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Improving the Evidence Base on Aflatoxin Contamination and Exposure in Africa

Sheila Okoth

Series: Agriculture and nutrition



Improving the Evidence Base on Aflatoxin Contamination and Exposure in Africa: Strengthening the Agriculture-Nutrition Nexus

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The Technical Centre for Agricultural and Rural Cooperation (CTA) is a joint international institution of the African, Caribbean and Pacific (ACP) Group of States and the European Union (EU). Its mission is to advance food and nutritional security, increase prosperity and encourage sound natural resource management in ACP countries. It provides access to information and knowledge, facilitates policy dialogue and strengthens the capacity of agricultural and rural development institutions and communities.

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About the Partnership for Aflatoxin Control in Africa (PACA)

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Acronyms

AATF	African Agriculture Technology Foundation
AF-alb	Aflatoxin–albumin
APTECA	Aflatoxin Proficiency Testing and Control in Africa
AUC	African Union Commission
BecA-ILRI	Biosciences eastern and central Africa-International Livestock Research Institute
BMGF	Bill and Melinda Gates Foundation
CIMMYT	International Maize and Wheat Improvement Centre
COMESA	Common Market for Eastern and Southern Africa
CTA	Technical Centre for Agricultural and Rural Cooperation
DRC	Democratic Republic of Congo
DTMA	Drought Tolerant Maize for Africa project
EAC	East African Community
EC	European Commission
ECOWAS	Economic Community of West African States
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FTA	Free trade area
GAP	Good agricultural practices
GC	Gas Chromatography
GMP	Good manufacturing practices
GST	Glutathione-s-transferase
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus
HPLC	High-Performance Liquid Chromatography

ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IFMP	Infant formula milk powder
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
ILRI	International Livestock Research Institute
ISO	International Organization for Standardization
LP	Locally processed
MBM	Maternal breast milk
MS	Mass Spectrometry
NAFDAC	National Agency for Food and Drugs Administration and Control (Nigeria)
NIR	Near Infrared
PACA	Partnership for Aflatoxin Control in Africa
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
REC	Regional economic communities
SADC	South Africa Development Community
TLC	Thin Layer Chromatography
UEMOA	Union Economique et Monétaire Quest Africaine
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
VCG	Vegetative compatible groups
WHO	World Health Organization

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Executive summary

This report reveals that substantial knowledge is available about the aflatoxin challenge that plagues African farmers, other agri-entrepreneurs, and governments. Commissioned by the ACP-EU Technical Centre for Agricultural and Rural Cooperation (CTA) in collaboration with the African Union Commission - Partnership for Aflatoxin Control in Africa (PACA), this literature review reveals that a wide range of commodities that are traded nationally, regionally and internationally are contaminated by aflatoxins. African citizens and economies are negatively impacted as a result.

Aflatoxins are a problem for agriculture, health and trade in Africa. At high doses, aflatoxins can cause acute poisoning and death, and at chronic lower-level doses they can cause liver cancer and chronic immunosuppression. They are also associated with kwashiorkor and poor growth in young children. The report shows that aflatoxin exposure in humans and livestock, including farmed fish, is pronounced due to:

- the widespread occurrence of the *Aspergillus* fungi that produce aflatoxins
- the wide range of agro-ecological conditions, temperature and humidity which favour the growth of *Aspergillus flavus* and *Aspergillus parasiticus* and other *Aspergillus* species (*A. flavus* and *A. parasiticus* are the two most economically important species)
- the wide variety of cereals (maize, millet, rice, sorghum, teff, wheat), and other crops (legumes [including groundnuts], roots and tubers [cassava], spices, and tree nuts) that are contaminated in the field or in storage by the *Aspergillus* fungi. The contaminated crops are also used in the production of processed foods and animal feeds, resulting in processed foods (e.g. peanut butter and vegetable oils) and foods of animal origin (e.g. eggs, milk and meat) being contaminated as well
- many aflatoxin-susceptible grains and legumes are essential staple foods for a wide-cross section of African consumers
- a low level of awareness of aflatoxin contamination, the health risks and potential mitigation measures. Low coverage of vaccination against hepatitis B and absence of vaccination against hepatitis A also increase the risk of developing liver cancer
- weak governance and legislative framework, nationally and regionally
- limited human resource capacity and access to up-to-date laboratory infrastructure for monitoring and evaluating levels of contamination and exposure
- numerous unsynergistic interventions driven by multiple interest groups.

The enabling environment for enhanced aflatoxin control is critical. Developing and implementing policy guidelines and national regulations to govern aflatoxin contamination are pre-requisites for success and will build consumer confidence. Accelerating the harmonisation of relevant policies and regulations within and across regional trading blocks and ensuring alignment, as far as is possible, with those of major international trading partners (e.g. the European Union) will enhance intra- and inter-regional trade and access to international markets. Improving capacity and laboratory infrastructure to ensure conformance to standards will also expand market opportunities for African farmers, traders, processors and other value chain actors. There is need to better coordinate the numerous development and research efforts, promote and share good practice, and mobilise stakeholders (especially farmers and other private sector actors) around a shared agenda for achieving greater impact in controlling aflatoxin.

Since farmers (both crop and livestock) are key stakeholders and the first target group in the aflatoxin control chain, priority should be given to building their capacity for adopting good agricultural practices (GAP) (e.g. selection of suitable varieties, site selection and preparation), improving post-harvest management (including proper drying and moisture control, rapid aflatoxin detection), and implementing good manufacturing practices during storage and distribution. Consumers also need to be made more aware of the health risks posed by exposure to aflatoxin-contaminated food and empowered to demand safe, quality food.

PACA has contributed towards improving the knowledge base by generating country-specific data on the state of aflatoxin contamination and exposure and has developed action plans in selected countries. These home-grown aflatoxin control action plans are in the process of being mainstreamed into national strategies and frameworks in pilot countries. Other activities include: mobilising support for building capacity in aflatoxin testing (efforts of the International Livestock Research Institute (ILRI) Biosciences eastern and central Africa (BeCA) Hub are noteworthy); compiling research and development activities and outputs; promoting good practice in creating databases accessible to stakeholders; and bringing researchers, policymakers, private sector actors and investors/financiers/donors together to identify strategic joint action and develop plans with clear targets and deadlines for aflatoxin control in Africa. CTA will continue to partner with PACA and other key African and international public and private sector partners in continued efforts to control aflatoxin contamination in Africa for enhanced agricultural performance, agri-business development, trade, nutrition and health.

1.0 Introduction

1.1 Aflatoxins: An overview

Aflatoxins are a group of mycotoxins that are classified in two broad sub-groups; the difurocoumarocyclopentenone series and the difurocoumarolactone series (Table 1). The major types of aflatoxins are; AFB₁, AFB₂, AFG₁ and AFG₂. In human beings and other animals, aflatoxins are reported to be carcinogenic, mutagenic and immunosuppressive. At high enough exposure levels, they can cause acute toxicity and, potentially, death in mammals, birds and fish. They display potency of toxicity, carcinogenicity and mutagenicity in the order of AFB₁ > AFG₁ > AFB₂ > AFG₂ as illustrated by their LD₅₀ (lethal dose that causes the death of 50% of subjects) values for day-old ducklings (Carnaghan, 1965; Kraybill, 1970; Jewers, 2015). Day-old ducklings are the most sensitive animals to aflatoxins. The minor aflatoxins have been described as mammalian biotransformation products of the major metabolites.

Aflatoxins are produced by *Aspergillus species* which are soil-borne fungi that are found worldwide (Table 2). About 20 *Aspergillus sp* assigned to three sections – *Flavi*, *Nidulantes* and *Ochraceorosei* – have been reported to produce aflatoxins (Baranyi *et al.*, 2013; Table 2). Most species produce B-type aflatoxins although species related to *A. parasiticus* and *A. nomius* are usually able to produce G-type aflatoxins.

The most economically important species are *A. flavus* and *A. parasiticus*. Both are saprophytic (living on dead or decaying material) during most of their life-cycle. They are also plant pathogens and are found on a wide variety of crops produced in Africa including cereals, legumes, oilseeds, roots and tubers, spices, and tree nuts.

A. flavus is divided into two morphotypes, the S and L strains (Cotty, 1989). Each morphotype is divided into many vegetative compatible groups (VCGs) that limit gene flow among dissimilar individuals (Papa, 1986; Bayman and Cotty, 1991). Both morphotypes and VCGs differ in many characteristics such as aflatoxin-producing ability (Bayman and Cotty 1993; Cotty 1997; Cotty and Cardwell, 1999). The S strain produces, on average, much higher aflatoxin concentrations than the L strain (Okoth *et al.*, 2012). Some isolates produce no aflatoxins at all and are termed atoxigenic (Cotty, 1990). Aflatoxin-producing ability tends to be similar among members of the same VCG (Bayman and Cotty 1993; Ehrlich and Cotty, 2004). The molecular basis for atoxigenicity is known in some cases (Ehrlich and Cotty, 2004; Chang *et al.*, 2005).

In 1993, the International Agency for Research on Cancer classified aflatoxin B₁ as a human Class 1 carcinogen (IARC, 2002). Aflatoxin B₁ is the most potent natural carcinogen known (Squire, 1981; IARC, 2012a) and is usually the major aflatoxin produced by toxigenic *A. flavus* strains.

Table 1: Major aflatoxins and the metabolites

Difuranocoumarins	Type of aflatoxins	Metabolites
Difurocoumarocyclopentenone series	Aflatoxin B ₁ (AFB ₁)	
	Aflatoxin B ₂ (AFB ₂)	
	Aflatoxin B _{2a} (AFB _{2a})	
	Aflatoxin M ₁ (AFM ₁)	Metabolite of aflatoxin B ₁ in humans and animals and comes from a mother's milk
	Aflatoxin M ₂ (AFM ₂)	Metabolite of aflatoxin B ₂ in milk of cattle fed on contaminated foods
	Aflatoxin M _{2A} (AFM _{2A})	Metabolite of AFM ₂
	Aflatoxicol (AFL)	Metabolite of AFB ₁
	Aflatoxicol M ₁	Metabolite of AFM ₁
	Difurocoumarolactone series	Aflatoxin G ₁ (AFG ₁)
Aflatoxin G ₂ (AFG ₂)		
Aflatoxin G _{2A} (AFG _{2A})		Metabolite of AFG ₂
Aflatoxin GM ₁ (AFGM ₁)		
Aflatoxin GM ₂ (AFGM ₂)		Metabolite of AFG ₂
AFGM _{2A}		Metabolite of AFGM ₂
Aflatoxin B ₃ (AFB ₃)		
Parasiticol (P)		
Aflatrem		
Aspertoxin		
Aflatoxin Q ₁ (AFQ ₁)		Major metabolite of AFB ₁ in in-vitro liver preparations of other higher vertebrates

Source: Adapted from Heathcote and Dutton, 1969; Bbosa *et al.*, 2013

Table 2: *Aspergillus* species - Type of aflatoxins and other mycotoxins produced and their occurrence

Species by section	Occurrence – countries where aflatoxins are commonly found	Type of aflatoxin produced	Other mycotoxins	References
<i>Aspergillus</i> section – <i>Flavi</i>				
<i>A.arachidicola</i>	Argentina, Brazil	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Kojic acid, aspergillic acid	Pildain <i>et al.</i> , 2008; Calderari <i>et al.</i> , 2013
<i>A.bombycis</i>	Japan, Indonesia, Brazil	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Kojic acid, aspergillic acid	Peterson <i>et al.</i> , 2001; Okano <i>et al.</i> , 2012; Calderari <i>et al.</i> , 2013
<i>A.flavus</i>	Worldwide	Aflatoxins B ₁ , B ₂	Cyclopiazonic acid, kojic acid, aspergillic acid	Varga <i>et al.</i> , 2009
<i>A.minisclerotigenes</i>	Argentina, USA, Australia, Nigeria, Portugal, Benin, Morocco, Algeria, Kenya	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Cyclopiazonic acid, kojic acid, aspergillic acid	Pildain <i>et al.</i> , 2008; Guezlane-Tebibel <i>et al.</i> , 2012; Probst <i>et al.</i> , 2012; Soares <i>et al.</i> , 2012; El Mahgubi <i>et al.</i> , 2013; Moore <i>et al.</i> , 2013; Probst <i>et al.</i> , 2014
<i>A.nomius</i>	USA, Japan, Thailand, India, Brazil, Hungary, Serbia	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Kojic acid, aspergillic acid, tenuazonic acid	Kurtzman <i>et al.</i> , 1987; Olsen <i>et al.</i> , 2008; Manikandan <i>et al.</i> , 2009; Okano <i>et al.</i> , 2012; Calderari <i>et al.</i> , 2013; unpublished observations
<i>A.novoparasiticus</i>	Columbia, Brazil	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Kojic acid	Gonçalves <i>et al.</i> , 2012
<i>A.parasiticus</i>	USA, Japan, Australia, Brazil, India, South America, Uganda, Portugal, Italy, Serbia	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Kojic acid, aspergillic acid	Varga <i>et al.</i> , 2009; Soares <i>et al.</i> , 2012; Baquião <i>et al.</i> , 2013
<i>A.parvisclerotigenus</i>	Nigeria	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Cyclopiazonic acid, kojic acid	Geiser <i>et al.</i> , 2000; Frisvad <i>et al.</i> , 2005

Species by section	Occurrence – countries where aflatoxins are commonly found	Type of aflatoxin produced	Other mycotoxins	References
<i>A.pseudocaelatus</i>	Argentina	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Cyclopiazonic acid, kojic acid	Varga <i>et al.</i> , 2011
<i>A.pseudonomius</i>	USA	Aflatoxin B ₁	Kojic acid	Varga <i>et al.</i> , 2011
<i>A.pseudotamarii</i>	Japan, Argentina, Brazil, India	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Cyclopiazonic acid, kojic acid	Ito <i>et al.</i> , 2001; Baranyi <i>et al.</i> , 2013; Calderari <i>et al.</i> , 2013
<i>A.togoensis</i>	Central Africa	Aflatoxin B ₁	Sterimatocystin	Wicklow <i>et al.</i> , 1989; Rank <i>et al.</i> , 2011
<i>A.transmontanensis</i>	Portugal	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Aspergillic acid	Soares <i>et al.</i> , 2012
<i>A.mottae</i>	Portugal	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Cyclopiazonic acid, Aspergillic acid	Soares <i>et al.</i> , 2012
<i>A.sergii</i>	Portugal	Aflatoxins B ₁ , B ₂ and G ₁ , G ₂	Cyclopiazonic acid, Aspergillic acid	Soares <i>et al.</i> , 2012
Aspergillus section - Ochraceorosei				
<i>A.ochraceoroseus</i>	Côte d'Ivoire	Aflatoxin B ₁	Sterigmatocystin	Frisvad <i>et al.</i> , 1999
<i>A.rambellii</i>	Côte d'Ivoire	Aflatoxin B ₁	Sterigmatocystin	Frisvad <i>et al.</i> , 2005
Aspergillus section - Nidulantes	Ecuador	Aflatoxin B ₁	Sterigmatocystin, terrein	Frisvad and Samson, 2004; Frisvad <i>et al.</i> , 2005
<i>A.astellatus</i> (= <i>Emericella olivicola</i>)	Italy	Aflatoxin B ₁	Sterigmatocystin, terrein	Zalar <i>et al.</i> , 2008
<i>A.venezuelensis</i> (= <i>Emericella venezuelensis</i>)	Venezuela	Aflatoxin B ₁	Sterigmatocystin, terrein	Frisvad and Samson, 2004

Source: Adapted from Baranyi *et al.*, 2013

Structure of aflatoxins

Aflatoxins have closely related structures and form a unique group of highly-oxygenated heterocyclic difuranocoumarin compounds. The compound is made up of five rings, having a furofuran moiety (rings B and C), an aromatic six-membered ring (A), a six-membered lactone ring (D), and either a five-membered pentanone or a six-membered lactone ring (E) (Figure 1 and Figure 2). AFM₁ and AFM₂ are hydroxylated products of aflatoxins AFB₁ and AFB₂, respectively, which bear a hydroxyl group at the junction of the two furan rings (Schuda, 1980). The minor aflatoxins have a hydroxyl group instead of a carbonyl group at ring E (AFR₀,

AFRB₁, AFRB₂ and AFH₁). In others, the D-ring (AFB₁, AFRB₂) or the E-ring (AFB₃) is opened (Figure 2). Other structural analogs (similar molecular structure) include AFP₁ and AFQ₁, which are AFB₁ metabolites found in urine and liver of rhesus monkeys, respectively (Dalezios *et al.*, 1971; Masri *et al.*, 1974; Sid *et al.*, 1974).

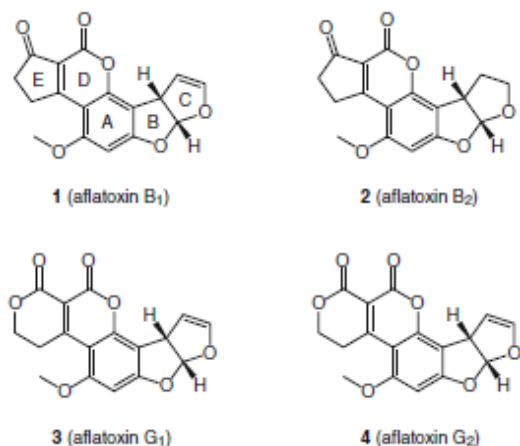


Figure 1: Chemical structure of major aflatoxins

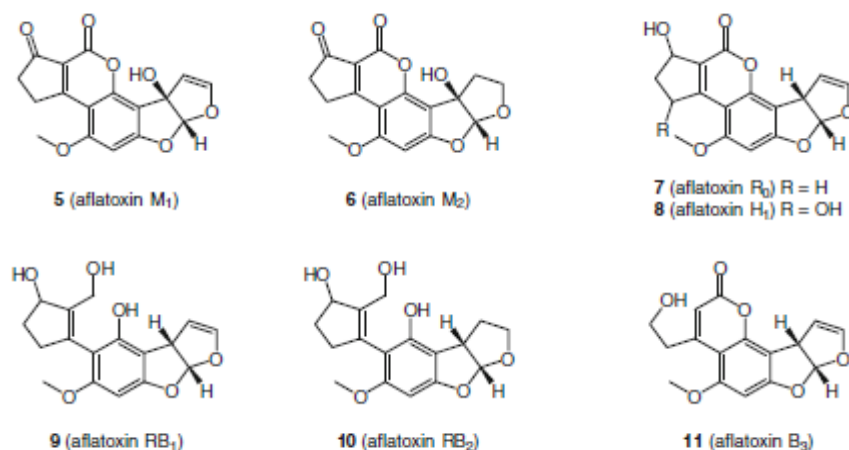


Figure 2: Chemical structure of other selected aflatoxins

Physical and chemical properties of aflatoxins

The reactions of aflatoxins to various physical conditions and reagents have been studied extensively to determine the utility of such reactions in the detoxification of aflatoxin-contaminated material. Aflatoxins are colourless to pale-yellow crystals that fluoresce intensively in ultraviolet light, emitting blue (AFB₁ and AFB₂) or green (AFG₁) and green-blue (AFG₂) fluorescence from which the designations B and G were derived, or blue-violet fluorescence (AFM₁). Aflatoxins are very slightly soluble in water (10–30 µg/ml), insoluble in non-polar solvents, and freely soluble in moderately polar organic solvents such as methanol, chloroform, acetone, acetonitrile and dimethyl sulfoxide (Cole and Cox, 1981). Their melting points are shown in Table 3.

