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Aflatoxins: Origin, Detection, Effect on Human Health and Safety, and Preventive Intervention Strategies (Focus on Developing Countries)

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ABSTRACT

Aflatoxins (AFs) are secondary metabolites of fungal origin produced by *Aspergillus flavus*. They contaminate agricultural commodities at pre- or post-harvest. Contamination of grains, peanuts and other dietary staples with AFs is a worldwide problem that affects both food safety and agricultural economies. Most countries have adopted regulations that limit the quantity of AFs in food and feed to ≤20 μg kg^{-1}. Environmental conditions, especially high humidity and temperature, favour fungal proliferation resulting in contamination of food and feed. The socio-economic status of the majority of inhabitants of sub-Saharan Africa predisposes them to consumption of mycotoxin-contaminated products either directly or at various points in the food chain. AF contamination has been linked to liver cancer, immunosuppression and impaired growth. Synergistic interactions between AF exposure and malaria, kwashiorkor and HIV/AIDS have been suggested. Methods to reduce AF contamination involving good agricultural practices such as early harvesting, proper drying, sanitation, proper storage and insect management, among others have been adopted. Other possible interventions include biological and chemical control, decontamination, breeding for resistance as well as surveillance and awareness creation. However, complete elimination of AF contamination might not be possible. Therefore efficient, practical and cost-effective approaches are needed in developing countries where the burden of liver cancer is highest. Chemoprevention strategies which alter AF disposition are a rational and pragmatic strategy to reduce the incidence of liver cancer in regions of the world with high dietary AF exposure. Modulation of AF disposition can be achieved through induction of conjugating and cytoprotective enzymes. Classes of chemopreventive agents such as oltipraz, epigalocatechin gallate, sulphoraphane, natural chlorophyll and kolaviron which induce cytoprotective enzymes have been identified. This new paradigm therefore raises the intriguing possibility of a novel therapeutic approach for hepatocellular carcinoma.

Keywords: *Aspergillus flavus*, chemopreventive agents, dietary staples, developing countries, hepatocellular carcinoma

CONTENTS

INTRODUCTION

Aflatoxins (AFs) are a family of highly toxic and carcinogenic toxins produced by several *Aspergillus* species. Pre- and post-harvest contamination of maize, peanuts, cotton, and tree nuts by members of the genus *Aspergillus* and subsequent contamination with the mycotoxin AF pose a widespread food safety problem for which effective and inexpensive control strategies are lacking (Holmes *et al*. 2008). Of the AFs, aflatoxin B_1 (AFB_1) has been implicated in the etiology of hepatocellular carcinoma (HCC) which is one of the most common cancers worldwide, causing nearly

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600,000 deaths each year (Yates and Kensler 2007). HCC is one of the most common cancers in Asia, Africa and in groups of Asian- and Hispanic-Americans and attacks people at an early age in high risk zones (Farombi 2006). The highest occurrence and the youngest people with this disease are in the hyper endemic areas of China, Taiwan, Thailand and sub-Saharan Africa (Kensler *et al*. 2003). Exposure to dietary AFs and chronic infection with hepatitis B virus (HBV) has been linked to more than 90% of HCC cases in these areas (Chen *et al*. 2003). In agreement with this observation, the synergistic interaction between HBV and AFs, especially AFB_1, has been observed in both animals (Bannasch *et al*. 1995) and humans (Lunn *et al*. 1997; Wang *et al*. 2001).

Since the discovery of AF as a potently carcinogenic food contaminant, extensive research has been focused on identifying methods of reducing its contamination of foods. Numerous diverse compounds and plant-derived phytochemicals containing activity inhibitory to AF biosynthesis have been reported (Nesci *et al.* 2007; Holmes *et al*. 2008). Using nonaflatoxigenic *A. flavus* isolates to competitively exclude toxigenic *A. flavus* isolates in agricultural fields has also become an adopted approach to reduce AF contamination (Chang and Hua 2007). However, the complete elimination of AF contamination might not be possible due to the socio-economic status of the majority of inhabitants of sub-Saharan Africa which predispose them to consumption of AF-contaminated products either directly or at various points in the food chain (Wagacha and Muthomi 2008). Therefore, chemoprevention, which utilizes non-toxic chemical compounds, synthetic or natural to reduce, attenuate or reverse the multistage process of carcinogenesis appears to be a more rational and pragmatic strategy to reduce the incidence of HCC in populations with high dietary AF exposure.

Thus understanding the mechanisms of AF-induced hepatocarcinogenesis provides the basis for evaluation of both exposures to AF, as well as modulation of AF disposition by chemopreventive agents (Yates and Kensler 2007).

The present article reviews the origin, detection, occurrence of AFs in foods in developing countries, effects on human health and safety and presents a detailed account of preventive intervention strategies involving the use of chemopreventive agents.

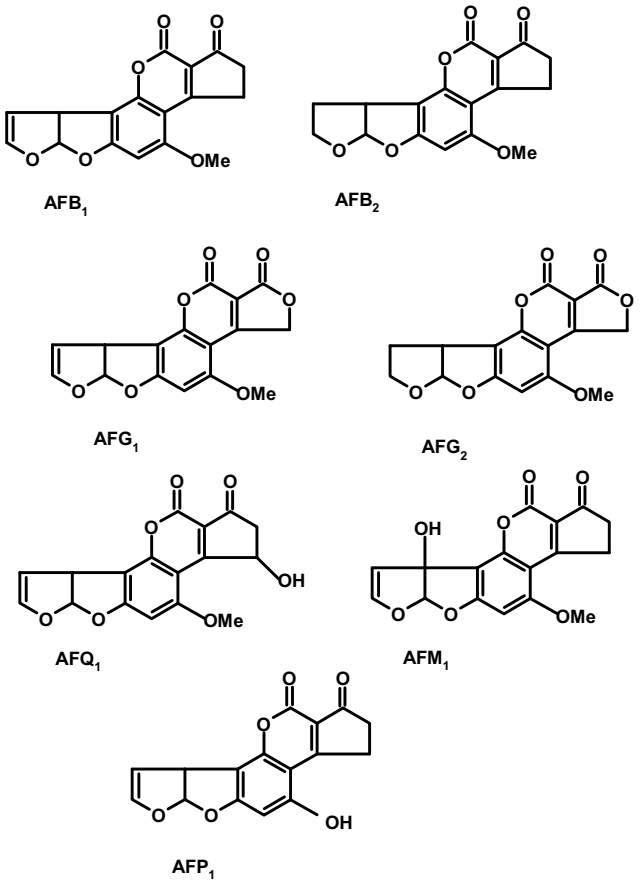


Fig. 1 The chemical structures of the major naturally occurring aflatoxins (AF) and the structures of aflatoxin M1, Q1 and P1; hydroxylated metabolites of aflatoxin B1.

HISTORY AND SOURCES OF AFLATOXIN

AFs are a family of closely related secondary metabolites (mycotoxins) produced by fungi viz., *Aspergillus flavus*, *Aspergillus parasiticus* which contaminate plants and plant products. Recent studies revealed that *A. nominus* and *A. tamari* strains are capable of producing the toxin (Goto *et al*. 1996, 1997). Very recently, Ito *et al*. (2001) isolated another strain, *A. pseudotamarii* capable of producing AF. At temperatures between 24 and 35°C and when the moisture content exceeds 7% (10% with ventilation) aflatoxins will grow within many commodities (Williams *et al*. 2004). There are four generally recognized AFs, designated B1, B2, G1 and G2 (**Fig. 1**). The metabolites, M1 and M2, which are found in milk (Thirumala *et al.* 2002), are shown in **Fig. 2**. The order of toxicity is B1 greater than G1, greater than G2, greater than B2. However, aflatoxin B1 is the major mycotoxin produced by most species under culture conditions (Ciegler *et al.* 1980). Because of this and its toxicity, AFB1 is the most frequently studied of the four. AFB1 and AFB2 are named because of their strong blue fluorescence under UV light, whereas AFG1 and AFG2 fluoresces greenish yellow. The B-toxins are characterized by the fusion of a cyclopentenone ring to the lactone ring of the coumarin structure, while G-toxins contained an additional fused lactone ring. Aflatoxin B1 and to a lesser extent AFG1 are responsible for the biological potency of aflatoxin-contaminated feed. These two toxins possessed an unsaturated bond at the 8, 9 position on the terminal furan ring. AFB2 and AFG2 are essentially biologically inactive unless these toxins are first metabolically oxidized to AFB1 and AFG1 *in vivo*. AFM1 and M2 are hydroxylated derivatives of AFB1 and B2 that may be found in milk, milk products or meat (hence the designation M1). They are formed by the metabolism of B1 and B2 in the body of the animals following absorption of contaminated feeds (Verma 2004). Aflatoxin M_1 (AFM1) is a metabolic hydroxylation product of AFB1, and can occur in the absence of the other aflatoxins. Human exposure occurs primarily via milk and milk products from animals that have consumed contaminated feed. International agency for research on cancer (IARC) concluded in 1993 that there was sufficient evidence in experimental animals for the carcinogenicity of AFM1 and inadequate evidence for the carcinogenicity of AFM1 in humans. Although AFM1 has been tested less extensively, it appears to be toxicologically similar to AFB1. AFM1 is considered to be a genotoxic agent, based on its activity *in vitro* and its structural similarity with AFB1. It is a less potent liver carcinogen, with a probable carcinogenic potency in laboratory animals within a factor of 10 of AFB1 (Cullen *et al.* 1987). No additional toxicological information on AFM1 has appeared in the literature since IARC (1993). Other major metabolites of AFB1 in human include AFQ1 AFM1, aflatoxicol (AFL), AFLH1, AFP1, AFB2á and AFB1-2, 2-dihydrodiol (Groopman *et al.* 1985). Both unmetabolized (B1, B2, G1, G2) as well as metabolized forms (aflatoxicol, M1 and M2) of aflatoxins get excreted in the urine, stool and milk (Coulter *et al*. 1986; Verma and Chaudhari 1997). AFs M_1 and M_2, the hydroxylated metabolites of AF B_1 and B_2, may be found in milk or milk products obtained from livestock that has ingested contaminated feed. Aflatoxins were first discovered in 1960s in England after the outbreaks of turkey disease that resulted in deaths and of cancer development in rainbow trout fed on rations formulated from peanut and cottonseed meals (Asao

