CHAPTER 7.

Intervention strategies to reduce human exposure to aflatoxins and fumonisins

This chapter reviews a broad range of interventions associated with the reduction of aflatoxin and/or fumonisin exposure that have proven health benefits at a community level and are suitable for implementation in rural Africa and Central America. The interventions vary in resources required, complexity, and useful scale. For effective implementation, all require social consent and political will. Some interventions are complicated and resource-intensive, and others are simple to implement on a community or even household scale. Nonetheless, all are unified by the need for cultural and sex-specific training, access to robust technology for implementation, and sustainability. Some of the interventions require further work to verify their efficacy in areas of high aflatoxin exposure.

The Working Group assessed the question of effective interventions in low-income countries using studies where there was reliable direct or indirect evidence of improvement of health, including reduced mycotoxin biomarker levels. The evaluation of evidence about public health interventions includes examining the credibility of the evidence as well as its completeness and its transferability at an individual, community, or national level. The "best quality" evidence (i.e. indicating that an intervention is ready for implementation) is for an approach that has reached a mature stage of development, results in significant intervention effects, and addresses the needs of important stakeholders (Rychetnik et al., 2002). Fifteen interventions were placed into one of four categories: (1) sufficient evidence for implementation, (2) needs more field evaluation, (3) needs formative research, and (4) no evidence or ineffective. Recommendations on how to approach the necessary further investigation and potential scale-up were also considered. The results of these evaluations are summarized in Table 7.1. The following text provides an analysis of the respective interventions.

Regulation

Although they are not explicitly discussed as interventions, corporate, international, and governmental regulatory frameworks can be important drivers in the reduction of mycotoxin levels in food and feed. The available evidence shows that the development of a functioning food safety system begins in the corporate sector, both for domestic consumption and for export crops

Table 7.1. Summary of the Working Group's evaluation of interventions associated with the reduction of aflatoxin and/or fumonisin exposure

Intervention	Category of evidence[a]	Context	Gap (research/translation)	Combination/issues/comments
Dietary diversity	—[b]	**Dose effect reduction of hepatocellular carcinoma**	• Investment in appropriate crops for the target region, both suitable for the climate and culturally acceptable	**Comment:** Difficult in food-insecure situations or in food-, arable land-, or water-insecure countries
Genetic resistance		**Contamination**		
Aflatoxin in maize	3		• Movement of resistance in agronomic lines • Identification of resistance genes	**Combination:** Biocontrol; agronomic and post-harvest practices **Issues:** Small research community; large environmental effect on phenotype expression; resistance is polygenic
Fumonisin in maize	2		• Movement of resistance in agronomic lines • Identification of resistance genes	**Combination:** Agronomic and post-harvest practices **Issues:** Small research community; large environmental effect on phenotype expression; resistance is polygenic
Aflatoxin in groundnuts	4		• Identification of sources of resistance • Movement into agronomic lines	**Combination:** Biocontrol; agronomic and post-harvest practices **Issues:** Large environmental effect on phenotype expression limits resistance expression over large areas; small research community; resistance is polygenic; resistance is not well described
Biological control		**Contamination**		
Atoxigenic strains	2		• Frequency and outcomes of genetic recombination • Consistency of efficacy evaluated across geography and users	**Combination:** Agronomic and post-harvest practices **Comment:** Ongoing translational research in Africa and the USA
Primary prevention		**Dose effect**		
Dioctahedral smectite clay	2		• Dose and duration on efficacy and safety • Effects on infants, children, and pregnant women	**Combination:** Clay amended with chlorophyllin and other trapping agents **Issue:** Formulation strategies **Comments:** Possible enhanced efficacy during outbreaks; potential to mitigate aflatoxins and fumonisins
Chlorophyllin	2			
Lactobacillus	3			
Yeast glucan	4			
Post-harvest		**Dose effect/ contamination**		
Package	1		• Knowledge translation is cultural • Modules need to be developed in partnership with farmers, area agricultural extension workers, traditional leaders, church groups, health workers, and civil society	**Comments:** Ready to be implemented; use in chronic-exposure situations as an ongoing intervention package; needs to be applied as a multifactorial intervention package
Sorting	1		• Done in all cultures for all crops; however, best practices need to be formally taught at the village level	**Issue:** Fate of the rejected food **Comment:** Important for complementary food
Nixtamalization	1		• Requires adequate water for washing • Has not been adapted in Africa or Asia	

Table 7.1. Summary of the Working Group's evaluation of interventions associated with the reduction of aflatoxin and/or fumonisin exposure (continued)

Intervention	Category of evidence[a]	Context	Gap (research/translation)	Combination/issues/comments
Chemoprevention		**Dose effect**		
Broccoli sprout extract	2		• To date, phase II clinical trials for efficacy; need for scaling to longer-term interventions • Translation to local, culturally acceptable foods with these enzyme inducers • Biomarker studies to date; no health end-point studies yet	**Comment:** Opportunity for use in acute-exposure situations; native plants; dietary diversification
Dithiolethiones	2			
Green tea polyphenols	2			

[a] Categories of evidence for public health interventions: (1) sufficient evidence for implementation, (2) needs more field evaluation, (3) needs formative research, and (4) no evidence or ineffective.
[b] This is a proven intervention (see text) but could not be designated as 1 (sufficient evidence for implementation) because of the complexity of achieving this goal in most circumstances.

(Reardon et al., 1999; Kussaga et al., 2014). As capacity and appropriate legal frameworks and enforcement structures are put in place, contamination levels in crops eventually decrease. However, the positive impact on subsistence farmers is usually limited, with the benefits generally going to larger farmers (Hansen and Trifković, 2014).

