Building an Aflatoxin Safe East African Community

Technical Policy Paper 9

Aflatoxin: Alternative Uses and Disposal Systems

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Situational Analysis for East Africa Region

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Authors:

Henry Njapau, National Institute for Scientific and Industrial Research, Lusaka, Zambia

Francesca Nelson, International Institute of Tropical Agriculture, Dar es Salaam, Tanzania

Timothy Wesonga, East African Community, Arusha, Tanzania

Victor Manyong, International Institute of Tropical Agriculture, Dar es Salaam, Tanzania

Contact IITA:

f.nelson@cgiar.org or c.njuguna@cgiar.org

IITA Tanzania East Africa Region Hub
Plot No. 25, Mikocheni Light Industrial Area
Mwenge, Coca Cola Road, Mikocheni B
PO Box 54441, Dar es Salaam, Tanzania

Cover: An ethanol plant. Production of ethanol fuel is one means of disposal of aflatoxin contaminated food and feed stuffs. *Courtesy Mott MacDonald.*

Foreword

It is estimated that by the year 2025 the population of the East Africa region will exceed 400 million people, with annual urban growth rates averaging 4 percent. Coupled with income growth, dietary transformation is likely to occur, increasing the demand for both processed foods and animal products. These events could have repercussions for the vast majority of low income consumers who, unlike their wealthier urban counterparts, will continue to rely on staple grains, roots, and tubers for sustenance. With commercial production of a single kilogram of beef requiring a feed input of 3.5 kilos of grain, demand for feed could out-compete supply for direct consumption by the poor. At the same time, global climate change (GCC) and poor land management practices will continue to pose challenges to providing adequate nourishment that keeps pace with population growth. One of the most cost effective and environmentally sustainable solutions is reduction of postharvest loss, currently estimated to be approximately 30 percent. However, levels of loss would increase significantly if current standards for aflatoxin contamination were enforced in the East Africa region.

In many countries, aflatoxin-prone crops are channeled into a processing system to maximize utilization and minimize waste. For example, loss of groundnuts due to aflatoxin contamination in the United States is a mere 2 percent, even under a regime of strict enforcement of the 10 parts per billion (ppb) standard. The most pristine nuts are taken first for foods. Nuts that exceed 20 ppb can be processed into oil which complies with standards, and the remaining groundnut cake is fed to animals, which have a higher tolerance level for aflatoxin. Grains such as maize exceeding the allowable limits for human consumption are blended or processed for animal feed. Disposal of waste products from all of these operations is expeditiously carried out with full adherence to public safety concerns.

To ensure the future food security of the East Africa region, a similar approach to protecting the public health while simultaneously maximizing food and feed availability is essential. Further, aflatoxin contaminated commodities that cannot be made safe for humans or animals may have a potential role in the development of biofuels within the region. To address the issues for alternative uses and disposal systems for aflatoxin contaminated crops in the region, and to explore solutions that provide maximum nutritional and economic benefit, we have established a knowledge platform specific to the East Africa region.

Table of Contents

Figures

Tables

Executive Summary

Contamination of agricultural commodities, including maize, groundnuts, cassava, milk, and cottonseed with aflatoxin poses a serious threat to human and animal health, and to the economies of the East Africa region. Physiologists and toxicologists have clearly established the hazard of exposure to aflatoxins, and epidemiologists have demonstrated a relationship between aflatoxin exposure through dietary consumption and human morbidity and mortality. This toxicological and epidemiological information has led to an increase in awareness of the hazards posed by aflatoxin to the public health, especially in the wake of numerous aflatoxicosis outbreaks across the East African Commmunity (EAC) resulting in loss of human life. Lower levels of chronic aflatoxin ingestion are also extremely detrimental to human health during the first 1000 days of life, for persons with compromised immune systems, and people suffering from liver disease. Persistent and chronic aflatoxin exposure has been shown widely prevalent throughout the five nations of the EAC, with elevated serum aflatoxin levels demonstrated across all geographical regions, within all socioeconomic and demographic groups, and among all ages and genders.[1]

It is, therefore, desirable that contamination be prevented to the greatest extent feasible. However, success in eliminating aflatoxin has eluded a legion of plant breeders, plant pathologists, and biochemists for several decades. Over the last 50 years, the environmental and host plant interactions, genetic constitution, and biochemical mechanisms that facilitate and encourage aflatoxin production have been explored thoroughly but still hold many mysteries. Aflatoxin contamination of food and feed continues to compromise the wellbeing of millions of households and their livestock. Episodes of human and animal aflatoxicosis, while periodic, still persist. Unless these issues are given serious attention and solved, aflatoxin will continue to affect human health and constrain the EAC's realization of key development objectives across the health, agriculture, trade, and environment sectors.

Increasing awareness of the hazardous nature of aflatoxins has led to the establishment of legal instruments by EAC partner states designed to minimize human and animal exposure to aflatoxin (e.g., EAS 2:2013). However, partner states still face a lack of secure and safe mechanisms for the disposal of aflatoxin contaminated food and feed. It is important that the EAC continues to explore and harmoniously adopt a policy framework to facilitate mechanisms for the safe, secure, and expeditious disposal of aflatoxin contaminated commodities.

In addition, some of these commodities may be appropriately diverted for alternative uses, such as animal feed and the production of energy. Processing technologies also

[1]There is no serum aflatoxin information currently available for Burundi and Rwanda.

exist that can reduce the aflatoxin content of the commodity to a level where it becomes safe to use for a particular category, such as stock feed.

This paper examines existing alternative uses and disposal systems, and discusses their potential in relation to the technical capability, ecological setting, and legal and regulatory framework among the EAC partner states.

In many countries, including the EAC partner states, there are no functional mechanisms for the disposal of aflatoxin contaminated agricultural commodities. In fact, secure and safe mechanisms for disposal of contaminated commodities have yet to be developed. While outright disposal may avoid the risk of harm to potential consumers of a contaminated commodity, it would often result in significant economic losses for the producer--and potential detriment to household, national, and regional food security. Disposal should always, therefore, be the option of last resort.

Another important consideration is that the severity of risk from aflatoxin differs substantially between humans and animals, as well as among animals. It further differs significantly within particular species of animals and among humans by age and health status. Hence, commodities unfit for human consumption can often be selectively diverted to animal feed for the appropriate type and category of livestock, within the appropriate timeframe of livestock development. Contaminated commodities can also be also processed to yield by-products that become fit for animal consumption. Similarly, products that may be classified as unsafe for infants may be tolerable for adults.

Chemical and physical processing can be used to bring certain commodities into compliance with animal feed standards. Alkaline treatment, including the use of ammonia and calcium hydroxide (nixtamalization), can reduce the levels of aflatoxins in maize and cottonseed. Physical processes—sorting, fractionation (wet and dry milling), and flotation—can also reduce aflatoxins by similar percentages.

There are many statutes, regulations, and practices among the EAC partner states, particularly those pertaining to the environment, that impact directly and indirectly on the handling of aflatoxin contaminated commodities. This paper examines potential alternative uses, processing techniques that reduce aflatoxin contamination in otherwise unsuitable commodities, disposal systems, and alternative uses. These aspects are discussed in relation to the technical capability, ecological setting, and legal frameworks in partner states of the EAC. We also forward policy recommendations to strengthen alternative uses and disposal systems for the East Africa region.

Introduction

In the East African Community, maize, groundnut and, to a lesser extent, cassava, are staple food crops, providing essential sources of energy, protein, and other nutrients for both humans and livestock. In many areas, milk is also a key source of protein, especially for children. Cottonseed is utilized primarily as a source of edible oil and animal feed protein.

Numerous mycotoxins, including the aflatoxins, naturally occur in agricultural commodities such as maize, groundnut, cottonseed, and cassava, as a result of unavoidable fungal infection and proliferation, particularly under warm and humid conditions such as those prevailing in the EAC (Diener and Davis 1977; Christensen et al. 2012). Of the mycotoxins isolated from food crops to date, aflatoxin is the most potent toxin and carcinogen (Sangare et al. 2014). When humans or livestock consume agricultural commodities contaminated by aflatoxin, they may experience various ailments, including impaired liver function, jaundice, acute hepatitis, impaired growth, impaired immune function. Acute aflatoxin poisoning can lead to death (Azziz-Baumgartner et al. 2005; Riley et al. 2008; Smith et al. 2012). Even when consumed at low levels, aflatoxin can be detrimental to human health, especially during the first 1,000 days of life, and for persons with compromised immune systems (Groopman et al. 2014).

Over the past 50 years, aflatoxin-associated morbidity and mortality has been documented in some of the EAC partner states (Peers and Linsell, 1973; Ngindu et al. 1981; Azziz-Baumgartner et al. 2005; Ocama et al. 2009; Shirima et al. 2014). Persistent, chronic aflatoxin exposure has been shown to be widely prevalent in the EAC partner states of Tanzania, Uganda, and Kenya, with elevated serum levels demonstrated across these regions, within all socioeconomic and demographic groups, and among people of all ages (Yard et al. 2013; Shirima et al. 2014; Asiki et al. 2014).

Consequently, the EAC has responded by establishing mechanisms that are aimed at restricting exposure to aflatoxin, such as regulations that stipulate tolerable limits or standards for aflatoxins in food (EAS 2: 2013). However, blanket enforcement of such regulations would result in substantial quantities of staple food crops being declared unfit for consumption by either humans or livestock and hence discarded. This would be particularly pronounced where agricultural practices are generally poor. Current protocols do not address issues related to aflatoxin contamination where food is consumed on the farm, as it is in over 50 percent of households across the region.

