as to the low response to vaccination in children from the tropical world (Korpe and Petri 2012).

Lunn and colleagues conducted extensive studies examining the relationship between EE and stunting in Gambian children (Lunn et al. 1991; Lunn 2000, 2002; Campbell et al. 2003, 2004). Over 95 percent of the infants were affected by leaky gut (Lunn 2000), which explained >50 percent of linear growth shortfall (Campbell et al. 2004).

Both aflatoxin exposure and EE are highly prevalent in the tropical world. Owing to many similarities, it has been hypothesized that aflatoxin may play a role in EE development, or act synergistically with EE on malnutrition (Gong et al. 2008, Smith et al. 2012). It is possible that the intestine is capable of metabolizing aflatoxin into toxic metabolites, which damage large molecules such as the intestinal junction proteins or epithelial nutrient transporters (Gong et al. 2008). Altered permeability was observed in a human intestinal cell monolayer following aflatoxin treatment in vitro (Gratz et al. 2007). Discovering the full effect of EE and aflatoxin exposure on child nutrition is critical for effective intervention strategy decisions.

Childhood Stunting

In the last two decades, the prevalence of stunting in children under five years old has decreased elsewhere in the world but remained high in sub-Saharan Africa and South Asia (Black et al. 2013). Nutrition-specific interventions, including micronutrient supplementation and fortification, as well as complementary feeding, have reduced stunting only to a certain extent (Lutter et al. 2011). It is likely that other factors, including aflatoxin exposure, may play a critical role in child stunting.

It is evident that aflatoxin exposure in humans occurs throughout life, including during gestation (Wild and Gong 2010). Aflatoxin exposure in utero not only affects birth weight but may also play a role in stunted growth in early childhood (up to 24 months). Higher levels of AF-alb in maternal blood are significantly associated with lower weight and height gain (Turner et al. 2007). Furthermore, it has been predicted that a reduction in maternal AF-alb level from 110 pg/mg to 10 pg/mg would lead to a 2 cm increase in height and a 0.8 kg increase in weight within the first 24 months of life. A strong negative correlation between levels of AFM1 in cord blood and maternal serum with birth weights

was observed in the United Arab Emirates (Abdulrazzaq et al. 2004) and low birth weight was seen in children of Ghanaian women with higher AF-alb level (Shuaib et al. 2010).

Because the first two years after birth is a fast growth period, the impact of aflatoxin exposure on growth is most critical during this period. In a cross-sectional study of 479 children aged between nine months and five years (Gong et al. 2002, 2003), 33 percent, 29 percent, and 6 percent of the children were found having stunting (height for age Z score HAZ<-2), underweight (weight for age Z score WAZ<-2), and wasting (weight for height Z score WHZ<-2), respectively. These definitions are based on WHO criteria (WHO 2006). Significant negative correlations between AF-alb and each of the growth parameters were observed. Another study by Turner et al. (2003) found that AF-alb levels were weakly associated with wasting, but not with stunting or underweight. Other cross-sectional studies examined this relationship (Mahdavi et al. 2010; Okoth and Ohingo 2004).

Gong et al. (2004) examined the effects of aflatoxin exposure on growth in 200 children from Benin (16-37 months old) followed up for eight months. Results showed that AF-alb levels were correlated with stunting and wasting. There was a difference in height of 1.7 cm over the eight-month period between the highest and lowest AF-alb quartile. This shows a temporal relationship indicating a potential causal effect.

Although the mechanisms that link aflatoxin exposure with impaired child growth are yet to be defined, it has been hypothesized (Gong et al. 2008; Smith et al. 2012) that: 1) aflatoxin-induced intestinal epithelium damage may contribute to EE; 2) aflatoxin-associated immune suppression could increase children's susceptibility to infections such as diarrhea; 3) liver toxicity of aflatoxin may reduce the production of insulin-like growth factors pathway proteins (IGFs) in the liver, leading to reduced IGFs in circulation and an adverse impact on child growth. Indeed, reduced levels of IGFs due to aflatoxin exposure have been demonstrated by recent data in vitro and in vivo (Castelino et al. 2014a).

It is worth noting that the observed effects of aflatoxin on growth could in part be due to effects of other mycotoxins that co-contaminate crops.

Immunosuppression

Children with AF-alb in sera have been found to be less resistant to infection (Adhikari et al. 1994). In Gambian children, dietary aflatoxin was associated with a reduction in salivary IgA (Turner et al. 2003), and certain subsets of cytotoxic T cells and B cells were reduced in Ghanaian adults with high AF-alb versus those with lower AF-alb (Jiang et al. 2005).

Allen et al. (1992) observed increased levels of AF-alb in Gambian children with *P. falciparum parasitaemia*, although there was no association with specific antibody responses. It has been proposed that the immunosuppressive properties of aflatoxin could make chronically exposed

individuals more susceptible to viral infections such as HBV or HIV, which are common in areas with high aflatoxin exposure (Wild and Montesano 2009).

HIV/TB

Since the epidemic started in the 1980s, 74 million people have been diagnosed with HIV/AIDS. Some 30 million have died and 34 million are living with HIV. About half of the people living with HIV/AIDS are women and around 10 percent are young children. A staggering 70 percent of the survivors are living in sub-Sahara Africa. According to FAO/WHO (2002), HIV/AIDS caused devastating damage to household food security and nutrition through its effects on the availability, stability, and access to food and its use for good nutrition.

Advances in treatment have made living with HIV more survivable. However, problems with cost and access to treatment mean that this is not as true in sub-Saharan Africa as elsewhere. Williams et al. (2004) proposed that aflatoxin exposure could contribute to three areas in relation to HIV/AIDS: increased infection with HIV, more rapid progression of HIV, and reduced survivability with AIDS. Aflatoxin exposure is known to increase the aggressiveness of infectious disease. Hendrickse et al. (1989) observed that the more rapid than normal progression of HIV and AIDS in heroin addicts in Scotland and the Netherlands could be due to the frequently found aflatoxin contamination of the heroin. The down regulation of the cytokine IL-2 caused by aflatoxin (Han et al. 1999), which could make CD4+ cells less effective, could increase susceptibility to HIV/AIDS.

Recent investigations of the interaction of aflatoxin exposure and HIV infection in Ghana show that viral load is higher in HIV-infected adults exposed to higher levels of aflatoxin (Jolly et al. 2013). Another study in Ghana (Jiang et al. 2008) reports that HIV+ participants with high AF-alb had lower levels of CD4+ T cells and B cells compared to those with low AF-alb, and that low perforin expression on CD8+ T cells was associated with AF-alb in both HIV+ and HIV- participants. An investigation of the association between AF-alb levels and opportunistic infections in Ghanaian HIV+ patients found an association between higher AF-alb levels and TB but not malaria, HBV, or pneumonia (Keenan et al. 2011). There is an increasing amount of evidence to support the potential role of aflatoxin exposure in increasing the risk of viral diseases such as HBV and HIV, but further work is needed and it should be recognized that other mycotoxins could contribute to these effects (Williams et al. 2010).