Where regulatory systems are established, implementation of intervention strategies and technologies is usually robust and foodborne exposure is low. Where regulatory systems are not fully functional, a basic developmental goal should be to put systems in place and get them operational. Enforcement of risk-based food law is critical to public health and economic viability, and drives the development and sustained use of intervention technologies.

Dietary diversity to mitigate mycotoxin exposure

Dietary diversity is a good way to improve nutrition and health (FAO, 1997; Frison et al., 2006; Lovo and Veronesi, 2014). Aspects important for a healthy diet include the number of different foods, the quantities, and the health (nutritional) value of those foods available for consumption (Drescher et al., 2007). Dietary data from the United Republic of Tanzania estimated the effect of crop diversification on child growth and projected a positive and significant impact on child nutritional status, particularly for girls and on children's height (Lovo and Veronesi, 2014).

A lack of dietary diversity is directly related to levels of mycotoxin exposure. In rural Africa and parts of Latin America, a high percentage of calories come from maize, which is commonly contaminated by aflatoxins and/or fumonisins. In East Africa, aflatoxin exposure has also been directly correlated with reported daily intake of maize, and fumonisin exposure occurs almost entirely from maize (Kimanya et al., 2008). Another major source of exposure to aflatoxin is through the consumption of groundnuts (Liu and Wu, 2010; IARC, 2012). Access to a greater variety of foods will lower the risk of exposure by lessening the intake of these commonly contaminated foods (Groopman et al., 2008). Replacing foods at high risk of mycotoxin contamination with those at lower risk would improve access to foods with better nutritional value.

An excellent example of improved health outcomes after a switch from a food source at high risk of aflatoxin contamination to one at lower risk occurred in Qidong, China. A government policy to grow foods that are eaten locally, combined with a prohibition on interregional shipments of food products, had forced residents of Qidong County to produce and consume primarily maize for several decades. Liberalization of the trans-boundary provincial trade policy allowed rice to be imported from other regions of the country, replacing maize as the staple cereal. Since aflatoxin contamination is much lower in rice than in maize, the result was reduced aflatoxin exposure and a precipitous drop in liver cancer incidence (Chen et al., 2013).

Food diversity and exposure risk can also be driven by socio-economic factors. In West Africa, Egal et al. (2005) reported that the average frequency of maize consumption is 5–7 days per week. Maize is currently the most common cereal staple, having displaced the native cereals sorghum and millet and other sources of starch (Miracle, 1966). Consumption of groundnuts, another common source of aflatoxins, was positively correlated with household and maternal wealth variables and varied by agro-ecological zone. In Ghana, Shuaib et al. (2012) showed interesting evidence of an inverse relationship between a woman's income and the level of aflatoxin biomarkers in her blood. This suggested that greater purchasing power may improve the opportunity for diversifying food choices.

Changing food preferences where there are no economic constraints can be a matter of social marketing and awareness. However, changing food preferences and access for people living in food-insecure conditions presents an enormous challenge. In 1950, by far the major source of dietary starch in sub-Saharan Africa was sorghum and millet (40%), followed by cassava (30%) and maize (15%) (Miracle, 1966). The subsequent shift towards maize is part of a global trend; over the past 50 years, consumption of sorghum and millets has declined by 50% and consumption of cassava by 40% (Khoury et al., 2014). In turn, this may have had a major role in increasing aflatoxin exposures. In West Africa, for example, aflatoxin concentrations in pearl millet and sorghum were substantially lower than those in maize (Bandyopadhyay et al., 2007).

Genetic resistance to aflatoxin and fumonisin contamination of maize

Aflatoxins

Genetic resistance to aflatoxin and fumonisin contamination exists in maize populations, but it is complex and involves multiple genes, and genetic engineering requires moving resistance genes into agronomically acceptable genotypes (Moreno and Kang, 1999; Eller et al., 2008; Warburton et al., 2013; Zila et al., 2013; Warburton and Williams, 2014).

Resistance to ear-feeding insects is associated with lower levels of aflatoxins and fumonisins (Miller, 2001; Munkvold, 2003). Transgenic expression of *Bacillus thuringiensis* (Bt) toxins reduces insect damage and fumonisin contamination (de la Campa et al., 2005; Barros et al., 2009; Ostry et al., 2010; Abbas et al., 2013; Pray et al., 2013). The effectiveness of Bt in reducing aflatoxin contamination is inconclusive (Abbas et al., 2013).

Proteomic, transcriptomic, and histological analyses of the interaction between the fungus and the maize seed show striking similarities to other well-characterized systems, suggesting that resistance is achievable. The new genetic technologies, along with improved breeding populations and phenotyping strategies, have dramatically increased the number of genetic markers associated with resistance to aflatoxins and fumonisins and have identified putative resistance genes and proteins (Lanubile et al., 2010; Brown et al., 2013; Campos-Bermudez et al., 2013; Dolezal et al., 2013, 2014; Warburton and Williams, 2014).

Progress has been made in selecting breeding lines of maize with resistance to aflatoxin accumulation that show high and repeatable resistance under different environments (Mayfield et al., 2012; Williams and Windham, 2012). Additional resistance has come from the Germplasm Enhancement of Maize Project, which is a public–private programme that uses exotic germplasm from across the world, including from the International Maize and Wheat Improvement Center (CIMMYT) (Li et al., 2002; Henry et al., 2013). Use of the maize core diversity panel, which captures most of the maize diversity in breeding programmes worldwide (Flint-Garcia et al., 2005), has identified more than 30 lines showing good resistance to mycotoxins in up to seven environments (Warburton et al., 2013; Warburton and Williams, 2014). These maize germplasm lines are publically available, and several already have been included in a joint United States Agency for International Development/United States Department of Agriculture (USAID/USDA) project, together with two CGIAR centres, with the goal of developing resistant hybrid cultivars (Warburton and Williams, 2014). As part of a USA–Africa collaborative strategy, the International Institute of Tropical Agriculture and USDA released six inbred lines adapted to Africa with enhanced resistance to aflatoxin accumulation (Menkir et al., 2006, 2008).