Table 3: Physical properties of aflatoxins

Aflatoxin	Molecular formula	Molecular weight	Melting point (°C)
B ₁	C ₁₇ H ₁₂ O ₆	312	268–269
B ₂	C ₁₇ H ₁₄ O ₆	314	286–289
G ₁	C ₁₇ H ₁₂ O ₇	328	244–246
G ₂	C ₁₇ H ₁₄ O ₇	330	237–240
M ₁	C ₁₇ H ₁₂ O ₇	328	299
M ₂	C ₁₇ H ₁₄ O ₇	330	293
B _{2A}	C ₁₇ H ₁₄ O ₇	330	240
G _{2A}	C ₁₇ H ₁₄ O ₈	346	190

Source: Adapted from O'Neil *et al.*, 2001

Aflatoxins are unstable; to ultraviolet light in the presence of oxygen, to extremes of pH (<3, >10) and to oxidising agents such as sodium hypochlorite, potassium permanganate, chlorine, hydrogen peroxide, ozone and sodium perborate. Aflatoxins are also degraded by reaction with ammonia, various amines and sodium hypochlorite. In a dry state, aflatoxins are stable to heat, up to the melting point. Aflatoxins are very stable and may survive quite severe processes.

A. flavus and *A. parasiticus* are semithermophilic (tolerant to relatively high temperatures) and semixerophytic (tolerant to arid conditions), growing at temperatures from 12 to 48°C and at water potentials as low as -35 megapascals (Kaushal and Bhatnager, 1998). The optimum temperature for growth is 25–42°C. Under conditions of high temperature and low water activity associated with drought, they become very competitive and may become the dominant fungal species in the soil. These two factors, more than any other, contribute to the epidemiology of these two fungi. The optimum temperature for biosynthesis of aflatoxins ranges between 28–35°C. Higher temperatures inhibit biosynthesis (O'Brian *et al.*, 2007; Yu *et al.*, 2011). Conditions in Africa are therefore ideal, favouring the growth of these two species and contamination of commodities across the continent.

1.2 Aflatoxin toxicity

Aflatoxin contamination has been reported in a wide range of commodities grown and consumed in Africa: cereals (maize, pearl millet, rice, sorghum, teff, wheat); legumes and oilseeds (groundnuts and peanuts, soybean, sunflower, cottonseed); root and tuber crops; spices (black pepper, chillies, coriander, ginger, turmeric) and tree-nuts (almonds, coconut, pistachio, walnuts); and several vegetables, fruits and even crops such as coffee, cocoa, tea and sugarcane (Sripathomswat and Thasnakom, 1981; Fukal *et al.*, 1987; Imwidthaya *et al.*, 1987; Juan-Lopez *et al.*, 1995; Vinitketkumnuen *et al.*, 1997; Vrabcheva, 2000; Reddy *et al.*, 2001; Thuvander *et al.*, 2001). Aflatoxin contamination occurs at every stage of the supply chain, from pre-production to post-harvesting, marketing and distribution. Aflatoxin accumulation during post-harvesting is a particular challenge for Africa (Miller, 1995; Bankole and Adebajo, 2003). Once contaminated at any stage in the value chain, commodities remain contaminated throughout all further stages of the chain.

Aflatoxicosis is the poisoning that results from ingesting aflatoxins, although exposure also occurs through dermal (skin) and inhalation routes. Two forms of aflatoxicosis have been identified: the first is acute severe intoxication, which results in liver damage and subsequent illness or death, and the second is chronic sub-symptomatic exposure. A review of the literature across all *Aspergillus* species provides clear evidence that the age of the victim and the dose and duration of exposure to aflatoxin have a major effect on toxicology and cause a range of consequences. While large doses lead to acute illness and death, usually due to liver cirrhosis, chronic sub-lethal doses have nutritional and immunological consequences. All doses have a cumulative effect on the risk of cancer.

Susceptibility to aflatoxin poisoning can be divided into three categories: those with a lethal dose that causes the death of 50% of subjects (LD₅₀) of 1 mg/kg body weight (bwt) or less; those with an LD₅₀ of 10 mg/kg bwt or more; and others that are resistant (Schoental, 1967). Dogs, turkey, ducklings, cats and rabbits are highly susceptible animals (Raney *et al.*, 1992; Agag, 2004; Dereszynski *et al.*, 2008). The acute lethal dose of AFB₁ in humans is estimated at 3 mg/kg bwt (Hsieh *et al.*, 1977), a value similar to that of rats (Newberne and Butler, 1969; Heathcote and Hibbert, 1978; Eaton and Heinonen, 1997). Similar pathologic findings have been reported in humans and rats (Serck-Hanssen, 1970; Krishnamachari *et al.*, 1975; Ngindu *et al.*, 1982; Chao *et al.*, 1991). Deaths have been reported mostly in humans, poultry, ducklings and dogs, in selected African countries. Human deaths have been linked primarily to the consumption of contaminated maize in Kenya and to a lesser extent due to the consumption of contaminated cassava in Uganda. More recently, deaths have been linked to consumption of contaminated cereals in Tanzania. It is also likely that many aflatoxin-related fatalities go unreported in Africa due to lack of capacity for testing the toxins.

1.3 Baseline information: Why this study?

Aflatoxins have gained increased recognition in Africa as more research reveals the negative impacts on health, food security and trade. Though produced in small quantities, the ease with which these highly potent, carcinogenic metabolites permeate African farmers' fields, is of grave concern. Most aflatoxin-susceptible commodities produced in Africa do not meet internationally accepted standards, (United States Food and Drugs Administration (FDA), international Codex Alimentarius limits, and European Union (EU) regulations). The contaminated produce is often rejected by major buyers, processors and traders and by international regulatory agencies before entry in key export markets. High consumption of aflatoxin-susceptible commodities in Africa is compounded because rejected produce that does not meet international standards enters African food and feed value chains, leading to increased risk of exposure to the toxins. Chronic exposure to aflatoxins contributes to the increased incidence and severity of many infectious and non-infectious diseases. Extended exposure is implicated in: immunodeficiency and immunosuppression; stunting and kwashiorkor and an interference with the metabolism of micronutrients in children; and liver cancer, especially in people with hepatitis B or C, or liver disease.

Absence of legislation in several African countries and limited capacity for enforcement where they do exist, do not only put consumers and value chain actors at risk, they limit intra-regional and international trade and do harm to businesses and economies. The extent of the problem, level of contamination and exposure is also under-reported, under-estimated and under-costed, hence compiling baseline information and existing knowledge (on occurrence,

management strategies, economic evaluation, exposure patterns and regulatory frameworks and capacities) can support efforts to:

- undertake risk assessments to identify gaps and design risk management strategies to improve processes to better formulate and implement appropriate control measures and set new research and legislative agendas
- support a more concerted collaborative approach among key partners (including governments, farmers and other private sector actors and knowledge institutes) for effectively managing aflatoxin contamination and reducing risks to human health (especially children under five) and trade (domestic, regional, international).

This study, which is based on available literature on Aflatoxin contamination across major value chains in Africa, was commissioned by the ACP-EU Technical Centre for Agricultural and Rural Cooperation (CTA) in collaboration with the Partnership for Aflatoxin Control in Africa (PACA). It complements the achievements that PACA has made in building an evidence base to inform policies and programmes for effectively managing aflatoxin contamination in Africa. It will also serve as a basis for facilitating more focused interventions including better coordinated research, education, training, agribusiness development, policy harmonisation and implementation. Building new and strengthening existing strategic partnerships towards a more holistic and integrated approach will contribute to achieving greater impact in controlling aflatoxin contamination in Africa.

2.0 Aflatoxin exposure and contamination of food and feed

This section presents available information on aflatoxin contamination of food and feed, and exposure levels in Africa. There is no comprehensive data set from which to evaluate the prevalence of exposure of humans in Africa and other developing countries. However, likely exposure could be estimated by collating the following:

- reports of acute poisoning incidences
- post-mortem reports in which the toxin has been measured in organs
- reports on contamination in foods sampled from households and markets
- reports on direct measurements of human biological exposure to aflatoxin.

Presence of aflatoxins in agricultural commodities (food and feed) suggests that chronic exposure is a possibility. Whether or not consumption of any particular commodity represents a risk factor is partly determined by how susceptible the commodity tends to be and the amount consumed daily. In most parts of Africa, evaluation of exposure in humans and animals have been reported using data from analysis of aflatoxins in food and feed samples collected from farms, markets, mills and stores. The most reliable measure of exposure, however, may be through analysis of samples of prepared meals because grains are normally sorted, though in varying degrees, to remove kernels that are considered unfit to eat.

Quantification of aflatoxins in food may not give exact exposure levels as the amount of aflatoxins present in raw foods may not be the same as that ingested. Some may be lost through processing and – in some cases – the interaction with other food components may affect bioavailability and hence the systemic dose of aflatoxins. The use of biological markers in epidemiological studies on dietary exposure to aflatoxins has enabled progress beyond the determination of levels in food and feeds.

2.1 Exposure assessment applying biomarkers

Albumin is the only serum protein that binds AFB₁ to form a high level of adducts in rats (Skipper *et al.*, 1985), while haemoglobin binds AFB₁ in a very low yield (Tannenbaum and Skipper, 1984). Moreover, albumin is readily extracted from human blood and provides a relatively non-intrusive measure of the biologically effective dose of ingested AFB₁ (Wild *et al.*, 1990). Identification of the metabolic fate of aflatoxins has provided a more authentic tool for exposure studies from the initial simple detection of free aflatoxins to a more reliable AF-alb adduct (Hendrickse *et al.*, 1982; Tsuboi *et al.*, 1984). The aflatoxin, which binds to albumin, can be AFB₁ and AFG₁ but not AFB₂ and AFG₂, which can be metabolised to 8, 9-epoxide (Egal *et al.*, 2005). However, the AF-alb adduct levels should be regarded as a measure of the AFB₁ levels ingested, due to the fact that the AFG₁ presence in the contaminated food is less prevalent.

The molecular markers developed and applied for aflatoxin analysis include the AFB₁ metabolites and AFB₁ macromolecular adducts. These involve AFM₁ and AFB₁-N₇-guanine in urine, as well as AFB₁-albumin adducts in serum (Wang *et al.*, 2001). The latter adduct is considered to better reflect the longer term intake of AFB₁ based on the longer half-life of albumin in humans (30–60 days) compared to urinary metabolites (Sabbioni *et al.*, 1987; Groopman *et al.*, 1994; Williams, 2004). The AF-alb adduct, as a biomarker, is more stable and fluctuates less compared with urinary excretion of aflatoxin metabolites over the same period of time (Wild *et al.*, 1992; Groopman *et al.*, 1994). It has thus been considered to have greater value as a biomarker and has been applied in epidemiological studies on human aflatoxin exposure in different countries (Wild *et al.*, 1990; Wild *et al.*, 1993). This approach may be useful for rapid screening of samples for acute exposures and it also reflects chronic exposure that is not available from other markers such as the aflatoxin-N₇-guanine adduct in urine.

Use of genetic markers to study epidemiology on dietary exposure to aflatoxins has been explored. The association between peanut butter intake as a source of aflatoxins and the gene for glutathione-s-transferase (GST) M₁ (GSTM₁) genotype in the etiology of the most common form of liver cancer, hepatocellular carcinoma (HCC) has been investigated in Sudan (Omer *et al.*, 2001). GSTM₁ is used as a genetic marker for cancer risk with regard to the homozygous deletion of the gene (GSTM₁ null) leading to a lack of corresponding enzymatic activity. The GST enzymes are involved in detoxification of several potentially carcinogenic compounds. The results showed that there was a positive association between peanut butter intake, a humid storage system, HCC incidence and GSTM₁ null genotype.

Biomarker studies in Africa

Surveys on aflatoxin exposure using biomarkers in Africa show that 85–100% of children have either detectable levels of serum AF-alb or urinary aflatoxins and high exposure levels of AFB₁ and AFM₁ in human milk (Tables 4, 5 and 6). This is exacerbated by the confirmation of multiple mycotoxin exposure (Jonsyn *et al.*, 1995; Abia *et al.*, 2013; Ezekiel *et al.*, 2014). Aflatoxin exposure begins from utero and continues in the post-natal period through breast-feeding. Cord blood samples in Ghana, Kenya, Nigeria and Sudan – collected from infants whose mothers had aflatoxins in their blood at the time of delivery – tested positive for aflatoxins (Maxwell *et al.*, 1989).

The highest levels of aflatoxin exposure observed worldwide using aflatoxin-albumin adducts

have been in West African countries. Children in Benin and Togo have exceptionally high aflatoxin exposure, with some individual levels of AF-alb greater than 1,100 pico gram (pg) aflatoxin-lysine equivalents/mg albumin (Gong *et al.*, 2002; Gong *et al.*, 2003; Gong *et al.*, 2004). The exposure is widespread (99%) and associated with child stunting, child mortality, immune suppression and childhood neurological impairment. Seasonal and geographical differences in aflatoxin exposure are also reported within Benin, Egypt, The Gambia and Senegal with serum levels higher during the dry seasons compared to wet seasons (Turner *et al.*, 2000; Gong *et al.*, 2004; Polychronaki *et al.*, 2007; Watson *et al.*, 2015). A cross-sectional serosurvey in Kenya confirmed regional influence on exposure patterns (Yard *et al.*, 2013). In this study, 600 serum specimens from the 2007 Kenya AIDS Indicator Survey – a nationally representative cross-sectional serosurvey – were analysed for aflatoxin levels. Seventy-eight percent of the sampled group was exposed to aflatoxins and the exposure varied by province; it was highest in Eastern (median = 7.87 pg/mg albumin) and Coast (median = 3.70 pg/mg albumin) provinces, and lowest in Nyanza (median = < limit of detection [LOD]) and Rift Valley (median = 0.70 pg/mg albumin) provinces. Sex, age group, marital status, religion and socioeconomic characteristics did not influence exposure. Although Kenya has experienced multiple aflatoxicosis outbreaks as previously mentioned and often resulting in fatalities, the extent of exposure in the country has, for a long time, remained unknown.

Several studies have reported correlation between demographic factors and aflatoxin exposure. In Benin, Ghana, Kenya and Togo, age, sex, socioeconomic status and agro-ecological zone and weaning status was significantly associated with aflatoxin-albumin concentration (Gong *et al.*, 2002; Jolly *et al.*, 2006; Leroy *et al.*, 2015). There is also a correlation between AFM₁ in human milk, AFB₁ in sampled food and socioeconomic status of mothers in Nigeria (Adejumo *et al.*, 2013). Low-income mothers were vulnerable to aflatoxins. Biomarkers in urine, have also shown that rural populations were more exposed to several mixtures of mycotoxins in Nigeria (Ezekiel *et al.*, 2014). Similar results on the influence of socio-economic status on exposure pattern to aflatoxins were reported from a longitudinal evaluation of two cohorts in southwestern Uganda (Kang *et al.*, 2015). From the 713 archived (between 1989 and 2010) serum samples from human immunodeficiency virus (HIV)-seronegative participants, 90% of samples were positive for AFB-Lys. There existed a correlation between the adduct levels and residential areas and occupations from further analysis of one of the cohorts sampled four times from 1999 to 2013 (Table 4). The exposure, however, did not vary by demographic parameters such as sex, age group and education level. Although a link between aflatoxins and hepatoma was suggested decades ago in Uganda (Alpert *et al.*, 1968; Serck-Hanssen, 1970; Alpert *et al.*, 1971), only a few other human exposure studies have been assessed in Uganda: a pilot investigation in a small number of children (Wild *et al.*, 1990) and a pilot study (Asiki *et al.*, 2014). A study from Ghana revealed that all of its participants were exposed to aflatoxins and that the exposure was very high among HIV-infected pregnant and early postpartum women. Serum levels were twice as high in HIV-infected women than in uninfected women and these high levels increased dramatically during pregnancy and early postpartum (Natamba *et al.*, 2016).

Aflatoxins have been implicated in the pathogenesis of kwashiorkor, a severe manifestation of protein energy malnutrition in children, since the 1980's (Hendrickse, 1984; Hendrickse, 1991). Though the mechanisms underlying this relationship is still unclear, studies in Africa (Ghana, Kenya, Liberia, Nigeria, South Africa, Sudan, Zimbabwe) have detected aflatoxins most frequently and at high concentrations in the sera and liver of children with kwashiorkor

who conversely showed aflatoxins least frequently in their urine and in concentrations that were disproportionately lower compared to sera/urine aflatoxin levels in other groups (Hendrickse *et al.*, 1982; Lamplugh *et al.*, 1982; De Vries *et al.*, 1986; De Vries *et al.*, 1990; Ramjee *et al.*, 1991; Oyelami *et al.*, 1998; Onyemelukwe *et al.*, 2012; Castelino *et al.*, 2015). Oyelami *et al.*, (1997) detected aflatoxins in autopsies of lung specimens from children who died from kwashiorkor in Nigeria. These findings indicate altered aflatoxin metabolism in kwashiorkor, supporting existence of a relationship that is further strengthened by a similar geographical distribution of kwashiorkor and aflatoxin presence in food and the similarities of the metabolic disturbance induced by both in animals. Synergisms of kwashiorkor and aflatoxin in causing intestinal function damage, reduced immune function and impaired liver function, are suggested hypotheses.