Measuring Exposure and Health Risk

Aflatoxin in the Diet: Global Mapping

Contamination of crops by *A. flavus* and *A. parasiticus* occurs at temperatures between 24˚C and 35˚C with 7-10 percent relative humidity (Williams et al. 2004). Contamination therefore predominantly affects the area between approximately 40˚north and 40˚south of the Equator, particularly in developing countries within the tropical region (Cardwell and Cotty 2002). Fungal contamination and toxin production can occur before harvest and continue to increase postharvest under hot and humid conditions. Contamination in the field often happens as a result of insect damage and lack of irrigation (Hell et al. 2000). Storage practices, which vary largely by the agro-ecological zone, can affect fungal growth and aflatoxin production in grains. In Benin, it was observed that aflatoxin production increased during three to five months of storage; and there was less contamination in maize stored in the bamboo basket (known as an Ago) in the south Guinea Savannah (Hell et al. 2000). Other techniques, including proper drying of grains, improved ventilation at storage, hand-sorting moldy grains, and pesticide usage, proved to be effective in aflatoxin reduction during the postharvest stage (Hell et al. 2000). Food processing may reduce aflatoxin contamination. Dry and wet milling segregated fractions of the commodity and hence reduced aflatoxin levels in the consumed fraction. Chemical processing, including ammoniation, may also greatly reduce aflatoxin levels (Park 2002).

Dietary staples including maize, rice, groundnuts, and cassava can be contaminated with aflatoxin. Animal products, especially milk, may contain aflatoxin when feeds are contaminated. Contamination levels vary in different food types. Maize and groundnuts are extremely susceptible to *Aspergillus*. Maize serves as a primary dietary staple more often in Africa and South America than on other continents. Groundnut is another staple, especially in the West African diet, usually consumed as a primary sauce in daily dishes (Prentice 1993). In addition, cottonseeds, other nuts, spices, and dried fruit can be affected, but to a lesser degree (Pitt et al. 2012).

In Asia, rice and wheat contamination from aflatoxin is frequently reported. Although incidence and contamination levels were found at lower levels in rice, wheat, and oats than in maize and groundnut (Reddy 2011), given the high consumption of rice and wheat in the Asian diet, it is possible that rice and wheat make significant contributions to aflatoxin exposure in these populations.

Grandmother and child at a health clinic in a coastal province of Kenya. USAID

Diet-Based Exposure Measurement

Aflatoxin exposure can be estimated by multiplying consumption data of a certain food item and the occurrence of aflatoxin in this food item (AFB_1 alone or total of aflatoxin); and then summing up the results from each food item consumed. A probable daily intake (PDI) in µg/kg bw/day is thus obtained. This PDI can be compared against recommendations and guidelines in order to assess the risk of exposure in a given population (Shephard 2008).

Food consumption data can be obtained either from national or regional food databases, or from purposely designed diet surveys in a population of interest. Typically, a food frequency questionnaire (FFQ) provides information on the frequency of each food consumed in the previous week, month, or year; a diet recall questionnaire records the amount of each food item consumed over the defined period, e.g., 24 hours, three days, or seven days. These methods can be informative, provided that the questionnaire is fully validated and tailored for the local diet, and the surveyors well trained.

Occurrence data is another part of exposure assessment. The quality of the occurrence data is highly dependent on representative sampling, standardized analytical methods, and well-calibrated equipment. Aflatoxin contamination is extremely heterogeneous. In large-sized food commodities such as groundnut and fig, often a few moldy nuts in a bin or bag may increase aflatoxin levels significantly. Thus, good sampling practice is critical. Analytical methods, as reviewed by Shephard (2009), typically employ either high throughput, rapid ELISA, or equivalent techniques if a suitable antibody is available, or liquid chromatography.

Chromatography, coupled with mass spectrometry, is highly sensitive and specific, and multiple chemicals can be measured simultaneously (Shephard et al. 2013). Method choice depends on the requirement of the project and availability of equipment and skills.

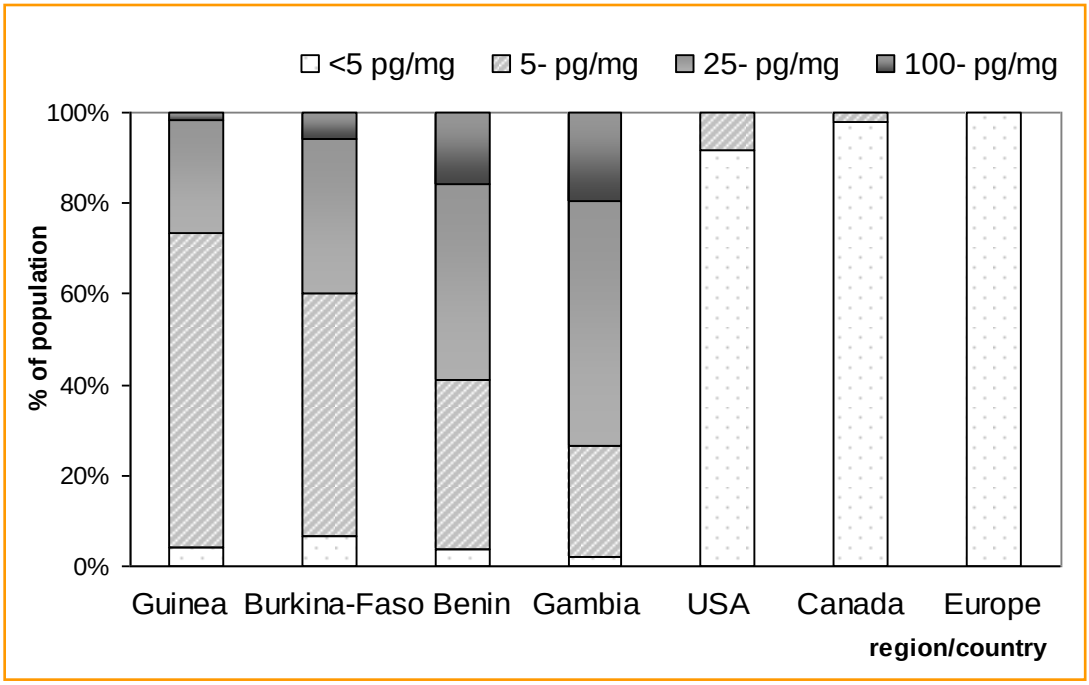
An accurate exposure assessment demands reliable food questionnaires. Both FFQ and diet recall data are prone to recall errors and often lead to poor accuracy in diet quantity estimates (Kohlmeier and Bellach 1995). Improvement can be achieved by various techniques, including using photographs of the dish to improve portion size estimate accuracy, collecting a duplicated dish to correctly measure portion size and the content of the dish, etc. To ensure occurrence data quality, standardized methods of sampling and analysis for aflatoxin in foodstuffs were set by the European Commission in 2006, serving as guidelines for good laboratory practice (Commission Regulation [EC] No. 401/2006). Generally speaking, sufficiently large sample volume and a multiple sites sub-sampling approach must be followed to ensure satisfactory occurrence results. From an analytical standpoint, the large variation in equipment types and experimental skills in different laboratories hinders comparability of reported occurrence data. This requires particular attention in low-resourced countries, as quality assurance may not be guaranteed even at the top national laboratories.