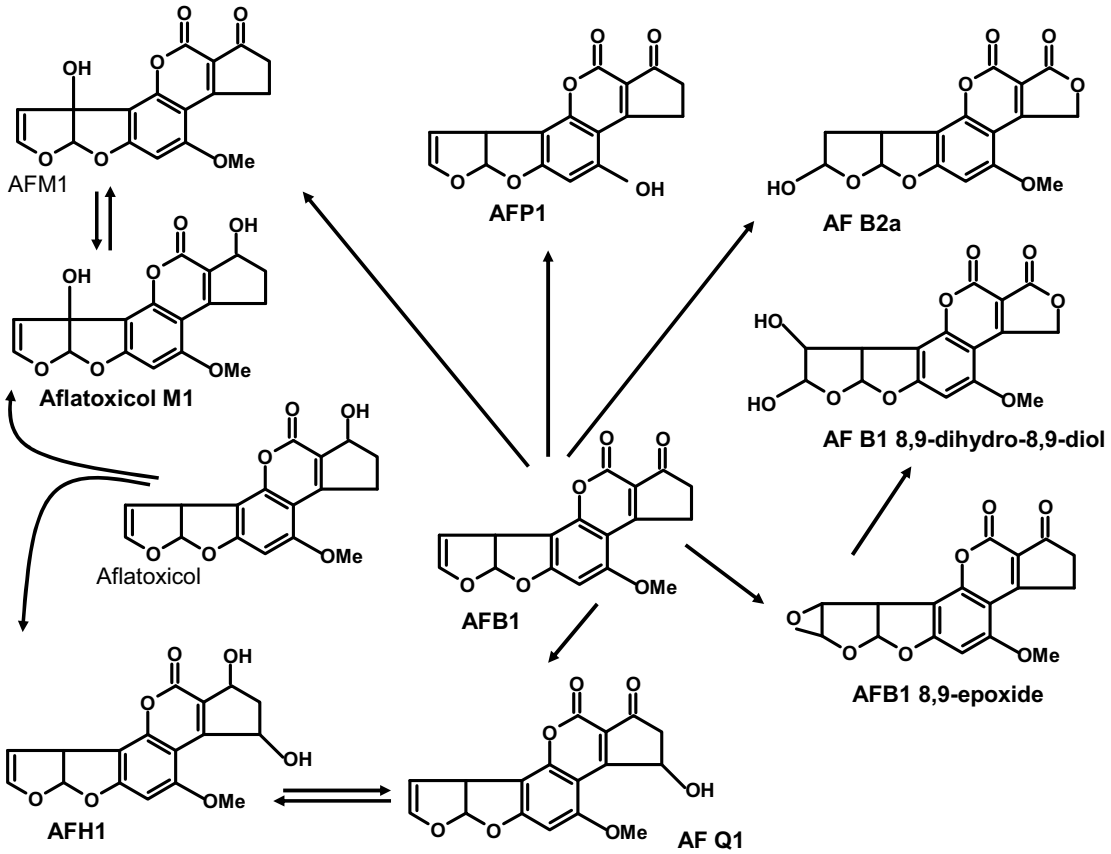


Fig. 2 Biochemical relationships between AFB1 and its metabolites. Adapted from Essigman *et al.* (1982).

et al. 1963). Of these six AFs, AFB_1 is the most frequent one present in contaminated samples and AFs B_2, G_1 and G_2 are generally not reported in the absence of AFB_1. Dietary intake of AFs arises mainly from contamination of maize and groundnuts and their products. Most commodities in the developing countries are therefore easily contaminated due to the environmental condition, poor processing and lack of proper storage facilities (Farombi 2006). There is therefore great health concern over AFs because of their high level of toxicity to humans and their potent carcinogenic effects in laboratory animals. AFs are toxic and carcinogenic to animals, including humans.

Among these, AFB1 is one of the most potential environmental carcinogens, with toxic effects on humans through its direct consumption in food products or as metabolic residues in animal tissues.

TOXICOLOGY AND METABOLISM

Of the AFs, AFB_1 is the most prevalent, the most occurring and also the most potent. Acute dietary exposure to AFB_1 has been implicated in epidemics of acute hepatic injury (Sudakin 2003). The liver is the primary site of biotransformation of ingested AFB_1. The predominant human CYP450 isoforms involved in human metabolism of AFB_1 are CYP3A4 and CYP 1A2 (**Fig. 3**). Both enzymes catalyze the biotransformation of AFB_1 to the highly reactive *exo*-8,9-epoxide of AFB_1 (Guengerich *et al.* 1998). CYP 1A2 is also capable of catalyzing the epoxidation of AFB_1 to yield a high proportion of *endo* epoxide and hydroxylation of AFB_1 to form AFM_1, which is a poor substrate for epoxidation (Guengerich *et al.* 1998), less potent than AFB_1 (Wild and Turner 2002) and generally considered a detoxification metabolite while CYP 3A4 can also form AFQ_1 a less toxic detoxification metabolite. CYP 3A5 metabolizes AFB_1 mainly to the *exo* epoxide and some AFQ_1 (Wang *et al.* 1999). Polymorphism studies with CYP 3A5 revealed that this isoform is not expressed by most people and in particular about 40% of African-Americans do not express this enzyme (Wild and Turner 2002). Studies with Gambian children revealed that AF can cross the placenta and be transported into the new born (Wild *et al.* 2000). Thus CYP 3A7, a major cytochrome P450 in human fetal liver, has the capacity to activate AFB_1 to 8,9-epoxide (Kitada *et al.* 1989).

Epoxidation of AFB_1 to *exo*-8,9-epoxide is a critical step in the genotoxic pathway of this carcinogen (**Fig. 3**). The epoxide is highly unstable and binds with high affinity to guanine bases in DNA to form afltoxin-N^7-guanine (Guengerich 2001). The afltoxin-N^7-guanine has been shown to be capable of forming guanine (purine) to thymine (pyrimidine) transversion mutations in DNA (Bailey *et al.* 1996). Studies *in vitro* and animal models as well as epidemiological studies have revealed a high incidence of this transversion mutation occurring at codon 249 of the p53 tumor suppressor gene (Li *et al.* 1993; Mace *et al.* 1997) a region corresponding to the DNA binding domain of the corresponding protein (Sudakin 2003).

The glutathione pathway has been shown to play a major role in the detoxification of AFB_1 (Johnson *et al.* 1997; Farombi *et al.* 2005a). The AFB_1 8,9-*exo*- and -*endo*-epoxides can be conjugated with glutathione resulting in the formation of AFB-mercapturate catalyzed by glutathione *S*-transferase (GST) (Johnson *et al.* 1997). The *exo*- and -*endo*-epoxide can also be converted non-enzymatically to AFB_1-8,9-dihydrodiol which in turn can slowly undergo a base-catalysed ring opening reaction to a dialdehyde phenolate ion (Guengerich *et al.* 1998). AFB_1 dialdehyde can form Schiff bases with lysine residues in serum albumin forming AF-albumin (AF-alb) complex (Sabbioni and Wild 1991). Furthermore, AF dialdehyde can be reduced to a dialcohol in a NADPH-dependent catalyzed reaction by AF aldehyde reductase (AFAR) (Knight *et al.* 1999).