Table 4: Human exposure to aflatoxins in Africa – results from biomarker studies

Country	Subject	Frequency of aflatoxin-positive samples (%)	Concentration of aflatoxin albumin levels (AFB ₁ -lysine equivalent pg/mg albumin)	Remarks	References
Benin and Togo	Children (9 months to 5 years)	99	5–1,064 (mean 32.8)	Greater in fully-weaned children	Gong <i>et al.</i> , 2002; Gong <i>et al.</i> , 2003; Gong <i>et al.</i> , 2004
Egypt	Pregnant women	35	Mean 4.9	Co-exposure	Piekkola <i>et al.</i> , 2012
Egypt and Guinea	Infants		Aflatoxin B ₁ , B ₂ , G ₁ , G ₂ , M ₁ , M ₂ , B _{2a} , G _{2a} , B ₃ , GM ₁ , and P as well as aflatoxicol		Hatem <i>et al.</i> , 2005; Polychronaki <i>et al.</i> , 2008
Guinea	Adult male	> 90	nd–385	Co-exposure of aflatoxin and hepatitis B or C viruses	Diallo <i>et al.</i> , 1995
The Gambia	Children (3–4 years)	100	2.2–459	Daily consumption of food with > 100 ppb of aflatoxins	Turner <i>et al.</i> , 2000
The Gambia	Children (6–9 years)	93	5–456		Turner <i>et al.</i> , 2003
The Gambia	Adults	99	5% >100		Wild and Hall, 2000

Country	Subject	Frequency of aflatoxin-positive samples (%)	Concentration of aflatoxin albumin levels (AFB ₁ -lysine equivalent pg/mg albumin)	Remarks	References
The Gambia	Maternal blood at pregnancy Cord blood Infants (16 weeks)		4.8–260.8 5–30.2 5–189.6	Effect on growth	Turner <i>et al.</i> , 2007
The Gambia	Children (3–4 years)	95	2.2–250.4		Wild <i>et al.</i> , 1993
The Gambia	Children	100	up to 720	Influence of ethnicity and villages	Allen <i>et al.</i> , 1992
The Gambia, Kenya	Adults	100	7–338		Wild <i>et al.</i> , 1990
Ghana	Pregnant women <i>During pregnancy</i> HIV-uninfected HIV-infected <i>Postpartum</i> HIV-uninfected HIV-infected	100	1.2 (±0.08) 1.91 (±0.12) 1.15 (±0.13) 3.8 (±0.19)	High levels of aflatoxins in HIV-positive women increased as pregnancy progressed	Natamba <i>et al.</i> , 2016
Ghana	Adults		0.12–3	Influence of demographic factors	Jolly <i>et al.</i> , 2006
Kenya	Women	100	7.47	4.7–7.1 times higher in poor compared to the best-off women	Leroy <i>et al.</i> , 2015
Sierra Leone	Cord blood during pregnancy Maternal blood at delivery	58 75		Co-occurrence	Jonsyn <i>et al.</i> , 1994
Tanzania	Children (12–22 months)	84	2.8–652	Greater in fully-weaned children	Shirima <i>et al.</i> , 2013; Shirima <i>et al.</i> , 2015

Country	Subject	Frequency of aflatoxin-positive samples (%)	Concentration of aflatoxin albumin levels (AFB ₁ -lysine equivalent pg/mg albumin)	Remarks	References
Uganda					Kang <i>et al.</i> , 2015
1989–2010		90	0.4–168 (mean 1.58)		
1999–2013		93	0.4–122.5 (mean 1.18)		

Table 5: Human exposure to aflatoxins in Africa – results from urinary biomarker studies

Country	Subject	Frequency of aflatoxin-positive samples (%)	Contamination rate/ concentration (AFM ₁)	Remarks	References
Cameroon	Adults (83% HIV-positive)	83	Detected	Co-exposure	Abia <i>et al.</i> , 2013
Cameroon	Kwashiorkor	Male 44, female 43,	0.109–2.84 µg/l		Tchana <i>et al.</i> , 2010
	Marasmic	Male 60, female 33,	0.109–0.864 µg/l		
	Kwashiorkor control	Male 15, female 6.3	0.007–0.15 µg/l		
Egypt	Pregnant women	48	19.7 pg/mg	Co-exposure	Piekkola <i>et al.</i> , 2012
Egypt and Guinea	Infants		Aflatoxin B ₁ , B ₂ , G ₁ , G ₂ , M ₁ , M ₂ , B _{2a} , G _{2a} , B ₃ , GM ₁ , P, aflatoxicol		Hatem <i>et al.</i> , 2005; Polychronaki <i>et al.</i> , 2008
Ghana	Adults		Non-detectable to 11,562.36 pg/mg	Peanut and maize consumption	Jolly <i>et al.</i> , 2006
Ghana	Children	100	24.7–8368.9 pg/mg	Weaning food	Kumi <i>et al.</i> , 2015
Nigeria	Children, adolescents, adults	50.8	Detected in samples from all age categories	Chronic lifetime exposure	Ezekiel <i>et al.</i> , 2014
Sierra Leone	Infants (urine, serum, stool)	100, 94, 94			Jonsyn <i>et al.</i> , 1999
Sierra Leone	Adults	98		Co-exposure	Jonsyn-Ellis <i>et al.</i> , 2000
South Africa	Adults	0	Not detected	Co-exposure	Shephard <i>et al.</i> , 2013

Table 6: Aflatoxin levels (AFM₁) in human milk in Africa

Region	Frequency of aflatoxin positive samples (%)	Contamination rate/concentration (AFM ₁)	Remarks	References
Egypt	20	Mean 2.75 µg/kg		Alla <i>et al.</i> , 2000
Egyptian	37	Detected		Polychronaki <i>et al.</i> , 2006
Egypt	36	10.27–21.43 pg/ml	Influence of socioeconomics	Polychronaki <i>et al.</i> , 2006
Egypt	56 July January	64 pg/ml (6.3–497 pg/ml) 8 pg/ml milk (4.2–108 pg/ml)	Seasonal pattern observed Influence of peanut consumption	Polychronaki <i>et al.</i> , 2007
Egypt	Milk powder (IFMP) Maternal breast milk (MBM)	74.413 ± 7.070 ng/l 9.796 ± 1.036 ng/l	Average daily exposure MBM = 52.684 ng/l IFMP = 8.170 ng/l	El-Tras <i>et al.</i> , 2011
Cameroon	4.8	0.005 – 0.652 µg/l		Tchana <i>et al.</i> , 2010
The Gambia	100			Hudson <i>et al.</i> , 1992
Nigeria	82	3.49–35 ng/l	Influence of socioeconomics	Adejumo <i>et al.</i> , 2013
Sierra Leone	91		Co-exposure	Jonsyn <i>et al.</i> , 1995
Sudan	54.2	Mean 0.401 ± 0.525 µg/kg Max. level of 2.561 µg/kg	Peanut butter, vegetable oils and rice influence AFM ₁ burden	Elzupir <i>et al.</i> , 2012
Sudan and Zimbabwe		10–50 ppt (0.01–0.05 ng/l)		Wild <i>et al.</i> , 1987
Tanzania		0–0.55 ng/ml	AFM ₁ Exposures 1.13–66.79 ng/k body wt/day	Magoha <i>et al.</i> , 2014

Deaths in humans, poultry, ducklings and dogs have been reported in many African countries due to aflatoxicosis outbreaks linked to the consumption of several contaminated products (Table 7).

Table 7: Reported aflatoxicosis outbreaks in Africa (1960–2016)

Year	Those affected	Numbers affected	Country	Sources of aflatoxins	Recorded effects with a focus on mortality	References
1960	Ducklings	16,000	Kenya - Rift Valley	Groundnut feed	Death	Peers and Linsell, 1973
1977/78	Dogs/poultry	Large numbers	Kenya - Nairobi, Mombasa, Eldoret	Feed	Death	Muraguri, 1981; FAO, 1988
1981	Humans	12	Kenya - Machakos	Maize	Death	Ngindu <i>et al.</i> , 1982
1984/85	Poultry	Large numbers	Kenya	Imported maize	Death	CIMMYT <i>et al.</i> , 1986; FAO, 1988
1988	Human	3	Kenya - Meru North	Maize	Death	Astrup <i>et al.</i> , 1987
1989 1991	Poultry	Large numbers	Morocco	Feed (2,000–5,625 µg/kg)	Death	Kichou and Walser, 1993
2001	Humans	3 26	Kenya - Meru North Kenya - Maua	Maize	Death (16)	Probst <i>et al.</i> , 2007
2003	Humans	6	Kenya - Thika	Maize	Death	Onsongo, 2004
2004	Humans	317	Kenya - Eastern, Central	Maize	Death (125)	Lewis <i>et al.</i> , 2005
2005	Humans	75	Kenya - Machakos, Makueni, Kitui	Maize	Death (32)	Azziz-Baumgartner <i>et al.</i> , 2005; Daniel <i>et al.</i> , 2011
2006	Humans	20	Kenya - Makueni, Kitui, Machakos	Maize	Death (10)	Mutire and Ogana, 2005; Daniel <i>et al.</i> , 2011
2007	Humans	4	Kenya - Kibwezi, Makueni	Maize	Death (2)	Wagacha and Muthomi, 2008
2008	Humans	5	Kenya - Kibwezi, Kajiado, Mutomo	Maize	Death (2)	Muthomi <i>et al.</i> , 2009
1967	Humans	3	Uganda	Cassava	Death (1)	Serck-Hanssen, 1970
1987, 2000, 2005	Dogs		South Africa - Gauteng Province	Dog food	Death	Bastianello <i>et al.</i> , 1987; Reyers and Miller, 2000; Naicker and Botha, 2005
2011	Dogs	Over 200	South Africa - Gauteng Province	Dog food (< 5–4,946 µg/kg)	Death	Arnot <i>et al.</i> , 2012

Year	Those affected	Numbers affected	Country	Sources of aflatoxins	Recorded effects with a focus on mortality	References
2016	Humans	67 9 (a family) Large number	Tanzania Dodoma, Manyara Chemba and Kondoa District, Dodoma	Cereals Cereals	14 confirmed deaths, 53 suspected cases	Buguzi, 2016

Source: Adopted from a Food and Agriculture Organization of the United Nations (FAO) report (Kangethe, 2011) and updated

2.2 Incidence of aflatoxin contamination of commodities (food and feed) by region

The data presented summarises the available literature on the occurrence of aflatoxins in a range of commodities (food and feed) across various regions in Africa as an indicator of the extent of the exposure. The data also reflects research efforts and various areas of interest across the continent. The data are not exhaustive.

Eastern Africa region

Among Eastern African countries, cereals (maize, rice, sorghum), peanuts, pulses, cassava and sweet potatoes are the major crops in terms of area planted and consumption. Overall, maize is the most worrisome followed by peanuts in terms of susceptibility and consumption of aflatoxin contaminated foods and feed (Table 8 and Table 9).

Kenya

Maize and other cereals such as millet and sorghum are staple foods depending on the region. Research on aflatoxins in Kenya has concentrated mostly on maize, peanuts and dairy farming. Maize-meal is consumed at a rate of about 258 g/person/day (ACDI/VOCAa, 2015) and has been the cause of all human aflatoxicosis outbreaks. Consumption of peanuts is at a lower level, estimated at 1.1 g/person/day. Both maize and peanuts also form major portions of the gruel used to wean children and these have been shown to be a source of aflatoxin exposure (Okoth and Ohingo, 2005; Nelson *et al.*, 2016). Most of the peanut samples tested are far beyond acceptable limits for aflatoxins as set by the Kenya Bureau of Standards (Table 8).

Regional variation in aflatoxin contamination of maize has been reported with drought-prone semi-arid eastern regions recording higher levels of contamination of up to 48,000 µg/kg (Daniel *et al.*, 2011; Kilonzo *et al.*, 2014) compared with the highlands and western Kenya that have recorded a high of 4,500µg/kg (Okoth and Kola, 2012; Mutiga, *et al.*, 2015). Sirma *et al.* (2015) reported levels of 0.17–5.3 µg/kg from 67% of maize collected from parts of the Rift Valley region, which is the major producer of maize in the country. Ninety-two percent of millet samples were positive for aflatoxins with a range of 0.14–6.4 µg/kg, while 50% of sorghum samples were positive with a range of 0.21–210.1 µg/kg. Home-grown maize has significantly lower levels of contamination than market samples, though those from eastern Kenya are still far above acceptable limits (Daniel *et al.*, 2011; Okoth and Kola, 2012; Mutiga *et al.*, 2015). In 2010, surveillance of maize resulted in the confiscation of 2.3 million 90 kg bags of aflatoxin-

contaminated maize harvested in the country by Kenyan authorities (Njoroge, 2010). This harvest was considered unfit for both human and animal consumption.

Commercially processed products are also a source of exposure. In October 2011, 25 t of contaminated Unimix (a high-protein mix containing maize flour) destined for relief efforts in drought-affected areas of Kenya was recalled (Menya, 2011). Traditional maize preparation methods (e.g. fermentation and dehulling) in eastern Kenya have been reported to reduce aflatoxins by up to 71% (Mutungi *et al.*, 2008).

Dairy farming is another source of aflatoxin exposure and high levels of AFB₁ have been recorded in feeds (Lanyasunya *et al.*, 2005; Kang'ethe and Lang'a, 2009) (see Table 8). Contaminated milk and milk products with aflatoxin AFM₁ is a concern. The list drawn up by Ochungo *et al.* (2016) of regions that are at risk of an aflatoxin outbreak from milk literally covers all production areas in the country. Protein-rich supplements (cottonseed cake, sunflower cake, fish-meal and other oil-seed by-products), cereal grains and their by-products (maize bran, maize germ, wheat bran and other grain milling by-products) are a rich source of nutrients for moulds. These fungi readily contaminate crop residues and homemade dairy concentrates as a result of poor handling and storage conditions in smallholder farms. This problem is worsened by farmers' widespread practice of using spoilt (pest- or mould-damaged) grains to formulate dairy rations.

Uganda

Plantains, cassava, maize, sweet potato and beans are – in that order – the most important staple foods in Uganda with consumption recorded at 172 kg, 101 kg, 31 kg, 82 kg and 16 kg/person/year, respectively (Haggblade and Dewina, 2010). Peanut consumption is lower, at 4.6 kg/year. Most research on aflatoxin contamination since the 1960s has focused on maize and peanuts. High incidence of liver cancer in the country has been attributed to consumption of aflatoxin-contaminated maize and peanut (Okobia and Bunker, 2003; Kaaya and Warren, 2005). Table 8 summarises comprehensive epidemiological studies conducted in Uganda on aflatoxin exposure from 1967 to 2001 (Kaaya and Warren, 2005). A greater proportion of these studies were conducted on foods sampled at the market-level than from farms, and they indicate that foods from the former are more contaminated with aflatoxins than from the latter, with some having levels above the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Codex Alimentarius recommended limits of 20 µg/kg (FAO/WHO Codex Alimentarius, 2004). Not much has been reported on aflatoxin contamination of livestock feed, but it is believed that spoilt maize is used in animal feed.

Tanzania

Tanzania has the highest maize and peanut consumption rate (349 g/person/day and 15 g/person/day, respectively) among Eastern African countries (Lamb *et al.*, 2015). Other staples are cassava, rice, wheat and sorghum. According to 2012 prevalence data from Abt Associates, in collaboration with the Tanzania Food and Drugs Authority, aflatoxin contamination is a concern in the eastern (Morogoro) and western (Shinyanga) zones of Tanzania. In the eastern zone 43% of maize samples were above regulated levels (5 µg/kg), with an average contamination of 50µg/kg; and in the western zone, 40% of samples were above 5 µg/kg, with average contamination of 28µg/kg. In the southern zone (Ruvuma), none of the samples were above 5µg/kg. Groundnut samples from the northern, southern (Mtwara)

and western zones were contaminated with AFB₁ levels above 5 µg/kg; with mean contamination of 20 µg/kg, 18 µg/kg and 20 µg/kg respectively. Other data are captured in Table 8.

Burundi and Rwanda

Beans, maize, cassava, sweet potatoes and plantains are the major crops produced in these two countries. Maize consumption is 155 g/person/day in Burundi and 39 g/person/day in Rwanda (Lamb *et al.*, 2015). Groundnut consumption is estimated to be much lower at 6.3 g/person/day in Burundi and 2.5 g/person/day in Rwanda. There is not much information on aflatoxin contamination of foods in Burundi and Rwanda, but levels as high as 425 µg/kg have been reported in groundnuts in Burundi (Constant *et al.*, 1984). High contamination levels of all staple foods have also been reported with maize having up to 3,219 µg/kg, peanuts 1,755 µg/kg, cassava 534 µg/kg and beans 154 µg/kg (Nyinawabali, 2013). Aflatoxin exposure (ng/kg bwt/day) in Eastern African countries, based on data from Global Environment Monitoring System/Food data, are estimated at: Burundi 10–180; Kenya 3.5–133; Rwanda (no data); Tanzania 0.02–50; Uganda 10–180 ng/kg bwt/day (Palliyaguru and Wu, 2014).

Sudan

In Sudan, wheat, sorghum or maize flour is used to make porridge, which is a staple food. Beans and fish are also popular. Sudan is a leading global producer of peanuts; a key ingredient in Sudanese cooking and the most researched for aflatoxin contamination in that country. There is conformance to the high standards for the European export market. Sorting results in the elimination of contaminated kernels, however, rejected kernels find their way into the local market, particularly for oil-processing. Varying levels of aflatoxin contamination have been reported in the peanut value chains; 2%, 64%, 14% and 11 % for kernels, butter, cake and roasted peanuts, respectively (Younis and Malik, 2003). Other vegetable oils such as cottonseed, sesame and sunflower oil, are produced in local factories and consumed by almost all segments of the population. These oils, including peanut oil as well as other products, are a source of aflatoxin exposure (Table 8).

Low aflatoxin levels have been recorded in cereal grains and legume seeds collected from retailers (Abdel-Rahim *et al.*, 1989) but a 54% prevalence of aflatoxin contamination was found in commodities, feeds and feed ingredients sourced directly from livestock farms and feed production sites (Rodrigues *et al.*, 2011).

Ethiopia

The major staple crops in Ethiopia include a variety of cereals (mainly teff, wheat and barley), pulses, oilseeds and coffee. Peanuts are one of the most valuable cash crops in eastern Ethiopia and are also consumed in large amounts. Aflatoxin contamination is endemic in Ethiopia due to predisposing factors such as end-season drought, harvesting methods and storage conditions, and low or limited knowledge of aflatoxins and related risks by value chains actors. Cereals, spices, pulses, peanut and dairy products are all high-risk commodities (Table 8 and Table 9). However peanut seems to be the most worrisome with high levels of aflatoxins reported in samples of peanuts from both storage facilities and markets (Eshetu, 2010; Chala *et al.*, 2013). Levels of AFB₁ as high as 738 µg/kg and 692 µg/kg have been found in peanuts and sorghum, respectively (Fufa and Urga, 2001). Use of less-susceptible varieties could reduce exposure as demonstrated by analyses of aflatoxin accumulation in different varieties

of peanut (Chala *et al.*, 2014a). Maize, millet, barley, red pepper and teff were most frequently found to have levels above 20 µg/kg. Exposure from consumption of staple cereals seems to be chronic, though at lower levels compared to exposure from peanut and its products (Chala *et al.*, 2014b; see Table 8).