Using Biomarkers to Map Global Exposure

The heterogeneity of aflatoxin contamination in food and variations in the intake of contaminated foods make it difficult to accurately assess individual exposure by the above-discussed dietary-based approach. The measurement of biomarkers in body fluids is more suitable for health-effect studies (Wild et al. 2002). Several aflatoxin-specific metabolites have been exploited as biomarkers of aflatoxin exposure in human populations (Routledge and Gong 2011). The AFB1-epoxide reacts with DNA to form the aflatoxin-DNA adduct, which can be detected in tissue, and the aflatoxin-guanine adduct excreted in urine can reflect recent exposure to aflatoxin (within two or three days) (Autrup et al. 1983; 1987; Groopman et al. 1992; 1993). The AFB1-epoxide also reacts with proteins to yield a protein adduct, namely AF-alb, in blood (Sabbioni et al. 1990). AF-alb can be used as a biomarker reflecting exposure during the previous two to three months (Wild and Gong 2010). It has been calculated that about 2 percent of the daily exposure of aflatoxin becomes AF-alb (Gan et al.1988).

The urinary DNA adduct has been used in a number of studies, particularly in China (Groopman et al. 1992; 1993), and urinary aflatoxin metabolites have been used as biomarkers of exposure in several studies in Southeast Asia and Africa (Polychronaki et al. 2008; Cheng et al. 1997). In the majority of studies, investigating aflatoxin exposure and/or the health effects of that exposure in human populations, the AF-alb has been applied, usually being detected by a well-established ELISA method (Chapot

and Wild 1991; Routledge and Gong 2011; McCoy et al. 2005). Most of these studies have been carried out in at-risk populations in China, Taiwan, or West Africa (Wild and Gong 2010; Obuseh et al. 2011; Castelino et al. 2014b). Only a few studies have been conducted in East Africa: in Kenya (Azziz-Baumgartner 2005; Gong et al. 2012; Yard et al. 2013; Castelino et al. 2014a), Tanzania (Shirima et al. 2013), and Uganda (Asiki et al. 2014). AF-alb has also been used as a biomarker of exposure in population studies in Egypt, Brazil, Thailand, and Malaysia (Wild and Gong 2010; Leong et al. 2012) and the United States (Johnson et al. 2010; Schleicher et al. 2013). The use of biomarkers such as aflatoxin DNA adducts and AF-alb has provided evidence for the exposure of human populations in various geographic locations and has been particularly helpful for investigating the health effects associated with this exposure, as discussed below.



Source: Gong et al. 2008

Figure 2: Aflatoxin exposure (blood AF-alb) worldwide.

The global comparative exposure data shows that in The Gambia and Benin, over 90 percent of young children have detectable levels of AF-alb and the exposure is high in all age groups, in strong contrast to the less than 1 percent detectable rate in the developed world (Gong et al. 2008). This exposure pattern clearly demonstrates a huge public health burden in sub-Saharan Africa with the magnitude of exposure varying from 3 to >1000 pg AF-alb adducts per mg albumin in children (Gong et al. 2003; Gong et al. 2004).

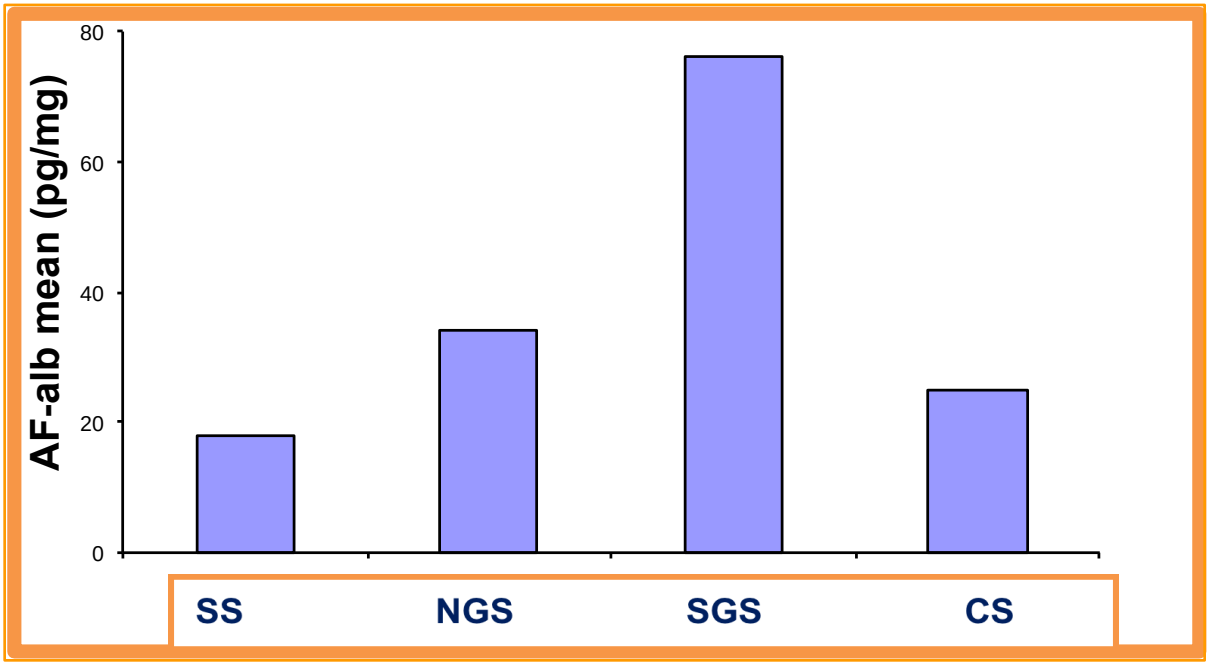


Figure 3: Aflatoxin exposure (AF-alb) in different agro-zones in Benin.

There is a large variation in exposure among the four different agro-ecological zones in Benin (Gong et al. 2003). Climate conditions, storage practice, and food type all contribute to the difference (Hell et al. 2000). Strong seasonal influence on exposure has been demonstrated in various countries. In The Gambia, research has repeatedly shown higher exposure in the dry season than the wet season. This is possibly because the dry season is shortly after the groundnut harvest; high consumption of groundnuts may have contributed to high aflatoxin exposure in this period (Wild et al. 2000; Castelino et al. 2014).