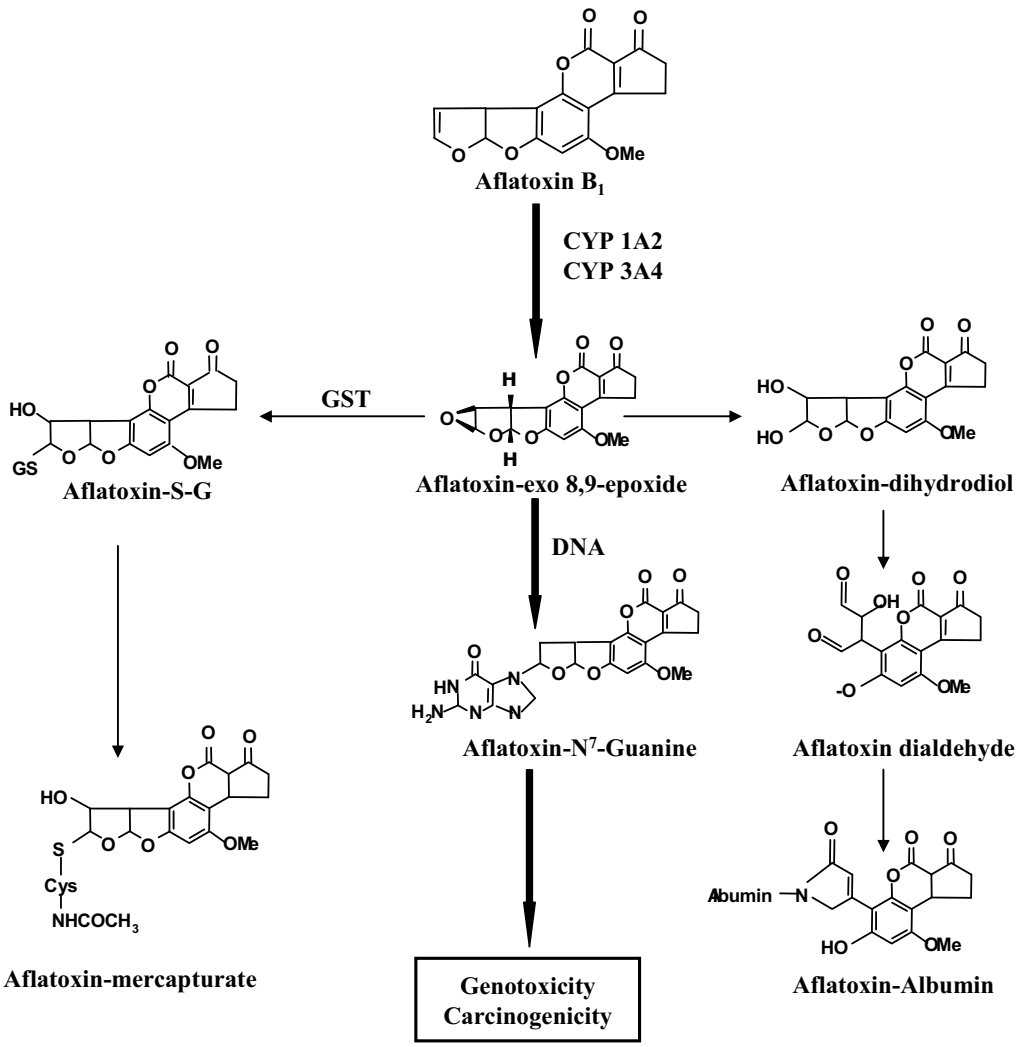


Fig. 3 Major mechanism of biotransformation of Aflatoxin B_1 (AFB_1) to AFB_1-exo 8,9-epoxide leading to the formation of AFB_1-DNA adduct (AFB_1-N^7-guanine), detoxification product AFB_1-mercapturate catalysed by glutathione *S*-transferase (GST) Adapted from Farombi (2006).

DETECTION OF AF

For epidemiologic studies, biomarkers in serum and urine provide a better estimate of AF exposure than food analysis. Testing food AFs is constrained by two limitations. First, obtaining a representative sample of food from subsistence farmers is difficult. Second, there is a lack of information about threshold levels associated with adverse health effects. Agricultural data of the relationship between concentrations of AFs in food and acute aflatoxicosis has resulted in a regulatory limit of 300 ppb for animal feed in the United States. Foods for human consumption in the industrialized world (including exports from developing countries) are enforced with regulatory limits varying from 4 to 20 ppb based on limited information from risk assessments of HCC (Henry *et al*. 1999; van Egmond 2002). Little information is available concerning AF concentrations between 300 ppb and 20 ppb. AF metabolites in urine reflect recent exposure (i.e. 2-3 days) whereas the measurement of AF-alb adducts in blood reflects exposure over a longer period (i.e., 2-3 months) (Groopman *et al*. 1994). These analyses, however, are labor-intensive and expensive (Wild *et al*. 1990; Sheabar *et al*. 1993 McCoy *et al.* 2005). There is also limited information regarding the interpretation and application of AFB adducts and urine immunoassays (Turner *et al*. 1998; Wild *et al*. 2001). The detection of AF metabolites or adducts in urine and serum indicate exposure but do not necessarily equate to adverse health effects. Some studies have correlated AF intakes to biomarker levels (Groopman *et al*. 1992; Wild *et al*. 1992) and to disease (Qian *et al*. 1994; Wang *et al*. 1996; Gong *et al*. 2004; Azziz-Baumgartner *et al*. 2005). More research is needed to determine AF levels in biological specimens that are associated with adverse health effects. Research must also clarify the relationship between AF levels in biological specimens and levels in food.

In developed countries, commercial crops are routinely screened for AF using detection techniques that are performed in a laboratory setting. Food supplies that test over the regulatory limit are considered unsafe for human consumption and destroyed. In developing nations, many people are exposed to AF through food grown at home. Inadequate harvesting and storage techniques allow for the growth of AF-producing fungus and homegrown crops are not routinely tested for the presence of AFs. As a result, an estimated 4.5 billion people living in developing countries may be chronically exposed to AF through their diet.

In May, 2006, an outbreak of acute aflatoxicosis was reported in a region of Kenya where AF contamination of homegrown maize has been a recurrent problem. CDC teams worked with the Kenyan Ministry of Health to trial a rapid, portable AF screening tool that could be used in the field to identify contaminated maize and guide urgent maize replacement efforts during an outbreak. To do this, the CDC teams used a portable lateral flow immunoassay; a test validated for use at commercial silo laboratories, and modified the methods for use in rural Kenya without electricity or refrigeration (CDC 2004).

Field methods used during the outbreak were compared to Vicam immunoaffinity methods currently used at the Kenya National Public Health Lab. Field screening methods showed a sensitivity and specificity of 98 and 91%, respec-

tively. This investigation demonstrates that rapid lateral flow immunoasssays may be modified to provide a simple, on-site screening tool that gives immediate results and facilitates timely interventions.

AF exposure cannot be measured accurately at the individual level through a combination of questionnaire-based approach and food analysis, primarily because the heterogeneity of toxin distribution within a particular food product makes representative sampling impractical. Exposure biomarkers have been developed to circumvent this problem, including serum AF-alb adducts that reflect recent past exposure (previous 2-3 months) (Wild and Turner 2002). In a cross-sectional study in Benin and Togo, young children showed a consistently high prevalence and level of AF-alb, with detection of the marker in 99% of children [geometric mean (GM), 32.8 pg/mg; 95% confidence interval (CI), 25.3-42.5]. Exposure was significantly related to weaning status in children 1-3 years of age, with mean AF-alb levels approximately 2-fold higher in fully weaned children compared with those receiving a mixture of breast milk and solid foods. Furthermore, the level of AF-alb was strongly associated with growth faltering, particularly stunting (Gong *et al*. 2002, 2003). Although breast milk may contain AFs (Zarba *et al*. 1992), these are generally less toxic metabolites (AFM_1) than are the parent toxins found in the diet (AFB_1, AFG_1), and they occur at lower levels. Thus, breastfeeding provides a period of relatively low AF exposure in a population whose primary weaning foods, particularly maize, are at high risk of contamination. Toxin exposure during the postweaning period may be a critical factor in young children in determining the adverse health effects of AFs in terms of growth, immune status, and eventually liver cancer risk.

Denning *et al*. (1991) reported the quantity of AFB_1 and AFG_1 in human cord sera obtained at birth and in serum obtained immediately after birth from the mother. The subjects of the study were residents of Songkhla, Thailand. Of the 35 samples of cord sera, 17 (48%) contained AF in concentrations from 0.064 to 13.6 nmol/ml, with a mean of 3.1 nmol/ml. By comparison only two (6%) of 35 maternal sera contained AF (mean 0.62 nmol/ml). These results demonstrate transplacental transfer and concentration of AF by the feto-placental unit, which may be of biological importance.

An enzyme-linked immunosorbent assay (ELISA) developed to determine AF in food was adapted to analyse rapidly human serum for AF (Wilkinson *et al*. 1989). Sera from subjects in the U.K., Nigeria and Nepal were studied. No AF was found in U.K. sera, whilst 76 and 100%, respectively of Nigerian and Nepalese samples were found positive for AF. A study was also made of maternal and cord sera from Thai subjects. Only 6% of maternal blood had detectable AF whilst 49% of cord sera were found positive for AF (Wilkinson *et al*. 1989). This is evidence of *trans*-placental transfer of AF in humans and possibly of the concentration of AF by the feto-placental unit. Maternal to child exposure of AFM_1 in breast milk is an under evaluated risk factor from dietary exposure to AFB_1 (Zarba *et al*. 1992; IARC 1993). A molecular dosimetry study in Gambia, West Africa, was initiated to explore the relationships between dietary intake of AFs during a 1-week period and a number of AF biomarkers including AF metabolite excretion into breast milk. Detection of AFM_1 in cord sera has been reported from Thailand, Ghana, Nigeria and Gambia. Several studies have demonstrated the presence of AFM_1 in human milk. It was observed that only a small percentage of dietary AF intake was excreted in milk (Lamplugh *et al*. 1988).