In summary, maize hybrids with improved resistance to *Aspergillus flavus* and aflatoxins are being used, but the level of resistance is not yet adequate to prevent unacceptable aflatoxin contamination in some fields. Putative resistance-associated genes have been identified by gene expression profiling studies and could be evaluated for their role in resistance to aflatoxin contamination.

Fumonisins

Many genotypes have been identified with some resistance to fumonisin accumulation (Mesterházy et al., 2012; Santiago et al., 2013), including germplasm lines adapted to Argentina (Presello et al., 2011), Central and West Africa (Afolabi et al., 2007), and South Africa (Small et al., 2011), but no hybrids are available with adequate resistance. Heritability for resistance to fumonisin accumulation is higher than that for resistance to aflatoxin contamination (Zila et al., 2013), and a moderate to high genotypic correlation between ear rot and fumonisin content suggests that resistance to the fungus and to fumonisin production may be closely linked (Eller et al., 2008; Presello et al., 2011; Zila et al., 2013). This correlation has allowed selection for resistance to fumonisin accumulation based on ear rot scores (Robertson et al., 2006; Eller et al., 2008; Santiago et al., 2013), thus making screening quicker and less expensive.

Genome-wide association studies on the maize core diversity panel have identified three novel loci associated with 3–12% of the genetic variation associated with resistance to ear rot (Zila et al., 2013). Three putative resistance genes co-localized with the genetic markers. The large number of genetic markers available on the diversity panel is allowing the dissection of complex quantitative traits, such as resistance to mycotoxin accumulation.

Fumonisin accumulation is consistently decreased when Bt maize hybrids effectively reduce insect damage. This can make the difference between maize products that are relatively safe and those that are not (de la Campa et al., 2005; Pray et al., 2013).

Genetic resistance to aflatoxin contamination of groundnuts

Genetic resistance to aflatoxin contamination in groundnuts is complex: heritability is low to moderate, there is a poor correlation between fungal growth and aflatoxin contamination, and results from in vitro seed assays do not correlate with those from field assays (Holbrook et al., 2008; Arunyanark et al., 2010; Girdthai et al., 2010b; Hamidou et al., 2014).

Germplasm with some resistance is available, but genotypes do not show consistent response across locations, due to large interaction effects between the genotype and environment on aflatoxin contamination (Liang et al., 2006; Arunyanark et al., 2010; Girdthai et al., 2010a, 2010b; Hamidou et al., 2014).

One key environmental effect is drought stress, and many programmes have focused on breeding for drought tolerance as a way to improve resistance to aflatoxin contamination. A field study in West Africa examined 268 genotypes over four locations and confirmed that drought stress intensity increases aflatoxin contamination; however, the investigators did not show a significant relationship between drought tolerance and aflatoxin contamination (Hamidou et al., 2014), possibly due to other site-specific environmental effects.

An improved understanding of resistance mechanisms should help improve selection of resistant germplasm. Genome sequences of the two diploid progenitors of groundnut are now available (http://peanutbase.org/browse_search), which may facilitate molecular mapping and breeding for disease resistance.

Biological control of aflatoxins

In the USA, biocontrol strategies have been developed to reduce aflatoxin contamination in cottonseed (Cotty, 1994), groundnuts (Dorner and Lamb, 2006), maize (Dorner et al., 1999), and pistachio nuts (Doster et al., 2014) using strains of *A. flavus* that do not produce aflatoxins (i.e. atoxigenic strains). In commercial practice in the USA, these atoxigenic strains are applied to the field during crop development (Cotty, 1994; Dorner and Lamb, 2006). Under appropriate conditions, the spread of the introduced strain throughout the field displaces the native, toxic strains (Mehl et al., 2012; Atehnkeng et al., 2014). Strains formulated into biological control products may be single clones (Bock and Cotty, 1999) or be composed of more than one strain to improve local adaptability (Atehnkeng et al., 2014).

Several factors have been identified that affect efficacy. Dew and moisture will allow for the atoxigenic strains to produce spores over several days (longer if conducive conditions persist). If the seeds are placed on dry soil, an adequate production of spores may not occur, but they will stay inert and viable until moisture is available (Bock and Cotty, 1999). A late application of atoxigenic strains on maize (after silking) may not be effective. In the event of a heavy rain shortly after the inoculum is spread, the biological control product may not stay evenly distributed on the surface of the field. In a review of the use of atoxigenic strains of *A. flavus* in the USA, Abbas (2011) indicated that this technology is emerging as a useful management practice for reducing aflatoxin concentrations in maize.

Use under African conditions

In one study in Nigeria, the inoculation of a mixture of four endemic atoxigenic strains of *A. flavus* in maize plots in four agro-ecologies over 2 years resulted in significant reductions in aflatoxin concentrations at harvest and after storage (Atehnkeng et al., 2014). At harvest, the reduction in aflatoxin ranged from 57.2% (27.1 ppb in untreated plots vs 11.6 ppb in treated plots) to 99.2% (2792.4 ppb in untreated plots vs 23.4 ppb in treated plots). The applied atoxigenic strains remained with the treated crop, and the reduction in aflatoxin concentration in grains after poor storage ranged from 93.5% (956.1 ppb in untreated vs 66.2 ppb in treated) to 95.6% (2408.3 ppb in untreated vs 104.7 ppb in treated).