Using Biomarkers for Risk and Intervention Assessment

Using biomarkers has also aided the evaluation of interventions to reduce aflatoxin exposure and the effects of aflatoxin ingestion. Interventions to reduce aflatoxin exposure have included changes to preharvest agriculture practices and postharvest storage practice to reduce aflatoxin contamination of crops, use of chemopreventive drugs or natural dietary components to alter metabolism of aflatoxin, and use of diet additives (chlorophyllin, probiotic bacteria, or clay) to reduce the bioavailability of aflatoxin in the gastrointestinal tract. Biomarkers have been used to assess the efficacy of all of these types of intervention except for preharvest intervention (Wild and Hall 2000). Turner et al. (2005a) demonstrate that low-technology postharvest intervention methods can reduce aflatoxin exposure by reducing aflatoxin contamination of crops during storage. The intervention, which was based in Guinea, included drying groundnuts on mats, and storing them in natural fiber bags, raised on wooden pallets to reduce pest access and improve

ventilation. Reduction in aflatoxin exposure was demonstrated using the AF-alb biomarker, which showed a 50 percent reduction in serum from people in the intervention villages compared to the control villages (who continued normal practices) at five months after the harvest. This community-based intervention has the advantage of reducing the contamination of the staple crop using relatively low-cost methods that can be reused in subsequent years.

The chemoprevention approach depends on the use of a chemical that boosts the body's defenses to the effects of aflatoxin. The drug oltipraz showed some efficacy in modifying activating enzymes and inducing glutathione S transferase (GST) enzymes responsible for detoxification of aflatoxin (Gross-Steinmeyer and Eaton 2012). Oltipraz was tested in an intervention study in a high-risk population in China. After administration of oltipraz at 125 mg/day or 500 mg/day for eight weeks, AF-alb in serum and urinary AFM1 were reduced at the higher dose and AFB-mercapturate metabolites in urine increased, suggesting that induction of GST had occurred (Kensler et al. 1998; Wang et al. 1999). However, the long-term use of a drug in the diet to reduce the effects of aflatoxin would be practically and economically difficult, and therefore not suitable for the the most seriously contaminated regions (Kensler et al. 2004).

A similar effect may be accomplished through dietary intervention with natural dietary components. For example, green tea contains polyphenol compounds that have been shown to be effective at inhibiting induction of aflatoxin-induced liver cancer in rats (Qin et al. 1997). In an intervention trial with green tea polyphenols (GTPs), there was a reduction in aflatoxin-albumin adducts over three months in sera from participants taking 500 mg or 1000 mg of GTPs, and there was a 42 percent-43 percent reduction in median levels of AFM1 in urine in treated groups compared to placebo (Tang et al. 2008). Hence, the biomarkers demonstrated the efficacy of the GTPs.

Glucosinolates, such as glucoraphanin in broccoli sprouts, can be metabolized by gut microflora to yield sulforaphane, which is an inducer of detoxification enzymes. In a trial based in the Qidong region of China, involving the nightly drinking of hot water infused with three-day-old broccoli sprouts, the measurement of metabolites in urine showed inter-individual variation in the bioavailability of the glucosinolates and no difference in aflatoxin-guanine adduct levels between high (400 micromolar) and low (<3 micromolar) glucoraphanin groups (Kensler et al. 2005). However, there was an inverse relationship between excretion of the metabolites of glucoraphanin and the DNA adducts, showing a protective effect in those individuals capable of metabolizing the glucosinolates.

Another approach to reducing the effects of aflatoxin is to reduce the absorption of aflatoxin in the gut. Chlorophyllin is a natural product found in green leaves with the capacity to bind to aflatoxin in the gastrointestinal tract, and has also been tested in a clinical trial in Qidong, China for efficacy at reducing aflatoxin-induced intermediate biomarkers (Egner et al. 2001). Chlorophyllin (100 mg/day) or placebo was given to 180 healthy adults for four months.

Median levels of aflatoxin-guanine adducts excreted in urine were found to be 55 percent lower in those taking 100 mg chlorophyllin with each meal compared to placebo, showing that the chlorophyllin was effective in reducing aflatoxin absorption from contaminated food. In a study in Guangzhou, China, the addition of probiotic bacteria to the diet twice a day for five weeks led to a reduction of 55 percent in levels of AFB-guanine adducts excreted in the urine compared to placebo (El-Nezami et al. 2006), showing the potential of probiotics in the diet to mitigate the effects of aflatoxin exposure. This is due to the bacteria binding the aflatoxin and so reducing bioavailability.

NovaSil clay is processed calcium montmorillonite clay commonly used as an anti-caking agent in animal feeds (Phillips et al. 2008). It has been shown to bind preferentially to aflatoxin in the gastrointestinal tract and thereby reduces the bioavailability of aflatoxin in the blood, liver, and other organs. Experiments in a number of animal species demonstrated the safety and efficacy of the use of NovaSil clay as a dietary additive to reduce the effects of aflatoxin (Phillips et al. 2006) and, following a short term safety testing trial in humans (Wang et al. 2005), a three–month double blind placebo controlled clinical intervention trial was carried out in the Ashanti region of Ghana. Three groups of 177 participants took part in the study, consuming respectively 0g, 1.5g and 3g of NovaSil per day as capsules. This showed the safety of using NovaSil in a human population (Afriyie-Gyawu et al. 2008) and its efficacy in reducing aflatoxin exposure (Wang et al. 2008). AF-alb levels in serum measured by radioimmunoassay were reduced in both low-dose and high-dose groups compared to the placebo at three months. There was also a significant decrease in levels of aflatoxin M1 in urine samples at three months in the high-dose group compared to placebo (Wang et al. 2008). It has been proposed that a uniform particle size NovaSil (UPSN) additive to food could be used to reduce absorption of aflatoxin from the diet (Marroquin-Cardona et al. 2011). Recently, it has been shown that AFM1 in the urine can be used as a biomarker to demonstrate that addition of UPSN to food reduces aflatoxin uptake (Mitchell et al. 2013) and that common African cooking processes do not alter the efficacy of the UPSN (Elmore et al. 2014).

Of course, the best intervention is to reduce exposure through reducing consumption of contaminated crops, if this is possible. There has been a dramatic reduction in liver cancer in the Qidong area of China in recent years as a result of changes to dietary patterns, improvements in regulation of aflatoxin in food, and also as a result of an infant HBV vaccination program in the early 1980s (Sun et al. 2013).