Analysis of food samples provides only an indirect evidence of AF ingestion, whereas, direct evidence can only be obtained by analysis of body fluids as already reviewed (Dorner and Cole 1989). Monitoring of their concentration in body fluids requires the determination of even trace amounts because of their potent biological activity. Also, their measurement in fluids would give a direct measurement of exposure. Due to the highly immunosuppressive and carcinogenic nature of AFB_1, even a low level of contamination is important. Conventional methods used for its detection viz., TLC, GLC, HPLC, etc., have limitations in terms of sensitivity, ease and duration time of test. Therefore, there is a need to develop highly sensitive, specific, simple and nonradioactive tests. Enzyme immunoassays have become established as routine procedures in many developing countries (Morgan *et al*. 1986; Wilkinson *et al*. 1988; Park *et al*. 1989). Chu and Ueno (1977) for the first time developed ELISA for AFB_1 detection with a sensitivity of 0·2-2 ng/0.5 ml sample. In the study, in dot-ELISA, sensitivity was improved from 500 pg to 1 pg by including an additional step of preincubation, as also reported earlier (Shashidhar and Rao 1988). Sensitivity limit obtained in dot-ELISA in the present study (1 pg) is much higher than the 20 ng limit reported by Singh and Jang (1987). In plate ELISA, a sensitivity of 100 fg was obtained as also reported by Morgan *et al*. (1986), but higher than the one achieved by Biermann and Terplan (1980).

Sekhon *et al*. (1996) described ELISA for the detection and quantitation of AFB_1 in poultry sera. Dot-ELISA is intended for screening samples at the field level for on-site monitoring of feed samples also. This is because the serum concentration of AF reflects the level of toxin found in food and subsequent consumption of this contaminated food. Also, the toxin ingested regularly, does not disappear rapidly, levels remaining significantly high due to release of toxin from tissue stores. Hence, it would be worthwhile to test this assay for AFB_1 detection in the tissues also. In the report presented by Lewis *et al.* (2005) AF contamination of commercial maize was analyzed using a slightly modified immunoaffinity method based on the Association of official Analytic Chemists (AOAC) method 991.3 (Trucksess *et al*. 1994). Briefly, the whole sample was ground to pass a No. 20 sieve, and a 50-g subsample was removed for analysis. Methanol: water (80:20) solvent (100 ml) and 5 g NaCl were added to the 50-g sub sample, and the mixture was blended at high speed for 1 min. The mixture was then filtered through a fluted filter paper (Whatman 2V) and the filtrate was diluted (1:4) with water and refiltered through a glass-fiber filter paper. Two ml of the glass-fiber filtrate was placed on an Aflatest P immunoaffinity column and allowed to elute at 1-2 drops/sec. The column was washed two times with 5 mL water, and AF was eluted from the column with 1 mL high performance liquid chromatography (HPLC)-grade methanol. A bromine developer was added to the methanol extract, and the total AF concentration was read in a precalibrated VICAMSeries-4 fluorometer set at 360 nm excitation and 450 nm emission.

INTERACTION OF AF WITH THE HEPATITIS B VIRUS

Studies have shown that concurrent infection with the Hepatitis B virus (HBV) during AF exposure increases the risk of HCC. As HBV interferes with the ability of hepatocytes to metabolize AFs, an AFM_1-DNA conjugate exists for a longer period of time in the liver, increasing the possibility of damage to oncogenes such as p53. This effect is synergistic with the resulting damage far greater than just the sum of AF or HBV individually (Williams 2004). The etiology of primary liver cancer is nowadays largely understood. **Table 1** summarizes the range and the point estimates of the attributable fractions in two different settings, the low-risk areas in Europe and the USA and the high-risk areas in Africa and Asia. In both scenarios, viral infections to hepatitis B or C virus are associated with liver cancer in a range from 65 to 100% of cases. In low-risk countries HBV predominates and the other relevant factors are alcohol, tobacco and oral contraceptives. In high-risk areas HBV predominates and AFs play a role, although quantification has been difficult. The evidence points to a synergistic interaction between HBV and AF in the etiology of liver cancer and some debate exists as to the independency of AF as an etiologic agent in humans. It is noteworthy that the large

Table 1 Casual factors of liver cancer and estimates of the attributable fractions.

Factor	Low-risk countries:				High-risk countries:	
	Japan and Europe		USA		Africa and Asia	
	Estimate	Range	Estimate	Range	Estimate	Range
Hepatitis B	<15%	4-50%	20%	18-44%	60%	40-90%
Hepatitis C[3]	60%	12-64%	50%	40-80%	10%	NE
Aflatoxin	Limited exposure	Limited exposure	Limited exposure	Limited exposure	Important exposure	Important exposure[1]
Alcohol	<15%[4]	-	<20%	11-30%[5]	NE	-
Tobacco	<12%[4]	-	40%	38-51%[5]	NE	-
Oral contraceptive	10-50%[2]	-	NE	-	NE	-
Other	< 5%	-	-	-	<5%	-

[1] Attributable risk not quantified. One study suggested attributable fraction close to 50%.
[2] Restricted to liver cancer in women. Likely to increase in future generations.
Uncertain if hepatitis infections (notably HCV) are necessary co-factors.
[3] Not including double infections with HBV and HCV. Very few studies available using second-generation assays.
[4] Estimates for the USA
[5] Estimates from three studies of LC in men
NE: not evaluated.
Note: attributable fractions do not necessarily add to 100% due to multiple exposures and possible interactions between risk factors.
Adapted from CDC 1989; Bosch and Munoz 1991; Thomas 1991; Tanaka *et al.* 1993; IARC 1994; Bosch 1995

majority of the available epidemiological studies including data on AF exposure are based on high-risk countries where both HBV and AF are highly prevalent. Since the nature of the interaction at low levels of exposure is unknown, extrapolation of results from available studies to other settings is questionable.

HCC as a result of chronic exposure has been well documented, generally in association with HBV or other risk factors (Quian *et al*. 1994; Wang *et al*. 1996; Chen *et al*. 2001; Henry *et al*. 2002; Omer *et al*. 2004). The International Agency for Research on Cancer (IARC) first recognized AFs as carcinogenic in 1976 and has subsequently reaffirmed naturally occurring mixtures of AFs and AFB_1 as Group 1 carcinogens (carcinogenic to humans) (IARC 2002). Additional effects of chronic exposure have not been widely studied but are thought to include immunologic suppression, impaired growth, and nutritional interference (Patten 1981; Cullen and Newberne 1994; Fung and Clark 2004; Williams *et al*. 2004). Food commodities affected by AFs are also susceptible to other types of mycotoxins and multiple mycotoxins can co-exist in the same commodity (Bankole and Mabekoje 2004; Fung and Clark 2004; Speijers and Speijers 2004).

Various cereals affected by AFs are also susceptible to contamination by fumonisins, trichothecenes (especially deoxynivalenol), zearalenone, ochratoxin A and ergot alkaloids. Maize can be contaminated with AFs, fumonisin, trichothecenes, zearalenone and, rarely, ochratoxin-A, while wheat can be contaminated with AFs, trichothecenes, ochratoxin-A, ergot alkaloids and zearalenone. Therefore individuals may be exposed to various combinations of mycotoxins. The health effects associated with exposure to multiple mycotoxins are not well documented. Related mycotoxins are thought to have an additive effect while unrelated mycotoxins may have a synergistic effect (Speijers and Speijers 2004). A better understanding of exposure to multiple mycotoxins and the health effects associated with the interactions of multiple mycotoxins would clarify the true health impact of mycotoxins.

Several studies in China have indicated combined exposure to HBV and AFs are associated with a much higher risk of HCC (Qian *et al*. 1994; Wang *et al*. 1996). This interaction has not been studied in other high risk areas such as sub-Saharan Africa and the molecular mechanism of the interaction between HBV and AFs is not known (Turner *et al*. 2002; Wild *et al*. 2002). Studies have reported that Chronic HBV infection may induce the cytochrome P450s that metabolise inactive AFB_1 to the mutagenic AFB_1-8, 9-epoxide. Hepatocyte necrosis and regeneration and the generation of oxygen and nitrogen reactive species resulting from chronic HBV infection increase the likelihood of the AFB_1-induced p53 249^{ser} and other mutations and the subsequent clonal expansion of cells containing these mutations. Nuclear excision repair, which is normally responsible for removing AFB_1-DNA adducts, is inhibited by HBV×protein, favouring the persistence of existing mutations. This protein also increases the overall frequency of DNA mutations, including the p53 249^{ser} mutation, and may contribute to uncontrolled cell cycling when p53 is non-functional (Michael 2003). Quantifying the proportion of HCC attributable to AF exposure, to HBV, and to the interaction of AF exposure and HBV will help identify the best public health strategy to reduce HCC, including the benefits and limits of widespread HBV vaccination.

AFs AND FOOD SAFETY

There is increasing concern about the levels of mycotoxins in human foods, both from vegetable and animal origin. Mycotoxins can contaminate agricultural products and threaten food safety. AFs are of particular public health importance because of their effects on human health. AFs have both carcinogenic and hepatotoxic actions, depending on the duration and level of exposure. Chronic dietary exposure to AFs is a major risk factor for HCC, particularly in areas where HBV infection is endemic. Ingestion of higher doses of AF can result in acute aflatoxicosis, which manifests as hepatotoxicity or, in severe cases, fulminant liver failure (Fung and Clark 2004). Contamination of food supplies by these and other naturally toxins are of particular concern in rural communities of developing countries (Bhat *et al*. 1997). AF remains an unavoidable and common contaminant of foods, particularly in the staple diets of many African and other developing countries. Methods used to ensure minimal contamination in Europe and other developed Western countries have been impracticable in developing countries because of the characteristics of the food systems and the technological infrastructure.

The outbreak of acute hepatotoxicity was identified among people living in Kenya's eastern and central provinces. Epidemiologic investigations determined that the outbreak was the result of aflatoxion poisoning from ingestion of contaminated maize. As of July 2004, 317 cases and 125 deaths had occurred; making this one of the largest and most severe outbreaks of acute aflatoxicosis documented worldwide (CDC 2004).