This paper presents the results of a literature review and consultations with representatives of EAC partner states and other international experts to establish a basis and procedures for alternative uses and disposal systems for aflatoxin contaminated commodities. Our review and consultations:

- Examined the ecological setting within the East Africa region that facilitates the invasion of target crops by and proliferation of *Aspergillus*, leading to the production of aflatoxin;
- Considered alternative uses of aflatoxin contaminated crops declared unfit for human consumption;
- Considered disposal options for the aflatoxin contaminated crops; and
- Reviewed the legal statutes that impact the utilization and disposal of aflatoxin contaminated crops.

This report provides the findings of the exercise and suggests a number of policy options that could assist regional, national and community management of aflatoxin contaminated agricultural commodities.

Ecology and Toxicity of Aflatoxin

Environmental Conditions Leading to Aflatoxin Contamination

Aspergillus species which produce aflatoxins occur in most subtropical soils (Hesseltine et al. 1966). The fungus first becomes associated with the crop in the field, and drought stress, conducive temperatures (13–37 °C), and insect pests enhance fungal invasion and formation of aflatoxin in the crop (Lee et al. 1989; Dowd. 1998). Proliferation of the fungus is governed by various factors, including the weather, soil chemistry, and agronomic practices (Riley and Norred1999). In crops in storage, the formation of aflatoxin is likewise facilitated by environmental parameters. Thus, ambient temperature (25-32° C), moisture percentage and biotic factors remain of great importance in the etiology of aflatoxin formation.

Toxicity of Aflatoxin

Aflatoxins, produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus*, are foodborne toxins that cause detrimental effects in humans and animals (Williams et al. 2004; Khlangwiset and Wu 2010). Aflatoxin came to international attention more than 50 years ago, following the Turkey X episode in Britain, which killed more than 100,000 turkey poults in the first known acute manifestation of aflatoxin toxicity. (Dickens and Jones 1963). It has since been established that consumption of food or feeds contaminated with aflatoxin leads to illness or even death in humans and livestock. (Goldblatt and Dollear 1977; Hendrickse et al. 1982; Azziz-Baumgartner et al. 2005; Jaykus et al. 2008; Turner 2013).

Equally significant to humans is the carryover of aflatoxin B_1 residues from livestock feed into meat and eggs, and the carryover of its metabolite aflatoxin M_1, from feed into milk. Consumption of aflatoxin M_1, though less potent than aflatoxin B_1 still remains hazardous (Jaykus et al. 2008). This puts children, the major consumers of milk, at great risk of aflatoxin exposure. Estimates of the fraction of aflatoxin B_1 carried over from feed into milk as aflatoxin M_1 have varied with milk yield, but are generally in the range of 1.4 – 4.5 percent (Agag 2004).

Status of Aflatoxin Contamination of Agricultural Commodities

Soon after the acute aflatoxicosis outbreaks on English farms in 1960 (Dickens and Jones 1963), the magnitude of the aflatoxin problem became apparent. Consistently, aflatoxins were and continue to be isolated from a variety of foodstuffs the world over (van Ransburg, 1985; Placinta et al. 1999; Kankolongo et al. 2009; Mudili et al. 2013; El-Shansoury et al. 2014; Darwish et al. 2014).

After realizing that aflatoxin was consistently present in foodstuffs, scientists sought a way to stop its production in food crops. Over the last 50 years, the biology of *Aspergillus flavus* and the mechanisms by which it interacts with the crops have been extensively studied (Bhat 1991; Christensen et al. 2012). Similarly, the pathways and biochemical processes through which aflatoxin is produced in crops have been described (Yu et al. 2004; Yu 2012). Agronomic practices that could prevent the occurrence of aflatoxin in maize, groundnuts, cassava, and cottonseed have equally been widely studied.

The focus has been on preventing contamination through practices that result in less fungal infection in the field (Heseltine 1966; Reddy et al. 2011; Fountain et al. 2014) and during storage (Passone et al. 2012; Villers et al. 2014), and the development of crop varieties resistant to fungal invasion and aflatoxin production (Brown et al. 2010; Wang et al. 2014; Asters et al. 2014). Consequently the literature is replete with agricultural practices and technologies that have been shown to have a potential for realizing aflatoxin-free or minimally contaminated agricultural commodities. Nevertheless, absolute elimination throughout the production and value chain has continued to elude the scientific community. Aflatoxin contamination continues to occur and is expected to be accelerated in some areas of the region by increases in humidity and temperature arising from global climate change (Jaykus et al. 2008; van Asselt et al. 2013). Thus, consideration of additional options for managing aflatoxin contamination, such as the adoption of alternative uses for contaminated commodities, and mechanisms for the disposal of those that cannot be utilized, is an important undertaking.

Considerations in Managing Aflatoxin Risk

The toxicity of aflatoxin and its prevalence in foods and feedstuffs are the reasons for efforts to prevent or minimize human exposure through the establishment of regulations by several countries. The establishment of these regulations is, however, influenced by both scientific and social economic factors (van Egmond 2013). Consequently, aflatoxin limits vary approximately nine-fold (Wu and Guclu 2012) among countries that have established regulations for aflatoxin contamination in human foods. Regulatory limits are often relatively higher in countries where food security is likely to be compromised or the impact on the local economy may be significant. In the same vein, enforcement of food safety regulations can be confounded by socioeconomic needs. The regulator has to delicately balance the two often competing sides, so that neither is adversely compromised. For instance, the U.S. Food and Drug Administration (FDA) exercised some science-backed flexibility in the enforcement of aflatoxin regulations when socioeconomic demands have required special consideration.

In 1964, soon after the identification of aflatoxin, the FDA set a 20 ppb aflatoxin action level for both humans and livestock (21 CFR, 1964). In 1982, the FDA increased the permissible level for aflatoxin in cottonseed meal used as a feed ingredient to 300 µg/kg for mature beef cattle, swine, and poultry. This change was based on the fact that cottonseed contamination was extraordinarily prevalent during that year, and that cottonseed meal usually makes up a relatively minor part (~11 percent) of an animal's diet (Park and Stoloff 1989). The FDA also cited scientific evidence that aflatoxin residues in edible muscle tissue were only 0.2 – 0.5 percent of that in the feed (Jacobson et al. 1978; Shreve et al. 1979), hence the adjusted standard still protected public health.

The FDA has also made temporary adjustments to permissible aflatoxin levels for feed on two other occasions. Severe droughts in the 1977 and 1988 maize-growing season in the Southeastern "maize belt", and more recently in 2012 across the mid-Western states of Illinois, Indiana, Iowa, Kansas, Kentucky, Nebraska, and Oklahoma, stressed the maize crop, leading to elevated aflatoxin levels. The FDA accordingly allowed the affected states to blend maize contaminated with aflatoxin (up to 500 µg/kg) with cleaner maize and use the blend for feedlot cattle. Whereas ordinarily blending as a means of lowering aflatoxin contamination is forbidden in the United States, the regulatory authority made adjustments to accommodate exceptional circumstances that prevented jeopardizing farmer incomes while still protecting the health of the public, the primary goal of the aflatoxin regulations. Such judiciousness is necessary in the management of a prevalent contaminant of food crops. A similarly flexible approach may be appropriate for East Africa.

Commodities Classified Unfit for Consumption

The rejection or classification of a commodity as being unfit for the intended purpose may arise from 1) the commodity being visibly spoiled owing to mold infection or physical damage arising from insect infestation, or 2) the commodity, while visibly clean, being determined, by a chemical analysis, to be aflatoxin contaminated above a tolerable limit for the intended purpose. Such material becomes a candidate for either diversion to an alternative use or outright disposal.

Moldy or Insect-Infested Commodities

Mold infection and insect infestation may occur in the field or during storage. Estimates of visible mold and insect damage vary from two to four percent of a crop (Snelson 1987; Utono 2013; Teferaet al. 2011). Sometimes, the bottom layer of maize that is in bags and stacked becomes discolored and caked due to heat and not necessarily mold.. Nevertheless, such maize, often mistakenly assumed to be aflatoxin contaminated, would esthetically be considered unfit for human consumption and would either be diverted to livestock feed or discarded outright.

The quantity of the commodity falling into this category depends on the total production of a country. For instance, for a country that produces 5 million metric tons of maize, 2 - 4 percent of the total production comes to 100,000 to 200,000 tons. The disposal of such large amounts of food not only impacts national food security but poses a disposal challenge.

Commodities Contaminated with Aflatoxin

Owing to the current inability to completely eliminate aflatoxins from food crops and the deleterious nature of aflatoxin to the health and productivity of both humans and animals, the majority of governments around the world have responded by establishing measures that limit the exposure of consumers to aflatoxin (Wu et al. 2013). Aflatoxin occurrence above the set limits are considered to render a commodity unfit for the intended purpose, e.g. consumption by humans or livestock. Table 1 illustrates aflatoxin contamination limits set for maize and groundnuts in the United States and the European Union (EU).

Table 1: Maximum aflatoxin levels, United States and United Kingdom.

Agricultural commodity	For consumption by	Tolerable Levels (>µg/kg total aflatoxin)	
		EU[a]	USA[b]
Maize	Humans	4	20
Maize (if to be sorted)	Humans	10	--
Groundnuts	Humans	4	20
Groundnuts (if to be sorted)	Humans	15	--
Maize	Immature animals	10	20
Maize	Mature animals	20	100
Maize	Mature feedlot cattle	20	300
Maize	Dairy cattle	5	20
Milk	Humans	0.05	0.5
Milk	Infants	0.025	0.5

[a]**European Commission Guidelines, EC 1881/2006 & EC 576/2006**

[b] **FDA Action Levels (FDA Policy Guides CPG 555.400 (Humans) & CPG 683.100 (Animal Feeds)**

Alternative Uses for Contaminated Commodities

"Alternative use" refers to the diversion of a commodity to uses other than those it was originally intended for. Often, this refers to the diversion of human food to animal feed. It also includes diversion of contaminated food commodities to the production of materials, such as the production of ethanol from contaminated maize.