Table 8: Frequency of aflatoxin contamination and concentration levels in household and market samples from Eastern Africa

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁ (µg/kg)	References	
Ethiopia	Peanut		34.7	Bisrat and Gebre, 1981	
	Peanut butter		105		
	Peanut		5–250	Amare <i>et al.</i> , 1995	
Ethiopia	Peanut		Storage samples	Chala <i>et al.</i> , 2013	
		Babile	87		293–11,865
		Darolabu	88		15–4,939
		Gursum	59		15–5,563
					Market samples
		Babile			15–9,765
Darolabu		15–1,977			
Gursum		16–10,087			
Ethiopia	Spices	8	250–252	Fufa and Urga, 1996	
	Legumes	13			
Ethiopia	Peanut	73	Trace: 447	Eshetu, 2010	
Ethiopia	Barley, sorghum, teff and wheat		Trace: 26	Ayalew <i>et al.</i> , 2006	
Ethiopia	Maize		< 26	Ayalew, 2010	
Kenya - Eastern Province	Maize kernels	45	18–480 Mean dietary exposure: 292 ± 1,567 ng/kg bwt/day	Kilonzo <i>et al.</i> , 2014	
	Dehulled maize (<i>Muthokoi</i>)	20	12–123 Mean dietary exposure: 27 ± 154 ng/kg bwt/day		

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁ (µg/kg)	References
	Maize-meal	35	6–30 Mean dietary exposure: 59 ± 62 ng/kg bwt/day	
Kenya - Nairobi	Market samples for feed	95	> 10 (5.13–1,123)	Okoth and Kola, 2012
	Maize and maize products for food	83	> 10 (0.11–4,593.93)	
Kenya and Malawi	Malted grains	29	Non-detectable: 1,020	Kenji <i>et al.</i> , 2000
Kenya - Nairobi, Western Province	Peanut (82 fresh samples)	0–26.3	0–2377.1	Ndung'u <i>et al.</i> , 2013
Kenya - Eastern Province 2005 2006 2007	Maize 36 samples 18 samples 24 samples		Non-detectable: 48,000	Daniel <i>et al.</i> , 2011
Kenya - Western Province	Maize (14 samples from National Cereal Produce Board warehouses)	57 43 2 samples	> 20 ≥ 100 > 1,000	Lewis <i>et al.</i> , 2005
Kenya - Western Province	Maize (samples from households and markets) Maize for relief efforts	335.5 20.1 10.6	> 20 >100 > 1,000 < 20	Mwihia <i>et al.</i> , 2008
Kenya - Western, Nyanza and Nairobi Provinces	Peanuts and peanut products: Supermarkets Informal markets	28 48	96% raw podded peanuts < 4 4% raw podded peanuts > 10 69% peanut butter and 75% spoilt peanuts > 10 68% of peanut samples stored in plastic jars >10	Mutegi <i>et al.</i> , 2013
Kenya	350 samples of maize and maize products from local markets and government warehouses	55 > 20 µg/kg 35 > 100 µg/kg 7 > 1,000 µg/kg	≤ 46,400 ≤ 1,800	CDC, 2004a,b; Lewis <i>et al.</i> , 2005; Mutegi <i>et al.</i> , 2013

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁ (µg/kg)	References
Kenya - Western Province	Peanut		≤10	Mutegi <i>et al.</i> , 2012
Sudan	Sesame	14.3	0.2–0.8	Idris <i>et al.</i> , 2010
	Groundnut	3.57	0.6	
Sudan	Groundnut paste	16.67	12.93	Kabbashi and Ali, 2014
	Peanut butter	97	< 10	
Sudan	Peanut butter (specialist health food outlets)	64 36 1 sample	< 10 16–318 345	Kabbashi and Ali, 2014
Sudan	Peanut, sesame, cottonseed oils from factories and traditional mills	14.3	43.7% sesame 0.2–0.8 3.57% groundnut 0.6	Yousif <i>et al.</i> , 2010
Sudan	Peanut butter and peanut samples		Average of 87.4 in west Sudan and 8.5 in central Sudan	Omer <i>et al.</i> , 1998
Sudan	Peanut butter	100	1–170	Elshafie <i>et al.</i> , 2011
Sudan	Peanut, sesame, sunflower and mixed oils from retailers and factories	36.8–100	0.43–339.9	Elzupir <i>et al.</i> , 2010
Sudan	Sorghum		11.13–120.2	Saeed, 2015
Tanzania	Maize	45–87	3–1,081	Kamala <i>et al.</i> , 2015
Tanzania	Maize	18	≤ 158 (12% > 10)	Kimanya <i>et al.</i> , 2008
Tanzania	Maize-based foods	32	0.11–386 Mean dietary exposure: 1–786 ng/kg bwt/day	Kimanya, 2014
		44	57–825 Mean dietary exposure: 0.38–8.87 ng/kg bwt/day	
Uganda	Groundnut	100	940	Osuret <i>et al.</i> , 2016
	Groundnut paste	100	720	

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁ (µg/kg)	References
Uganda	Maize, peanut and cassava	50	Positive	Kaaya and Miduuli, 1992
Uganda	Peanut (varieties)			Kaaya <i>et al.</i> , 2001
	Kumi - newly harvested	28	0–5	
	Kumi - stored 7 months	48	0–22	
	Mayuge - newly harvested	50	0–10	
	Mayuge - stored 5 months	40	0–18	
Uganda	Peanut (farm level)	60	7.3–12.4	Kaaya <i>et al.</i> , 2006
Uganda - southwest	Peanut, cassava, millet, sorghum flour, <i>eshabwe</i> sauce (90 food samples)		0–550	Kitya <i>et al.</i> , 2010
Uganda - mid-altitude (moist)	Maize kernels	83	9.7	Kaaya <i>et al.</i> , 2006
Uganda – mid-altitude (dry)		70	7.7	
Uganda - highland zone		55	3.9	
Uganda	Peanut		15% > 1 ppm 3% > 10 ppm	Lopez and Crawford, 1967
Uganda	Beans Maize Sorghum Groundnut Millet Cassava Rice (480 samples)	72 45 38 18 16 12	29%: 1–100 (all sample types except cassava) 8%: 100–1,000 (maize, beans, sorghum, peanut and cassava) 4% > 1,000 (beans, sorghum, peanut and cassava)	Alpert <i>et al.</i> , 1971
Uganda Produce Marketing Board and Animal Feed Mill	Maize, peanuts, soy beans, poultry feed	100	Trace - > 20	Sebunya and Yourtee, 1990
Uganda	Maize		0–50	Ssebukyu, 2002

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁ (µg/kg)	References
Uganda	Heinz mixed cereals, Cerelac, cornflakes Wheetabix, porridge oats Baby soya, <i>Kayebe</i> , <i>Mwebaza</i> rice porridge, jacinta millet and <i>Mukuza</i>		Non-detectable 10–20 20–50	Nakamya, 2008

Table 9: Aflatoxin contamination in feed and dairy products in Eastern Africa

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁	References
Kenya	Milk (processed) Raw	100	0.012–0.127 µg/kg 0.0002–0.013 µg/kg	Obade <i>et al.</i> , 2015
Kenya	Dairy products	59 32 9	< 2 ppt 2–50 ppt > 50 ppt	Sirma <i>et al.</i> , 2014
Kenya	Animal feed (830 samples) Milk samples (613 samples)	86 72	67% > 5 µg/kg 20% > 0.05 µg/l	Kang'ethe and Lang'a, 2009
Kenya	Milk samples Animal feed	45.5 98.6	49% > 0.05 µg/l 83% > 10 µg/kg	Kang'ethe <i>et al.</i> , 2007
Sudan	Groundnut cakes (18 samples)	11	0.013 µg/kg 0.014 µg/kg	Walaa <i>et al.</i> , 2015
Sudan	Cow milk (35 samples) Powdered milk (12 samples)		100% > 0.05 µg/kg 77% > 0.5 µg/kg 50% > 0.05 µg/kg 33% > 0.5 µg/kg	Ali <i>et al.</i> , 2014
Sudan	Cattle milk	95	0.22–9.60 µg/l	Elzupir and Elhussein, 2010

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁	References
Ethiopia	Animal feed	All samples	0.028–4.98 µg/kg	Gizachew <i>et al.</i> , 2015
	Milk (dairy farmers)	8.2	≤0.05 µg/kg	
	Milk (traders)	26.3	>0.5 µg/kg	
	Feed (dairy farmers)	All samples 10.2	7–419 µg/kg ≤ 10 µg/kg	
	Feed (feed producers, processors and traders)	26.2	> 100 µg/kg	
	Wheat bran		31 µg/kg	
Noug cake		290–397 µg/kg		

EU limit of AFM₁ in milk = 0.05 µg/kg

Codex Alimentarius (Codex) limit of AFM₁ in milk = 0.5 µg/kg

WHO/FAO (Codex) limit of AFM₁ is 0.05 µg/kg

West Africa region

Staple foods in West Africa are maize, cassava, yam, rice and plantains, served with beans, legumes, meats, fish, peanut sauce, palm oil, spices and chillies. Countries include Benin, Burkina Faso, Cameroon, Cape Verde, Côte d'Ivoire, Equatorial Guinea, Gabon, The Gambia, Ghana, Guinea, Guinea Bissau, Liberia, Mali, Nigeria, Senegal, Sierra Leone and Togo. Generally there is a wide variation in climate conditions ranging from tropical hot and humid conditions in most of West Africa to desert and arid conditions in the northern parts of Mali and Niger. The varying temperatures and humidity are favourable for the growth of toxigenic fungi and mycotoxin production. Tables 10, 11 and 12 provide an overview of aflatoxin contamination in West Africa as well as data on the export notifications from West Africa to Europe based on literature reviewed. Aflatoxin contamination has significantly constrained exports of peanut from West Africa to European markets.

Nigeria

Numerous surveys on aflatoxin contamination of food and feed in Nigeria have been carried out (Table 10) which indicate a wide range of commodities are impacted. Recorded aflatoxin readings are as high as 1,000–5,000 µg/kg in groundnut, maize, rice and millet beer. The Nigeria Mycotoxin Awareness and Study Network has created mycotoxin maps of the country that summarise the occurrence of aflatoxin contamination in maize and peanuts (Figure 3 and 4) respectively. The Southern Guinea Savanna and Sudan Savanna zones in Nigeria have been reported to have significantly higher aflatoxin contamination than other cooler dryer agro-ecological zones (Udoh *et al.*, 2000).

Table 10: Aflatoxin contamination in household and market samples from Nigeria

Commodities contaminated	Frequency of contamination	Range of concentration ($\mu\text{g}/\text{kg}$)	Mean concentration ($\mu\text{g}/\text{kg}$ or $\mu\text{g}/\text{l}$)	References
Groundnut		100–2,000		Darling, 1963
Maize	100%	30.9–507.9		Atehnkeng <i>et al.</i> , 2008
Stored maize grains		137.33–596.85		Adetuniji <i>et al.</i> , 2014
Stored groundnut	35%	100–2,000		Peers, 1965
Palm wine				Bassir and Adekunle, 1969
Bitterleaf		> 94		Bassir, 1969
Groundnut		> 900		Okonkwo and Nwokolo, 1978
Dried fish		600–700		
Cereals (millet sorghum and rice)		150–300		
Sorghum		< 20		Dada, 1978
Sorghum	8/8 8/8	30.32–211.2 2.4–208		Uriah and Ogbadu, 1982
Groundnut		0–600		Abalaka and Elegbede, 1982
Groundnut oil		> 98		
Cottonseed oil		> 65		
Poultry feed made of groundnut cake (aflatoxicosis in pekin ducklings)			3,000	Ikwuegbu, 1984
Livestock rations		4–340		Gbodi <i>et al.</i> , 1984
Poultry feed	69/120	0.57–2.55		Oyejide <i>et al.</i> , 1987
Maize	27/64 21/64 5/6 43/64	0–960 0–543 0–83.33 0–23.53	0.27–372.49 1.5–113.2 2–203 92–13.33	Gbodi, 1986
Acha	4/24 2/24	0–20 0–12		
Cottonseed	3/8 3/8 2/8 1/8	0–271 0–36.6 0–183 0–9.1	52.25 24.85 38.13 1.14	

Commodities contaminated	Frequency of contamination	Range of concentration (µg/kg)	Mean concentration (µg/kg or µg/l)	References
Millet beer	10/10	500–5,000		Obasi <i>et al.</i> , 1987
Peanut cake	18/20 29/29	20–455 13–2,824	236.69	Akano and Atanda, 1990; Ezekiel <i>et al.</i> , 2012
Cowpea	3/268	0–48	31.6	Opadokun, 1992 (samples collected from 1962–1985)
Maize	81/281	0–1,250	74–218	
Millet	10/275			
Rice	13/279	0–160	42	
Sorghum	22/318	0–40	5	
Cottonseed	17/28	0–40	5	
Groundnut	414/634	0–8,000	105	
Groundnut oil	56/57		151–767	
Melon seed	22/30	0–40	9.5	
Palm kernel	41/55	0–53	19	
Restaurant dishes (<i>gari</i> , bean with soup) Dried okra Dried pepper	17/17	31.2–268.32		Obidoa and Gugnani, 1990
Maize Maize cake Maizeroll snack		25–777 15–1070 10–160	200 233 55	Adebajo <i>et al.</i> , 1994
Red hot chili pepper		> 2.2		Adegoke <i>et al.</i> , 1996
Maize	144/288	234–908 234		Tijani, 2005
Human milk Cow milk Ice cream	5/28 3/22 2/6		4 3.02 2.23	Atanda <i>et al.</i> , 2007
Mouldy rice	97/196	0–1,642	200.19	Makun <i>et al.</i> , 2007
Mouldy sorghum	91/168	0–1,164	199.51	Makun <i>et al.</i> , 2009a Makun <i>et al.</i> , 2009b
Powdered milk Bean Wheat	7/100 29/50 27/50	0–0.41 0–137.6 0–198.4	0.016–0.325 59.29 85.56	Makun <i>et al.</i> , 2010

Commodities contaminated	Frequency of contamination	Range of concentration (µg/kg)	Mean concentration (µg/kg or µg/l)	References
Rice	21/21 21/21 21/21 19/21 21/21	4.1–309 1.3–24.2 5.5–76.8 3.6–44.4 27.7–371.9	37.2 8.3 22.1 14.7 82.5	Makun <i>et al.</i> , 2011
Melon seed	30/120	2.3–15.4		Bankole and Mabekoje, 2004
Maize	20/103	3–138		
Fonio millet	13/16 4/16 4/16	0.08–1.4 0.07–0.1 0.2–2	0.4 0.08 0.6	Ezekiel <i>et al.</i> , 2012
Commercial poultry feed	44/58 29/58 35/58 6/58	6–1,067 10–114 8–235 10–20	198 34 45 13	Ezekiel <i>et al.</i> , 2012
Rice Beans Cassava flour Semovita Yam Wheat meal Maize Gari Human milk	19/21 15/17 3/4 2/6 6/7 2/3 3/3 13/18 41/50	*nd –0.3 nd–0.89 nd–0.07 nd–0.17 nd–0.27 nd–0.06 0.11–0.2 nd–0.69 nd–92.14(ng/l)	0.14 0.15 0.05 0.09 0.14 0.04 0.16 0.25 15 (ng/l)	Adejumo <i>et al.</i> , 2013
Dried yam chips	97.5%	190		Abiala <i>et al.</i> , 2011
Rice samples	18.4%	Mean of 5		Abdus-Salaam <i>et al.</i> , 2015
Maize Maize cake Maize roll snacks	45% 80% 12%	25–770 15–1,070 10–160		Adebajo <i>et al.</i> , 1994
Pre-harvested maize	18.4%	3–138		Bankole and Mabekoje, 2004
Maize Maize-based gruels	45% 25%	25–770 0.002–19.716		Williams <i>et al.</i> , 2004
Maize	2–19%	716 4.6–530		Oyelami <i>et al.</i> , 1996

Commodities contaminated	Frequency of contamination	Range of concentration (µg/kg)	Mean concentration (µg/kg or µg/l)	References
Muscle tissue/beef		Fresh samples 21.7 Sundried samples 2.9		Oyero and Oyefulo, 2010
Liver		Fresh samples 33.9 Sundried samples 3.1		
Heart		Fresh samples 55.9 Sundried samples 27.9		
Kidney		Fresh samples 85.2 Sundried samples 75.8		
Pepper	12/20	0.05–19.45	0.39–2.21	Makun <i>et al.</i> , 2012
Milk types	100%	0.15–0.96		Okeke <i>et al.</i> , 2012
Poultry feed	100%	0–67.9	15.5	Adebayo-Thato and Etta, 2010
Peanut cake		20–455		Akano and Atanda, 1990
Peanut cake	90%	> 20		Ezekiel <i>et al.</i> , 2012
Rice	64/86 41/86	4–292 0.4–27.2	157.34 5.17	Olorunmowaju, 2012
Groundnut	72/82 61/82	4–188 0.4–38.4	53.06 8.08	Ifeji, 2012

* Not detectable

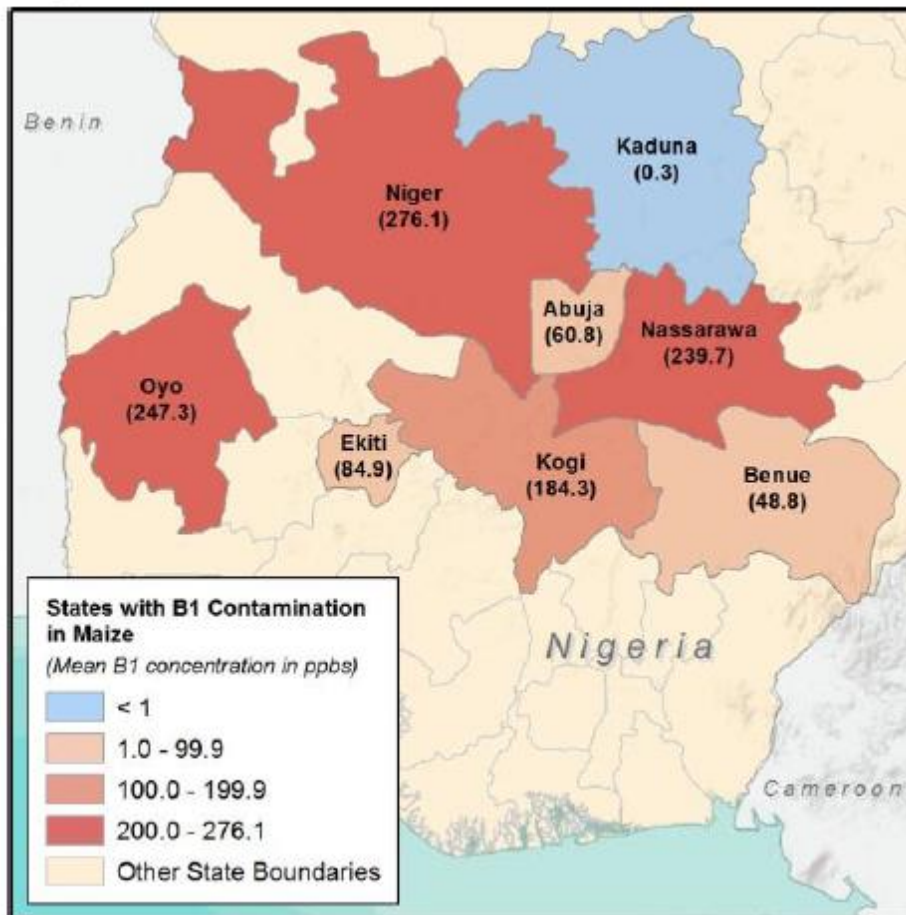


Figure 3: Aflatoxin B₁ contamination in maize in Nigeria

Source: Adapted from: Abt Associates, 2012a

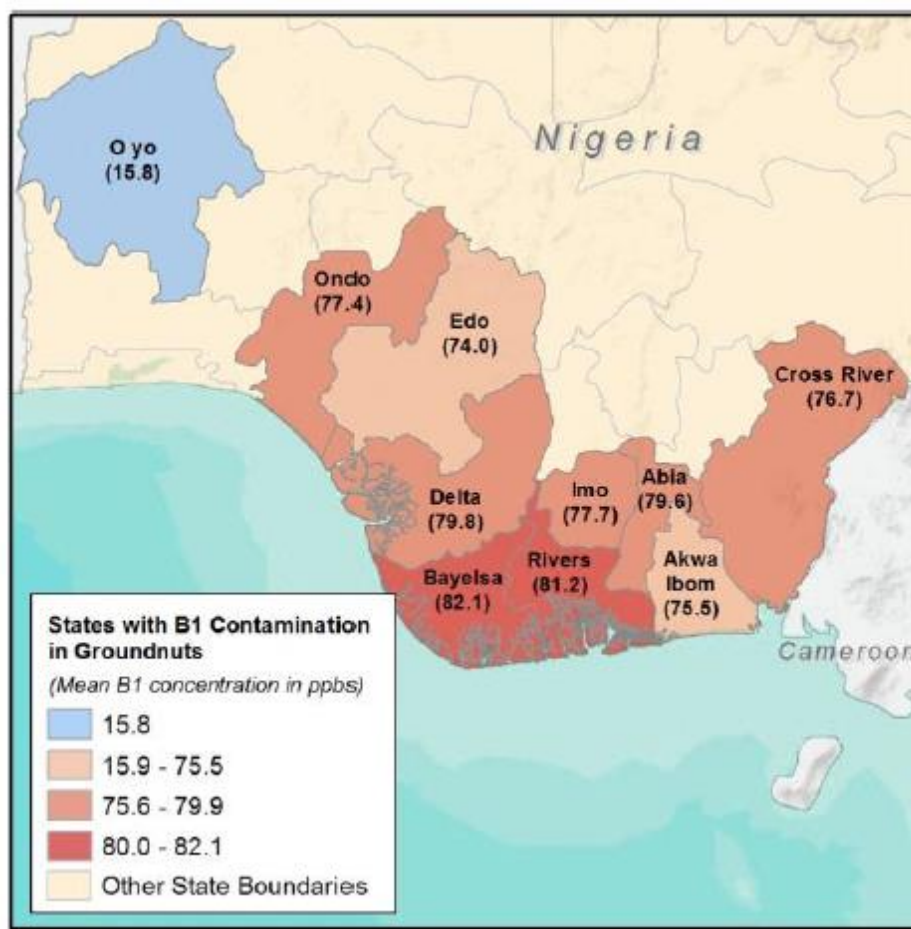


Figure 4: Aflatoxin B₁ contamination in groundnuts in Nigeria

Source: Adapted from: Abt Associates, 2012a

Benin and Togo

In Benin and Togo, maize is a staple food, consumed and stored across all agro-ecological zones and a major source of aflatoxin contamination. There have been reports of higher aflatoxin accumulation from the south to the northern drier parts in Benin (Setamou *et al.*, 1997) and in other commodities (e.g. dried yam, okra and pepper) (Table 11).