In Nigeria, a similar percentage of maize samples were contaminated by both aflatoxin and fumonisin (Adetuniji et al., 2014; Adetunji et al., 2014), which is not uncommon. In situations where conditions are permissive for both aflatoxin and fumonisin in the field, interventions that are effective for both toxins are needed. Aside from Bt maize, which is not yet widely used in Africa, there are few interventions for pre-emptive prevention of fumonisin in the field. Preliminary trials have shown potential for development of biological control treatment for *Fusarium verticillioides* (Sobowale et al., 2007).

Genetic recombination with *A. flavus* has been shown to increase genetic variation within the populations (Olarte et al., 2012; Horn et al., 2014). Sexual recombination leading to the acquisition of toxin genes is possible, but the implications of this are not clear with respect to biological control (Abbas et al., 2011). Studies to date show that aflatoxin production is heritable and is not lost during sexual recombination; however, hybridization between toxic and atoxigenic strains produced progeny of no or lower aflatoxin production (Olarte et al., 2012).

Cyclopiazonic acid can also be produced by A. flavus

Cyclopiazonic acid (CPA) has been shown to be toxic and immunosuppressive in various strains of mice and rats as well as swine and poultry (Burdock and Flamm, 2000; De Waal, 2002; King et al., 2011). One of the commercial atoxigenic strains used in the USA, AF36, produces CPA. It is possible to select for strains of *A. flavus* that produce neither aflatoxin nor CPA (King et al., 2011). Efforts need to be made to minimize or eliminate CPA production in biological control strains before use (Abbas et al., 2011; King et al., 2011).

Research needs

The use of atoxigenic strains to help manage aflatoxin in maize and groundnuts in Africa, and other parts of the world, will require an investment to optimize, adapt, and deploy the technology in a sustainable manner.

Given the large number of exploratory investigations in Africa, studies are needed to evaluate the impact of the low rate of genetic recombination, which will then inform the deployment of the technology in diverse settings.

Sorting

In developed countries, sorting and grain cleaning techniques are required to reduce mycotoxin contamination, notably in grains contaminated by ergot and in nuts. Ergot sclerotia are removed by specific gravity seed cleaning equipment, a practice that has been in place for a long time. In groundnuts, after basic clean-up of the crop by commercial farmers, high-capacity electronic optical sorters are used to remove nuts contaminated by aflatoxin (Whitaker et al., 2005). For maize, normal grain cleaners reduce aflatoxin and fumonisin by 50–60% (Malone et al., 1998; Pacin and Resnik, 2012), far less than the reduction from hand sorting (Brekke et al., 1975).

Soon after the discovery of aflatoxin in 1961, sorting emerged as a regular and effective practice to improve safety for groundnuts. The need for efficient ways to remove aflatoxin-contaminated nuts prompted experiments on the concentrations of aflatoxin in kernels from shells that were not visibly mouldy. This revealed that visual sorting was an efficient way to segregate more- versus less-contaminated kernels in the laboratory. However, parts of some nuts that appeared sound contained substantial levels of aflatoxin (Cucullu et al., 1966). In the USA, after 3–4 hours of training on visual signs of contamination with *Aspergillus*, people with no prior experience were asked to visually sort samples of groundnuts that had already been classified according to their quality (sound, damaged, intermediate) by federal inspectors according to the official grading procedure. In the best grade of groundnuts, misclassification occurred, which the authors ascribed to mostly false-positives, with some false-negatives and sampling error (Dickens and Welty, 1969).

By 1968, another step was introduced into the United States inspection system: examination by the inspectors of the damaged kernels for *Aspergillus*. After training, each inspector was given a folder with two sets of coloured photographs that showed what to look for and what not to look for. Before the development of the current methods of inspection, this low-technology approach was proven useful (Goldblatt, 1973). Whitaker et al. (1998) demonstrated that visual sorting of groundnuts provided a practical first-action regulatory method. They found that sound mature kernels and sound half kernels contained about 7% of the aflatoxin, with the damaged kernels containing the rest. Studies on grains contaminated with *Fusarium* toxins indicate that these strategies work best where there is ongoing training (Desjardins et al., 2000; van der Westhuizen et al., 2010). A study in the Philippines found that manual sorting reduced aflatoxin concentrations in lots of raw groundnuts from 300 ng/g to less than 15 ng/g (Galvez et al., 2003). Research conducted in Kenya (and Haiti) demonstrated that manual sorting of groundnuts purchased at local markets could reduce lot aflatoxin concentrations by about 98% (Filbert and Brown, 2012).

In the case of maize in Africa, manual sorting is moderately effective at the village level for segregating kernel lots for decreased concentrations of aflatoxin. Removing visibly mouldy, insect-damaged, and broken grains by hand reduced aflatoxin concentrations by 40%, based on reports from a study in Benin (Fandohan et al., 2005). Studies in South Africa and the United Republic of Tanzania have demonstrated that hand sorting of maize kernels by local farmers by removing the visibly infected or damaged kernels reduced fumonisin concentrations by 20% (Kimanya et al., 2009; van der Westhuizen et al., 2010).

The willingness to hand sort grains and nuts has been shown to depend on the available supply (Kimanya et al., 2008; van der Westhuizen et al., 2010; and references cited therein). A study in Ghana found that household income and agricultural training increased the quality of the nuts consumed (Adu-Gyamfi, 2013). In South Africa, the effectiveness of hand sorting on fumonisin reductions has been documented by biomarkers (van der Westhuizen et al., 2011).