The economic costs of various intervention strategies have been reviewed by Khlangwiset and Wu (2010), who suggest that biocontrol (the application of atoxicogenic strains of *A. flavus* in the field to out-compete toxicogenic strains) and postharvest community interventions (such as that used by Turner et al. 2005) are cost-effective from a health economics viewpoint but that education at all levels (from farmer to government policy makers) is

essential to ensuring the successful reduction of aflatoxin exposure in developing countries (Wu and Khlangwiset 2010).

Biomarkers of aflatoxin exposure, especially those that are well validated intermediate end points, have shown their usefulness in evaluating postharvest interventions. However, to date there has been no application of biomarkers to the evaluation of preharvest agricultural interventions to reduce field contamination of crops (e.g., genetically modified crops, irrigation methods, use of insecticide, or biocontrol with atoxigenic fungi strains).

Situational Analysis

Human health effects of aflatoxins in the EAC region were reported as early as the 1960s. In a meeting conducted in Dar es Salaam in 1964, a scientist reported on the correlation between aflatoxin exposure through groundnuts and liver fibrosis and a high incidence of liver carcinoma in Tanzania (Latham 1964). A study conducted in Uganda in 1967 reported that the frequency of aflatoxin contamination in food was particularly high in provinces with a high hepatoma incidence, or where cultural and economic factors favored the ingestion of moldy food (Kaaya and Warren 2005). In the early 1970s, a study in Kenya reported a correlation between aflatoxin B_1 ingestion and the incidence of primary liver cancer in the sampled population (Peers and Linsell 1973).

Outbreaks of Aflatoxicosis and Aflatoxin Exposures

In the EAC region, an outbreak of acute aflatoxicosis was reported for the first time in Uganda in 1967 and was associated with the death of a 15-year-old boy (Kaaya and Warren 2005). A sample of the cassava eaten by sickened children contained 1700 ppb of aflatoxin, far higher than the maximum allowed contamination of 20 ppb based on U.S. Food and Drug Administration (FDA) standards. Generally, however, Kenya is the country which has been most affected by outbreaks of aflatoxicosis. In Kenya, the first reported human aflatoxicosis outbreak occurred in Machakos in 1981, and the most severe aflatoxicosis incident ever reported occurred in Eastern Province of Kenya in 2004 (Obura 2013). This outbreak resulted in 317 cases and claimed 125 lives, a case fatality rate (CFR) of 39 percent. Table 1 shows the trend of aflatoxicosis outbreaks (including those involving animals) in Kenya from the year 1960.

Table 1: Aflatoxicosis outbreaks in Kenya, 1960-2010.

Year	Outbreak
1960	16,000 ducklings die on one Kenyan farm.
1977	A large number of dogs and poultry die in various parts of the country.
1981	12 people die in Machakos District after consuming aflatoxin contaminated maize. A large number of dogs and doves also die.
1984/85	Large numbers of poultry die on many farms after consuming imported maize.
1988	3 people die in Meru North after consuming contaminated maize.
2001	26 people are admitted to Maua Methodist Hospital; 16 of them die from aflatoxin poisoning. 3 people die in Meru North after consuming moldy maize.
2002	Large numbers of dogs and poultry die at the coast after consuming contaminated feed.
2003	6 people die in Thika after consuming moldy maize.
2004	331 cases including 125 deaths after consuming aflatoxin contaminated maize in Eastern and Central Makueni Kitui.
2005	75 cases of acute poisoning with 32 deaths in Machakos, Kitui from consumption of contaminated maize.
2006	20 people had acute poisoning after consuming contaminated maize in Makueni, Kitui and of these 10 die.
2007	4 people affected in Kibwezi, Makueni and 2 die after consuming contaminated maize.
2008	5 people affected in Kibwezi, Kajiado, and Mutomo after consuming contaminated maize; 3 hospitalized and 2 die.
2010	Suspected food poisoning due to contaminated maize in 29 districts. Unconfirmed dog cases; prices spiral down and grain trade collapses.

Source: FAO 2011

The distribution of acute outbreaks of aflatoxicosis in Kenya is consistent with the geographical distribution of aflatoxin exposure. In 2011, the U.S. Centers for Disease Control carried out aflatoxin studies as part of the Kenya Aids Indicator Survey (KAIS) and found that exposure varied regionally and was highest among participants from Eastern Province and lowest in Rift Valley and Nyanza Provinces (CDC 2012). In this assessment, approximately 80 percent of participants had detectable levels. The extent of exposure was similar across the spectrum of age, gender, and socioeconomic status. In Makueni, high levels of exposure were seen in school age children in 2002 (mean = 114.5 pg/ml albumin) with even higher levels in 2004 (mean = 539.7 pg/mg albumin), the year of the aflatoxicosis outbreak in Kenya (Gong et al. 2012). In a cross-sectional exposure assessment conducted in Kenya in 2007, Yard et al. (2013) detected AFB1 in 78 percent of the blood specimens tested. The exposure was fairly universal across several socioeconomic characteristics, including highest level of education and employment status.

EAC Vulnerability to Aflatoxins

The EAC is faced with a number of ailments that have a synergistic effect with aflatoxins in the development of negative health outcomes, including liver cancer, HBV, HIV, and under nutrition. While information on prevalence and severity of liver cancer in EAC partner countries is incomplete, one can project the vulnerability of EAC population to aflatoxin exposure by referring to the high prevalence of under nutrition and HIV/AIDS in the region. Recent surveys carried out within the EAC partner states show a relatively high prevalence of HIV/AIDS in these countries, as shown in Table 2. It may be that the chronic exposure to aflatoxin in the EAC is leading to a greater susceptibility to HIV/AIDS in these populations.

Table 2: Prevalence of HIV/AIDS in EAC partner countries.

Reference group	Prevalence (percent)				
	Kenya	Tanzania	Rwanda	Uganda	Burundi
Total	5.6	7.0	3.0	7.3	3.2
Men	4.4	6.3	2.2	6.1	2.6
Women	6.9	7.7	3.7	8.3	3.8
Children	0.9	N.A.	N.A.	N.A.	N.A.
Urban	6.5	N.A	7.1	8.7	9.4
Rural	5.1	N.A.	2.3	7.3	2.5

There is limited information on HBV prevalence in EAC countries. In Uganda, 10 percent (more than 3 million Ugandans) are living with chronic HBV infection. The highest burden has been recorded in northern Uganda, with 20 percent to 25 percent of the population infected. Other infected areas are West Nile, north central, and northeast Uganda. About 4 percent to 7 percent of people infected are in the southern and southwestern regions of the country (MOH 2011). A review carried out by Kiire (1996) on HBV prevalence in sub-Saharan Africa indicated a high prevalence of HBV in Kenya and to some extent in Burundi. Also, findings of prevalence of HBV or HBV/HIV co-infection among patients with HCC and with resultant high mortality rates have been reported in Kenya, Uganda, Rwanda, and Tanzania (Mwangi and Gatei 1993; Ocam et al. 2011; Muriuki et al. 2013; Rusine et al. 2013; Hawkins et al. 2013). Further research is required to determine the role of aflatoxin exposure in these viral diseases.