AF contamination of crops is a widespread serious problem particularly in most groundnut-producing countries where the crop is grown under rain-fed conditions. The contamination of crops by AF does not affect crop productivity but it makes produce unfit for consumption as toxins are injurious to health. The marketability of contaminated produce, particularly in international trade is diminished to nil due to stringent standards of permissible limits on AF contamination set by the importing countries.

This can occur in the field before harvest, during post-

harvest drying and curing, and in storage and transportation. The semi-arid tropical environment is conducive to preharvest contamination when the crop experiences drought before harvest, whereas in the wet and humid areas, post harvest contamination is more prevalent.

There is little or no information in this regard because of the complex nature of the problem and lack of qualified personnel and appropriate infrastructure. Nevertheless, some countries have been regularly monitoring groundnut and its products for AF at different stages (farm, markets, and storage). AF contamination can be minimized by adopting certain cultural, produce handling, and storage practices. However, these practices are not widely adopted particularly by the small farmers in the developing countries, which contribute about 60% to the world (Upadhyaya *et al*. 2001) groundnut production.

The problems caused by mycotoxins have trade and economic implications. In domestic markets economic losses occur at various levels, from the commodity producers to the brokers, the processors and the animal producers (Akande *et al*. 2006). The need for setting maximum levels of AFs in foods and feeds is generally recognized. Several countries, particularly some industrialized ones, have already set specific regulations. Limits for AFB_1 in foodstuffs of 0 to 30 µg/kg, while those for total AFs range from 0 to 50 µg/kg. Economic pressures have created a double standard for allowable contamination of commodities destined for human and animal consumption. Human foods are allowed 4-30 ppb AF, depending on the country involved. In Africa, 15 countries, accounting for approximately 59% of the continent's population, were known to have specific mycotoxin regulations in 2003 (Henry *et al*. 1999).

EFFECT OF AF ON ANIMAL HEALTH

The effects of AFs on animal health have been observed in many species for over forty years (Patten 1981) beginning with the documentation of the Turkey X disease in 1960 (Asao 1963). Acute effects include hemorrhagic necrosis of the liver and bile duct proliferation while chronic effects include HCC. In animals, suppression of immunity, growth retardation, and increased susceptibility to infectious disease due to AF exposure is well-documented (Patten 1981). The effects of AFs on humans, as with animals, are dependent upon dosage and duration of exposure. Acute exposure can result in aflatoxicosis, which manifests as severe, acute hepatotoxicity with a case fatality rate of approximately 25% (Cullen and Newberne 1994). Early symptoms of hepatotoxicity from aflatoxicosis can manifest as anorexia, malaise, and low-grade fever. Acute high level exposure can progress to potentially lethal hepatitis with vomiting, abdominal pain, jaundice, fulminant hepatic failure, and death. Outbreaks of acute aflatoxicosis are a recurring public health problem throughout the world (Krishnamachari *et al*. 1975; Ngindu *et al*. 1982; Lye Ghazali *et al*. 1995; CDC 2004).

THE IMPACT OF AFs ON HUMAN HEALTH

AF-associated health effects pervade the developing world despite the fact that these effects could be mitigated or prevented with the current state of agricultural knowledge and public health practice. The discussion of this problem and its remedies must be held in the context of the associated question of food insufficiency and more general economic challenges in developing countries. Outbreaks of acute AF poisoning are a recurrent public health problem. In 2004, one of the largest, most severe aflatoxicosis outbreaks occurred in Kenya followed by another outbreak in 2005 (CDC 2004). Given that diseases in the developing world often go unreported, the Kenya outbreaks are likely to be an underestimation of the problem; furthermore, the burden of disease attributable to chronic AF exposure (e.g. HCC, impaired growth, immune suppression) remains undefined. These outbreaks emphasize the need to quantify and control AF exposure in developing countries and highlight the potential role of public health.

Cancer can be caused by a variety of factors including oncogenic viruses and other biological agents. To date the only clearly established non-viral biological occupational carcinogens are the mycotoxins. These occur in industries in which mould-contaminated materials are handled (Anonymous 1998). Perhaps the best-known carcinogenic mycotoxin is AF from *A. flavus*, which is an established human carcinogen particularly with regard to liver cancer (Hayes *et al*. 1984; Sorenson *et al*. 1984).

There is no doubt that the presence of mycotoxins in grains and other staple foods and feedstuffs has serious implications for human and animal health. But it is interesting to note that the World Health Organization (WHO) does not recognize AFs as a high-priority problem from their analysis of factors contributing to the burden of disease across the world, even in developing countries where a short lifespan is prevalent (Abarca *et al*. 1994). However, because of the immunologic and nutritional effects of AF as indicated in several studies, the probability that the six top WHO risk factors (which account for 43.6% of the disability-adjusted life years (DALYs) in countries where the short lifespan is prevalent), as well as the risks of liver cancer, are modulated by AF has been suggested (William *et al*. 2004). It is of prime importance therefore to adequately document the toxicity profile of AFs and the broad consequences of human exposure and also ensure deliberate efforts at managing the problem in developing countries.

Additional health effects associated with chronic AF exposure have not been well studied. Without knowing the relationship between chronic exposure and health, the true human health impact and the resulting burden of disease in developing countries are not known. Preliminary evidence suggests that there may be an interaction between chronic AF exposure and malnutrition, immunosuppression, impaired growth, and diseases such as malaria and HIV/AIDS. Experimental animal evidence suggests that chronic exposure to AFs may lead to impaired immunity, reduced uptake of nutrients from the diet, and growth retardation (Hall and Wild 1994). The extent to which factors such as immune suppression contribute to the overall burden of infectious disease is difficult to quantify, but is undoubtedly significant (Shephard 2008). These effects are only now being investigated and characterized in human populations.

Several studies of children in Benin and Togo have shown an association between AF-alb adducts levels and impaired growth (Gong *et al*. 2002, 2003, 2004). In a recent study in Ghana, higher levels of AFB_1-alb adducts in plasma were associated with lower percentages of certain leukocyte immunophenotypes (Jiang *et al*. 2005). A study in Gambian children found an association between serum AF-alb levels and reduced salivatory secretory IgA levels (Turner *et al*. 2003). While the effects on immunity suggest the possible influence of AFs on susceptibility to infectious disease, further investigation is needed.

It has been hypothesized that kwashiorkor, a severe malnutrition disease, may be a form of pediatric aflatoxicosis (Hendrickse 1997). In a study with Egyptian infants with protein-energy malnutrition (PEM), AFs were found to be significantly higher in serum and urine of infants with kwashiorkor and marasmus. The study demonstrated a are highly prevalence of AFs in the study population and show a high degree of correlation with severe PEM (Hatem *et al*. 2005).

AFLATOXIN CONTAMINATION OF FOOD IN DEVELOPING COUNTRIES

Aspergillus flavus contaminates many crops with AFs, including corn, peanut, sorghum, millet, beans cottonseed, and tree nuts (almonds, pistachios, etc). These usually occur during growth, harvest, or storage. Secondary exposure, through the consumption of products derived from animals that consumed AF-contaminated feed also occurs (Williams

Table 2 Examples of food commodities and aflatoxin (AF) contamination levels reported in the literature.

Country	Reference	Commodity	Frequency of AF-positive samples	Contamination rate/concentration
Botswana	Mphande *et al.* 2004	Raw peanuts	78%	12- 329 µg/kg
Nigeria	Bankole *et al.* 2004	Pre-harvest maize, dried yam chips, melon seeds	*A. flavus* in 65% of samples	Total AFs in maize = 3-138 µg/kg in positive samples; Mean concentration of AFB_1 in yam chips is about 27.1 ppb; AFB_1 above 5 µg/kg in 32.2% of samples
Malaysia	Ali *et al.* 1999	Peanut	65%	50 µg/kg aflatoxin level
Senegal	Diop *et al.* 2000	Peanut oil	AFB_1 in >85% of samples	Mean contents about 40 ppb
Thailand	Waenlor *et al.* 2002; Lipigorngoson *et al.* 2003	Peanut, corn and milk	38.9% contained aflatoxin	73 µg/kg 102 µg/kg
South Africa	Odhay *et al.* 2002	Traditionally brewed beers	2/6 commercial beer samples contained AFs	200 and 400 µg/l
Philippines	Ali *et al.* 1999	Peanut	40%	375 µg/kg aflatoxin level
		Corn	All the maize samples	130 µg/kg aflatoxin level
Brazil	Freitas *et al.* 1998	Peanut	Aflatoxins in 8-80%	2-16,862 µg/kg
China	Li *et al.* 2001	Corn and peanuts	70.27% of corn samples contained AFB_1	27.44-1098.36 µg/kg
Turkey	Dogan *et al.* 2006	Cacao	55% contained aflatoxins	0.065-25.753 ppb

et al. 2004). AF production also occurs on soybeans (Sinha *et al.* 1990) and is often considered a post harvest problem. Many studies of AF-producing fungi and AF contamination have been conducted in agricultural areas of some developing and South America (SA) countries. These regions have predominantly tropical and subtropical continent and provides environmental conditions favorable for fungus growth on food crops, especially the species *Aspergillus flavus and Aspergillus parasiticus*. Depending on the grain and weather conditions in certain regions of SA, high levels of AFs can be produced during harvesting or storage. That is a real problem in most of the continent. **Table 2** summarises the examples of food commodities contaminated with aflatoxin (AF) in some developimg countries