In developed countries, sorting of contaminated grains is the primary tool used to reduce mycotoxin contamination in grains and nuts after harvest and can be effective at all scales of production.

Research needs

There is a need to adapt commercial optical sorting equipment for groundnuts for the African value chain for both large and small operations.

Targeted training in manual sorting for rural women would appear to be a good investment. In Africa, food security is the major barrier to implementation of sorting (Fandohan et al., 2008). Safe alternative uses for rejected lots need further research (e.g. Filbert and Brown, 2012).

Nixtamalization

In Mexico and Central and South America, nixtamalization has been the usual practice for millennia. Hydrolysis of fumonisin during commercial production of masa virtually eliminates fumonisin. Masa is made by boiling maize meal with the addition of lime, which is then washed out. The ratio of maize to lime to water used and the boiling, soaking, and rinsing practices vary (De La Campa et al., 2004).

In the USA, fumonisin concentration is low in commercial tortillas from major companies (Voss et al., 2001). In contrast, in the USA, masa products from artisanal production facilities often contain some fumonisin (De La Campa et al., 2004; Dvorak et al., 2008). Where there is sufficient washing of the lime-treated product in the traditional process before consumption, concentrations of fumonisin and aflatoxin are lowered (De Arriola et al., 1988; De La Campa et al., 2004; Méndez-Albores et al., 2004; Guzmán-de-Peña, 2010). In Latin America, variability in the process means that there can be residual parent fumonisin in the tortillas (e.g. Dombrink-Kurtzman and Dvorak, 1999; Meredith et al., 1999) that leads to fumonisin exposure (Gong et al., 2008a).

Research needs

In Latin America, nixtamalization has been shown to reduce exposure to aflatoxin and fumonisin. A knowledge translation package based on factors known to reduce fumonisin in the residual masa (De La Campa et al., 2004) would be beneficial.

Post-harvest storage intervention strategies to reduce aflatoxin and fumonisin exposure

Mycotoxin contamination of crops can occur in the pre- and post-harvest agricultural system due to inadequate agricultural practices. Fungal growth and toxin production can occur in the field (e.g. fumonisin, aflatoxin), in storage (aflatoxin), or in both. High humidity (> 85%), high temperatures (> 25 °C), insect and rodent activity, improper drying of crops, and water infiltration in the storage structure will result in the growth of *A. flavus* and *Aspergillus parasiticus* and aflatoxin accumulation (Adegoke and Letuma, 2013).

Most developing countries are located in the world's tropical zones and are subjected to monsoons and high temperature and humidity levels, which contribute to large post-harvest crop losses.

Inadequate storage practices account for 20–50% of these losses. Despite being a major United Nations priority since 1946 (Schulten, 1982), such losses remain a global problem, increasing the risk of food insecurity (food availability, hunger, and nutritional value) and poverty (Hell et al., 2008; Jayas, 2012; Kimatu et al., 2012; Gitonga et al., 2013; Guillou and Matheron, 2014). The double burden of both chronic exposure to mycotoxins and food insufficiency increases both mortality and morbidity, especially in children (Bryden, 2007; IARC, 2012). Adequate post-harvest measures that are practical, economic, and culturally acceptable will therefore address food safety and security and improve public health.

In subtropical climates, maize in the field is typically infected by *A. flavus*, and unless it is dried very quickly, aflatoxin concentrations increase after harvest (IARC, 2012). The stored post-harvest crop ecosystem is therefore an integral part of mycotoxin prevention strategies (Marín et al., 2004; Choudhary and Kumari, 2010; Chulze, 2010). Most of the conditions associated with the post-harvest period can be controlled, unlike those affecting the pre-harvest phase. Strategies to reduce mycotoxin levels during storage mainly consist of: adequate drying of crops before storage; using clean, dry, and enclosed storage facilities; proper water drainage; well-aerated stores; and eliminating insect activity and other pests such as rodents and birds (Lanyasunya et al., 2005; Turner et al., 2005; Hell et al., 2008).

Before storage, harvested field crops should be dried as soon as possible to reduce fungal growth; safe moisture levels recommended for cereals are 10–13% and for oilseeds are 7–8% (Hell et al., 2008). Common storage practices for crops include: on the field; on the floor in homes; on top of or under the roof of houses; in jute or polypropylene bags, wire cribs, pits, and metal bins; and in conical structures or other constructed structures, with or without roofing, made from wood, bamboo, thatch, or mud (Hell et al., 2010; Narrod, 2013; Abass et al., 2014).

Evidence-based post-harvest storage intervention strategies among subsistence farmers are limited. Turner et al. (2005) conducted a field study among groundnut farmers in West Africa (600 volunteers from 20 villages) to reduce aflatoxin exposure by implementing a specific intervention package, and to assess the impact of the intervention by monitoring aflatoxin B_1 (AFB_1) levels in groundnuts and blood aflatoxin–albumin adducts (AF–alb) as a measure of exposure. The intervention package included hand sorting of kernels (with removal of damaged kernels), drying kernels on natural fibre mats, estimating the completeness of a sun-drying period, storing kernels in natural fibre bags, supplying wooden pallets to store the bags on, and using insecticide (acetilite). Significant reductions in both AF–alb in blood (58% reduction) and groundnut contamination levels (70% reduction) were observed. This is the only study of its kind that showed the reduction of aflatoxin exposure in the groundnut-consuming population (Turner et al., 2005).

In Africa, maize is matured under dry conditions and is commonly left in the field to dry on the stalk, whereas in South-East and East Asia, maize is sometimes harvested wet and piled in stacks and left on the field to dry for a period of time (Pitt et al., 2013). Maize may also be shelled, and this, together with drying practices, increases aflatoxin levels. However, crops dried adequately away from the field and off the ground are less susceptible to insect damage and fungal growth.