Traditional processing of maize for the preparation of maize-based foods (*makume*, *akassa*, and *owo*) in Benin was shown to reduce aflatoxin content by up to 93% (Fandohan *et al.*, 2005). Effective methods for significant aflatoxin removal were sorting, winnowing, washing and crushing combined with dehulling of maize grains (Fandohan *et al.*, 2006). Fermentation and cooking showed little effect (Fandohan *et al.*, 2005).

Ghana

Peanuts and maize are the crops most researched when looking at aflatoxins in Ghana. Peanut samples from the 1994 crop season in six locations in southern Ghana contained aflatoxin concentrations at levels ranging from 12–110 µg/kg (Awuah and Kpodo, 1996). A higher range of contamination (5.7–22,000 µg/kg) was obtained from damaged kernels sampled during a nationwide survey covering 12 markets in all 10 regions of Ghana, demonstrating the importance of sorting as a useful method of aflatoxin management.

Aflatoxins were not detected in 50% of visibly undamaged kernels tested and were present at low levels (0.1–12 µg/k) in the remaining undamaged kernels (Awuah and Kpodo, 1996). High levels of aflatoxins were recorded in maize collected from silos and warehouses and fermented maize dough samples from major processing sites and markets (Kpodo *et al.*, 1996; Kpodo, 2001; see Table 10). In a separate study, implementation of good manufacturing practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) in traditional processing of *kenkey* in a plant in Accra was shown to reduce aflatoxins. Levels of aflatoxins in *kenkey* samples reported at the plant before implementation of GMP and HACCP were between 64.1 and 196 µg/k, and after implementation dropped to between 14.5 and 17.2 µg/k (Amoa-Awua *et al.*, 2007).

Other West African countries (Senegal, the Gambia and Guinea Bissau)

Peanut is the principal export crop constituting 80% and 66% of the earnings from agricultural exports in Senegal and The Gambia, respectively. Its production, handling, processing and marketing employ 86% and 70% of the active labour force in Senegal and Gambia, respectively. The UK imports most of Senegal’s peanut exports (86%) (Caswell, 1985). In The Gambia, on average, 45% of agricultural land is annually allocated to peanut, with production fluctuating around 107,000 t. Guinea Bissau does not export peanuts. Being a staple food, consumption of contaminated peanut results in chronic aflatoxin exposure, as confirmed by high levels in cooked food in The Gambia (Hudson *et al.*, 1992; Turner *et al.*, 2000; Wild and Hall, 2000).

Table 11: Aflatoxin contamination in household and market samples from Nigeria

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁ (µg/kg)	References
Benin	Dried yam	98	2.2–220 (mean 14)	Bassa <i>et al.</i> , 2001
Benin	Cereals		≤ 14 ≤ 58 AFG ₁	Bouraima <i>et al.</i> , 1993
Benin	Cashew nuts		Not detectable	Lamboni <i>et al.</i> , 2016
Benin	Yam chips	20 80	> 15 > 4	Mestres <i>et al.</i> , 2004
Benin (four agro-ecological zones)	Cowpea		Not detectable	Houssou <i>et al.</i> , 2009
Benin	Cassava chips		Not detectable	Gnonlonfin <i>et al.</i> , 2012
Benin - North zone Benin - South zone	Maize	56 25	2–2,500 (mean 220) (mean 100)	Hell <i>et al.</i> , 2000a; Hell <i>et al.</i> , 2000b

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁ (µg/kg)	References
Benin, Mali and Togo	Dried okra Dried hot pepper		Mean 6 Mean 3.2	Hell <i>et al.</i> , 2009
Benin and Togo	Maize, peanut	91	3.6% > 20	Egal <i>et al.</i> , 2005
Burkina Faso	Maize Groundnuts Animal feed	50 50 22		Kpodo <i>et al.</i> , 2000; Warth <i>et al.</i> , 2012
Burkina Faso, Niger, Senegal	Peanut		1–450 (mean 143)	Waliyar <i>et al.</i> , 1994
Côte d'Ivoire	Oilseeds		<20	Kershaw, 1982
Côte d'Ivoire	Peanut	13	>50	Wyers <i>et al.</i> , 1991; Kouadio <i>et al.</i> , 2014
Côte d'Ivoire	Maize	12.6 7.9 4.4 73	≤ 360 > 250 > 1,000 > 10	Pollet <i>et al.</i> , 1989
Côte d'Ivoire	Cereals	86	> 20	Sangare-Tigori <i>et al.</i> , 2006
The Gambia	Groundnut sauce	90	19–944 (mean 162)	Hudson <i>et al.</i> , 1992
	Maize	90	2–35 (mean 9.7)	
	Millet	100	1–27 (mean 9.8)	
	Sorghum	25	2–16	
	Rice	70	2–19 (mean 7.9)	
	Leaf sauces	100	21–34	
Ghana	Peanut	31.7	12.8	Williams <i>et al.</i> , 2004
Ghana	Weaning food	100	7.9- 500	Kumi <i>et al.</i> , 2015
Ghana	Peanut		3–220	Mintah and Hunter, 1978
Ghana	Maize		355 2–662	Kpodo <i>et al.</i> , 1996; Kpodo <i>et al.</i> , 2000
Ghana (silos and warehouses)	Maize		20–355	Kpodo <i>et al.</i> , 1996
Ghana (processing sites)	Fermented maize dough		0.7–313	

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁ (µg/kg)	References
Ghana	<i>Kenkey</i>	77	≤ 200	Kpodo, 2001
	Maize kernel	66	≤ 2,000	
Ghana	Weanimix (weaning food from beans, groundnut and maize)	100	7.9–500	Kumi <i>et al.</i> , 2014
Ghana	Peanut paste (100 samples)	86	65 samples > 30 (highest = 3,300)	Kpodo, 1997
Ghana	Sorghum	3	7.5 8 8.1	Bankole and Kpodo, 2005
	Soya beans	5	≤ 36	
	Cassava	4	4–21	
	Cashew paste	3	≤ 370	
	Rice		< 2	
	Maize cake		< 8	
	<i>Agushie</i>		< 15	
Mali	Peanut		47–2,100	Soler <i>et al.</i> , 2010
Mali	Peanut butter		2.34–189.34	Babana <i>et al.</i> , 2013
Mali	Peanut		≤ 20	Waliyar <i>et al.</i> , 2015
Niger (1989, 1900 and 1991)	Peanuts (26 lines)	37	1–750	Waliyar and Hassan, 1993
Senegal	Peanut oil	80	57–82 (mean 40)	Diop <i>et al.</i> , 2000
Senegal	Peanut butter	90	2.3–189.3	Keita <i>et al.</i> , 2013
Senegal	Maize Sesame		120 1.2	Diedhiou <i>et al.</i> , 2011
Senegal	Peanut oil	85	40	Williams <i>et al.</i> , 2004
			4.6–530	
Sierra Leone	Fish, fermented food			Jonsyn 1989; Jonsyn and Lahai, 1992

Table 12: Export notification of peanut from West African countries to Europe

Notified by	Countries concerned	Subject
France	France (D), Togo (O)	Aflatoxins (B ₁ = 128.1; total = 133.2 µg/kg) in raw peanuts in shell from Togo
Lithuania	The Gambia (O), Lithuania	Aflatoxins (B ₁ = 101; total = 144 / B ₁ = 30; total = 46 µg/kg) in groundnut kernels from The Gambia
Poland	Poland, Senegal (O)	Aflatoxins (B ₁ = 80.56; total = 95.57 / B ₁ = 76.77; total = 90.94 µg/kg) in raw groundnut kernels from Senegal
UK	Poland, Senegal (O)	Aflatoxins (B ₁ = 39.57; total = 43.77 / B ₁ = 30.14; total = 33.44 µg/kg) in groundnut kernels from Senegal
UK	Nigeria (O), Spain, UK	Aflatoxins (B ₁ = 65; total = 84 µg/kg) in groundnut oil from Nigeria
UK	Nigeria (O), Spain, UK	Aflatoxins (B ₁ = 19; total = 23 µg/kg) in peanuts from Nigeria
UK	Ghana (O), Spain, UK	Aflatoxins (B ₁ = 100; total = 120 / B ₁ = 28; total = 30 µg/kg) in groundnut paste (peanut butter) and groundnuts
UK	Ghana (O), Spain, UK	Aflatoxins (B ₁ = 76; total = 112.7 µg/kg) in peanut butter from Ghana

O = origin; D = destroyed

European Commission (EC), Rapid Alert System for Food and Feed (RASFF) Portal: <https://webgate.ec.europa.eu/rasff-window/portal>

Source: Adapted from Senghor (2015)

Central Africa region

Central Africa consists of Cameroon, Central African Republic, Chad, Republic of the Congo and the Democratic Republic of Congo (DRC). The staple foods of this region are cassava, rice, millet, sorghum, squash, pumpkin and plantain. There is little information on aflatoxin contamination in this region except for Cameroon (Table 13). High levels of aflatoxins were reported in peanuts collected from rural areas in Kinshasa in DRC (Kamika and Takoy, 2011). AFB₁ levels increased from the dry season to the rainy season with values ranging from 1.5 to 390 and 12 to 937 µg/kg, respectively. Seventy percent of the peanut samples from both seasons exceeded the maximum limit of 5 µg/kg. In a separate study, 75% of peanut samples exceeded the maximum limit (Ilunga, 2014).

Table 13: Aflatoxin contamination in household and market samples from Cameroon

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/total concentration of AFB ₁ (µg/kg)	References
Cameroon	Maize	9	≤ 2–42	Kana <i>et al.</i> , 2013
	Peanut meal	100	39–50	
	Poultry feed mixtures	93.3 (broiler)	2–52	
		83 (layer feed)	2–23	
Cameroon	Eggs		7.86	Speijers and Speijers, 2004
Cameroon	Cassava chips	33	5.2–14.5	Essono <i>et al.</i> , 2008
Cameroon	Dried food commodities	51	Mean 2.6	Njobeh <i>et al.</i> , 2010
Cameroon	Maize	74	6–645	Ediage <i>et al.</i> , 2014
	Peanut	62	6–125	
	Cassava	24	6–194	

North Africa region

Algeria, Egypt, Libya, Mauritania, Morocco, Sudan and Tunisia make up North Africa. Their major staples are wheat, barley, rice, nuts, herbs, beans and pulses. Spices are also used extensively to flavour food and as medicines.

Egypt, Libya and Morocco

The preservative and antioxidant properties of spices make them highly valuable. Spices are reported to be a significant source of aflatoxin exposure due to the tropical climatic conditions in areas where they are grown. Furthermore they are usually dried on the ground in the open air in poor hygienic conditions that promote growth of moulds and production of mycotoxins (Martins *et al.*, 2001). In Cairo, aflatoxins were detected in processed meat that contained spices but aflatoxins were absent in fresh meat (beefsteak and minced meat), canned meat, salami. The contamination of processed meat with aflatoxin was shown to be correlated with the addition of spices to fresh meat (Nagy and Youssef, 1991). Aflatoxins ranged from 8–35 µg/kg in spices and 2–150 µg/kg in processed meat. When 120 samples of 24 different spices were examined in Egypt, an aflatoxin range of 8–35 µg/kg was recorded in 16 samples of anise, black pepper, caraway, black cumin, fennel, peppermint, coriander and marjoram (El-Kady *et al.*, 1995). Similar levels have been reported in Morocco. Peanuts and dairy products are also of concern in this region (Table 14).

Table 14: Frequency of aflatoxin contamination and concentration levels in household and market samples from North Africa

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/concentration ($\mu\text{g}/\text{kg}$)	References
Egypt	Hazelnut	90	25–175	Abdel-Hafez and Saber, 1993; Williams <i>et al.</i> , 2004
	Peanut and watermelon seeds	82	Positive	
	Soybean	35	5–35	
	Spices	40	> 250	
	Walnut	75	15–25	
Egypt	Milk	38	0.023–0.073	Amer and Ibrahim, 2010
Egypt	Raisin	3	220–300	Youssef <i>et al.</i> , 2000
Egypt	Peanut		1,056 (AFM ₁)	El-Gohary, 1996
Egypt	Milk	20	3–6 (AFM ₁)	Alla <i>et al.</i> , 2000
	Cheese	10–20	0–0.5 (AFM ₁)	
Egypt	Cereal, pulses, fenugreek, peanut, and cottonseed cake	33	3–12	Girgis <i>et al.</i> , 1977
Egypt	Maize		9.75	Madbouly <i>et al.</i> , 2012
	Rice		5.15	
Libya	Milk	71	0.03–3.13 ng/ml (AFM ₁)	Elgerbi <i>et al.</i> , 2004
	Cheese	17	0.11–0.52 ng/ml (AFM ₁)	
Libya	Maize	9	7.1–13.9	Youssef, 2009
Libya	Commercial baby cereals	2.4	19–70	Kofi <i>et al.</i> , 2011
Morocco	Milk	88.8	0.001–0.117 $\mu\text{g}/\text{l}$ (mean 0.0186 $\mu\text{g}/\text{l}$) 3.26 ng/person/day	Zinedine <i>et al.</i> , 2007b
Morocco	Poultry feed	4 17 1 farm	\leq 110 110–200 2,000–5,625	Kichou and Walser, 1993
Tunisia	Sorghum	38	15.4	Oueslati <i>et al.</i> , 2014

Southern Africa region

Maize, groundnuts and cassava are major food and cash crops in Southern Africa and are the crops most researched regarding aflatoxins. Samples of a range of commodities from selected

countries (Botswana, Malawi, Zambia and Zimbabwe) have been found to contain unacceptable levels of aflatoxins (Table 15). Levels of contamination and exposure in the Republic of South Africa based on literature reviewed are profiled in Table 16.

Botswana, Malawi and Zambia

In Botswana, Mphande *et al.* (2004, 2014) reported the presence of aflatoxins greater than 20 µg/kg in half of the samples of peanut meals. In Malawi levels of up to 1,020 µg/kg were reported in grains (Glaston *et al.*, 2000). In a separate study AFB₁ was detected in 45.3% of the maize samples with 12.3% of them exceeding 5 µg/kg. The traditional flour production procedures reduced AFB₁ significantly in this order: soaking of dehulled maize (72.4±5.4, 75.4±3.5 and 80.9±5.3% for 24, 48 and 72 h soaking periods, respectively) > dehulling of maize (mean 29.3±5.4%) > sun drying (11.7% max). Sun drying followed pseudo-first order kinetics in AFB₁. A maximum AFB₁ reduction of 88.1 ± 3.1% was achieved using a sequence of dehulling, soaking for 72 h and sun drying the flour for 4.5 h (Matumba *et al.*, 2009). Njapau *et al.* (1998) also observed that village processing techniques in Zambia reduced aflatoxin content of maize and peanut products. In 30 trials, maize kernels were dehulled, soaked for 24 h, washed and dried before grinding into flour and boiling in water to a thick consistency (*Nshima*). Shelled peanuts were either dry-roasted as whole kernels or ground into peanut meal and cooked. Dehulling, following by 24 h soaking (steeping) and subsequent washing significantly reduced the aflatoxin B₁ content of maize flour from 900 to 150 µg/kg, and similarly that of aflatoxin G₁ from 929 to 114 µg/kg. Preparation of *Nshima* did not result in a substantial reduction in aflatoxin content, and neither did extension of the cooking duration of 2 h. Whereas boiling peanut meal yielded a moderate reduction in the content of aflatoxins B₁ and G₁, roasting whole peanut kernels greatly reduced ($P<0.001$) the concentrations of the toxins from that in raw kernels (AFB₁ = 8600 µg/kg and AFG₁ = 6200 µg/kg) to 1300 and 1200 µg/kg, respectively.

All malt and beer samples in Malawi, and 15% and 43% of the sorghum and *thobwa* (opaque sweet beverage) samples, respectively, collected from the southern region of Malawi during the humid month of January 2010, were contaminated with aflatoxins. The sorghum malt prepared for beer brewing had a significantly higher total aflatoxin content (average 408 ± 68 µg/kg Standard Error of the Mean [SEM]) than any other type of sample. The average aflatoxin content in the beer was 22.32 µg/l, which is higher than the permissible maximum level in ready to eat foods set by the Codex Alimentarius Commission (10 µg/kg). Thus consumption of opaque sorghum-based traditional beer poses a risk of aflatoxin exposure.

In a separate study, traditional maize based opaque beers collected from tribal (*chewa*) rituals and commercial village brewers from Lilongwe and Dowa districts in Malawi in August 2012 were analysed for aflatoxins. With exception of one beer sample, all the beers contained aflatoxins at a mean concentration of 90 ± 95 µg/kg. Consumption of 1.0–6.0 l of the traditional beer from this study translates to daily aflatoxin exposure of 1.5–9.0 µg/kg bwt/day for a 60 kg adult (Matumba *et al.*, 2014).