Commodities deemed unfit for a particular use can be directly channeled to other uses with less stringent requirements, processed for the purpose of bringing the commodity into compliance with an alternative use, or processed into a byproduct compliant with aflatoxin limits, such as peanut oil from groundnuts (Fonseca and Regitano-d'Acre 1993; Banu and Muthumary 2010; Idris et al. 2010) or starch from maize (Aly 2002). In some processing operations, such as the production of ethanol from contaminated maize, a more toxic byproduct may also be produced. Whereas the distillate (ethanol) will contain no aflatoxin, the stillage and fine grain particles (DDGS) and the coarse unfermented grain (Dried Maize Solids [DCS]) used as animal feed may contain a higher concentration of aflatoxin than the original maize (Johnston et al. 2012; Inoue et al. 2013). The increased aflatoxin concentration in these byproducts could make them unfit for use as animal feed as well, and necessitate their disposal or further reprocessing.

Direct Alternative Utilization

Visibly moldy, discolored, or insect-infested maize may not be fit for direct user processing, depending on the level of moldiness and intensity of insect infestation or discoloration. If the maize is heavily moldy, black, and caked, or powdery as a result of heavy insect infestation, it would be appropriate to discard it. Alternatively, if the maize is not very discolored and remains intact, its aflatoxin content could be determined and thereafter categorized appropriately to fit a livestock feed category.

Cascading Direct Utilization

The cascading principle reflects the fact that the severity of the response to aflatoxin differs among humans and animals by health and nutritional status (Gradelet et al. 1998) as well as the occurrence of other mycotoxins in the food or feed (Milicevic et al. 2010). For instance, soon after the establishment of an association between aflatoxin and hepatocellular carcinoma (HCC) (primary liver cancer), it was demonstrated that infection by hepatitis B virus increases the risk of developing the cancer approximately 30-fold (Henry et al. 2002). On the other hand, populations that consume diets rich in vegetables and fruits seem to be at lower risk for succumbing to the deleterious effects of aflatoxin (Gradelet et al. 1998).

Similarly, in animals there is great diversity of tolerance among species (cattle, chickens, pigs, fish) and age groups (chicks versus market-ready broilers, or calves versus mature feedlot beef cattle) (Wogan 1966; Roebuck and Wogan 1977; Pier 1992; Wild and Gong 2010). Generally, when species that have been studied are arranged in order of decreasing susceptibility (or increasing resistance) to aflatoxin, they rank as follows: rabbit>duck>pig>dog>man>turkey>guinea pig>sheep>chicken>monkey>mouse. The rabbit and the duck are the most susceptible, while the mouse is the most resistant to aflatoxin. The median lethal dose of the duck is 0.3 mg per kilogram of body weight (kg/bw), whereas that of the chicken is 18 mg/kg/bw (Williams et al. 2004).

In the "cascading utilization" process, a commodity would be considered for remediation through processing only if: 1) it is not fit for use for any of the other categories or 2) it is desired for use in a category requiring less aflatoxin contamination.

To illustrate the "cascading utilization" principle, Table 2 shows the options for using aflatoxin contaminated maize based on the European Union, EAC, and U.S. aflatoxin limits. In the United States, there are multiple options for direct use of aflatoxin contaminated maize, up to an aflatoxin contamination level of 300 µg/kg. As mentioned earlier, in special circumstances where the weather has been particularly unfavorable, the FDA may authorize blending of contaminated maize (up to 500 µg/kg) with

clean maize to achieve the desired level for animal feed. Therefore, commodities determined to be unfit for consumption by, for instance, swine, can be diverted to an appropriate age category for poultry feed.

In the European Union and the EAC on the other hand, options are limited to utilizing commodities with less than 10 ppb aflatoxin. Under these circumstances, enforcement of the standard could lead to the rejection and disposal of a substantial quantity of an agricultural commodity. Where direct cascading is not practical or not permitted, contaminated commodities can also be processed to yield byproducts that become fit for consumption by one of the species.

Table 2: Utilization options for aflatoxin contaminated maize in the EU and U.S.

Lot No.	Total aflatoxin contamination (µg/kg)	EU	USA
1	4	Direct human consumption	Direct human consumption
2	10	▪ Subject to sorting for humans ▪ Direct immature animals consumption	Direct human consumption
3	20	Direct mature animal consumption	▪ Direct human consumption ▪ Direct immature animal consumption ▪ Direct dairy consumption
4	100	Reject for all classes	Direct mature animals
5	300	Reject for all classes	Direct mature feedlot cattle
6	500	Reject for all classes	Seek authorization to blend for mature feedlot cattle
7	>500	Reject for all classes	Reject for all

Table 3 shows the feed-to-tissue ratios obtainable with the various types of livestock. The feed-to-tissue ratio refers to the amount of aflatoxin that is retained in the muscle of an animal that ingests it. For example, when a chicken is fed a ration containing 33,800 ppb of aflatoxin, the muscle (meat) of the chicken contains only 1 ppb aflatoxin. Hence in terms of the public health of U.S. consumers of meat, aflatoxin levels shown in the Feed/Tissue Ratio column of Table 3 would be considered safe for all the indicated categories of livestock.

Table 3: Ratios of aflatoxin in feed to that in edible animal (livestock) tissues

Animal	Tissue	Aflatoxin	Feed/Tissue ratio (ppb)
Chicken (Layer)	Egg	B_1	2,200[a]
Chicken (Broiler)	Muscle	B_1	33,800[b]
Swine (Pigs)	Muscle	B_1	182[b]
Cattle (Dairy)	Milk	M_1	75[a]
Cattle (Beef)	Muscle	B_1	500[b]

[a]Adapted from Park and Liang. 1993; [b]Adapted from Manning et al. 2005

Batch-Based Direct Utilization (Salvaging)

Accurately estimating the concentration of aflatoxin in agricultural commodities that are bulked is extremely difficult, if not impossible. The difficulty arises from errors associated with the steps involved in determining the concentration of the aflatoxin in the lot, i.e. sampling, subsampling, comminution, and analysis (Whitaker 2003). The source of the largest error is the sampling step, more so if the lot is static and the sample size is small, as is most often the case in testing regimens in less technologically advanced settings. This is so because often less than 1 percent (<1 percent) of the kernels in a lot may have aflatoxin (Johansson et al. 2000), albeit those possibly at extremely high levels. Whitaker and co-workers have further demonstrated that when lots of groundnuts (mean concentration 10-20 ppb) were tested using an official sampling scheme (5.4 kg composite sample), approximately 30 percent of 10 samples from one lot would give results above the mean aflatoxin concentration of the lot (Whitaker et al. 1972; Whitaker et al. 2005). When the mean aflatoxin concentration was between 40 and 100 ppb, approximately 50 percent of 10 samples would yield a result above the mean. These statistics inform us that when only one sample is collected from a lot of, for example, 5,000 90-kg bags, and analyzed for aflatoxin content, the result has only a 70 percent chance of accurately depicting the true average aflatoxin concentration of the lot if it were 10 ppb and appropriate sampling is carried out. Three out of ten samples would lead to a rejection of the lot. With inappropriate (non-representative) sampling, which is more the norm, the error rate is likely to be higher. Whitaker and Slate (2012) have also demonstrated that increasing the ratio of sample size to lot size by decreasing the size of the lot and maintaining the sample size would increase the accuracy of the test result.

Therefore, when a bulked and stacked lot of maize is declared to violate a set standard on the basis of a result from a single sample or from multiple samples around the periphery, there is a high probability that the result is erroneous. It would therefore, be prudent to subdivide the lot into small batches of, for instance 500 x 90 kg bags, and

resample at the same rate. There is a high probability that the majority of the batches will yield results below the stipulated limit, hence "salvaging" back into the food or feed channel a larger portion of a commodity that would otherwise have been discarded.

Treatment or Decontamination Methods

When a commodity cannot be used directly through cascading or salvaging, it can be processed by removing contaminated parts of, or whole, kernels, or removing the aflatoxin from, or inactivating the aflatoxin within, the commodity (Bothast et al. 1991). These remediation processes can be biological, chemical, or physical, and some of their byproducts would be a commodity suitable for a particular use. The ideal process would achieve complete removal or inactivation of the aflatoxin without leaving toxic residues in the processed product. It should also be cost-effective and easy to use or adapt, either at the household or industrial level.

Removal of whole kernels can be achieved by sorting or flotation, while removal of portions of a kernel can be achieved through dry or wet milling (maize), or blanching (groundnuts). Removal of the toxin from the commodity can be achieved by solvent extraction, and toxin inactivation can be attained by either physical (heatingand/or irradiation) or chemical (nixtamalization and ammoniation) means. Numerous procedures for reducing the content of aflatoxin in contaminated commodities are thus available, each with varying degrees of efficacy and applicability. The selection of the most suitable procedure will depend on its overall suitability for the local setting, as each requires a different degree of technological sophistication.

Physical Processing Methods

Physical methods that can reduce aflatoxin content include sorting, winnowing, heating, fractionation, irradiation, extrusion, ozone treatment, and solvent extraction.