There is some evidence that aflatoxin exposure is associated with stunting and other forms of malnutrition (Gong et al. 2004; Abdulrazzaq et al. 2004; Turner et al. 2007; Shuaib et al. 2010; Wild and Gong 2010; Castelino et al. 2014a). This may partly explain the high prevalence of malnutrition in the EAC partner countries, especially in some areas, such as

parts of western Uganda, that are considered food baskets for other regions (RDHS 2010; BDHS, 2012; KNNA 2012-2017; NBS & ICF Macro 2011; UBOS and ICF 2012).

Measuring a patient's CD4 count at the Kyabugimbi Health Center in Uganda.

Aflatoxins in Staple Foods

Aflatoxin exposure is due to the consumption of aflatoxin-contaminated food in the daily diet. In the EAC region, maize and groundnuts are the main sources of aflatoxin exposure. Other foods commonly consumed in the EAC that are potential sources of aflatoxin are milk, cassava, millet, sorghum, rice, beans, poultry, fish, and dried fruit.

As early as 1966, aflatoxin contamination was reported in a number of foods in Uganda (Kaaya and Warren 2005). Results from a survey carried out in Uganda between September 1966 and June 1967 indicated that more than 25 percent of the sampled foods contained 1–100 ppb; 8 percent, between 100–1000 ppb; and 4 percent, more than 1000 ppb of aflatoxins (Alpert et al. 1971). Aflatoxins occurred most frequently in beans, followed by maize and sorghum. Groundnuts, millet, and cassava were contaminated least frequently. Additional surveys of aflatoxin contamination of foods in Uganda were conducted in 1990, 1991, 1992, and 1999

and results consistently indicated more than the maximum allowed level at that time (20 ppb) for total aflatoxins (Sebunya and Yourtee, 1990; Kaaya and Muduuli 1992).

Of the foods sampled at that time (maize, groundnuts, soybeans, cassava chips, formulated baby foods, and animal feeds), the most susceptible were groundnuts and groundnut products. Of particular concern is the occurrence of contamination in the locally manufactured and more affordable baby foods. The sampled foods had aflatoxin levels exceeding 20 ppb (Kaaya and Warren 2005).

In Kenya, Mutegi et al. (2010) carried out a study on aflatoxin contamination in groundnut samples. Of all samples tested, 38 percent were contaminated at levels exceeding the maximum permissible level of 10 ppb. The least-contaminated product was raw podded groundnuts; the most contaminated product was groundnut flour. Groundnuts sold directly by farmers were less contaminated than groundnuts moving through the value chain.

Maize is the major staple consumed in Kenya and is frequently contaminated with aflatoxin at levels that significantly exceed the allowable standards. During outbreaks of acute aflatoxicosis, the levels of aflatoxins can be abnormally high, as experienced in Makueni District where the mean contamination was as high as 53 ng/g and the maximum contamination as high as 5400 ng/g (Shephard 2008; Lewis et al. 2005). A three-year (2005–07) comparative study on maize aflatoxin levels from different sources in eastern Kenya reported a higher prevalence of aflatoxin in maize that was consumed on farm relative to that supplied by relief agencies or purchased from the open market (Daniel et al. 2010). As much as 64 percent of the maize consumed directly by the household had aflatoxin levels higher than the recommended 20 ppb. This indicates the serious problem facing subsistence farmers in rural areas.

In Tanzania, maize is also the staple food and is frequently contaminated with aflatoxin. A study by Kimanya et al. (2008) reported aflatoxin contamination of maize in all the four regions surveyed in Tanzania (Table 3).

Table 3: Occurrence of aflatoxins in maize in Tanzania.

Region	Samples above 10 ppb (percent)	Highest contamination level (ppb)
Tabora	30	158
Kilimanjaro	7	80
Ruvuma	3	26
Iringa	7	58

A countrywide aflatoxin assessment, conducted in Tanzania by the Tanzania Food and Drug Administration (TFDA) in conjunction with Abt Associates, Inc., summarized the occurrences of aflatoxin B_1 in Tanzanian groundnuts and maize as follows:

- Eastern zone (Morogoro): 43 percent of the maize samples were above 5 ppb, the maximum limit for aflatoxin B_1 (AFB1)
- Western zone (Shinyanga): 40 percent of the samples were above 5 ppb
- Northern zone (Manyara): 9 percent of the samples were above 5 ppb
- Southern Highlands (Iringa, Mbeya, and Rukwa): only 4 percent were above 5 ppb
- Southern zone (Ruvuma): none of the samples were above 5 ppb.

Other studies have reported aflatoxin contamination in maize (Kimanya et al. 2014) and other locally prepared foods and beverages (Shephard 2003). Cured fish (salted, sun-dried, and smoked) have all been found to contain aflatoxin levels in the range of 6.7–18 µg/Kg (Mugula and Lyimo 1992). Of particular concern are locally brewed alcoholic beverages, due to the reportedly large quantities consumed. In some studies, samples of local brew in Tanzania had AFB1 levels of 10–50 µg/L (Nikander et al. 1991).

Worth noting is the fact that, apart from Kenya, where aflatoxicosis cases have been highly publicized, there is very low awareness by EAC households of aflatoxin in foods and its health effects (Kaaya and Warren 2005; Daniel et al. 2011; Abt Associates, Inc. and TFDA 2012). However, even the relatively high awareness in Kenya did not translate into significantly lower consumption of aflatoxin-contaminated foods. The authors attributed this unfortunate finding to a lack of supportive infrastructure and perhaps food insecurity in the affected regions (Daniel et al. 2011). As discussed by Williams et al. (2004), scenarios like the relatively high occurrence of aflatoxin in crops consumed on farm, the poorly developed food systems, and food insecurity in the region will require aflatoxin abatement approaches that are different from those applied in the more developed world.

Consumption Patterns for Contaminated Foods in EAC

Another important factor influencing the extent of aflatoxin exposure is the amount of contaminated food consumed by an individual daily. As previously stated, maize is a staple in Kenya and Tanzania. On average, Tanzanians eat 144 grams of maize per person per day (NBS and FAO 2010). Kenya has the highest maize consumption rates in the region. It is estimated at 400g/person/day (Muriuki and Seboe 1995). Ofwona (2013) analyzed the food consumption trends in Kenya based on data from the 2005-06 Kenya Integrated Household Budget Survey. The study found that cereals, particularly maize, are the most popular food consumed in Kenya. This indicates that even low levels of aflatoxin contamination of maize will have a measurable health impact in Kenya and Tanzania. The very high reliance on maize in the Kenyan diet may partially explain the relatively high incidences of aflatoxicosis in Kenya relative to other EAC partner states.