Cassava is the second most important staple (after maize), nourishing over 30% of the population in Malawi and Zambia. As cassava is highly perishable, it is often processed into dry forms such as *kadonoska*, *kanyakaska*, *makaka*, and fermented and unfermented flour to increase shelf life. *Kadonoska*, *kanyakaska* and *makaka* are processed into flour for *nsima* (in Malawi) or *nshima* (in Zambia), confectioneries or stored for later use. A study was conducted

to assess the level of fungal and mycotoxins' contamination in commonly processed cassava products. A total of 92 and 88 samples of processed cassava products comprising *makaka*, flour, *kanyakaska*, *kadonoska*, scrapes and grates were collected in the rainy season of 2008 and 2009 in Malawi, respectively. Further, 22 samples of processed cassava products comprising dried cassava chips and flour were collected in the rainy season of 2009 in Zambia. None of the samples in 2008 were contaminated with aflatoxins. Similar results were obtained in 2009, with almost all the samples in Malawi and Zambia having aflatoxin levels much lower ($<2.0 \mu\text{g}/\text{kg}$ in Malawi and $<4.2 \mu\text{g}/\text{kg}$ in Zambia) than the Codex Alimentarius Commission maximum permissible level of aflatoxins of $10.0 \mu\text{g}/\text{kg}$, implying that the cassava products analysed were safe for human consumption (Chiona *et al.*, 2014).

Peanut is also an important crop in Malawi both as food and a cash crop. A total of 1,397 groundnut samples collected from farm homesteads, local markets, warehouses and shops in 2008 and 2009 had 46% and 23% of the total samples respectively contaminated with levels greater than 4 ppb, and 21% of the samples in 2008 and 8% in 2009 were above 20 ppb. Similarly high AFB₁ contamination, was recorded across the country with 11–28% of all samples collected from the warm low to mid-altitude ecologies, recording contamination ≥ 20 ppb and low contamination (2–10% of samples) in the mid to high altitude cool ecologies (Monyo *et al.*, 2012). In a recent study, samples of locally (Malawian) processed and imported maize- and groundnut-based food products (peanut butter, roasted groundnuts, peanut based therapeutic foods, instant baby cereals, maize puffs and de-hulled maize flour) were collected from popular markets in Lilongwe and analysed for aflatoxins. No aflatoxins were detected in all samples of imported baby cereal and locally processed de-hulled maize flour. However, all locally processed maize-based baby foods had aflatoxins above the EU maximum tolerable level of $0.1 \mu\text{g}/\text{kg}$ set for food for health purposes. In 75% of locally processed maize puffs, aflatoxins were detected at levels of up to $2 \mu\text{g}/\text{kg}$. Peanut based therapeutic foods had aflatoxin level between 1.6 and $2.9 \mu\text{g}/\text{kg}$. Locally processed peanut butter had aflatoxin levels in the range of $34.2\text{--}115.6 \mu\text{g}/\text{kg}$, which was significantly higher than their imported counterparts ($<0.2\text{--}4.3 \mu\text{g}/\text{kg}$). Samples of locally processed skinned and de-skinned roasted groundnuts contained aflatoxin levels in the range of $0.5\text{--}2.5 \mu\text{g}/\text{kg}$ and $0.6\text{--}36.9 \mu\text{g}/\text{kg}$, respectively. These results show that publicly marketed foods are a source of exposure (Matumba *et al.*, 2014).

Table 15: Frequency of aflatoxin contamination and concentration levels in household and market samples from Southern Africa

Region	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/concentration ($\mu\text{g}/\text{kg}$)	References
Botswana	Peanut	78	12–329	Mphande <i>et al.</i> , 2004
Botswana	Sorghum	40	1–64 (mean 0.3)	Siame <i>et al.</i> , 1998
	Peanut	71	1.64 (mean 23)	
	Maize	0		
Botswana	Opaque beer	0		Nkwe <i>et al.</i> , 2002
Botswana	Peanut butter	0	0	Mupunga, 2013
Malawi	Grains	12.3	1,020 > 5	Glaston <i>et al.</i> , 2000
Malawi	Opaque beer	99	90 \pm 95	Matumba <i>et al.</i> , 2014
Malawi	Sorghum malt for <i>thobwa</i>	100	6.1–54.6 $\mu\text{g}/\text{l}$	Matumba <i>et al.</i> , 2011
	Sorghum malt for beer	100	4.3–1138.8 $\mu\text{g}/\text{l}$	
	Beer	100	2.1–7.1 $\mu\text{g}/\text{l}$	
	<i>Thobwa</i>	43	8.8–34.5 $\mu\text{g}/\text{l}$	
Malawi	Peanut 2008 2009		46% > 4 23% > 4	Monyo <i>et al.</i> , 2012
Malawi	Maize	100	2–150	Mwalwayo and Thole, 2016
Malawi	Imported baby cereal and locally processed (LP) de-hulled maize flour	100	Not detectable	Matumba <i>et al.</i> , 2014
	LP maize-based baby foods	100	> 0.1	
	LP peanut-based foods	75	1.6–2.9 34.2–115.6	
	LP peanut butter		< 0.2–4.3	
	Imported peanut butter		0.5–2.5	
Malawi and Zambia	Cassava flour (2008 and 2009)		Malawi < 2 Zambia < 4.2	Chiona <i>et al.</i> , 2014

Region	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate/concentration ($\mu\text{g}/\text{kg}$)	References
Zambia	Peanuts	55	0.014–48.67	Bumbangi <i>et al.</i> , 2016
Zimbabwe	Peanut Peanut butter	100	6.6–622 6.8–250 (mean 73.5)	Mupunga, 2013

South Africa

South Africa has both a well-developed commercial farming system and subsistence farming. Maize and groundnuts are the crops of interest for aflatoxins, especially in rural areas. Individual samples of agricultural commodities specifically submitted over a 10-year period (1984–1993) to a commercial testing service found aflatoxin contamination in 229 of the 1,602 submitted samples, with levels in maize, other cereals, oilseeds, poultry feed, animal feed, forage and soybean in the range of 1–500 $\mu\text{g}/\text{kg}$ (Dutton and Kinsey, 1996). Surveys carried out by the South Africa Maize Board (SAMB) since 1986 shows low incidence of aflatoxin contamination in local maize. Analytical reports from SAMB showed that contamination of unprocessed commercial maize was nil or < 2 $\mu\text{g}/\text{kg}$ in the following years: 1986 (n=456), 1987 (n=496), 1988 (n=277), 1990 (n=55) and 1991 (n=166) (Viljoen *et al.*, 1993; Rheeder *et al.*, 1995). From 1992 onwards, higher levels have been reported as shown in Table 16. Surveys on maize and groundnuts produced by subsistence farmers during the 2005/06 and 2006/07 seasons in KwaZulu-Natal, recorded an increased area with contamination of up to 20 $\mu\text{g}/\text{kg}$ compared to the 2004/05 season. Incidences of > 20 $\mu\text{g}/\text{kg}$ were also reported. Surveys of commercial sorghum during the 2007/08, 2008/09 and 2009/10 seasons recorded mean contamination of up to 0.9 $\mu\text{g}/\text{kg}$ (Flett *et al.*, 2015). Aflatoxin contamination in peanut butter used in a national school-feeding programme showed contamination of 271 $\mu\text{g}/\text{kg}$ (PROMEC Unit, 2001). Generally, overall contamination levels are low, but high levels are sporadically observed. Regular monitoring of commodities is paramount.

Contamination of feed and animal products is also of interest in South Africa due to previous aflatoxicosis outbreaks in animals following consumption of contaminated feed. Analysis of samples of feeds, forage, maize and milk taken at nine dairy farms, and processed milk collected from the dairies to which the farms had delivered their fresh milk showed aflatoxin contamination. All milk samples from the dairy farms were positive for AFM₁, ranging from 0.02–1.5 $\mu\text{g}/\text{l}$. Milk available on the retail market was also frequently contaminated with AFM₁, at levels of 0.01–3.1 $\mu\text{g}/\text{l}$ (Dutton *et al.*, 2012; Mulunda and Mike, 2014).

Table 16: Aflatoxin contamination of food and feed in South Africa from 1992–2007

Year	Commodity	Sample size	Aflatoxin concentration (µg/kg)	Reference
1992	Maize	5/118	20	Viljoen <i>et al.</i> , 1993
1993	White maize		2	Rava <i>et al.</i> , 1996
	Yellow maize		50	
1994/95	Maize	291	< 1–6	Rava, 1996
		156	< 1	
		62 (feed)	< 1	
1996	Baked or boiled areca nuts		0.1	Van der Bijl <i>et al.</i> , 1996
	Raw areca nuts	10	3.5–26.2 (mean 8.9)	
1989	Maize (for export to Taiwan)		< 0.5	Rheeder <i>et al.</i> , 1994
1987	Maize	90	Nil	Marasas, 1988
1999/00	Maize	1/50	22	Anon, 2001
2006/07	Peanut in: KwaZulu-Natal Mpumalanga Limpopo		≤ 131	Ncube <i>et al.</i> , 2010
			≤ 160	
			≤ 2	

Summary

In summary, aflatoxin exposure is ubiquitous among the African countries studied, with a wide number of commodities having relatively high levels of aflatoxins far above Codex limits. Variations in aflatoxin exposure that exist between countries are largely a function of diet as well as economic status. An average estimation of exposure rates based on annual consumption, as is appropriate for cancer risk because of the cumulative nature of this response, indicate that aflatoxin exposure was 3.5–14.8 ng/kg/d in Kenya, 11.4–158.6 ng/kg/d in Swaziland, 38.6–183.7 ng/kg/d in Mozambique, 16.5 ng/kg/d in former Transkei South Africa (Eastern Cape), and 4–115 ng/kg/d in The Gambia (Ding *et al.*, 2015). The exposure in Ghana, as measured from peanut consumption alone, is estimated to be 9.9–99.2 ng/kg/d (Awuah, 2000). Prevalence testing is needed in other areas where data are not currently available to establish a fuller picture for the country.

3.0 Aflatoxin control in Africa: State of knowledge

3.1 Awareness

Poor awareness about aflatoxins, appropriate control measures to control contamination in the field and in storage, and the negative health effects of aflatoxin consumption are reported in most African countries (Narrood *et al.*, 2011; N'dede *et al.*, 2012; Abt Associates, 2013a; Ephrem *et al.*, 2014). In a study on farmers in Nigeria, out of 2,689 respondents, only 860 (32%) knew what mycotoxins were (Idahor and Ogara, 2010). Other studies have reported similar findings (Ezekiel *et al.*, 2013). In Benin, Ghana and Togo awareness rates have been reported as follows: 20.8% among farmers, 26.7% among traders, 60% among poultry farmers and 25.2% among consumers (James *et al.*, 2007). In a separate study among health workers in Ghana, 80.6% of respondents knew about aflatoxin poisoning through lectures and reading but none had ever told their patients about the risk of aflatoxin ingestion (Ilesanmi and Ilesanmi, 2011). Surveys in Kenya and Mali revealed that most farmers who had heard of aflatoxin obtained that information via local language radio and extension workers, and a lack of understanding contributed to poor control of aflatoxin in the region (Unnevehr and Grace, 2013). In Kenya, households in the drylands, where aflatoxicosis outbreaks occurred in 2004, had a higher perception of risk, as expected, but low knowledge on safety attributes and necessary measures to minimise exposure to aflatoxin. In most parts of Tanzania, knowledge of aflatoxins is low and farmers do not discard aflatoxin-contaminated harvests (Abt Associates, 2013a). Neither do they receive lower prices for aflatoxin-contaminated food since markets do not differentiate between aflatoxin-free and aflatoxin-contaminated food. In Ethiopia, 98.7% of farmers, 96.7% of traders and 70% of consumers were unaware of aflatoxin contamination and its consequences (Ephrem *et al.*, 2014). Moreover, there was no significant difference in responses between farmers (97.3%) and traders (96.7%) in knowledge of long-term exposure to aflatoxigenic fungi and aflatoxin.

Nyangaga (2014) reported that 56.6% of traders in major open markets in Nairobi County were aware of aflatoxin contamination but cattle feed traders were more aware than food traders. Recommendations by Nyangaga (2014) included raising awareness, improving storage facilities and providing guidelines to control aflatoxin levels. Households that are more market-oriented (i.e. sell more than 25% of their produce) and have assets are more willing to pay for aflatoxin risk-reducing technologies in both Kenya and Mali (Tiongco *et al.*, 2011a; Tiongco *et al.*, 2011b; N'dede *et al.*, 2012). In Kenya's drylands, where outbreaks of aflatoxicosis had occurred, respondents were more willing to pay for improved seeds, and tarpaulins and metal silos for drying and storing grain, compared to other regions. Of grave concern, is the fact that there is "no set 'agenda' for agricultural extension services to include aflatoxins, mycotoxins, food safety, or GAP in their messaging" (Abt Associates, 2013a), the full adoption of which can contribute to improving farmers' knowledge and awareness on aflatoxin control.

3.2 Traditional practices

In the context of aflatoxin control in Africa, it is important to examine some traditional practices. In Benin, local maize varieties were determined to have lower aflatoxin levels than imported varieties (Hell *et al.*, 2008). Planting maize varieties that are less susceptible to fungal growth has been reported to be one of the best methods to help alleviate the effects of mycotoxin-producing fungi (Brown *et al.*, 2001). Grains harvested with the husks when mature during dry

weather, and early removal of any damaged maize kernels or cobs, has also been demonstrated to be an effective method for aflatoxin control applied in rural African settings (Hell *et al.*, 2008). Traditionally, sorting was done manually or even through winnowing to get rid of the lighter grains, which were assumed to be lighter due to insect or mould damage. Methods such as visual sorting, winnowing, washing, crushing and dehulling have been found to contribute up to a 40–80% reduction in aflatoxin levels in grains (Whitaker, 2003; Fandohan *et al.*, 2005; Waliyar *et al.*, 2008a). Sorting is highly recommended for reducing aflatoxin contamination, especially in groundnuts, in countries such as Benin, Ghana and Togo (Park, 2002; Turner *et al.*, 2005; Hell *et al.*, 2008; N'dede *et al.*, 2012). Water has also been used to sort grains – allowing floating grains presumed to be damaged to be removed and heavy grains that sink to be cooked. In Kenya and Malawi, soaking and cooking in magadi soda, malting and roasting are other methods that have been used to reduce the levels of aflatoxins in maize (Glaston *et al.*, 2000; Makokha *et al.*, 2002; Fandohan *et al.*, 2005; Mutungi *et al.*, 2008).

In Morocco, grapes have been dipped in sieved ash solutions with quicklime salt and water, to prevent rot formation and fermentation (Mazhour, 1983). Some communities in western Kenya use ash to reduce insect pests that have been documented to increase the effects of fungi in maize kernels (Avantaggiato *et al.*, 2003; Munkvold, 2003).

Use of plant products

Many farmers use local plant products, either in their pure form or as oil or water extracts, to control insects during storage. *Ocimum gratissimum*, *Aframonium* spp., *Zingiber officinalis*, *Xylopia aethiopica*, *Monodera myristica*, *Ocimum basilicum*, *Tetrapleura tetrapeta* and *Piper guineense* have all been tested for their ability to inhibit the mycelia growth of *A. flavus*, while *P. guineense* inhibits the growth of all tested maize pathogens. Essential oils from *Azadirachta indica* and *Morinda lucida* inhibit the growth of toxigenic *A. flavus* and significantly reduced aflatoxin synthesis in inoculated maize grains (Bankole, 1997; Nguefack *et al.*, 2004). Ground *Aframomum danielli* (Zingiberaceae) has been shown to control moulds and insect infestation in stored maize and soybeans for up to 15 months under ambient conditions in southwestern Nigeria (Adegoke *et al.*, 2000). The role of natural botanical products in controlling post-harvest aflatoxin contamination, however, is underdeveloped and under-researched. Further tests are needed to determine inhibition mechanisms and identify the active ingredients of the natural products that inhibit fungal growth for commercialisation.

Fermentation

Fermentation (mainly associated with lactic acid bacteria and yeast) not only improves the taste of food, but has also been shown to increase the availability of specific nutrients and to reduce the accumulation of mycotoxins (Nout *et al.*, 1993; Mokoena *et al.*, 2006; Oluwafemi and Da-Silva, 2009; Chelule *et al.*, 2010; Okeke *et al.*, 2015). North and West Africa have a rich tradition of using fermentation to prepare traditional staple foods; maize, millet or sorghum (Ross *et al.*, 1992; Bankole and Adebajo, 2003; Benkerroum, 2013) and aflatoxin-decontamination effects of fermentation have been documented (Fandohan *et al.*, 2005). In South Africa, sour gruel referred to as *amahewu* and the beverage *Incwana* are cereal-based fermented foods (Chelule *et al.*, 2010). While fermented foods have deep cultural values, their role in mitigating risk from aflatoxin contamination need further study and technological improvements to standardise processes if they are to be promoted widely.

Smoking

Smoking has been used not only to reduce the moisture content in grains, but also to reduce the effects of insects and to act as a fungicide (Daramola, 1986; Hell *et al.*, 2008). In Nigeria, smoking has been applied to reduce moisture content in food crops and meat and has been shown to reduce aflatoxin levels in stored maize and fish (Udoh *et al.*, 2000).

Use of contaminated grains as animal feed

Grains found to be contaminated with aflatoxins were traditionally converted to animal feed by farmers, to minimise financial losses. This practice is not recommended especially given the levels of exposure linked to the consumption of derived products such as contaminated milk as has been previously reported.

4.0 Capacity for detection and quantification of aflatoxins

Specific, sensitive and simple analytical methods for detection and quantification of aflatoxins are needed given their presence in very low concentrations in foods and feed. Accurate determination of the aflatoxin content of a commodity is influenced by the way each step in the evaluation process from sampling to extraction, clean-up and quantification is carried out.

4.1 Sampling framework

The general approach to sampling for aflatoxin testing is by collecting representative samples from which inferences can be made. Variability in results from sampling is much higher than variability in sample preparation and analysis, thus adequate sampling is extremely important (IARC, 2012c). The physical state of samples determines the sampling strategy employed, i.e. whether the sample is a grain, fine or coarse powder, or liquid. Different sampling procedures have been developed to obtain representative samples for laboratory testing. Additionally, several regulatory bodies and agencies provide guidelines on appropriate procedures compliant with regional and international best practices. A manual on effective sampling methodologies for detection of mycotoxins in foods highlights various sources of errors in sampling, including: inadequate sample sizes, biased sampling procedures, inadequate sample comminution, and improper subsampling for analysis (Whitaker *et al.*, 2011).

Sampling grains and flour

Aflatoxin concentrations show a skewed or uneven distribution in whole kernels, making it difficult to collect a sample that accurately represents mean concentration. Thus distribution of ingredients within an analysed sample is a critical aspect (Cheli *et al.*, 2012). When sampling grains for aflatoxin testing, it is preferable to take a homogenised sample from harvesting or handling operations. Therefore it is better to sample shelled maize rather than ear maize, and to sample ground maize rather than shelled maize (Davis *et al.*, 1980).