Sorting

Physical separation of visibly discolored and/or moldy kernels and separation of fines are simple sorting methods that can be applied at the rural household and industrial levels. The sorting is based on the appearance of the kernel to the naked eye or to an apparatus such as an electronic sorter (Whitaker 1997). The change in appearance of the kernel could be a result of direct fungal infection and growth or a result of fungi-induced biochemical changes (da Gloria 2011). In some instances, obvious discoloration or Bright Greenish-Yellow Fluorescence (BGYF), though not definitive, are used as surrogates for aflatoxin contamination.

According to Whitaker (1997), when color sorting of groundnuts is supplemented by blanching, the aflatoxin content of the nuts is reduced by 87–91 percent. Various devices that aid in the sorting of groundnuts have been developed in recent times (Yao et al. 2010). Nevertheless, hand-sorting is still a reliable tool.

Size and Density Segregation

Size and density have also been employed to separate aflatoxin contaminated kernels from sound ones. Size sorting (Piedade et al. 2002) is often achieved through the use of sieves, while winnowing and flotation rely on the weight of the kernels or their components when subjected to a stream of air or placed in a liquid, respectively. Removal of small and shriveled (lighter) peanut kernels has been shown to substantially reduce the mean aflatoxin content of the remaining larger sound kernels (Whitaker et al. 2005; Dorner 2008). Huff (1980) recorded a 60 percent reduction in aflatoxin in maize through flotation. Similarly, da Gloria (2011) reported substantial reductions of aflatoxin in groundnuts using flotation as a separation mechanism.

Sorting and flotation yield two products: a "clean" or low-aflatoxin fraction and a "dirty" or high-aflatoxin-content fraction. For instance, Piedade et al. (2002) found that the aflatoxin concentration of particulates segregated on the basis of size was approximately three times that of the original maize. If the high-aflatoxin fraction is not discarded, which could easily happen if the sorting were the result of an external demand such as a buyer or importer requirements, consumption of this fraction poses a greater danger than the original material. The efficiency of hand sorting and density segregation as aflatoxin-reduction tools depends on the product type, product size (quantity), and the way the technique is applied. Hand sorting is labor intensive and therefore unlikely to be adopted by mid-level traders and commercial storage facilities, unless demanded by law or the benefits justify its use. However, this method can be useful and effective at the rural household level where quantities in the range of 10 - 15 kg are processed for grinding maize into flour for home consumption.

Dry and Wet Milling

Dry and wet milling are widely used in processing maize. Studies have shown that during dry milling, aflatoxin is primarily segregated in the bran and fines fraction (Njapau et al. 1998; Mutungi 2006), and less than 12 percent of the aflatoxin in the original material remains in the main product (endosperm/grits and low fat flour) (Scott 1984). In wet milling, aflatoxin is segregated primarily in the steep water (40 percent) and fiber and germ (30–42 percent) (Wood et al. 1982; Njapau et al. 1998). When wet milling is used to produce starch, 1 percent or less of the original quantity of aflatoxin ends up in the starch (Yahl et al. 1971). The ability of these processes to yield acceptable products is highly dependent on the initial level of toxin in the original material. Severely contaminated starting material could yield products that may still contain

unacceptable levels of aflatoxin. For example, where a 95 percent reduction is expected, the original material should be at 200 ppb or less for the final product to contain 10 ppb or less.

Heating

Processing operations such as baking, cooking, frying, and roasting utilize heat. Aflatoxin is, however, known to be relatively stable, decomposing at 269°C (Quadri et al. 2010). Heat-induced degradation of aflatoxin has also been shown to be dependent on the matrix and its moisture content (Njapau et al. 1998; Shapira 2004). Thus the extent to which aflatoxin is destroyed during heating is dependent on the commodity and the process applied. Ordinary boiling in water destroys less than 50 percent of the aflatoxin content of a commodity (Christensen et al. 1977; Njapau et al. 1998). When maize flour is cooked into the edible thick maize porridge consumed widely as a staple food throughout Africa, the aflatoxin content of the flour is reduced by about 10–18 percent (Njapau et al. 1998; Mutungi 2006). The cooking step should therefore not be assumed to further reduce aflatoxin substantially. Extrusion cooking can afford similarly low or higher reductions depending on the conditions under which the process is undertaken. Dry roasting groundnuts can reduce aflatoxin by 50–80 percent depending on the temperature and duration of roasting (Njapau et al. 1998; Ogunsanwo et al. 2004). Similar effects are obtained when roasting cocoa beans at 250° C for 15 minutes. Roasting at the temperatures and durations studied affords significant reductions in the content of aflatoxin in groundnuts; however, the product is likely to be discolored to an extent that it becomes less attractive for direct human consumption. But the roasted nuts could be used as a safe protein source for animal feed.

Other Physical Treatments

Solvent extraction relies on the solubility of aflatoxin in various organic solvents such as ethanol, isopropanol, and methanol, and can be an effective means of removing aflatoxin from a food matrix. Aflatoxin extraction from meals using organic solvents is routine and forms the basis for most laboratory analysis methods. However, the process could also remove vital nutrients and alter or diminish desirable sensory characteristics of the remaining food material. It is not practical in a rural setting due to the potential hazard of handling the solvents as well as the associated costs (Shapira 2004).

Several physical procedures can therefore be utilized to reduce the level of aflatoxin in some contaminated commodities. However, procedures such as solvent extraction, irradiation, extrusion, and ozonation are not feasible at the household level. Fractionation, as in dry and wet milling, is feasible for less contaminated commodities; it does not completely eliminate the aflatoxin. Because a fraction of the aflatoxin remains in the food, the safety of the final product depends on the original concentration of aflatoxin.

In addition, the production of alcohol from maize by the wet milling process concentrates the aflatoxin in the spent grain, often used as animal feed. Therefore, of the physical procedures discussed, only sorting and dry and wet milling have potential for application at the rural household level and in less industrialized settings. Winnowing generates maize dust which often contains aflatoxin that may be at levels hazardous to humans in the vicinity of the winnowing operation (Autrup et al. 1991; Desai and Gosh2003; Lai et al. 2014). Once inhaled, aflatoxin dust has detrimental effects which in some aspects are similar to those arising from oral exposure.

Chemical Processing Methods

Several chemical processes have been employed to reduce aflatoxin contamination in maize, groundnuts, and cottonseed. The specific chemicals used include alkalis, acids, and oxidizing and reducing agents. The most widely used and easily adaptable chemical methods are the alkali treatment process, nixtamalization (de Arriola 1987), and ammoniation (Park 1988). Nixtamalization is applied to human food whereas ammoniation is used on commodities destined for animal feed.

Nixtamalization

Nixtamalization is a centuries-old alkaline treatment process for preparing dough (*masa*) for tortillas--flat maize bread commonly consumed throughout Latin America. The traditional process involves boiling maize in hot lime (calcium hydroxide), soaking (steeping), straining, washing, grinding, dough softening, dough shaping, and baking (de Arriola et al. 1987). Over the years, multiple patents have been granted for the original procedure and subsequent modifications of it, including US 6,428,528 B1 of 2002; US 6,265,013 B1 of 2004 and US 8,110,239 B2 of 2012. According to several studies, it is a simple and low-cost decontamination process reported to eliminate 68 – 90 percent of aflatoxin in maize (de Arriola et al. 1987; Perez-Flores et al. 2011). Modifications to the process, such as the use of calcium sulphate (Maya-Cortes et al. 2010), and microwave heating (Perez-Flores 2011), increase its effectiveness, limiting acid-induced reversions to 3 percent as compared to the 33 percent reported by Mendez-Albores (2003) when using the traditional method of tortilla production. However, a recent study has reported cytotoxic and genotoxic activity of tortilla extracts lending support to the earlier observations of acid-induced reversions of the altered aflatoxin molecule to the original toxic chemical structure (Vazquez-Duran et al. 2014).

Overall, the alkali-based process of nixtamalization is a simple and effective decontamination tool that can be applied to maize for human consumption with minimal technology and training. However, consideration should be given to the strength of the alkali solution, duration of exposure to the alkali and the process temperature during the alkali exposure. The lower these parameters are, the less likely that a permanent destruction of aflatoxin molecule would be achieved.

Ammoniation

Ammoniation, like nixtamalization, is an alkali-based decontamination process that can be applied to maize, groundnuts, and cottonseed using gaseous or liquid ammonia. It similarly alters or breaks down and inactivates the aflatoxin molecule (Park1993). The ammoniation process has been extensively studied (Anderson 1983; Park et al. 1988) since it was first patented in 1942 (Patent No. US 2,293,845). It has been used to treat maize, groundnuts, and cottonseed to reduce levels of aflatoxin or increase the non-protein nitrogen content of the product. The ammonia can be applied to whole kernels or meals using two processes that differ by the operating pressure, temperature, and duration. One version uses a short duration (20-60 minutes), high pressure (45-55 psi) and high temperature (80-120°C) (HP/HT) process; the other employs ambient pressure and ambient temperature (AP/AT) and requires 14-28 days duration. Both lead to aflatoxin reductions of up to 99 percent (Table 4).

Table 4: Parameters of the HP/HT and AP/AT ammoniation processes.

	High Pressure/High Temperature (HP/HT)	Ambient Pressure/Ambient Temperature (AP/AT)
Ammoniation level (percent)	0.5 - 2.0	1.0 - 2.0
Pressure (psi)	45 - 55	Atmospheric
Temperature (C)	80 - 120	Ambient
Duration	20 - 60 min	14 - 28 days
Commodities	Maize, groundnuts, cottonseed and meals	Maize, groundnuts, cottonseed, and meals
Infrastructure required	Treatment plant	Bag, tent, or open flat space

Source: Adapted from Park et al.