Compared to Kenya and Tanzania, the population of Uganda consumes a more diverse diet. In a survey carried out by Harvey et al. (2010), cereals accounted for only 20 percent,

14 percent, and 13 percent of the human food energy supply in the South Western Uganda, Kampala, and Northern Regions, respectively. Root crops, tubers, and plantain (cooking banana/matooke) accounted for the highest proportion of energy intake at 25 percent, 35 percent, and 40 percent for the same regions, respectively. There was however, a relatively high intake of groundnuts, ranging between 8 percent and 14 percent in the surveyed regions and ranking as the third highest consumed food in the study population. Given the extreme susceptibility of groundnuts to aflatoxin contamination, their high intake is a cause of concern for Uganda. Information on food consumption in Rwanda and Burundi indicates a pattern similar to Uganda, with tubers and pulses being most consumed by the population (NISR 2009; Burundi CFSV 2008). The six most consumed foods in Rwanda are dry beans (11.2 percent), sweet potato (9.0 percent), Irish potato (8.4 percent), cooking banana (6.0 percent), cassava (3.5 percent), and fresh beans (2.6 percent). According to the 2010/11 Rwanda Integrated Household Living Conditions Survey, maize accounted for only 2.2 percent of all foods consumed by the households in Rwanda. Results from the 2008 Burundi Food Security and Vulnerability Analysis (CFSV) showed that 85.7 percent of households consumed tubers at least five days a week; 70.3 percent consumed pulses at least five days a week; 47.5 percent consumed oil at least five days a week; and 35.0 percent consumed vegetables at least five days a week. Households reported less frequent consumption of food items in the categories of cereals, animal products (meat, fish, and poultry), fruits, and milk.

The source of food consumed is an important consideration in the design of aflatoxin interventions. In Uganda, about half of the food consumed is provided through household production, while the remaining half is purchased at market (UBOS 2013). In Rwanda, the situation is slightly different. Markets account for 52 percent of consumption, home production 45 percent, with other sources contributing the remaining 3 percent (NISR 2009). More than half (62.5 percent) of the population in Burundi accesses its supplies of cassava (the main staple) at market (CFSV 2008). In Kenya, 30 percent of the food consumed by rural households is purchased, while 70 percent is derived from home production. The picture in urban areas is quite different: 98 percent of food consumed in Kenyan urban areas is purchased, and only 2 percent is produced at home (MOA 2009). In Tanzania, 55.3 percent of maize is accessed from the market (NBS 2010).

Health Sector Based Interventions for Aflatoxin Control

Each of the EAC partner states has a policy framework to aid formulation and implementation of programs to address public health issues of importance. The following policy documents are available on the Internet.

- Kenya Food Security Main Paper, August 2009
- Kenya National Food and Nutrition Security Policy, 2011

- Kenya National Nutrition Action Plan, 2012–2017
- Rwanda National Food and Nutrition Policy, October 2013
- Rwanda National Nutrition Policy, October 2005
- Tanzania National Nutrition Strategy, July 2011/12–June 2015/16
- The Food and Nutrition Policy for Tanzania, July 1992
- The Uganda Food and Nutrition Policy, 2003
- Uganda Nutrition Action Plan, 2011–16
- Burundi National Health Development Plan, 2011–2015
- Kenya Health Sector Strategic and Investment Plan (KHSSP), 2012–2018
- Kenya Health Policy, 2012–2030
- Rwanda Health Sector Policy, 2005
- Rwanda Third Health Sector Strategic Plan, July 2012–June 2018
- Tanzania Health Sector Strategic Plan III, July 2009–June 2015
- Uganda Health Sector Strategic and Investment Plan, 2010/11–2014/15.

Apart from those in Kenya, the enabling policies do not directly refer to aflatoxin abatement as a priority health and food safety issue. Nevertheless, all the countries' food and nutrition policies do emphasize food safety. These existing policy frameworks could be instrumental in stepping up aflatoxin abatement programs as part of a unified campaign to improve health and nutrition.

In addition to the policy frameworks, many EAC countries have food-safety institutions and regulatory bodies that can be used for aflatoxin abatement. These include the one-stop central food safety authority in Tanzania and the relevant departments and agencies mainly in the ministries responsible for health, agriculture, and trade within the EAC countries. Often, the lead ministry responsible for food safety is also responsible for health. Apart from Tanzania, where the TFDA has been established to lead overall food safety initiatives, all the countries have multiple institutions sharing the food safety roles as per their mandates (WHO 2002; WHO 2009; Jasper Oloo 2010; ABT Associates, Inc. and TFDA 2012). However, even in Tanzania, these various institutions have maintained their parallel operations as per their mandates in the country.

Findings

EAC consumers are highly exposed to aflatoxin, through contaminated staple foods (maize and groundnuts). HIV/AIDS, HBV, and malnutrition in the region may interact synergistically with aflatoxin exposure, further increasing the vulnerability of the population.

High levels of on-farm consumption of aflatoxin-prone foods, especially maize, is a concern, because such foods cannot be checked for aflatoxin contamination. This is because enforcement of regulations is possible only in communities where food is traded. The best approach to minimizing aflatoxin contamination in maize is to control contamination at each

stage from farm to fork. This would involve educating farmers, producers, processors, and consumers about appropriate handling and storage methods at all stages. Other recommended approaches include the use of aflatoxin-resistant maize varieties, practicing crop rotation, the use of fertilizers, well-timed planting, timely harvests, and the use of appropriate drying and processing techniques such as sorting, cleaning, and milling (Hell and Mutegi 2011).

While these aflatoxin control measures are well known, most are beyond the reach of small scale farmers in the region. Awareness alone without follow-up provision of necessary infrastructure and indeed tackling the underlying food insecurity will not be sufficient to sustainably control aflatoxin in the region. In the follow-up policy recommendations, the authors have presented this holistic approach.