The techniques routinely used to sample grains for aflatoxin analysis include: probe, stream and field sampling, ranked in that order of preference. The choice of sampling technique is dependent on the aim of the sampling exercise. Probe sampling is done using commercially available probes and is appropriate for grains that are properly blended; in sampling a bag of maize, it is better to take portions from top, middle and bottom and combine into a sample. Stream sampling involves taking small portions from a moving stream of grains at periodic time intervals and combining the portions into a sample. Field sampling, coordinated with harvesting, ensures a very large number of maize ears are represented in the sample of shelled maize.

Whenever possible, initial sampling should yield a minimum quantity of 4.54 kg to be ground and passed through a No. 14 sieve, be thoroughly blended, and then properly sub-divided into a 1 kg sample. The 1 kg sample is then ground, passed through a No. 20 sieve, thoroughly blended, and properly subdivided to yield five to nine analytical samples (Ware, 1991). As a rule of thumb a larger sub-sample is required for coarsely-ground material than for finely-ground material.

Cereal millers, middlemen and silos provide large storage facilities that require a sampling plan. However in Africa, smallholder farmers often store their harvest in granaries made out of local plant material that may be iron-roofed. Others store in bricks, either in sacks or

polypropylene bags stacked onto each other. Others still keep unshelled maize grains in stores on the store floor. Sampling in this case can be varied to best suit the situation.

Sampling biological fluids

Bio-monitoring of aflatoxins occurs by analysing the presence of aflatoxin metabolites in blood, milk and urine. Additionally, excreted DNA adducts and blood protein adducts can also be monitored (Bennett and Klich, 2003). When working with human biological materials, the Helsinki declaration and good clinical practice are the cornerstones that form the principles and ethics of samples collection (WMA, 2004). The protocol for sampling and testing should be submitted to an ethical review committee for consideration, comment, guidance and, where appropriate, approval. For research animals, it is a must that their welfare be respected.

Sampling methods to be used to determine AFM₁ levels in milk are specified by the EC Directive (EC, 1998) and Decision (EC, 1991). From a batch of milk mixed by manual or mechanical means, a minimum sample of 0.5 L is collected, composed of at least five increments. The batch is accepted if the concentration of AFM₁ in the sample does not exceed the permitted limit (IARC, 2012c).

EC Directive 2002/98/EC sets quality and safety standards for the collection, testing, processing, storage and distribution of human blood and blood components. Whole blood is collected using blood collection tubes. Urine is collected in clean bottles and kept refrigerated or frozen depending on period of storage before analysis.

4.2 Analytical methods – access and accuracy

Methods for detecting and quantifying aflatoxins in agricultural food crops, feed and samples from human and animal subjects can be grouped as:

- 1) Chromatographic methods
 - a) Thin Layer Chromatography (TLC)
 - b) High-Performance Liquid Chromatography (HPLC)
 - c) Gas Chromatography (GC)
- 2) Spectroscopic methods
 - a) Fluorescence Spectrophotometry
 - b) Frontier Infrared Spectroscopy
- 3) Immunochemical methods
 - a) Radioimmunoassay
 - b) Enzyme-Linked Immunosorbent Assay (ELISA)
 - c) Lateral Flow Devices (Immunodipsticks)
 - d) Immunosensors

Chromatographic methods, such as TLC and HPLC, are the most widely used techniques in aflatoxins analysis and regarded as the official analytical techniques, and are mounted with various detectors. The most recent of these methods use immunoaffinity columns (IACs) for sample extraction and clean-up before HPLC analysis. The highly specific nature of mass spectrometry (MS) eliminates need for extract purification (IARC, 2012c). The development of multi-analyte HPLC-MS/MS methods has enabled analytical chemists to combine analytical steps with a confirmatory test by measuring the mass spectrum of the HPLC peak.

Immunoassays have emerged as better alternatives for routine and on-site detection of aflatoxins. These adapted rapid-screening methods include ELISAs, fluorometric methods, lateral flow devices, and a range of tests that give a yes/no result for contamination above or below a set control level. These methods have been developed for situations where quick decisions are required, such as at granaries, silos and factories (IARC, 2012c). Quantitative or semi-quantitative ELISAs have the advantage of not requiring sample extract purification and can handle many samples in a single experiment. Its disadvantages include cross-reactivity with related mycotoxins, matrix interference problems, possible false positive/negative results and that confirmatory liquid chromatography (LC) analysis is required (Pascale and Visconti, 2008). Some of the very sensitive immunoassay methods require skilled and well-trained operators.

A number of authors have published updates on the developments in mycotoxin analysis covering limit of detections and recovery percentages, and the advantages and disadvantages of various methods (Pascale and Visconti, 2008; Maragos and Busman, 2010; Shephard *et al.*, 2011; Shephard *et al.*, 2013; Berthiller *et al.*, 2014; Wacoo *et al.*, 2014).

In Africa, most researchers use immunoassays, which are considered as screening methods. For more accurate and precise identification and quantification of mycotoxins present, chromatographic methods are used, e.g. HPLC or LC-MS/MS. These methods are not only precise and accurate but they can be used to analyse multiple mycotoxins in a single run whilst using a very simple method for sample preparation (e.g. QuEChERS). Despite these benefits, chromatographic methods require a stable electricity supply and ready availability of special reagents, consumables and spare parts, as well as trained personnel to operate and maintain them. This makes routine use of these analytical methods more likely in well-developed modern laboratories and less likely in laboratories hosted by government-run national institutions in Africa.

4.3 Capacity for aflatoxin determination and quantification

The challenges associated with mycotoxin testing in Africa include lack of political commitment, infrastructure, trained personnel, sustainable supplies, instrument maintenance and repairs, and laboratory quality control and assurance schemes. Considering cost, speed of analysis, availability of personnel and facilities, as well as the characteristics of the tests (sensitivity, specificity and reproducibility) – TLC, HPLC, ELISA and other immunoassays have been identified as the preferred methods for the African region (FAO/WHO, 2005a). Validation of the methods is often carried out by official laboratories or regulatory bodies, and supported by international organisations such as the Association of Official Analytical Chemists and International Organization for Standardization (ISO). In most studies in Africa, direct competitive ELISA procedures are applied unless there is collaboration with foreign laboratories to use the more advanced techniques. The few laboratories that conduct aflatoxin testing using chromatographic methods are costly and are inaccessible to local researchers. Promotion of local development of antibodies and immunoassay kits can help obviate commercial costs, but caution needs to be exercised in maintaining quality of such kits.

Laboratories under the CGIAR Consortium are well equipped and have highly-skilled staff who carry out mycotoxin testing. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), a CGIAR centre, has developed a simple, robust, versatile, low-cost, effective competitive ELISA (cELISA) test for the detection of aflatoxin (ICRISAT, 2009). The

CGIAR centres have the benefit of favourable funding, which supports the development of their diagnostics capability. The International Livestock Research Institute (ILRI), for example, through an Australian Agency for International Development (AusAID) funded project has established an aflatoxin research and capacity-building platform at the Biosciences eastern and central Africa (BecA-ILRI) Hub in Nairobi, Kenya, which is open to biosciences researchers focused on improving food security in Africa. At the platform there is a laboratory team working to develop new aflatoxin diagnostics, such as the electronic nose, that are more suited to the African context. Another technology being worked on at the BecA-ILRI Hub is Near Infrared (NIR) to predict the presence of aflatoxin in samples. NIR is supplied by South Africa-based Bruker® who offer technical support. Compared to other African countries, South Africa is advanced in aflatoxin-testing technology and is well equipped. NIR, if operational, will be non-destructive, requiring no sample preparation or extraction solvents, making it quick and reliable for quantitative and qualitative analysis. However, a need still exists for methods that are applicable to small-scale farms, where resources are limited and rapid decisions are needed concerning contamination (Harvey *et al.*, 2013).

5.0 Regional collaboration and mitigation activities

Food safety can only be guaranteed by policies and legislation that safeguard consumers, minimise economic and health risks, and ensure functioning of food markets in an orderly manner (Jabbar and Delia, 2012). Many African countries have established food laws and regulations mainly based on Codex Alimentarius standards to allow exports to be accepted in different international markets (Mutasa and Nyamandi, 1998). Crop exports from Africa cannot easily access European markets due to the strict sanitary and phytosanitary requirements imposed on their products (Otsuki *et al.*, 2001). Standards still remain important barriers to trade (Bhat and Vashanti, 1999; Rios and Jaffee, 2008; Unnevehr and Grace, 2013).

Standards for 'acceptable' aflatoxin levels in food vary widely across African countries, from 0–50 ppm. Studies on the status of food and feed safety legislations in Africa identified significant gaps, inadequate linkages between strategies, and outdated and overly prescriptive laws that failed to address a whole range of food safety concerns (FAO/WHO, 2005a; Jabbar and Delia, 2012; Hell, 2015). Failure to meet standards for aflatoxin have had a negative impact on African trade, with a loss of up to US\$750 million annually (Jabbar and Delia, 2012). Africa's inability to meet the regulatory standards set by many importing countries, especially the EU, is cause for concern. PACA, in collaboration with regional economic communities (RECs), is working toward improving the enabling policy and institutional environments to support countries to update and harmonise legislation (Waliyar *et al.*, 2008b; Ayalew *et al.*, 2013), to enforce standards and maintain larger trading blocks that can negotiate with importing countries and larger bodies, such as the World Trade Organization.

5.1 Regional trading blocks

There are 14 RECs in Africa that are officially recognised by the African Union (AU), some of which overlap in membership (Hell, 2015). The RECs include Arab Maghreb Union, Communauté Economique et Monétaire des Etats de l'Afrique Central (EMAC), Communauté de EtasSahêlo Sahariens, Common Market for Eastern and Southern Africa (COMESA), the East African Community (EAC), Economic Community of Central African States (ECCAS), Economic Community of West African States (ECOWAS), South Africa Development Community (SADC), Southern Africa Customs Union (SACU), and Union Economique et Monétaire Quest Africaine (UEMOA). Aflatoxin contamination undermines the free trade agreements of RECs in Africa.

ECOWAS

ECOWAS is a regional integration group of 15 member countries, eight of which have food safety legislation in place (Hell, 2015). Only Benin, Ghana and Nigeria have specific standards for aflatoxin while the other five use Codex limits as required by major trading partners (Hell, 2015). Most research data on levels of aflatoxins and affected commodities have been collected from Benin, Ghana and Nigeria, but smaller studies have occurred in Burkina Faso, Côte d'Ivoire, The Gambia, Guinea, Mali, Senegal and Togo. Little or no research has been documented from other countries including Cape Verde, Guinea-Bissau, Liberia, Niger and Sierra Leone. ECOWAS countries are at different levels on instituting GAP to reduce mycotoxin through post-harvest and processing interventions. Examples include the use of good practices and Purdue Improved Crop Storage (PIC) bags; good post-harvest practices

in maize (Guinea); special sorting of groundnut to reduce aflatoxin; screening technology; and reduction of post-harvest losses of grains and pulses (Hell, 2015).

Some Western and Central African countries have also set up food testing laboratories under the French Protocol which is an agri-food quality programme (Tulasne, 2002). Twenty-three laboratories are currently participating in the regional network, creating openings for product certification for national, regional and international markets.

UEMOA

UEMOA is a regional organisation of eight francophone (Benin, Burkina Faso, Côte d'Ivoire, Guinea Bissau, Mali, Niger, Senegal and Togo) countries in West Africa that share a common currency. A programme of SPS harmonisation began in early 2003, focusing on the preparation of a legislative framework and associated treaties, training of officials to interpret and implement the treaties, and strengthening of quality control laboratories. With EU support, UEMOA established legal and regulatory frameworks for food safety; a regional certification scheme and harmonisation of 36 national standards; enhanced the competitiveness of enterprises that complied with international trade rules and technical regulations; and developed national and regional infrastructure for quality, standardisation and conformity assessment.

SADC

SADC represents 15 member states (Angola, Botswana, DRC, Lesotho, Madagascar, Malawi, Mauritius, Madagascar, Malawi, Mozambique, Namibia, Seychelles, South Africa, Tanzania, Zambia and Zimbabwe). There is good cooperation over food contamination emergencies in SADC. Varying laboratory capacities have been identified between member states, so the upgrading of existing facilities into regional centres of excellence has been recommended (FAO/WHO, 2005b). Some countries are sharing facilities amongst several states as a more cost-effective and sustainable arrangement to deal with the problem of poor laboratory facilities.

The SADC Secretariat has carried out a number of activities aimed at improving the capacity of member states to implement the SPS Annex to the SADC Protocol on Trade. The activities were funded through an EU-sponsored Regional Economic Integration Support programme.

In Malawi, Mozambique and Zambia – with collaborators from the USA and UK – the Peanut and Mycotoxin Innovation Lab) 5-year project, which is expected to end in 2017 is implementing aflatoxin management interventions, education, and analysis at various steps along the peanut value chain; production, post-harvest handling, and processing issues that impact aflatoxin contamination levels, yield and profitability.

EAC

EAC represents six states in Eastern Africa (Burundi, Kenya, Rwanda, South Sudan, Tanzania and Uganda). Several workshops have been held to raise awareness and build capacity on aflatoxin control in the EAC. The United States Agency for International Aid Development (USAID) is very active:

- USAID Leveraging Economic Opportunities (LEO) project is evaluating SPS trade policy constraints within the maize and livestock/animal-sourced products value chains in Eastern Africa.
- USAID's Feed the Future initiative: capacity building activities with the EAC to strengthen laboratory diagnostics and quality assurance, as well as methods to augment surveillance for plant disease and control of product contaminants such as aflatoxin.

Technical assistance activities that are addressing aflatoxin include:

- The Kenya-based East Africa Trade and Investment Hub project funded by the USAID regional mission developed harmonised guidelines for sampling, testing and grading procedures and methods for the East African States 2013 Staple Food Standards. The programme – Development Alternatives Inc. Trade Africa: East Africa Trade and Investment Hub (2014–2019) – has several project goals, including a strong SPS initiative to increase EAC inter-regional trade in staple foods by 40%. The project builds on the policy environment with EAC integration in trade and investment.
- Developed and implemented in Kenya, the International Food Policy Research Institute (IFPRI)-run 'Aflacontrol Survey', which targeted maize (Kenya) and groundnut (Mali) was funded by the Bill and Melinda Gates Foundation (BMGF).
- The 'Aflastop Storage Drying for Aflatoxin Prevention' maize storage and drying programme, funded by USAID and BMGF, offered farmers and traders practical technological options to store and minimise the risk of aflatoxin contamination.
- BMGF also funded research to create a low-cost diagnostics test for aflatoxin. The aflatoxin test-kit was piloted in 2013 but funding was not sustained.
- Run by the International Institute for Tropical Agriculture (IITA), the Aflasafe project develops and tests biological control products for 11 sub-Saharan African countries and has developed and proposed protocols for aflatoxin sampling in maize and groundnuts which are being piloted. The project is also assisting the World Bank's AgResults Aflasafe commercialisation pilot in Nigeria. Funded by BMGF, through PACA, the project also leverages funds from several other donors including USAID and United States Department of Agriculture (USDA).

COMESA

COMESA represents 19 member states (Burundi, the Comoros, DRC, Djibouti, Egypt, Eritrea, Ethiopia, Kenya, Libya, Madagascar, Malawi, Mauritius, Rwanda, Sudan, Swaziland, Seychelles, Uganda, Zambia and Zimbabwe). Maize traded in the COMESA region is duty free, but borders are often closed if there is a perceived shortfall of the crop in exporting countries. Aflatoxins in COMESA countries therefore challenge regional and international trade. COMESA, in collaboration with the Kenya Plant Health Inspectorate Services, has worked towards the harmonisation of aflatoxin sampling and testing protocols in the region through capacity building (28–29 February 2012, Nairobi, Kenya). The aim was to develop a regional action plan to lead to agreed regional protocols for aflatoxin sampling and testing procedures (Byanyima, 2012b). Another workshop in Uganda in 2013 and supported by USDA, established priorities for SPS capacity building using Multi-criteria Decision Analysis. A 2014 African Agriculture Technology Foundation (AATF)/AUC/COMESA/IITA/PACA/USAID regional workshop looked at the challenges posed by aflatoxins and opportunities to improve health, trade and food security through regional efforts to mitigate aflatoxin contamination.

A major achievement of COMESA has been the creation of the tri-partite free trade area (FTA), by merging EAC, COMESA and SADC FTAs. The tri-partite FTA agreement laid out a legally binding coordination mechanism to enable the three RECs to harmonise SPS programmes and implement risk-based SPS measures, ensuring smooth flow of food and agricultural products across the tri-partite region.

The coordination of activities of regional bodies in relation to aflatoxin control is challenging given the overlapping mandates, yet the efforts by PACA to harmonise regulations can possibly lead to sustainable outcomes and greater impact. Coordinating legislation and standards is currently under way to address aflatoxin control across the value chain by enforcing GAP and paying attention to production practices by smallholder farmers. However equal attention should also be given to the adoption and implementation of good manufacturing practices to reduce the levels of aflatoxin in contaminated foods and feed.

5.2 Mitigation activities: Feasibility of interventions and uptake in Africa

Aflatoxin mitigation requires a multifaceted approach since contamination can take place anywhere along the value chain. Mitigation interventions can be primary, targeting the prevention of the occurrence of the toxins in food and feed, or secondary, which attempt to prevent exposure of humans and animals to the toxins after ingestion of contaminated food or feed. Developing mitigation strategies for the prevention or reduction of aflatoxins requires a good understanding of the factors that influence the infection process and the conditions that influence toxin formation.

Soil type and condition, and the availability of viable spores, are important factors. Environmental factors that favour *A. flavus* infection in the field include high soil and/or air temperature, drought stress, nitrogen stress, crowding of plants and conditions that aid the dispersal of conidia during silking (Diener *et al.*, 1987). Factors that influence the incidence of fungal infection include presence of invertebrate vectors, grain damage, oxygen and carbon dioxide levels in stores, inoculum load, substrate composition, fungal infection levels, prevalence of toxigenic strains, and microbiological interaction (Horn, 2003). Crop rotation and management of crop residues are also important in controlling *A. flavus* infection in the field. Significant inroads are being made in establishing various promising pre- and post-harvest control strategies in Africa.

5.3 Primary interventions

Pre-harvest management strategies

Pre-harvest technologies are seen as the most promising, cost-effective and easy-to-use technologies for farmers (Bhatnagar-Mathur *et al.*, 2015). A combination of strategies is needed to adequately prevent mycotoxin contamination in the field. Plants may be developed that resist fungal infection and/or reduce the toxic effects of the mycotoxins themselves, or interrupt mycotoxin biosynthesis.

Breeding

Plant resistance is generally considered a highly desirable approach to reducing or eliminating *A. flavus* infection and subsequent accumulation of aflatoxin. Potential biochemical and

genetic resistance markers have been identified in crops, particularly in maize, and are being utilised as selectable markers in breeding for resistance to aflatoxin contamination. Efforts to enhance plant resistance to aflatoxin contamination have mainly focused on the fungus, inhibition of aflatoxin production and resistance to insects, and drought tolerance, by applying both conventional and transgenic technology. Gene clusters housing the genes governing formation of aflatoxins have been elucidated and are being targeted in strategies to interrupt the biosynthesis of these mycotoxins. The focus on resistance to insects and drought tolerance is because of a suggested correlation between them and aflatoxin contamination in some studies.