Over the last 40 years, several studies have demonstrated the efficacy of the ammoniation process. Gardner et al. (1971) reduced aflatoxin in contaminated cottonseed from 5,000 ppb to les than 10 ppb, using anhydrous ammonia at 68°C and 42 psi for 15 minutes. Similarly, Park and co-workers (Park et al. 1984) demonstrated the reduction of aflatoxin levels in cottonseed using the HP/HT process. Using the AP/AT process, Jorgensen and Price (1981) reduced aflatoxin B_1 in cottonseed from 800 ppb to below 20 ppb in 15 days. In follow-up studies, Price et al. (1982) and Bailey et al. (1994) observed similar AP/AT derived reductions of aflatoxin in cottonseed. Comparable observations have been made in studies with maize (Brekke et al. 1977a; Martinez et al. 1994; Weng et al. 1994) and groundnut meal (Neal et al. 2001). More recently, Nyandieka et al. (2009) observed aflatoxin reduction of 88-96 percent in maize ammoniated using the AP/AT process. In the studies with maize (Martinez et al. 1994), a portion of the ammoniated

material was exposed to 0.2 N HCl at 37°C for two hours, at pH 2.0 to simulate stomach or abomasal digestion and acidity in animals. The reversibility of the ammonia-treated aflatoxin molecule to its parent form, based on aflatoxin B_1 analysis, was 0–0.5 percent of the original concentration. Overall, these studies have demonstrated the efficacy of both versions of ammoniation.

Source: Courtesy of Peter Cotty

Figure 1: Ambient pressure/ambient temperature (AP/AT) ammoniation of cottonseeds. Left, inserting temperature sensors; right, ammoniation in process.

Ammoniation has been adopted and is routinely used to reduce levels of aflatoxin in cottonseed and groundnuts in Brazil, France, Mexico, Senegal, South Africa, and Sudan--achieving efficacies similar to those reported by the studies cited above. In the United States, the states of Alabama, Arizona, California, Georgia, North Carolina, and Texas, routinely use the ammoniation process to treat aflatoxin-contaminated cottonseed and maize. To date there have been no reports of detrimental effects to the livestock fed the ammonia-treated materials. When ammonia-treated feed ingredients are fed to dairy cows, the carryover into milk of ammonia treatment related aflatoxin byproducts is less (0.35 percent) than that ordinarily observed (1.4 percent) with untreated feed ingredients (Hoogenboom et al. 2001; Brits et al. 2013; Ronchi et al. 2005).

Table 5: Selected studies showing efficacy of ammoniation.

Type	Initial aflatoxin concentration (ppb)	Final aflatoxin concentration (ppb)	Percent reduction	Reference
Maize				
AP/AT	1000	10	99	Brekke et al. 1977
AP/AT	750	3	99	Hughes et al. 1979
AP/AT	617	~80	87	Bothast et al. 1982
HP/HT	1000	~20	98	Anderson, 1983

Type	Initial aflatoxin concentration (ppb)	Final aflatoxin concentration (ppb)	Percent reduction	Reference
HP/HT	7500	2	>99	Weng et al. 1994
Cottonseed				
HP/HT	120	ND	>99	*Gardner* et al. 1971
AP/AT	800	10	99	Jorgensen et al. 1981
AP/AT	5200	10	99	Bailey et al. 1994
HP/HT	1200	ND	>99	Bailey et al. 1994
AP/AT	700-800	5-20	97-99	* al. 2002

AP/AT - ambient pressure/ambient temperature
HP/HT - high pressure/high temperature
ND – None detected
***Unpublished**

Nevertheless, ammoniation has not yet been approved by the FDA as a means of removing aflatoxin from agricultural commodities. It is however approved as a means of increasing non-protein nitrogen in cottonseed meal (21CFR573.140), rice hulls (21CFR573.160), and maize silage (21CFR573.180) for use as ingredients in the feed of ruminant animals. The FDA's reluctance to approve ammoniation as a means of deactivating aflatoxin in agricultural commodities is partly based on what the agency considers as insufficient data on the safety of two byproducts of the process--aflatoxin D_1 and a molecular weight 206 compound. The two compounds constitute less than 1 percent of the aflatoxin in the original material (Shapira 2004), and their mutagenicity is ~400 times less than that of the parent aflatoxin B_1 (Park et al. 1988).

On a weight-of-evidence basis, therefore, ammoniation is a highly efficacious process that can be used to substantially decrease levels of aflatoxin in contaminated maize or groundnuts for use as livestock feed ingredients. It requires materials and safety procedures that may not be attainable in a rural setting, however, limiting the application of ammoniation to a semi-industrial scale.

Chemical Binders/Absorbents

Studies have been underway for several decades on the use of clay-based chemical binders, such as calcium bentonite, in the aflatoxin contaminated diets of animals, with very positive results. However, further study is needed to establish the parameters under which feed treated with calcium bentonite binding products, such as NovaSil™, can produce the strongest protective effects against aflatoxicosis. NovaSil belongs to a class of chemical absorbents, including aluminosilicates, phyllosilicates, and activated carbon, that reduces the absorption of aflatoxin from the gastrointestinal tract. The most widely studied of these enterosorbents

are NovaSil and the organo-sulfur dithiolethione Olipraz™ (Kensler et al. 2011). Phillips (1999) reviewed the principle and use of hydrated sodium calcium aluminosilcate (HSCAS) as an enterosorbent for chemoprevention of aflatoxicosis. He concluded that HSCAS was efficacious and showed great promise as a tool for the amelioration of aflatoxin toxicity. The safety (Morris et al. 2014) and efficacy (Afriyie-Gyawu et al. 2005) of NovaSil in humans were demonstrated in subsequent studies although substantial research on larger study groups would be required to consider its safety and related ethical issues for application to the general population. In other studies, other clays such as montmorillonite have been shown to bind aflatoxin in the gastrointestinal tract reducing the absorption of aflatoxin by 88 percent (Mitchell et al. (2014). Similarly, glucamannon (MTB-100™) has been shown to bind up to 80 percent of aflatoxin in distillers' dried grains with solubles (DDGS) (Johnston et al. 2012).

Working under the hypothesis that the addition of clay binders to animal diets tightly binds and immobilizes aflatoxins in the gastrointestinal tract, Abdel-Wahib et al. (1998) found that the "addition of bentonite or HSCAS to the AF-contaminated diet [of rats] diminished most of the deleterious effects of the aflatoxin. Pathological examinations of liver and kidney proved that both bentonite and HSCAS were hepatonephro protective agents against aflatoxicosis."

In 1999, Abdel-Wahib et al. used the pregnant rat as an in vivo model to compare the potential of HSCAS and bentonite to prevent the developmental toxicity of aflatoxin. The method was as follows: "Aluminosilicates (HSCAS) and bentonite were added to the diet at a level of 0.5 percent (w/w) and fed to the pregnant rat throughout pregnancy (i.e. days 0-20). Test animals were fed an aflatoxin contaminated diet (2.5 mg kg (-1) diet) with or without sorbents during gestation days 6-15. Evaluations of toxicity were performed on day 20. These included maternal (mortality, body weights, feed intake and litter weights), developmental (embryonic resorptions and fetal body weights) and biochemical (ALT, AST and AP) evaluations. Sorbents alone were not toxic and aflatoxin alone resulted in significant maternal and developmental toxicity. Animals treated with phyllosilicate (plus aflatoxin) were comparable to controls following evaluations for resorptions, live fetuses and fetal body weights, as well as biochemical parameters. While bentonite plus aflatoxin resulted in significant reduction in fetal body weight, none of the fetuses from HSCAS or bentonite plus aflatoxin-treated groups had any gross, internal soft tissue or major skeletal malformations."

A 2000 study found that addition of sodium bentonite to the diets of chickens was effective in ameliorating the negative effect of aflatoxicosis on the percentage and mean of phagocytosis" (Ibrahim et al.).

The long-term effect of enterosorbants on humans remains under scrutiny. Wang et al. (2005) undertook a study to determine the experimental conditions under which NovaSil clay can be

safely tested in humans to determine if it can diminish exposure and health risks from aflatoxin contaminated food. Adult volunteers (n=50) were divided into two groups, one receiving a low dose (13.5 grams) and the other a high dose (27 grams) of NovaSil clay in tablet form for two weeks. No significant differences were shown in hematology, liver and kidney function, electrolytes, vitamins A and E, and minerals in blood tested before and after the trial in either group. While these results may demonstrate the relative safety of NovaSil clay in a very limited number of otherwise healthy adults living in the U.S. over a short time exposure, many ethical questions remain for applications in developing countries. This would imply the willful encouragement of consumption of toxic and contaminated foods. Additionally, there is no substantial research regarding long-term use among human subjects with compromised health status as one might encounter in developing country settings.

Jaynes and Zartman (2011) produced a highly informative summary of the status of research on the history and chemistry of bentonite and other clay additives in the fight against aflatoxin. This study serves well as a starting point for researchers and officials who seek to understand the benefits of enterosorbant technologies in detail.

To date, the FDA has yet to approve the use of chemical absorbent clays specifically as a means of removing aflatoxin and other mycotoxins from agricultural commodities for either animal or human consumption. Bentonite is approved for use as a feed additive when used in accordance with good manufacturing or feeding practice (21CFR§582.1155). The FDA has also approved bentonite as a food additive, affirming "this ingredient is generally recognized as safe (GRAS) as a direct human food ingredient… based upon the … current good manufacturing practice conditions of use (21CFR§184.1155)."