Sun-drying of maize and groundnuts is common practice in Africa and, together with the use of platforms, has been shown to reduce the growth of toxigenic fungi such as *Aspergillus*, *Fusarium*, and *Penicillium* (Hell et al., 2008). In Ghana, the method of inverted windrowing of groundnut pods after harvest ensures exposure to direct sunlight and circulating air. This cost-effective method dries the pods rapidly and sufficiently to ensure reduction of aflatoxin levels (Amoako-Attah et al., 2007). For groundnuts, drying on raised surfaces or on mats to a kernel moisture content of 8% is required to reduce the risk of aflatoxin contamination (Waliyar et al., 2013).

Kaaya and Kyamuhangire (2010) investigated the effect of biomass-heated natural convection dryers on maize quality during storage in Uganda. During that study, insect damage, mould infection, aflatoxin contamination, and the maize germination potential were determined. The use of these dryers proved to be protective against insect damage, reduced mould and aflatoxin contamination, and had no effect on the grain germination potential. They also were shown to be highly effective in eliminating crop loss due to insect damage. Additional benefits included the reduced need for insecticides to protect the crop, the extension of crop storage duration by 1.8–2.4 months, the improvement of availability of food by more than 1 month, and an increase in jobs and income.

A suggested replacement for sun-drying is the use of solar dryers, because they dry crops faster and more efficiently and provide a controlled environment that offers improved sanitation (Sharma et al., 2009; Ogunkoya et al., 2011). The lack of success of using solar-based drying among rural commercial farmers has been attributed to the cost, complicated operational procedures, and the reluctance to change from traditional methods (Ekechukwu and Norton, 1999). Small-scale farmers require solar dryers that are more affordable to purchase or construct and need little maintenance (Ogunkoya et al., 2011). Of the solar drying technologies available, including the active (forced-convection) solar dryers and the passive (natural-circulation) types, the use of a ventilated greenhouse dryer has been suggested for rural small-scale farmers, due to its low cost, simplicity, and on-site construction and operation (Ekechukwu and Norton, 1999).

The use of hermetically sealed storage bags, such as those of the Purdue Improved Crop Storage project, is apparently effective for insect control, increasing insect mortality by 95–100% in stored maize (Baoua et al., 2014; Hell et al., 2014). The efficiency of hermetic technologies to prevent fungal growth and consequent mycotoxin contamination seems to be dependent on the type and specific characteristics of the crop. Storage of groundnuts in Super Grain Bags (bags made of multilayer polyethylene that have a two-track zipper and are sealed using a zipper slider) reduced the growth of aflatoxin-producing fungi during an experimental study (Navarro et al., 2012). Mutegi et al. (2013) showed that groundnuts stored in polyethylene bags were 7–13% more contaminated than samples stored in polypropylene and jute bags. Jute bags are considered more feasible compared with polyethylene and polypropylene only if crops are properly dried before storage; polyethylene and polypropylene bags are poorly aerated and do not absorb moisture. The use of natural fibre jute bags has been suggested to be more suitable to maintain crop quality (Turner et al., 2005).

Research needs

Strategies to improve post-harvest storage of crops should be an urgent research priority (Anankware et al., 2012). Ideally, technologies should be economically feasible, require low labour intensity, be practical and sustainable, reduce the need to use chemicals, and be convenient, widely available, and easy to transport (Hell et al., 2010; Baoua et al., 2014). The interventions should also be developed for both rural small-scale and commercial farmers. In sub-Saharan Africa, 80% of farms are smallholder, mostly subsistence farms (Mboya and Kolanisi, 2014), and a distinction should be made as to what technologies are feasible for commercial versus small-scale farmers in rural areas.

The cultural acceptability of a proposed intervention in the different agricultural systems is also important. Therefore, post-harvest strategies in developing countries should be comprehensively field-tested and validated to assess their efficacy, economic feasibility, cultural acceptability, and sustainability (Strosnider et al., 2006; De Groote et al., 2013; Jones et al., 2014). To ensure compliance, it will be important to monitor large-scale implementation.

Apart from the lack of feasible and inexpensive strategies, other obstacles to improving post-harvest storage of crops include the absence of governmental commitment and the shortage of trained personnel, such as agricultural extension workers (Hell et al., 2010). Establishing strategies to safeguard crops during storage will inevitably require cooperation and communication between governments, research entities, nongovernmental organizations, other stakeholders (market agencies, farmers' and consumer groups), manufacturers, and the farmers.

In Africa, farmers' awareness of the health risks associated with aflatoxin and how to reduce exposure is influenced by their socioeconomic status, education, farm size, extension participation, market orientation, economic motivation, and perceptions (Kumar and Popat, 2010; Adegoke and Letuma, 2013). The role of women in rural agro-ecological zones in developing countries should also be considered, because they play an important role as mothers, educators, and businesswomen managing household nutrition, farming, and the selling of smallholder crops. Women in certain areas of Ghana and Nigeria were able to produce less maize compared with men. This was due to a lack of access to fertile soil and new technologies or innovations (Udoh et al., 2000; Adu-Gyamfi, 2013). In Ghana and Nigeria, women have less influence on decision-making compared with men (Ogunlela and Mukhtar, 2009; Adu-Gyamfi, 2013). In South Africa, the situation is different; women head 60% of the rural households in the Eastern Cape Province and manage the farms (Burger et al., 2010). More research on gender and mycotoxin management is needed to properly develop education campaigns and ensure equitable access to information by both men and women.