Various programmes funded by the International Maize and Wheat Improvement Centre (CIMMYT) are screening maize germplasm for resistance to aflatoxin accumulation in Africa. The University of Nairobi, Kenya Agricultural and Livestock Research Organization (KALRO), Stellenbosch University and the Agricultural Research Council, funded by CIMMYT, have screened inbred lines from Kenya and South Africa for resistance to aflatoxin and fumonisin accumulation. Breeding trials with inbred lines identified as having low susceptibility to *Aspergillus* ear rot and aflatoxin accumulation are ongoing. The Agricultural Research Council of South Africa is also carrying out separate trials. ICRISAT has also reported new peanut varieties with low pre-harvest aflatoxin contamination (Nigam *et al.*, 2009).

In Malawi and Tanzania, the McKnight Foundation and Collaborative Crop Research Programme-funded groundnut breeding programme worked from 2010 to 2013 to identify lines with low aflatoxin contamination. In Malawi the project was implemented by ICRISAT, the National Agricultural Research Services and the National Smallholder Farmers' Association of Malawi, while in Tanzania it was implemented by Naliendele Agricultural Research Institute.

ICRISAT has reported the following breeding lines to be resistant to *A. flavus* seed infection and colonisation: ICGVs 87084, 87094, 87110, 91278 and 91284. The following peanut cultivars have shown stable resistance to *A. flavus* across locations: J 11, 55-437, and PI 337394F. Some of these breeding lines also possess aflatoxin resistance and are also high yielding. Efforts have been made to develop aflatoxin-resistant transgenic peanut plants (Waliyar *et al.*, 2015) as an effective long-term genetic approach to the problem.

For maize, the transgenic approach has focused mainly on expression of recombinant insecticidal proteins from *Bacillus thuringiensis* (Bt), expression of antifungal peptides and proteins, and the use of Host Induced Gene Silencing technology. No product is currently on the market except for Bt maize which is grown only in South Africa where it was approved for commercial use in 1998 (Marnus *et al.*, 2006). Conflicting reports have been documented on the ability of Bt toxin technology against the European corn borer to reduce aflatoxin levels in maize. One study has shown no significant effect of Bt maize while other studies have shown mixed results (Wu, 2007; Ostrý *et al.*, 2015). The effect Bt maize has on lowering levels of aflatoxins is not pronounced compared with fumonisins and this should be expected because of the different infection pathways of the fungi producing these toxins. These data therefore require further research studies to be conclusive (Diaz-Gomez *et al.*, 2016). The development of transgenic plants expressing genes that protect against fungal infection, for example, would be a more effective strategy.

Environmental stresses, such as drought and heat, have been shown to promote aflatoxin production through the accumulation of reactive oxygen species within the host plant tissues,

which in turn initiate toxin production by *A. flavus* (Chen *et al.*, 2004; Guo *et al.*, 2008; Fountaina *et al.*, 2015). Drought- and insect-resistant maize and groundnut varieties have been reported to have relatively lower pre-harvest aflatoxin contamination than the check cultivars in other parts of the world (Holbrook *et al.*, 2000; Tubajika and Damann, 2001; Guo *et al.*, 2008; Williams *et al.*, 2015). Breeding for stress-tolerant cultivars is therefore seen as another strategy for management of aflatoxins. CIMMYT, jointly with IITA, launched the 'Drought Tolerant Maize for Africa' (DTMA) project in 2006 to work in close collaboration with national agricultural research systems in participating nations to mitigate drought and other constraints to maize production in sub-Saharan Africa. DTMA has released several such drought tolerant varieties in 13 African countries (CIMMYT, 2015).

Biocontrol

Atoxigenic biocontrol of *A. flavus* that can outcompete closely related, toxigenic strains in field environments, consequently reducing levels of aflatoxins in crops, is commercially packaged and sold as Aflasafe™. IITA, in partnership with the USDA–Agricultural Research Service and AATF developed Aflasafe™, which is already registered in Kenya and Nigeria. IITA has reported consistent reduction of aflatoxin contamination in maize and groundnuts by 80–90%. The product is ready for registration in Burkina Faso and Senegal and is being tested in Zambia. Trials are being expanded in Ghana, Malawi, Mali, Mozambique, Tanzania and Uganda. In Kenya and Burkina Faso, IITA has identified separate sets of four competitive atoxigenic strains isolated from locally-grown maize to constitute a biocontrol product called aflasafeKE01™ and aflasafe-BF01, respectively (IITA, 2012). The use of other organisms, such as bacteria and yeasts, as biocontrol agents are still under experimentation (Aliabadi *et al.*, 2013). Wu and Khlangwiset (2010) describe the use of pre-harvest Aflasafe™ in Nigeria and post-harvest management in Guinea as cost-effective in terms of improved health outcomes: the monetised value of lives saved and quality of life gained by reducing aflatoxin-induced hepatocellular carcinoma compared with the cost of the two interventions. However, the long-term consequences of the use of these biocontrol products to both farmers and the environment have been questioned (Ehrlich, 2014; Ehrlich *et al.*, 2014).

Good Agricultural Practices (GAP)

GAP aims at reducing the load of the fungus in soil, reducing environmental stresses on plants and ensuring growth of healthy plants. The following practices are being promoted to reduce aflatoxin contamination: selection of healthy seeds, early planting, avoidance of mono-cropping, treatment of foliar diseases, application of lime or gypsum, mulching, maintenance of optimal density of plants in the field, avoidance of end season drought through irrigation, and removal of dead plants from the field before harvest (Waliyar *et al.*, 2013).

Post-harvest management strategies

Although aflatoxins can contaminate commodities anywhere along the value chain, dramatic increase is normally observed in storage. Crop management practices at harvest and post-harvest are an effective way of avoiding, or at least diminishing, infection by *A. flavus* and subsequent aflatoxin contamination. These include, harvesting at maturity, avoidance of damage of kernels, rapid drying on platforms to avoid contact with soil, drying seeds to 8% moisture level, appropriate shelling methods to reduce grain damage, sorting, use of clean and aerated storage structures, control of insect damage, and avoidance of long storage

periods. Several methods and tools are being promoted and tested in collaboration with farmers in Africa. Examples are outlined below:

Storage and drying options by AflaSTOP

The AflaSTOP project in Kenya is testing storage and drying devices to reduce aflatoxin accumulation on smallholder farms (ACDI/VOCAb, 2015). Three different storage devices are currently being evaluated for commercialisation: GrainPro Grain Safe II manufactured by GrainPro; metal silos made out of aluminium by local artisans; and Purdue Improved Crop Storage initially introduced to West Africa by Purdue University and currently manufactured in Kenya by Bell Industries Metal Silo. Hermetic bags have also been used by ICRISAT in Mali and GrainPro company is now manufacturing them in Kenya (Villers, 2015).

AflaSTOP is testing the shallow bed dryer, which is reportedly showing promising results. It is a completely new device that has never gone on the market. The basic configuration of this mechanical dryer is a furnace, a heat exchanger and a supply of air (provided by fan). The heat from the furnace moves through the heat exchanger and through the raised bed that contains the grain. Mobile maize dryers are already being used by grain handlers in Kenya (Wanjiru, 2011).

Storage methods by Cultivate Africa's Future

CultiAF is a joint programme of the Australian International Food Security Research Centre and Canada's International Development Research Centre. The project is testing the efficacy of air-tight metal storage silos and thick plastic 'super bags' for storing grains to reduce aflatoxin accumulation in maize grains (ACIAR, 2015).

Storage practices to reduce aflatoxin contamination

IFPRI worked with households in Meru, Kenya, to test the effect of inexpensive improved post-harvest and storage practices on aflatoxin contamination of maize and the effect of reduced consumption of contaminated maize on growth of children under 2 years by swapping contaminated maize with clean maize (Hoffmann *et al.*, 2014). The results were positive.

Sorting options

Electronic devices such as Near-Infrared Hyperspectral Imaging and high-speed dual-wavelength sorters have been tested to remove maize and peanuts contaminated in the field with aflatoxin (Pearson *et al.*, 2004; Wei *et al.*, 2015). These methods are expensive and not suitable for smallholder farmers.

Detoxification and decontamination

Segregation and detoxification of aflatoxin-contaminated commodities has been suggested. In Malawi segregation of aflatoxin-contaminated peanuts by visual or mechanical means has been attempted. In Senegal and Sudan, industrial detoxification of aflatoxin-contaminated peanut oilseed cake by processes such as ammonia and formaldehyde treatment are in place (Grenier *et al.*, 2012). Industrial plants with decontamination capacities ranging from 0.5 t to 600 t are in operation (Bhat, 1991). However, such products (detoxified seed cakes) are useful only as animal feed and the possible deterioration of animal health by excessive residual

ammonia in the feed remains a concern and regulatory measures permitting the marketing of such detoxified products have yet to be formulated.

Detoxification of crude oil by binding aflatoxin in groundnut oil and cake has been presented as a possible method for use at the small-scale industry or household level (Mehan, 1995). The use of red clays in West African countries has been found to be effective in binding aflatoxin in contaminated groundnut cake. In Senegal, it was found that exposure to sunlight for 18–24 hours in transparent and translucent containers destroyed 100% of the toxin in contaminated oil (Kane, 1996). The method is simple and is suggested for use by oil processors at the village level. Other aflatoxin management initiatives are listed in Table 17.

Table 17: Aflatoxin management and reduction projects in Africa

Project initiative	Country of implementation	Project objective	Project link/references
Feed the Future Innovation Lab	Malawi, Mozambique, Zambia	Technologies along the peanut value chain, and education	http://pmil.caes.uga.edu/research/NCSU202/index.html 2013–2017
Platform for African European Partnership on Agricultural Research for Development (PAEPARD)-CRF project	Malawi	Practices in peanut farming, knowledge management, and policy	http://www.fanrpan.org/documents/d01766/ 2014–2017
Safe Food Safe Dairy, Government of Finland	Kenya	GAP	http://safefood.uonbi.ac.ke/
Food Africa, Government of Finland	CGIAR Research Programme in Benin, Ghana, Cameroon, Kenya, Senegal, Uganda		https://portal.mtt.fi/portal/page/portal/mtt_en/projects/foodafrica
World Food Programme	Uganda	Technologies along maize value chain, and education	http://documents.wfp.org/stellent/groups/public/documents/special_initiatives/WFP265205.pdf 2013–2014
Flemish Interuniversity Council-Insitutional University Cooperation (VLIR-UOS) funded project 2011–2016	Tanzania	Effective strategies for minimising exposure of mycotoxins in maize based complementary foods in Tanzania	Kamala <i>et al.</i> , 2015; Kamala <i>et al.</i> , 2016

Diagnostics for aflatoxin detection

Testing contaminated lots of commodities can be seen as a post-harvest technology that can be used to manage aflatoxin exposure. A set of diagnostic solutions is required that can be

used by both smallholder and commercial farmers in the field during harvest, in village and commercial mills, and in silos. These should be inexpensive and portable. A number of initiatives seek to address the lack of diagnostic tools characterised by a few inaccessible laboratories. Some of these include Agristrips, Dipstrips and E-nose, but none are being used by smallholder farmers. In Kenya the Aflatoxin Proficiency Testing and Control in Africa (APTECA) programme, hosted by the mycotoxin diagnostics platform at the BecA-ILRI Hub, is contributing to the availability of safe maize on the market through partnership with the commercial maize milling sector. APTECA trains millers in sampling and testing and provides routine proficiency testing and verification of mill results by the ISO accredited Texas A&M AgriLife laboratory housed at the BecA-ILRI Hub (Herman, 2016).

5.4 Secondary interventions: Adsorbents/binders

Addition of adsorbents (also named binders or sequestering agents) to livestock and poultry feed is practiced in Africa. These agents bind aflatoxins in the gastrointestinal tract and are capable of reducing its availability (Huwig *et al.*, 2001; Phillips *et al.*, 2002). Research with mycotoxin binders has been conducted for over 20 years with strong evidence about the performance of some of them. Substances used as mycotoxin binders include indigestible adsorbent materials such as silicates, activated carbons, and complex carbohydrates. Several of these adsorbent materials are recognised as safe feed additives and are used as flow agents and pellet binders, but not specifically as aflatoxin binders or for treatment of aflatoxicosis (Grenier and Applegate, 2015).

Use of binders in humans has been suggested for Africa and trials have been carried out with human subjects in Ghana (Phillips *et al.*, 2008), and as recently as 2015 in eastern parts of Kenya by the Centre for Disease Control (Awuor *et al.*, 2016). However the binding capacity of adsorbents has raised controversial questions regarding their influence on the utilisation of nutrients such as carbohydrates, proteins, vitamins and minerals, and their use in human diets has stirred even more reactions.

Diversifying diets and consumption of probiotics have been promoted to reduce aflatoxin exposure (Liu and Wu, 2010; Nduti *et al.*, 2016). The synergistic effect of Hepatitis B Virus (HBV) and aflatoxin in inducing hepatocellular carcinoma by thirty-fold has been documented (Groopman and Kensler, 2005). HBV vaccination has also been seen as a practical intervention for reducing the risk of aflatoxin-induced liver cancer and cirrhosis (Kuniholm *et al.*, 2008).

6.0 Conclusion

This literature review demonstrates that a wide range of commodities that are produced in Africa and traded domestically, regionally and internationally are contaminated with aflatoxins. Commodities can be attacked by the *Aspergillus* sp. anywhere along the value chain and once infected the aflatoxins remain. No single technology or intervention emerges as a standalone strategy for wide-scale adoption in Africa. Each has its unique benefits and drawbacks. The report also demonstrates that significant investments have been made, especially by the research, academic and donor community, in investigating the aflatoxin challenge and exploring possible solutions to control contamination, with varying measures of success. However, despite the vast knowledge base, the challenge of controlling aflatoxin contamination persists, with continued negative impacts on human health, agri-businesses, trade and socio-economic development.

A major factor that contributes to the pronounced exposure to aflatoxins in humans (as well as livestock, including farmed fish) in Africa, is the wide range of agro-ecological conditions, temperature and humidity, which favour the growth of *Aspergillus flavus*, *A. parasiticus* and other *Aspergillus* species. This is further complicated by the fact that the variety of cereals and other crops (roots and tubers, spices, legumes) that are contaminated in the field or in storage by the *Aspergillus* fungi are essential staple foods for a majority of Africans. For example aflatoxin levels as high as 138,000 µg/kg have been reported in pre-harvest maize samples in Nigeria and 48,000 µg/kg in stored maize. Contaminated crops are also used to produce a range of processed products (e.g. peanut butter and local brews) and animal feeds, resulting in both food and feed being contaminated.

In Africa, contamination levels of foods and feeds commonly exceed internationally acceptable standards. Aflatoxins found in feeds can also be efficiently converted to toxic metabolites in milk, meat and eggs. No country is immune and African consumers and livestock remain at risk. Exposure levels have been found to be up to 1,064 pg/mg aflatoxin albumin levels in blood samples based on biomarker studies, although this analytical technique is relatively new.

Apart from causing acute poisoning and death at high doses in both humans and animals, at chronic lower-level doses aflatoxins cause liver cancer, immunomodulation, stunting and kwashiorkor in young children. Reports of death resulting from severe aflatoxin poisoning and/or presence of aflatoxins in organs have been reported in both humans and animals in Kenya, Nigeria, South Africa, Tanzania and Uganda. The effects of chronic exposure cannot be quantified, but correlation with stunting in children in West Africa has been reported. Liver cancer causes about 26,000 deaths annually in sub-Saharan Africa. Ample evidence of other effects on humans due to chronic exposure have been deduced from animal studies.

Awareness of aflatoxins and the associated risks among African consumers and value chain actors (e.g. farmers, traders) is low. Knowledge is only high in areas where outbreaks have occurred and more so among educated populations. Inclusiveness of actors along commodity value chains in the fight against aflatoxins is imperative. Investing in public education and designing and implementing an effective communication strategy along value chains must be a priority for countries in the fight against aflatoxins. Studies have shown that consumers and buyers (processors, traders, exporters) are willing to pay a premium for aflatoxin free products. The premiums, however have to ensure that a wider cross-section of Africans can purchase

safe food at affordable prices; the demographic data shows that poorer people are more exposed and at greater risk.

There is little evidence that the biology of the *Aspergillus sp.* under diverse environmental conditions and the prevailing context of smallholder farming systems is well understood and applied in developing new technologies and piloting innovative solutions to control aflatoxin contamination in Africa. Adopting GAPs and controlling moisture content, especially during storage and transport, have been shown to be very effective in managing fungal growth and aflatoxin contamination and must be promoted widely in Africa. Farmers should be the primary target group in the fight against aflatoxin contamination in Africa. They need to be empowered with knowledge and appropriate technologies and given incentives and rewards for adopting good practice.

The report points to promising pre-harvest innovations that depend on the manipulation of the fungal population ecology (e.g. Aflasafe™), and reproduction and gene manipulation (e.g. breeding for resistance), but consideration should be given to the potential environmental impact of these products. For example, the possibility of atoxigenic biocontrol strain acquiring aflatoxin pathway genes through vegetative fusion and sexual reproduction could exacerbate the aflatoxin contamination problem. Breeding of *Aspergillus* Ear Rot-resistant maize to reduce aflatoxin and fumonisin accumulation is widely accepted as a safe and easy-to-use option. However, the aflatoxin resistant genes are polygenic, therefore requires gene pyramiding using numerous genotypes with novel genes which could take many breeding seasons to come up with a resistant variety. Greater knowledge of gene function and expression under a range of environmental conditions is a necessity given the knowledge of host-induced environmental reactions. Further, some of the resistant varieties are not adapted to, or do not yield well in the agro-ecologies endemic to aflatoxin contamination in Africa.

Weak governance and legislative framework is a major drawback in the fight against aflatoxins. Enforcement of regulations at every stage of a commodity's value chain is not possible, especially because small-scale farming systems and informal markets and trade predominate. The ideal workable situation is for countries to develop and apply stringent standards and enforce regulations backed by GAPs and appropriate sampling and testing to drive innovations to control aflatoxin contamination, as is the case in developed countries. Country governments are encouraged to invest in building certified and accessible infrastructure for training manpower, and testing and grading commodities. Another option is to provide incentives such that all enterprises (small, medium and large) can self-regulate to ensure conformance with relevant local, regional and international standards. However, absence of standards and ineffective implementation of regulations is not an option if Africa is to effectively address the aflatoxin challenge.

Harmonisation of legislation within trading blocks and strictness in upholding the rules will also contribute to mitigation of aflatoxin contamination. While international exports are easier to control (as recipient countries have well equipped accredited testing laboratories to ensure adequate enforcement and limit what comes into their countries), Africa must seek to do the same. This will give Africa a competitive advantage as the EU and other major trade partners lower the tolerable limits for aflatoxins and regulations and enforcement becomes stricter.

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