Despite the fact that the FDA does not allow feed additive companies to claim that bentonite and related clay binders function as mycotoxin absorbents, NovaSil and other products are used widely for this purpose across the United States and Europe, where NovaSil is marketed as NovaSil/Plus. While it has also been reported that clay binders are currently being used throughout the commercial dairy and poultry sectors across East Africa, hard data to corroborate this is not available at this time. Feed industry experts in the U.S. report that FDA approval for specific use against aflatoxin and other mycotoxins may soon be coming, which may serve to boost the research and development and marketing efforts of the feed additive industry. The state of Texas is leading the way. In 2011, Texas approved NovaSilPlus-TX™ for use as an additive in aflatoxin-contaminated feed, as long as the user files specific paperwork with the Office of the Texas State Chemist.

Some research has been devoted to the hypothesis that the chlorophyll in green vegetables can enhance aflatoxin binding. The modulation of aflatoxin toxic effects has been demonstrated with leafy green vegetables (Egner et al. 2001), broccoli sprouts (Kensler et al. 2013), and green tea (Tang et al. 2008).

Disposal of Aflatoxin Contaminated Commodities

Aflatoxin is classified by the World Health Organization (WHO) as a Class I carcinogen (IARC2012). It is also highly toxic to most living organisms. Once formed, aflatoxin may remain stable in the native matrix for an extended period of time. It has been reported to be stable for at least two years in groundnuts, posing a serious risk to birds, animals, and other organisms that may consume the nuts at a disposal site. In this regard, material containing aflatoxin and destined for disposal should be classified as toxic waste, thereby placing such material under the auspices of a country's health and environmental management authorities. Regulations on the classification and disposal of aflatoxin commodities are sparse outside of the U.S. and the EU. In the U.S., however, aflatoxin-contaminated crops are not classified as toxic waste as defined by the U.S. Environmental Protection Agency (40CFR 261.24). Furthermore, there seems to be no specific protocol prescribed for the disposal of aflatoxin-contaminated commodities at the federal level. Chapter 7 of the U.S. Code of Federal Regulations has guidelines on "disposal of meal contaminated by aflatoxin," (7 CFR, 1446.413). However, the instructions state only that the "the meal be disposed of for non-feed purposes only" but does not elaborate how the disposal should be carried out. A similar situation seems to exist in the regulations of the EU. Therefore, aflatoxin contaminated agricultural commodities generally remain under the umbrella of agricultural waste, the disposal of which would not have stringent restrictions.

On the contrary, within the East Africa region, a large quantity amounting to hundreds of thousands of tons of contaminated maize presents a different disposal scenario. Aflatoxin-contaminated maize or groundnuts could be buried in the soil, incinerated, or discarded into the seas. But there are problems with each approach.

First, it would require considerable amounts of money to incinerate, openly burn, grind and disperse, or bury it. Of the options listed, open-air burning may violate environmental laws as it would generate considerable amounts of smoke. In Zambia for instance, the Environmental Management Act of 2011 prohibits open-air burning and discharge of untreated toxic waste into the environment. In addition, disposal efforts can be costly. In a now familiar initiative by individual states, the *Los Angeles Times* of May 19, 1990 reported that the Iowa Department of Natural Resources spent USD $90,000 to dispose of 13,000 bushels of maize contaminated at 32,000 µg/kg.

The foregoing underscores the need for straightforward assignment of responsibilities regionally and for each of the EAC partner states, coupled with clear protocols on disposal of potentially large quantities of contaminated agricultural products. Such protocols do not currently exist. Instead, most regulations refer to disposal "in a safe manner" and "neutralization", "conditioning," or "treatment," without elaboration. For a number of reasons, it would then appear that processing prior to final disposal is the preferred system.

Disposal by Burying

Aflatoxin contaminated agricultural commodities can be disposed of by burying. Soil contains numerous microorganisms, some of which have been shown to degrade aflatoxin. *Flavobacterium auranticum* degrades aflatoxin in soil with some strains of this bacteria degrading aflatoxin within 72 hours (Wu et al. 2009). Similarly, *Mycobacterium fluorantheni vorans* (McCormick 2013), *Psuedomona saeriginosa* (Sangare et al. 2014) as well as *Aspergillus niger* (Zhang et al. 2014) degrade aflatoxin. Furthermore, aflatoxin has been shown to bind tightly to some clays, such as those rich in sodium calcium aluminosilicates (Williams et al. 2004). Such binding can sequester the aflatoxin, making it less harmful to susceptible soil biota. Microbial degradation is possibly responsible for the rapid conversion of aflatoxin in soil reported by Angle (1987). In the study by Angle, aflatoxin B_1 is reported to have been rapidly converted to the less toxic aflatoxin B_2 within 6 days, followed by the subsequent degradation of the aflatoxin B_2 in 77 days. Angle further reported that aflatoxin decomposed more rapidly in silt loam soils. The efficacy of the silt loam soil could be attributed to a higher prevalence of the aflatoxin degrading bacteria or aflatoxin binding clay that made the toxin less extractable from the soil.

Disposal of aflatoxin contaminated material by burying may also have its disadvantages. There is evidence, though not conclusive, that some plants may take up aflatoxin from the soil. Hariprasad et al. (2013) found aflatoxin in green leafy vegetables and the soil in which they were growing. The uptake of aflatoxin from soil by the vegetables was confirmed in subsequent greenhouse experiments. The phenomena was later demonstrated and confirmed by the same research team in sugarcane (Hariprasad et al. 2014) and hydroponic experiments with groundnuts (Snigdha et al. 2015). Therefore, burial of aflatoxin contaminated commodities may have to be done at depths below the root levels of food crops. If the contaminated material is buried at sufficiently deep levels, this form of disposal is unlikely to raise any environmental concerns.

Disposal at Sea

Disposal at sea is feasible given the quantities of the water bodies and the likely dilution effect. However, the material could become feed for marine species in the disposal area, some of which are susceptible to aflatoxin contamination, such as the rainbow trout (Williams 2012). Other studies have demonstrated the susceptibility of different species of fish to aflatoxin. Lim et al. (2001) exposed Tilapia (*Oreochromismoss ambicus*) in aquaculture to palm kernel based diets containing 75 to 100 ppb aflatoxin and demonstrated a reduction in growth rates of 30 and 40 percent, respectively. Similarly, Raghavan et al. (2011) showed that 50 percent of hybrid sturgeon raised in fiberglass tanks and exposed to diets containing 40 ppb aflatoxin died over a period of 35 days. The

growth rates of the surviving fish was, however, not different from controls. Al-Faragi et al. (2014) showed that common carp (*Cyprinus carpio*) fed diets containing 4,000 and 6,000 ppb gained only half the weight of controls and had 25-30 percent less survival. Al-Faragi did not expose the fish to levels less than 4,000 ppb.

On the other hand, other studies have not shown any detrimental effects of aflatoxin to aquatic species. Jantrarotai and Lovell (1990) showed that channel catfish *(Ictalurus panctatus*) tolerated aflatoxin levels in the range of 2,000 ppb and only showed depressed growth rates at 10,000 ppb. In addition, Huang et al. (2011) did not observe any differences in survival rates and body weight gains in Gibel Carp (*Carassius gibelio*) fed up to 1,000 ppb aflatoxin diet. Furthermore, Manning et al. (2005) and Hayat et al. (2013) showed that aquaria-raised Channel Catfish (*Ictalurus panctatus*) and Nile Tilapia (*Oreochromis niloticus*) fed diets containing 220 ppb and 25 ppb, respectively, did not differ from their controls in weight gains. Clearly, different species of fish will respond differently to aflatoxin challenges. However, aflatoxin levels that seem to elicit a negative response are high and unlikely to be attained in the aquatic environment of material disposed of at sea. The disposal of contaminated milk (0.5 ppb) in the Arizona case was hence unlikely to pose any danger to aquatic biota. It should however be pointed out that disposal of toxic waste into the sea is currently not permitted in the EAC.

Disposal by Incineration

Incineration, if carried out to completion, is probably the most effective disposal process, as it completely destroys the aflatoxin molecule. Aflatoxin decomposes at 269°C (Quadri et al. 2010) and incineration temperatures often reach upwards of 500°.

Incineration can be carried out as an open air operation or in kilns. In practice however, incineration is difficult to perform effectively. Several thousand tons of maize or groundnuts are unlikely to burn easily in the open. It is likely to take several days or even weeks to burn maize or groundnuts to completion by open-air incineration. The use of kilns is more efficient but limited by the capacity of the kiln. There were several reports of interest at a November 2014 EAC meeting of experts convened to review this paper:

- Incineration of contaminated barley has been successfully carried out in Uganda.
- In Kenya, a facility in a cement factory is incinerating several thousand tons of contaminated maize in kilns as a source of energy to generate steam for mill operations at the factory.
- The maize was being mixed with combustible oils to enhance the efficacy of the incineration process. This use of contaminated maize, if optimized, has incidental benefits and minimizes environmental pollution as the dispersal of the emissions is

controlled. Incineration residue, largely minerals, could be used as a supplement to agricultural fertilizer.

On the other hand, open-air incineration raises environmental concerns in a number of countries. Emissions from open-air burning are often not controlled and permeate the surrounding environment, conveyed and dispersed by prevailing winds. The products of incineration of plant materials are reported to include highly toxic polyaromatic hydrocarbons that can be detrimental to the biota in the vicinity of the operation (Lemieux1997; Lemieux et al. 2004; Derrough et al. 2013). Because of this, countries such as Zambia prohibit open-air burning of waste (ZEMA 2011). This form of disposal is hence only suitable for very small quantities of materials.