Post-harvest interventions to reduce mycotoxin exposure should include education programmes and awareness campaigns that will facilitate best practices. Working in rural South Africa, Mboya and Kolanisi (2014) (260 smallholder farm households) found that few people understood the health risks associated with mycotoxins. This was also the case in a much larger study (sample size, 2400) in Benin, Ghana, and Togo (James et al., 2007). Sustainability of appropriate agricultural practices will be more effective through continuous campaigns (Strosnider et al., 2006; Jolly et al., 2009). The emphasis should be on crop quality rather than on productivity in domestic markets (Kumar and Popat, 2010).

The following is a summary of recommended procedures using evidence-based, culturally acceptable, and validated prevention strategies.

- Develop knowledge translation modules in partnership with farmers, area agricultural extension workers, traditional leaders, church groups, health workers, and civil society, with a focus on women.
- Be prepared to implement prevention strategies on a larger scale.
- Use recommended procedures in chronic-exposure situations as an ongoing intervention package.
- Apply as a multifactorial intervention package to include hand sorting, rapid and proper (elevated) drying, proper storage, elevation of stored crops, and insect control.
- Consider that designs for solar or biomass dryers and storage structures preferably built from locally available materials are urgently needed.

Interventions useful in emergencies

A distressing number of cases of acute aflatoxicosis have occurred, notably in the past decade. Those who are most likely to consume foods contaminated with aflatoxins suffer the most severe effects, including disease and death after acute exposure (Lewis et al., 2005). Thus, feasible interventions and therapies to diminish human and animal exposure to aflatoxins during aflatoxin outbreaks are imperative.

Numerous strategies to sequester aflatoxins in the gastrointestinal tract and reduce their bioavailability have been evaluated for their potential as practical, cost-effective, and sustainable solutions to the aflatoxin problem. Aside from avoiding ingestion of contaminated food, none of these primary intervention strategies provides complete protection. However, a refined calcium montmorillonite clay (NovaSil [NS]) and chlorophyllin have been widely studied in animals and humans for safety and efficacy, with promising results. Similar research is under way to evaluate the efficacy of other enterosorption strategies, including various bacteria and indigestible carbohydrates such as glucans, glucomannans, cellulose, and peptidoglycans.

Aflatoxin enterosorbents

Studies describing materials that can tightly adsorb aflatoxins onto internal and/or external surfaces, causing a reduction in toxin uptake and bioavailability, have been recently reviewed (Kensler et al., 2013; Miller et al., 2014). The technical feasibility, costs, and efficacy of various mitigation strategies (including the use of enterosorption and trapping agents) have also been reported (Khlangwiset and Wu, 2010). It has been suggested that inclusion of toxin enterosorbents in the diet can decrease morbidity and mortality during outbreaks of acute aflatoxicosis. The most common materials used as toxin enterosorbents and trapping agents are discussed briefly below.

Chlorophyll/chlorophyllin

Chlorophyll and chlorophyllin are naturally occurring constituents of the human diet that have been shown to be effective anticarcinogens in several animal models (Dashwood et al., 1998). They are hypothesized to act as interceptor molecules by trapping carcinogens, such as AFB_1, thereby diminishing bioavailability by impeding their absorption (Breinholt et al., 1995).

In a 4-month clinical trial in China, ingestion of 100 mg of chlorophyllin at each meal led to an overall 55% reduction in median urinary levels of aflatoxin–N7-guanine adducts compared with placebo (Egner et al., 2001). In a crossover study among four human volunteers in the USA, data suggested that chlorophyll or chlorophyllin consumption may limit the bioavailability of aflatoxins, as shown in animals (Jubert et al., 2009). Prophylactic therapy with chlorophyllin or supplementation of diets with foods rich in chlorophylls may represent a practical measure to reduce the likelihood of developing aflatoxicosis (Kensler et al., 2013).

Clays

The use of clay-based products as enterosorbents for aflatoxins is a frequent strategy to reduce aflatoxin exposure in animals. Dioctahedral smectite clays (especially montmorillonite) are the common sorbents used for this purpose. Earlier studies showed that inclusion of a calcium montmorillonite clay (NS) in animal feed reduced the adverse effects associated with aflatoxin exposure in multiple animal species and decreased the level of aflatoxin M_1 (AFM_1) in milk from lactating dairy cows and goats (Phillips et al., 2008). Equilibrium adsorption isotherms, molecular modelling, and in vivo studies have been used to demonstrate that NS binds AFB_1 and fumonisin B_1 in the gastrointestinal tract, thereby reducing systemic bioavailability

(Phillips et al., 2008; Robinson et al., 2012).

Initial human trials in Ghana and in Texas (USA) showed no adverse health effects in humans (Phillips et al., 2008; Johnson et al., 2009; Mitchell et al., 2013). Based on animal and human studies, NS clay does not significantly alter the levels of vitamins and minerals. Overall, use of NS clay during outbreaks of acute aflatoxicosis appears to be a safe and practical strategy for vulnerable populations at high risk for exposure (Mitchell et al., 2014).

Other aflatoxin-sequestering materials that have been investigated include lactic acid bacteria (El-Nezami et al., 2000, 2006; Hernandez-Mendoza et al., 2009; Dalié et al., 2010; Pizzolitto et al., 2011) and yeast (Baptista et al., 2002; Diaz et al., 2004; Stroud, 2006; Kutz et al., 2009; Pizzolitto et al., 2011; Fruhauf et al., 2012).

Research needs

The young of all species are the most vulnerable to aflatoxins; thus, children are the most likely to suffer the consequences of aflatoxin outbreaks. The trials reported to date have been in adults, and there is a knowledge gap in emergency strategies for protecting infants and children.