Research is currently being conducted in countries like Brazil, Argentina, Colombia, Venezuela, and Uruguay; the major exporters of grains in SA to determine the levels of naturally occurring AFs in a range of locally processed foods. Most contaminated food commodities in SA include peanut and peanut products, followed by corn. The regions most affected by AF contamination in SA include mainly the peanut-producing countries of northern SA as well as Brazil, Argentina, Uruguay, and Paraguay. AF contamination of feeds and foodstuffs seems greater in Colombia and Ecuador. On the other hand, AFs in corn is high in Venezuela (see review (Scussel 2004). There is rather little information about the natural occurrence of mycotoxins in feedstuffs in Argentina. In a prelimnary data reported by Dalcero *et al.* (1997) on the occurrence of mycoflora and aflatoxin B1, zearalenone and deoxynivalenol in poultry feeds in Argentina. Three hundred samples of poultry feeds from 5 factories of Río Cuarto, Córdoba taken from May 1995 to May 1996 were analyzed. Fungal counts of poultry feeds ranged 104 to 106 CFU $g^{-}1$. The lowest counts were obtained on the first months from the sampling (May to September 1995) with mean values significantly different from those found at the last of the sampling (October 1995 to April 1996). Within the *Aspergillus* species: *A. parasiticus* (33%) and *A. flavus* (8%) were the most prevalent species identified. In poultry feeds AFB1 was the most significant mycotoxin with levels ranging from 17 to 197 ng/g. For deoxynivalenol (DON) the levels ranged from 240 to 410 ng/g. Only three out of 300 samples were contaminated with zearalenone (ZEA) in concentrations of 30, 120 and 280 ng/g.

A monitoring study on AF contamination in grains and grain products, carried out in Guatemala in 1976, showed a high incidence of contamination. On the southern Pacific coast of Guatemala the temperature and the relative humidity can get very high, conditions that favour AF contamination. As could be expected, the highest incidence of contamination (260/0 of the samples analyzed) was found in this region (Campos *et al.* 1980). Because of the potential danger involved when mold-infested food is consumed, various countries have established maximum limits for AFs in food. In the United States, the FDA applies a 20 ppb (parts per billion) action level for all affected foods, except peanuts where 15 ppb has been proposed (Campos *et al.* 1980). Guatemala has not yet established any maximum limits for AFs, but in those cases it is usual to employ the FDA regulations or those of the Codex Alimentarius Commission as guidance. The occurrence of AFs and fumonisins in Incaparina, a high-protein food supplement containing mixtures of corn and cottonseed flour and marketed in the US and Guatemala has been reported (Trucksess *et al.* 2002). In this study, eight samples of Incaparina manufactured in Guatemala were examined for fungal contamination. All samples contained AFs, ranging from 3 to 214 ng g^{-1} and <2 to 32 ng g^{-1} for AFB_1 and AFB_2, respectively: and one sample contained AFG_1 (7 ng g^{-1}). Total AFs present ranged from 3 to 244 ng g^{-1}. Appropriate regulatory action was recommended for the import of Incaparina and has been in effect since 22 December 1998. In another study, thirty-six samples of nine varieties of newly harvested corn (4 samples each variety) were analyzed for AFs by TLC and HPLC, and also cultured for the presence of *Aspergillus flavus.* Of the 36 samples studied, one was contaminated with 1290 ppb AFB1, which is 258% the concentration suggested by WHO, placed at 5 ppb in food for human consumption. Culture of the 36 samples of corn resulted in growth of 55 colonies of *A. flavus* from all but two (1 and 6) of the 9 varieties. Of the 55 colonies of *A. flavus* obtained, 15 (27.3%) were toxigenic. The implication of these findings on public health requires attention as suggested by the authors (Rojas *et al.* 2000).

There are few data available for common commodities from Nepal, which shows that mycotoxin are present in food and feeds (Desjardins *et al.* 2000). To find out the level of AF in common food and feed in Nepal a study was conducted in 16 districts of the Eastern region of Nepal. Samples were collected from retailers and whole sellers from 1995 to 2003. Common food items like cereals, pulse, nuts, vegetable oil and fat grown in Nepal as well as food products imported from other countries that had high chances of infestation were collected and the AF contamination detected by TLC method. Comparison of toxicity levels for AF was done by comparing fluorescence of sample spot with fluorescence of standard. A maximum value of 30 ppb was considered to classify the food items as contaminated more than the recommended level. Of the 832 samples analyzed for AF detection and estimation, One-third of the samples were found to be contaminated with AF. The highest percentage of contamination was found in peanut butter/ vegetable oil (42.5%) and the lowest in areca nut (25%). Highest proportion of cornflakes samples were found to be contaminated with AF by more than the recommended

value (30 ppb) and contamination in peanut was the lowest. The People of Nepal are possibly exposed to AF by consuming these food commodities. It is therefore of high importance for the Nepal department of food technology and quality control to give attention to this important public health issue because even in small doses, continuous consumption can lead to many health problems (Koirala *et al.* 2005). To conclude, the authors suggested that contamination of commonly used food and feed is an important unrecognized risk to public health and can have long-term health implications. A study conducted among the Philippine people with liver cancer showed 440% higher consumption of AF as compared to controls (Bulatao-Jayme *et al.* 1982). Time to time there has been outbreaks of AF toxicity caused by the consumption of moldy grain. A recent outbreak of jaundice due to AF toxicity occurred in Kenya. The mortality rate was as high as 39% (CDC 2004). The problem is more in this part of the world where there is no proper place to keep the grains dry for long time. It is very difficult to keep all foodstuffs in airtight containers. In an outbreak, 106 people died and 291 showed symptoms of hepatic dysfunction after consumption of moldy maize (Krisnamachari *et al.* 1975). Although liver, kidney, and muscles are commonly affected by AF, the brain is also affected. Autopsy of Thai children who died due to encephalopathy showed AF in their specimen (Shank *et al.* 1971). There are reports of the presence of aflatoxin in the blood of people from Nepal, who consumed contaminated food and feed (Denning *et al.* 1990). Commodities are often contaminated with B1 but much less frequently with B2, G1, G2. Although most people are at risk of exposure to mycotoxins, the individual effects of consumption are not the same because of differences in dietary habits and levels of contamination. In a study more than 11% child with kwashiorkor showed AF in their blood as compared to none of the controls (Coulter *et al.* 1986). It has been shown that the birth weight of the baby is also affected if mothers have taken AF in their food during pregnancy (Abdulrazzaq *et al.* 2002). A strong negative correlation between AF and birth weight was also observed by these authors (Abdulrazzaq *et al.* 2004). Apart from grains, nuts, butter and vegetable oil are also a source of this toxin (Ram *et al.* 1986). Because grains and oils are frequently imported and exported from one country to other, there are chances of spreading of toxicity by these products. Nepal is a country with limited resources so it needs to import these consumable items from different countries. In a study (Koirala *et al*. 2005), 832 samples were collected from 1995 to 2003. Although few of the food and feed were imported, majority were produced locally. Out of total samples more than half (52%) were cereal and pulses, and the rest (48%) were nuts, vegetable oil/butter. One-third samples (32.8%) were found to be contaminated with AFB1 or B2. Peanut butter (42.5%) was leading the list of contaminated food item followed by peanut (34%), maize grit and flour (31.9%), and cornflakes (31.5%). Areca nut was the only food item having AF level less than the recommended value (30 ppb) [**Table 3**]. Eighteen percent (151) of the total sample were found to be contaminated with the AF level even more than 30 ppb (recommended value). It has been observed that the highest proportion of cornflakes samples (26.3%) were contaminated with AF level by more than 30 ppb among all food items. One-fourth sample of wheat flour was also contaminated with more than the recommended value followed by peanut, peanut butter/vegetable oil and maize grit/flour. The level of AFB1 ranged from 54 to 1806 and that of B2 from 4 to 1560 among the samples. Peanut got the least proportion of contamination above the recommended level (**Table 4**).

Maize and wheat are the staple food in Nepal and are grown in this country. Although rice is also consumed in large quantities, it is seen that milled rice in Asian countries usually contains no or very low AF. However the lack of proper storage facility enhances chance of the rice getting infected with *Aspergillum*. In addition to these, the seasonal temperature and humidity accelerates the growth. It is seen

Table 3 Food and food products contaminated with aflatoxin.

Food items	Contaminated	Not contaminated	Total number of samples
Maize grit and flour	92 (31.9)	196 (68.1)	288
Peanut	68 (34.0)	132 (66.0)	200
Peanut butter/ vegetable oil	43 (42.5)	58 (57.5)	101
Cornflakes	18 (31.5)	39 (68.5)	57
Wheat flour	32 (30.1)	74 (69.9)	106
Areca nut	20 (25.0)	60 (75.0)	80
Total	273 (32.8)	559 (67.2)	832 (100)

Figures in parentheses are percentages
Adapted from Tan 2007

Table 4 Food and food products containing aflatoxin more than recommended level.