Situational Analysis: The East Africa Region

Geography and Climate

The EAC region is located between latitude 50 N and 120 S and 28 E and 42 E longitude, generally straddling the area between the East African Rift Valley and the Indian Ocean. It comprises Burundi, Kenya, Rwanda, Tanzania, and Uganda, covering a total area of approximately 1.8 million square kilometers, of which about 5.6 percent is in the form of water bodies (EAC 2014). The climate varies from warm, humid coastal regions, traversing the mild plateau region to the Rift Valley on the western edge of the region. The temperature and precipitation similarly vary, from 14°-29° C and from 10 to 244 mm respectively (EAC 2014), offering favorable conditions for aflatoxin producing *Aspergillius flavus* to flourish.

Aflatoxin in Food in the EAC

The contamination of maize and groundnuts by aflatoxin is widespread in the EAC (Kaaya et al. 2005; Daniel et al. 2011; Yard et al. 2013). Initial research on aflatoxin was centered in Kenya and Uganda, focusing on affirmation of the postulated relationship between exposure to the aflatoxins and the occurrence of HCC (Korobkin and Williams, 1968; Peers and Linsell, 1973; Cameron and Warick 1977). Thereafter, an association with other human and animal health conditions began to emerge. The 2004 aflatoxicosis outbreak in Kenya with its high number of fatalities sparked a renewed interest in aflatoxins in the EAC. Kaaya and Warren (2005) have extensively reviewed aflatoxin research in Uganda, while several investigators have reported on various aspects of aflatoxin contamination in Kenya (Kiswii 2006; Mutegi 2010; Strosnider et al. 2006; Kang'ethe and Lang 2009). Currently the focus is on the molecular and physiological mechanisms of response to

exposure to aflatoxins, particularly in infants and young children (Shirima et al. 2013; Yard et al. 2013; Castelino et al. 2014; Magahi et al. 2014).

The EAC has promulgated regulations that stipulate that food and feed cannot contain more than 10 ppb of aflatoxin (EAS 2: 2013). Non-compliant food or feed must be disposed of. Table 6 shows data from several surveys for aflatoxin contamination of maize and groundnuts in Kenya and Uganda over several years. The data includes total EAC maize and groundnuts production for each year studied, how much of the crop was contaminated above the statutory limit of 10 ppb, and a percentage transformation of the contaminated quantities. The geometric mean prevalence of contamination for maize over the years studied was calculated to be 41 percent. In other words, close to half the maize produced over the indicated years would have been declared unfit for human and livestock consumption if the 10 ppb standard were to be enforced. Approximately 3.3 million metric tons would be destined for disposal or an alternative use each year.

Table 6: Aflatoxin contamination of maize and groundnuts in the EAC.

Crop	Study year	EAC maize production (mMT)	Quantity above 10 ppb (mMT)	percent of maize above 10 ppb	Reference
Maize	1990	5.6	1.7	30	Kaaya et al. 2005
Maize	2004	8.5	4.3	51	Lewis et al. 2005
Maize	2005	7.4	3.0	41	Daniel et al. 2011
Maize	2006	8.1	4.1	51	Daniel et al. 2011
Maize	2006	8.0	6.7	83	Okoth & Kola 2012
Maize	2007	8.0	1.3	16	Daniel et al. 2011
Maize	2013	11.0	5.0	45	Kilonzo et al. 2014
Groundnuts	2003	0.6	0.3	49	Kaaya et al. 2006
Groundnuts	2009	0.7	0.2	32	Mutegi et al. 2010
Groundnuts	2013	1.1	0.8	73	Osao 2014

Processing for Alternative Use

Given the large quantities of maize and groundnuts that are likely to be declared unfit for direct use under enforcement of the 10 ppb standard, the EAC needs mechanisms that will facilitate the use of such commodities for purposes other than direct human food and animal feed. This includes identifying procedures and best practices that could be applied to reduce the content of aflatoxin in the commodity.

The techniques vary from simple hand sorting, and dry and wet milling, to more sophisticated and technologically demanding processes such as High Pressure and High Temperature (HP/HT) ammoniation, and the use of chemical adsorbents. The suitability of these techniques to the indigenous EAC setting may differ from one partner state to the other. Hand sorting and dry and wet milling are simple, cheap, and technologically feasible for use at the household level, provided there are adequate controls to remove the rejected commodities, foods and byproducts from consumer reach. While hand sorting is practiced routinely by households across the region, the best portion is often sold, and the second quality consumed by the household, with the damaged and contaminated lot dedicated for animal feed and home brewed alcoholic beverages. This poses issues of transfer rates from animal products to humans, lower animal productivity, and aflatoxicosis from beverages. The production of *muthokoi*, a dehulled and cracked maize kernel similar to grits produced by a semi-dry/semi-wet milling process is widespread across Kenya (Mutungi 2006). The production process has been shown to substantially reduce the aflatoxin in the final product (Mutungi 2006; Kilonzo et al. 2014). The process is feasible and efficacious, particularly if applied at a household level where it could be preceded by hand sorting to further reduce contamination levels.

Of the chemical processes that have been shown to reduce aflatoxin contamination in maize, groundnuts, and cottonseed, the most applicable to conditions in the EAC are nixtamalization and ammoniation. Both are alkali based and are similar to the use of *iati*, a locally made bicarbonate solution. Mutungi (2006) indicates that a solution of 0.5 percent *iati* applied to *muthokoi* reduced the content of aflatoxin by 86 percent. Furthermore, Nyandieka et al. (2009) have investigated the feasibility of using ammoniation to reduce aflatoxin in Kenya maize and observed an 88 percent destruction of aflatoxin B_1. These investigations need to continue to form the basis of a strengthened regulatory environment for alternative uses and disposal systems, while also providing more beneficial information.

Overall, the EAC is encouraged to promote the use of hand sorting at the household level provided there are adequate controls on disposal of contaminated lots, and to further explore the potential of nixtimalization and ammoniation.

Disposal of Aflatoxin Contaminated Commodities in the EAC

Disposal of aflatoxin contaminated commodities, especially in the quantities shown in Table 6, has yet to be considered in EAC policy-making. It poses serious food security, financial, and logistical challenges. Further complicating such a mammoth task are the legal statutes of the EAC partner states, some of which forbid certain alternative uses and disposal methods. Nevertheless, contaminated agricultural commodities that are

determined unsuitable for any purpose could be, as earlier discussed, buried or incinerated depending on the feasibility of the process as well as the environmental statutes.

Each of the five partner states has national statutes that impact diversion to other uses or outright disposal. The spectrum of legislation relates primarily to food, health, and the environment. The laws relating to food have more of a hygiene and/or consumer safety role in all cases, and are not likely to seriously impede waste disposal.

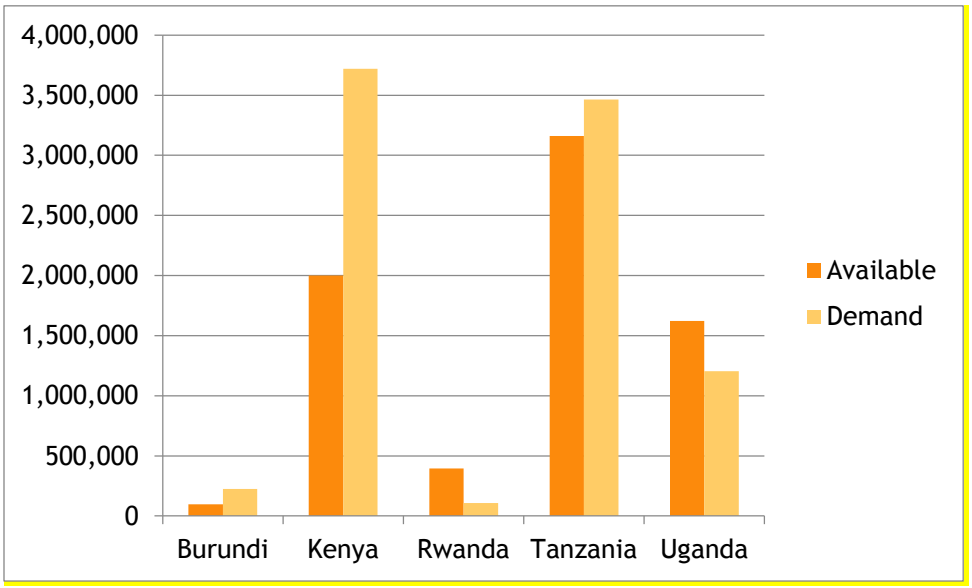
Conversely, environmental laws and regulations will have a serious bearing on the disposal of aflatoxin contaminated commodities. In the Kenyan Environmental Management and Coordination Act No. 8 of 1999, the definitions of "hazardous waste" and "pollutant" encompass, though they do not explicitly state, aflatoxin contaminated commodities. Sections 42 (1) and 93(1) prohibit their disposal into the environment, in particular, the aquatic environment, without specific authorization. Uganda's National Environment Act (Cap 153) defines hazardous waste in a way that could be interpreted to include aflatoxin contaminated commodities. Thus, their disposal into the environment becomes severely restricted (Sections 34, 52, and 56). The Tanzanian Food, Drugs and Cosmetics Act (2003) can be read to be somewhat accommodating to aflatoxin contaminated commodities. Section 32(2) of the act refers to disposal "in a prescribed manner." A follow-up government gazette notice, namely, the Tanzania Food, Drugs and Cosmetics (Treatment and Disposal of Unfit Food) Regulations 2006, offers more detail regarding alternative use and disposal. Section 5(1) permits "reconditioning of food in order to make it fit for human consumption or diversion to other uses." Section 9(4) of the same regulation alludes to a form of disposal that could fit either burying or incineration. The section stipulates that the food so rendered unfit should be "completely removed from channels of commerce and rendered impossible to be eaten."