Policy Recommendations

1. Develop and implement five-year, regional and national road maps to address aflatoxin issues in each key area of human health--reflecting short, medium, and long-term goals, objectives, program activities, and milestones. Improving public health, where the impacts of aflatoxin are ultimately felt, should be considered a priority sector.
2. The EAC will assume the leadership role in the development and coordination of regional policies and programs critical to aflatoxin abatement within this five-year initiative, while donors and ministries can take the lead for national programs.
3. The partner states will strengthen national cancer registries and other relevant epidemiological surveillance systems to better understand and adequately monitor the impact of chronic consumption of foods exceeding allowable aflatoxin limits on human health, as well as the demographics, frequency, severity, and geography of acute outbreaks of aflatoxicosis.
4. Donors and national governments will take steps to collaborate with the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), the Global Fund to Fight Malaria, Tuberculosis and AIDS, (The Global Fund), One Health initiatives, relevant ministries and institutions, and universities to more clearly define the interactions between aflatoxin and HIV/AIDS, and design and implement risk reduction protocols accordingly.
5. Partner states will develop a comprehensive curriculum on the science, etiology, diagnosis, and management of aflatoxin and aflatoxicosis. The curriculum should be included in the training of community health workers, undergraduate and post-graduate university medical sciences, and schools of midwifery, nursing, nutrition and dietetics, food safety specialists, and public health.
6. A program for aflatoxin prevention, dietary diversification, and food safety will be developed and integrated into primary and secondary school-based science, health promotion, and school feeding. This could be done with support of donors such as the World Bank, the USDA's McGovern–Dole International Food for Education and Child Nutrition Program, and the World Food Programme (WFP), which currently support these programs across the East Africa region.
7. Ministries of Health throughout the region will give special attention to vaccination campaigns that dovetail with aflatoxin control efforts. Specifically,

hepatitis B vaccination protocols currently approved for infants and young children, especially the birth dose, and expanded coverage rates should be emphasized. In addition, adolescent and adult immunization campaigns should be launched in conjunction with supporting communication messaging programs. The introduction of the hepatitis A vaccine should be pursued in accordance with the WHO recommended protocol. HCV screening should be strengthened, as this is also a highly vulnerable group in the East Africa region.

8. EAC partner states shall encourage a rigorous public health nutrition program to encourage and guide dietary diversity for the purpose of reducing the risks of aflatoxin ingestion, without compromising sound nutritional practices. Vulnerable groups such as children in the first 1,000 days and people living with AIDS (PLWAs) will be given first priority.

9. Organizations, ministry departments, and private sector stakeholders involved in the enrichment, fortification, and biofortification of aflatoxin-prone foods should convene to review current protocols, take into consideration the risk of inadvertently increasing aflatoxin ingestion among the general population, and address these issues.

10. EAC partner states shall intensify food safety monitoring systems for food products that are susceptible to high levels of aflatoxin contamination, such as groundnut butter, maize meal, maize-based infant foods, and milk.

11. EAC partner states should integrate seasonal mapping and early warning systems to predict high risk zones for aflatoxicosis outbreaks into food security forecasting models such as the Famine Early Warning Systems Network (FEWSNET) and the FAO early warning systems. This can then be used to initiate quick response mechanisms to reduce the consumption of dangerously high levels of aflatoxin, especially those associated with on-farm consumption.

12. EAC partner states, in the development of all policies, programs, regulations, and practices should be cognizant of and responsive to differences in aflatoxin abatement approaches required for on-farm consumption vs. purchased foods, formal and informal trading systems, and the larger context of food insecurity across the region.

List of Abbreviations and Definitions

Term	Definition
Acute aflatoxicosis	Diseases caused by short term exposure to aflatoxin
Adduct	A covalently bound addition product from the reaction of a chemical, such as aflatoxin, with DNA or proteins in the body
AF-alb	The aflatoxin albumin adduct formed in plasma that can be used as a biomarker of exposure to aflatoxin
Aflatoxin 8,9-epoxide	A metabolite of aflatoxin, produced by cytochrome P450 enzymes in the liver, that can react with DNA and proteins to form covalently bound adducts
AIDS	Acquired immunodeficiency syndrome
Ammoniation	Treatment of aflatoxin containing crops with ammonia under high pressure to detoxify the crops
Atoxigenic fungi	Strains of *Aspergillus* fungi that do not produce aflatoxin
Biomarkers	Chemicals found in blood, urine, or other body fluids and tissues that can be used to indicate interaction (e.g., exposure, effect, disease) between the chemical and the body
CFSV	Burundi Comprehensive Food Security and Vulnerability Analysis
Chemoprevention	The use of naturally occurring or man-made chemicals to reduce the risk of disease caused by exposure to chemicals such as aflatoxin
Chronic aflatoxicosis	Disease caused by long term exposure to aflatoxin (over at least three months)
Dietary staple	A food that is eaten routinely and in such quantities that it makes up a dominant portion of the diet for a given population
ELISA	Enzyme linked immunosorbent assay, a technique for measuring quantities of a chemical that utilizes the binding of the chemical with a specific antibody
Enteropathy	A chronic sub-clinical inflammation of the gastrointestinal tract that reduces nutrient absorption
EE	Environmental enteropathy
FAO	United Nations Food and Agriculture Organization
FFQ	Food frequency questionnaire, used to record how frequently different food items are consumed during a given period of time
GST	Glutathione *S* transferase, a detoxification enzyme that helps to remove reactive metabolites of chemicals such as aflatoxin by catalyzing their binding to glutathione, which is then excreted
GTPs	Green tea polyphenols, chemicals present in green tea that have chemopreventive properties as they can induce detoxification enzymes
HAV	Hepatitis A virus

Term	Definition
HBV	Hepatitis B virus, a virus that causes liver disease, including liver cancer, that has been shown to interact with aflatoxin to increase risk of liver cancer in exposed populations
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV+	A patient who is infected with the human immunodeficiency virus, which causes acquired immunodeficiency syndrome (AIDS)
HIV/AIDS	Human immunodeficiency virus/acquired immunodeficiency syndrome
IARC	The International Agency for Research on Cancer, a World Health Organization agency that is responsible for determining the likely carcinogenicity of naturally occurring and man-made chemicals
IGF	Insulin like growth factor, a hormone that is produced in the liver and is involved in the regulation of growth
KAIS	Kenya AIDS Indicator Survey
malnutrition	Lack of proper nutrition, caused by not having enough to eat, not eating enough of the right things, or being unable to use the food that one does eat
MS	Mass spectrometry, an analytical technique for determining the identity of chemicals by measuring the atomic mass of the chemical and its component parts
N7-guanine adduct	The aflatoxin epoxide reacts with guanine bases in DNA at the N7 position of the guanine molecule, to form this DNA adduct, a reaction product that is the aflatoxin molecule covalently bound to the guanine
p53	A protein integral to protecting cells from transforming into cancer cells, the p53 gene is often found to be mutated in human tumors, including liver tumors caused by exposure to aflatoxin
PEPFAR	The U.S. President's Emergency Plan for AIDS Relief
stunting	Reduced growth that is defined as giving a height for age Z score that is at least 2 standard deviations below the median of the reference population
TB	Tuberculosis, an infectious disease caused by the bacterium *Mycobacterium tuberculosis* that often attacks the lungs and is commonly fatal if not adequately treated.
TFDA	Tanzania Food and Drug Administration
USDA	U.S. Department of Agriculture
underweight	Reduced growth that is defined as giving a weight for age Z score that is at least 2 standard deviations below the median of the reference population
wasting	Reduced growth that is defined as giving a weight for height Z score that is at least 2 standard deviations below the median of the reference population

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