Food commodity	Proportion (%) of sample having aflatoxin >30 ppb (number)	Range of aflatoxin B1 detected
Maize grit and flour	19.7 (57)	64-859
Peanut	16.0 (32)	54-1806
Peanut butter/ vegetable oil	19.8 (20)	64-1736
Cornflakes	26.3 (15)	60-163
Wheat flour	25.4 (27)	109-693
Total	151*	-

* 18% of total sample. Adapted from Koirala *et al.* (2005)

that 22% of maize samples from foothills of the Nepal Himalayan Mountain contains mycotoxin (Desjardins *et al.* 2000). Koirala *et al.* (2005) demonstrated that peanut, its product, and maize were the main items contaminated with AF. African countries experienced these problems frequently in peanuts and maize (Mphande *et al*. 2004). A survey conducted in Nigeria showed only slightly higher percentage of contamination in maize and its products (Bankole *et al.* 2004). Contrary to the present study, which showed cereals, nuts contaminated (more than recommended value) with AF, developed countries also showed contamination in the same food items but below the recommended value (Blesa *et al*. 2004). The reason for this may be because of the better storage facilities and proper screening and regular monitoring for these contaminants in those countries. In comparison to developed countries, the third world has limited resources and the surveillance system thereby leading to chances of getting more contaminated food and food products. It was observed that the level of AF in maize ranged from 47 to 859 ppb. This finding is supported by another study from Bangladesh where AF level was ranged from 33 to 480 ppb (Dawlatana *et al.* 2002). When peanut and its products were analyzed for AF, 36% contained more than the recommended level of AF (Mphande *et al.* 2004). To conclude, the authors suggested that contamination of commonly used food and feed is an important unrecognized risk to public health and can have long-term health implications.

In a survey conducted at a poultry feed production unit in Kuwait for AF and other mycotoxin contamination in the samples of yellow maize, soybean meal, wheat bran etc individual AFs were detected and the average levels of AFs in maize at 0.27 ppb (range 0 to 1.69 ppb), soybean meal at 0.20 ppb (range 0 to 1.27 ppb), wheat bran at 0.15 ppb (range 0 to 1.07 ppb), prepared poultry feed for broiler starter at 0.48 ppb (range 0 to 3.26 ppb), broiler finisher at 0.39 ppb (range 0 to 1.05 ppb), and layer mash at 0.21 ppb (range 0 to 1.30 ppb). Although their concentrations were found to be lower than the permissible levels, wherever defined, for the poultry feed, other mycotoxins such as ochratoxin A, fumonisin and zearalenone appeared to coexist with AFs in the various commodities and prepared feed samples (Beg *et al.* 2006). In a report based on about 800 samples analyzed over a 12-month period from October 2005 to September 2006 for the aflatoxins (Tan 2007). The samples were received primarily from Asia and the data

Table 5 The occurrence and concentration of aflatoxins in all feed samples analyzed according to geographical regions.

	North Asia	South East Asia	South Asia	Oceania
No. of Test	414	220	31	62
No. Positive	23	88	17	2
Percent positive	6	40	55	3
Median (µg/kg)	< 4	< 4	15	< 4
Average (µg/kg)	51	37	39	4
Maximum (µg/kg)	494	306	139	4

North Asia (includes China, Japan, Taiwan and Korea)
South East Asia (includes Indonesia, Malaysia, Philippines, Thailand and Vietnam)
South Asia (mainly India)
Oceania (mainly Australia
Adapted from Tan 2007

analyzed from two perspectives; first by geographical regions where the samples were originally from, and second by means of commodity types (**Tables 5, 6**). The geographical regions were grouped as follows; North Asia (China, Japan, Korea and Taiwan), South-East Asia (Indonesia, Malaysia, the Philippines, Thailand and Vietnam), South Asia (primarily India) and Oceania (primarily Australia). The sample types are classified as feed ingredients (such as corn, soybean meal, wheat, rice, Distiller Dried Grain Soluble (DDGS), etc.) and finished feed samples. The occurrence of AFs was 6% in North Asia and the highest level detected was 494 ppb in a corn sample from China. The prevalence of AFs contamination in South-East Asia was 40%. Although the number of samples analyzed from the South Asia was comparatively smaller (total 31), the prevalence of AF (55%) was evident. More than half of the samples analyzed in Oceania were straws/hay; the prevalence of AF was 3%. About 180 corn samples analyzed, AFs were found in 20% (high of 494 µg/kg; average 72 µg/kg), Of the 80 soybean meal samples analyzed, AFs were found in 1% (only one sample was found at 5 µg/kg); More than 40 wheat/bran samples were analyzed. No AFs were found in all wheat/bran samples tested. The corn gluten meal samples analyzed show 16% AFs (maximum of 82 µg/kg, average 37 µg/kg). The use of DDGS as feed ingredient is gaining popularity within the region. AF was found in 25% of the samples (maximum 89 µg/kg; average 27 µg/kg). 25% of rice samples analyzed were found to be contaminated with AFs (maximum of 37 µg/kg; average 19 µg/kg). Finished feed comprises mainly poultry and swine feed samples. AF was found in 22% (maximum 139 µg/kg; average 23 µg/kg) of the samples. More than 40 straw/hay samples were analyzed and AF was not detected.

LEVELS, RELEVANCE AND CONTROL OF AFLATOXINS

Contamination of foods in Mexico

AFs are an important health hazard in Mexico for the following reasons: (i) Mexico has one of the highest per capita consumptions of corn in the world (≈ 325 g/day); (Elias-Orozco *et al.* 2002) (ii) Mexico imports 6 million tons of corn per year (often of dubious quality) at a cost of 550 million dollars (Guzman-de-Pena *et al*. 2005) representing 11% of total North American exports; (iii) storage conditions for corn in Mexico are insufficiently developed and there is no regular monitoring of AF contamination (Mendez-Albores *et al.* 2003) and (iv) laws regulating the domestic and international trade of corn contaminated with AFs have not been formulated. AFs have been found in different commodities such as corn, common beans, sorghum, peanuts, tortillas etc at concentrations significantly above levels permitted in the USA (Guzmán-de-Peña 1989). Concentrations of AFs in corn (mostly of corn grown in Mexico) ranged from 15 to 250 µg/kg in 1986. Similarly, nixtamal-flour contained AF levels that ranged from 2.7 to 17% µg/kg in a survey performed in 2004 (Guzman-de-Pena *et al*. 2005). Given that Mexico has the highest per capita consumption of corn in the world, these data suggest that the Mexican population is constantly exposed to the harmful effects of AFs.

Several actions to decrease the AF contamination in corn should be undertaken. For international transactions it is recommended that the grain should contain no more than 20 µg/kg of AFB1, at the selling site, during transportation, and final storage. This particular period of time is crucial in maintaining the quality of grain, since the level of contamination may significantly increase due to poor management and storage conditions. Undoubtedly, the lime treatment given to corn in the process of tortilla making, known as "nixtamalización", which has been practiced in Mexico since pre-Hispanic times, reduces by 95% the concentration of AF in the final product (Guzmán-de-Peña *et al.* 1995; Méndez-Albores *et al.* 2004). Unfortunately, "traditional nixtamalización" is not used in modern procedures in which corn is directly used to make flour, flakes, food additives, etc. Nevertheless, data from Kenya, Mozambique, the Philippines, Swaziland, Thailand and Uganda, show a positive association between high intakes of AFs and high incidence rates of liver cancer especially in adult men (Williams *et al.* 2004). The reports of Mexican patients with viral hepatic disease and high levels of AFB1 in urine have been published (Alvarez *et al.* 2000). A comparatively recent study of an AF-alb adduct in blood serum, for example, showed that exposure to AFs is significantly higher in Gambia, Kenya and parts of China compared with Thailand and Europe (Wild *et al.* 1992). Indeed, the incidence of liver cancer in many of these studies is a linear function of the log of dietary AF intake (Wang *et al.* 1999). Human fatalities have also occurred from acute AF poisoning in India (in 1974), for example, when unseasonal rains and a scarcity of food prompted the consumption of heavily contaminated maize (Krishnamachari *et al.* 1975). If the immunosuppressive action of the AFs in livestock is similarly manifested in humans, it is possible that AFs (and other mycotoxins) could play a significant role in the cause of human disease in some developing countries, where a high exposure to these toxins has been reported.

With regard to national corn production, attention should be paid, during growth and harvest of corn, particularly in those geographic zones in which high AF contamination has been reported. It is also very important to reinforce the use of "traditional or industrial *nixtamalización*"

Table 6 Occurrence of aflatoxins in the feed samples according to the commodity type

	No. of tests	No. Positive	Percent positive	Median (µg/kg)	Average (µg/kg)	Maximum (µg/kg)
Corn	177	36	20	< 4	72	494
Soybean meal	80	1	1	< 4	5	5
Wheat/bran	42	0	0	< 4	ND	ND
Corn gluten meal	19	3	16	< 4	37	82
Rice/bran	16	4	25	< 4	19	37
DDGS	16	4	25	< 4	27	89
Other feed Ingredients	71	12	17	< 4	52	133
Finished feed	324	70	22	< 4	23	139
Stray/Hay	47	0	0	< 4	ND	ND

Finished feed comprises mainly poultry and swine feed samples
Adapted from Tan 2007

as a means to reduce AF contamination in corn for human consumption. Since the "North American Free Trade Agreement" (NAFTA) came into force, the volume of corn imported from the USA grew by 140% (Guzman-de-Peña *et al.* 2005). According to the USA standards for corn, Grade 2 is usually imported which corresponds to grain of the following characteristics: a) Broken corn and foreign material, 3% (maximum limit); b) Damaged kernels, 5% maximum limit; c) Moisture content 15%; d) AF content, (20 µg/kg maximum allowed limit). 20 µg/kg corresponds to the maximum level permitted in the USA for International Trade. It has been estimated, for example, that annual loss in the US and Canada as a result of mycotoxins are about $5bn. The agency responsible for exports of corn in the USA is the Federal Grain Inspection Service (FGIS). The primary task of the Agency is to carry out the provisions of the U.S Grain Standards, to ensure integrity in the inspection, weighing, and handling of American grain. In addition to FGIS, private and state agencies may, upon application, be authorized to perform official services under the authority contained in the Act (United States Government Manual 1985). However, there is no agreement among the different laboratories on the use of a single, standardized method to measure AFs. Comparisons therefore are difficult to make, with the result that AF levels are often underestimated.