However, although the Kenyan and Ugandan statutes present a more difficult scenario for disposal of aflatoxin contaminated material, and are not as clearly accommodating as the Tanzanian regulations, they both refer to "authorization." It appears therefore that burying and incineration can still be used as a form of disposal provided permission is granted by the authorities. The burying could be done at depths below the reach of the roots of food crops to prevent xylem-facilitated uptake. Incineration has to be carried out in a manner that minimizes toxic smoke coming into contact with humans, particularly workers at the facility.

Disposing of Contaminated Maize

In 2013, the EAC population stood at 143.5 million (EAC 2014). Some 68 percent of this population residing in Kenya, Tanzania, and Burundi, uses maize as a staple food. The annual per capita consumption of maize was estimated to be 66 kg during the last decade (CYMMIT 2007), rising recently to146 kg (ILRI 2013). The total maize production for 2013 was

estimated at 12.3 million metric tons. Between 18-21 percent of this quantity is used for livestock feed (Shiferaw et al. 2011). Based on an estimated level of 41 percent of regional maize supply exceeding the maximum tolerance level of 10 ppb, Figure 2 compares the availability of maize against demand within each of the five partner states of the EAC for 2013. This reflects current standards used for both human food and animal feed.



Source: Adapted from FAOSTAT2013

Figure 2: Maize meeting the 10 ppb limit compared to maize required for human consumption in EAC 2013 (thousand metric tons).

Figure 2 illustrates that, had the standard been fully enforced during the year 2013, it would have created a dire food security crisis, both nutritionally and economically. Available maize fit for human consumption would have totaled 42 percent of the demand for Burundi; 53 percent of the demand for Kenya; 91 percent of the demand for Tanzania; 135 percent of the demand for Uganda; and 368 percent of the demand for Rwanda.

Rwanda and Uganda would have had excess maize, but the total excess from those two countries is only 20 percent of the deficit for the other three partner states. This does not address the need for adequate economic demand for this maize based on higher prices that would be needed to motivate traders to move the grain from areas of surplus to deficit regions. Additionally, the quantities to be disposed of would significantly affect food security and the nutritional well-being of a majority of residents of the EAC, while also presenting an insurmountable disposal problem. Immediate measures to adequately address contamination in the staple food supply, from field to fork, are a priority. Similarly, the phasing in of enforcement of the 10 ppb standard warrants deep reflection and a rational implementation plan weighing both benefits and consequences.

Conclusion

Since food is life, its availability and safety are paramount. Maize and groundnuts are major staple food crops in the EAC. The safety of EAC maize and groundnuts is often compromised by contamination with aflatoxin, as is cassava, rice, fish, fruits and milk. Aflatoxin is produced in these crops by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, which readily infect the crops as a result of the favorable EAC climate, and aflatoxin is virtually ubiquitous in susceptible food crops in the region.

Consumption of foods contaminated with aflatoxin may result in ailments including liver disease, stunting and low birth weight in infants and young children, compromised immune systems and sometimes death. Public health concerns often lead national authorities to deny entry to aflatoxin contaminated foodstuffs from other countries. Aflatoxin contamination is therefore, not only a public health issue; it is also a threat to attaining the key Millennium Development Goals of eradicating poverty and hunger through economic growth, and reducing childhood morbidity and mortality. Consequently, the EAC and its partner states should strengthen mechanisms to minimize human exposure to aflatoxin. One such mechanism is to establish a maximum acceptable level of aflatoxin in foods and feed.

Since aflatoxin is ubiquitous in crops and food products, often at levels deemed hazardous to humans and livestock, enforcement of the standard would lead to the segregation of “clean” and contaminated foods and feed. This classification could result in the accumulation of large quantities of staple foods that could not be used for human or animal consumption, hence requiring mechanisms for alternative usage or disposal. Potentially, the 10 ppb standard would classify approximately 41 percent of the EAC maize and groundnuts as unsuitable for either humans or livestock in an average year.

Aflatoxin contaminated commodities destined for alternative uses can be used directly, as in the cascading system, or undergo processing prior to use. Several physical and chemical procedures have been examined for before-use processing. Of these, only a few would be suitable for application at the household and semi-industrial level. Hand sorting, and dry and wet milling, are efficacious physical processing techniques that can be used at a rural household level. They are easily adaptable since the processes are similar to the production of *muthokoi* from maize, commonly practiced in Kenya. The cooking of maize meal is not effective in further reducing the aflatoxin content.

Of the chemical processes, nixtamalization is easily adaptable at the rural household level. It is simple and similar to a bicarbonate process (*iati*) widely used in Kenya. However, under some circumstances, nixtamalized products may be as toxic as their parent products. Ammoniation is efficacious but is more appropriate at a semi-industrial level because it has attendant safety requirements.

There are three potential disposal options: burying, incineration, and putting out to sea. Disposal into any water bodies is illegal in the EAC. Burying is possible but must be deep enough to prevent absorption of the toxin by food plants through the roots. With the huge quantities that would be disposed of, the difficulties of digging large enough pits to accommodate the material may become a constraint to the adoption of this mode of disposal. Incineration affords complete destruction of the aflatoxin, and the residue can be used for soil enrichment as supplemental fertilizer. The process may however produce toxic chemical constituents in the smoke emissions, and these may pose a health hazard to humans. Furthermore, ordinary open-air incineration of large quantities of maize is not efficient, as the material burns slowly and may smolder for weeks.

The disposal of aflatoxin contaminated material therefore remains a huge challenge. Since no official disposal protocols are currently available, the EAC will have to develop its own guidelines. Notwithstanding the health benefits of reducing exposure to aflatoxin, enforcement of standards for contamination, and resultant diversion of foodstuffs, would need to take into account the socio-economic and food-security needs of the population.

Further complicating such a mammoth task are the legal frameworks that exist within the statutes of the EAC and partner states. The existing legal frameworks are not supportive of either alternative uses or disposal. The environmental statutes in most partner states lead to the classification of aflatoxin as polluting toxic waste. The specific laws that may seriously affect disposal are:

1. Kenya Environmental Management and Coordination Act No. 8 of 1999.
2. Uganda National Environment Act (Cap 153).
3. Tanzanian Food, Drugs and Cosmetics Act (2003), and the Tanzania Food, Drugs and Cosmetics (Treatment and Disposal of Unfit Food) Regulations, 2006.

Overall, the EAC is encouraged to work closely with authorities in the partner states to develop and harmonize alternative use and disposal mechanisms. The success of these endeavors depends on how the EAC works with critical stakeholders in all the partner states. The health and environmental laws will affect the success of alternative use and disposal efforts, respectively. Hence, putting in place clear guidelines and regulations that satisfy health and environmental authorities is paramount. There is also need to develop specific statutes in the health and environment arenas that clearly refer to and classify aflatoxin and aflatoxin contaminated materials in relation to their use and handling. Another key to success is the interfacing of the proposed mechanisms with related existing systems. As Wu and Khlanwiset (2010) point out, there will be need to closely monitor governmental and community acceptance; identify potential social and cultural impediments; understand the cost of the processes to be introduced; and identify and mitigate potential health and ecological risks.

Policy Recommendations

The EAC and partner states are encouraged to:

1. Develop and adopt harmonized policies and program frameworks (codes of practice) for alternative uses and disposal systems for aflatoxin contaminated commodities. The policies and programs should further be reflected at the national level within individual EAC partner states.
2. Mainstream aflatoxin issues into national development priorities so as to promote public awareness and political will to address aflatoxin issues, thereby strengthening policy implementation and the appropriate integration of alternative use and disposal systems programs.
3. Review and modernize current regulations determining allowable standards, practices, and alternative uses of contaminated commodities to maximize their potential economic value, while also protecting public health.
4. Prioritize opportunities to support the livestock and fisheries industries, and the production of energy through biomass, in the modernization process of alternative uses.
5. Support initiatives to address the different needs of formal and informal trade and agriculture, which will influence the effectiveness, sociocultural relevance, and economic viability of proposed alternative use and disposal systems.
6. Develop special alternative use and disposal systems models, and consider social safety nets, for subsistence farmers and other small-scale producers with high levels of on-farm consumption of aflatoxin-prone foods and use of contaminated feeds.
7. Build capacity to conduct scientific research into novel and appropriate technologies to use agricultural products moderately contaminated with aflatoxin, and to establish, monitor, and manage rapid disposal systems for highly contaminated commodities.
8. Develop an agenda of short-, medium- and long term goals to modernize alternative use and disposal systems, while balancing food and feed safety with potential consequences on national food security and household nutrition.
9. Collaborate with the private sector food and feed industry to utilize all effective processing techniques that covert unsafe food and feed to edible and safe products.
10. Strengthen the EAC Food Safety Coordination System to ensure adequate formation of regional harmonized food safety standards at the regional and national levels.

List of Terms and Definitions

Term	Definition
AP	Atmospheric Pressure
ALARA	As Low As Reasonably Achievable
AT	Ambient Temperature
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
DDGS	Distillers' dried grains with solubles
EAC	East African Community
EPA	Environmental Protection Agency
EU	European Union
FDA	U.S. Food and Drug Administration
GCC	Global Climate Change
GRAS	Generally recognized as safe
HCC	Hepatocellular carcinoma
HP	High pressure
HT	High temperature
kgbw	kg body weight
ppb	Parts per billion
USDA	U.S. Department of Agriculture
USA	United States of America
WHO	World Health Organization

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