Further studies are warranted to assess the effects of aflatoxin dose and duration of exposure on efficacy and the safety of NS clay and chlorophyllin in the vulnerable, including malnourished infants, children, and pregnant women.

Other research needs include: determining the effects of mixtures of NS, chlorophyllin, and other enterosorbents; assessing the effectiveness of combinations of aflatoxin enterosorbents and chemoprotectants; identifying sustainable and effective delivery strategies to treat acute aflatoxicosis; and conducting phased clinical trials.

Chemoprevention studies

Dithiolethiones (oltipraz)

Oltipraz, a substituted 1,2-dithiole-3-thione, was originally developed by the pharmaceutical industry as a possible treatment for schistosomiasis and was extensively evaluated in clinical trials in the early 1980s. Subsequent studies demonstrated that oltipraz and some structurally related 1,2-dithiole-3-thiones were potent inducers of enzymes associated with the maintenance of reduced glutathione pools, as well as enzymes important to carcinogen detoxification, in multiple tissues of rats and mice (Ansher et al., 1983, 1986).

Aflatoxin biomarkers were used as intermediate end-points in a phase IIa chemoprevention trial of oltipraz in Qidong, China (Kensler et al., 1998; Wang et al., 1999). This was a placebo-controlled, double-blind study in which participants were randomized to receive placebo, 125 mg of oltipraz daily, or 500 mg of oltipraz weekly. In participants receiving the 500 mg weekly dose, urinary AFM_1 levels were reduced by 51% compared with the placebo group. Median levels of aflatoxin–mercapturic acid (a glutathione conjugate derivative) were elevated 6-fold in the 125 mg group but were unchanged in the 500 mg group. Increased aflatoxin–mercapturic acid levels reflect induction of aflatoxin conjugation through the actions of glutathione *S*-transferases. The apparent lack of induction in the 500 mg group probably reflects masking caused by diminished aflatoxin-8,9-epoxide formation for conjugation through the inhibition of CYP1A2 seen in this group. This initial study demonstrated for the first time that aflatoxin biomarkers could be modulated in humans in a manner that would predict decreased disease risk.

Sulforaphane

Although the oltipraz clinical trial demonstrated the proof of principle for increasing pathways leading to aflatoxin detoxification in humans, the practicality of using a drug-based method for prevention in developing countries is limited. Fortunately, oltipraz is not the only agent that affects enzyme changes through the Nrf2-Keap1 pathway. Many foods have high levels of these enzyme inducers (Talalay and Fahey, 2001; Fahey and Kensler, 2007).

A beverage formed from hot water infusions of 3-day-old broccoli sprouts, containing defined concentrations of glucosinolates as a stable precursor of the anticarcinogen sulforaphane, was evaluated for its ability to alter the disposition of aflatoxin (Kensler et al., 2005). Sulforaphane has been extensively examined for its chemopreventive properties and is a potent activator of the Nrf2-Keap1 pathway, leading to increased expression of carcinogen-detoxifying enzymes (Fahey et al., 2002; Dinkova-Kostova et al., 2007). In a study in Qidong, China, 200 healthy adults drank infusions containing either 400 μmol or less than 3 μmol of glucoraphanin nightly for 2 weeks. Urinary levels of aflatoxin–N7-guanine adducts were similar between the two intervention arms. However, the measurement of urinary levels of dithiocarbamates (sulforaphane metabolites) indicated striking interindividual differences in bioavailability. This outcome may reflect individual differences in the rates of hydrolysis of glucoraphanin to sulforaphane by the intestinal microflora of the study participants. Accounting for this variability, a significant inverse association was observed for excretion of dithiocarbamates and aflatoxin–N7-guanine adducts in individuals receiving broccoli-sprout glucosinolates (Kensler et al., 2005).

This preliminary study illustrates the potential use of an inexpensive, easily implemented, food-based method for secondary prevention in a population at high risk of aflatoxin exposure. A follow-up intervention seeking to minimize the interindividual variability in the pharmacokinetics of the glucoraphanin precursor is currently in progress.

Green tea polyphenols

Many studies have demonstrated that green tea polyphenols (GTPs) inhibit various chemically induced cancers in experimental animals (Moyers and Kumar, 2004; Yang et al., 2006). Qin et al. (1997) studied the effects of GTPs in drinking-water for 2 or 4 weeks to protect against the development of AFB_1-induced hepatocarcinogenesis in the rat. The data on GTPs in experimental animals provided the impetus to translate this strategy to human clinical trials. In an initial study in an aflatoxin-exposed high-risk group in Guangxi, China, the effects of GTPs were assessed in urine samples collected from a randomized, double-blinded, placebo-controlled phase IIa chemoprevention trial (Luo et al., 2006). All participants tested positive for AF–alb and took GTPs capsules daily at a dose of 500 mg or 1000 mg, or a placebo, for 3 months. Analyses were performed on blood and urine samples collected during this clinical trial to evaluate the efficacy of GTPs in modulating aflatoxin biomarkers; reductions in AF–alb and urinary AFM_1 levels were observed (Tang et al., 2008). After the 3-month trial, both of the GTPs intervention groups were found to have reduced AF–alb levels compared with the non-intervention controls.

Research needs

This research has established that chemoprevention with the above-mentioned agents is effective in relevant animal models and that the mechanism applies in humans. Similar plant polyphenols and sulforaphanes occur in several plant species found in developing countries that are affected by aflatoxin. Research is needed to determine which locally grown and consumed plants contain sufficient levels of these naturally occurring chemopreventive agents to induce protection from aflatoxin exposure, and to conduct experimental trials.