This decade-long surge of corn imports into Mexico, the diverse climatic conditions under which corn is stored, as well as the likelihood that the AF levels increase during transport and storage, has lead to an increased risk of hepato-cellular carcinomas. Thus the only way to ensure a safe supply of corn is to develop regulations and policies that will be applied throughout the processes of purchasing, transportation, distribution, storage and consumption.

AFLATOXINS IN COTTONSEED, PEANUT AND GROUNDNUT

Whitten *et al.* (1972) studied the incidence of AF in cottonseed in the US during the 1964-65 season, and found that 4% of the samples contained over 30ppb of AFB1. The authors also reported that small lots of cottonseed with low AF contamination when stored at 15, 18 and 22% moisture at 80 and 85 F had Maximum AF content reached within 30 days' storage. Aeration caused about a five-fold mean increase in AF content. In another study (**Table 7**), 99 samples of ginned cottonseed were analyzed for AF. From these 99 samples, 4 cultures of aspergillus and one of penicillium capable of producing aflatoxin were isolated. In only one case was an aflatoxin producer isolated from an aflatoxin contaminated sample (Schneider *et al.* 1972).

AF contamination of peanuts occurs during post harvest curing and storage, the most significant contamination usually occurs prior to harvest during periods of late season drought stress as peanuts are maturing. Mould infection of badly harvested and or poorly stored peanuts occurs around 20 to 25°C. When these mouldy peanuts are eaten or processed into food or feed, AF poisoning occurs. Increasing water stress during crop growth increases AF contamination in peanut. The toxins from the peanuts can be removed by extraction using polar solvents to which has been added 0.5% hydrogen peroxide or 0.2% sodium hypochlorite. *Aspergillus* section *flavi* strains isolated from peanuts, wheat and soybean grown in Argentina revealed *Aspergillus flavus* as the predominant species in all substrates, although there was almost the same proportion of *A. flavus* and *Aspergillus parasiticus* in peanuts. *Aspergillus nomius* was not found. Incidence of aflatoxigenic *A. flavus* strains was higher in peanuts (69%) than in wheat (13%) or soybeans (5%). Isolates of *A. flavus* able to produce simultaneously AF type B and cyclopiazonic acid were detected in all substrates, suggesting the possibility of co-occurrence of these toxins. Five of 67 strains isolated from peanuts showed an unusual pattern of mycotoxin production (AFs type B and G simultaneously with CPA). These strains also produced numerous small sclerotia like S strains of *A. flavus* detected in cottonseed in Arizona and in soils of Thailand and West Africa which were considered atypical strains, are not widely distributed in Argentina but were found uniquely in peanuts (Vaamonde *et al.* 2003). The risk of AF poisoning has been reported in Senegal where peanut oil and pastry are commonly consumed. One Study shows that artisanal pastry sold in different market of Dakar (Senegal) where most contaminated by AFs. According to the authors, 40% of these samples contained mean values of AFB1 (the most dangerous) widely over allowable EEC specifications (5 ppb) (Diop *et al.* 2000). Groundnuts and groundnut products are widely contaminated with AFs and contribute extensively to human AF exposure. It has been realized that an ideal situation of absolute elimination of AFs contamination of groundnuts can never be achieved, at least not yet, and many countries and the international community, have attempted to lower exposure by imposing regulatory limits that are as low as reasonable achievable. South Africa is one of the big producers and traders of groundnuts and, like all the other producers, have experienced problems in recent years relating to AF contamination in peanuts and peanut butter, with the School Feeding Programme receiving substantial media coverage. This led the Directorate: Food Control to establish a Steering Committee, which has representatives from both Government and the industry so as to come up with a programme to address the situation. A wide range of gaps were identified and these included, *inter alia*, a need for: a) a thorough investigation of exposure, b) identification of the unscrupulous processors, c) integrated and coordinated approach from farm to fork, d) effective law enforcement and monitoring, and e) strengthening of the mycotoxin legislation to meet international standards. Internationally, the Codex Alimentarius Commission, which is a joint Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO) Food Standards Programme, has established a level of 15 µg/kg for peanuts intended for further processing. This standard is accompanied by a sampling guideline intended for peanuts traded in the export market, because the contamination of grains is non-homogeneous and sampling for enforcement and control has proven to be problematic. In South Africa, AF contamination is regulated by regulation No. R. 313 of 1990, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972). Under these regulations, all foodstuffs containing more than 10 µg/kg AFs, of which B_1 should not be more that 5 µg/kg, are deemed contaminated, impure or decayed (National Monitoring Programme Report 2003-2004). In Nigeria, one study (Akano *et al.* 1989) had reported the presence of AFB, in groundnut cake ('kulikuli') purchased from four major markets in Ibadan, Oyo State. In all but two of the samples AFB, concentrations were between 20 and 455 pg/kg. The authors suggested that groundnut cake on sale in Ibadan markets is unacceptable for animal feed rations and human consumption and there is a need for some form of quality control and decontamination before usage. In another study, Bankole *et al.* (2005) reported that samples of dry roasted groundnuts

Table 7 Aflatoxin in cottonseed.

Sample No.	Country	Month	Aflatoxins µg/kg B1	Aflatoxin µg/kg B2	Aflatoxin µg/kg G1
3	Elsalvador	November	50	15	
79	Nicaragua	January	30	-	-
95	Elsalvador	March	30	9	5
97	Guatemala	April	60	-	-

Adapted from Schneider *et al.* 1972

(DRG) purchased from street hawkers, markets and retail shops in southwestern Nigeria were analysed for moisture content, fungal populations and AF contamination. The moisture content varied from 2.1 to 3.6% while the mould counts, using the dilution plating method, ranged from 2.9×10^2 to 6.3×10^2 colony-forming units/g. AFB_1 was found in 64.2% of samples with a mean of 25.5 ppb. AFs B_2, G_1 and G_2 were detected in 26.4, 11.3 and 2.8% of the samples with mean levels of 10.7, 7.2 and 8 ppb, respectively, in contaminated samples. It is concluded that the regular consumption of DRG by Nigerians might present potential health hazards to consumers.

AFs AND SORGHUM

Sorghum is a relatively poor substrate for AF production compared with high-risk agricultural commodities like maize and groundnut, even though it is susceptible to fungal attack. Thus research on AFs in sorghum has been limited. In a study to determine the growth of *A. flavus* and its potential for production of AF on high-moisture sorghum and corn during short incubation. The authors inoculated samples of ground, cracked, or whole kernels of sorghum or corn with *Aspergillus flavus.* The samples were incubated at 25 or 30°C and 90% relative humidity for 48 or 72 h. In all treatments, the 72-h samples contained more AFs B1 and B2 than the 48-h samples. Cracked sorghum at 30°C for 72 h and whole sorghum at 25°C for 72 h contained more AFs than any other treatment. Even in these short incubation periods, enough AFs could be produced to be harmful to livestock (Winn *et al.* 1978).

Fungal infestation of sorghum results in a varied biochemical composition of the deteriorated grain as was reported in a study (Ratnavathi *et al.* 2000). Six sorghum genotypes (red-AON 486, IS 620; yellow-LPJ, IS 17 779; white-SPV 86, SPV 462) were inoculated with a toxigenic strain of *Aspergillus parasiticus* (NRRL 2999) in order to evaluate the changes in the activities of various hydrolytic enzymes (α- and β-amylases, protease and lipase) in comparison with those in uninfected grains. Enzyme activities were measured at different times after fungal infestation, and the enzymatic activities were correlated with the AF production. α-amylase activity was observed to be greater than β-amylase activity in all six genotypes under both healthy and infected conditions. The increase in α-amylase activity during the period of infection was higher in white genotypes than in red sorghum genotypes. α-amylase activity in all the genotypes increased up to day 6 after fungal infection, but was significantly lower in infected grains than in healthy grains. The variability in the basal enzyme activities among the six sorghum genotypes was quite high compared with the amount of induction of each specific enzyme due to infection and germination. Higher protease activity was observed in the infected grains than in healthy grains. The enzyme activities in high tannin red genotypes were less than those in yellow and white genotypes. The α- and β-amylase activities were positively correlated ($r=0.406$ and 0.436; $P<0.05$) to AF production. Inherent lipase activity was highest (on day 0) in AON 486, SPV 462 and SPV 86, as compared with the activity in infected grains. The total AFs produced (quantified by TLC-fluorodensitometry) were lower in red genotypes than in yellow and white genotypes, suggesting that red genotypes were least susceptible to AF elaboration among the various genotypes tested. All four AFs, (B_1, B_2, G_1 and G_2) were present in five genotypes (IS 620, LPJ, IS 17 779, SPV 86 and SPV 462) at all the stages of infection, but, AF could not be detected in the red genotype AON 486 on day 3 after infection. White genotypes SPV 86 and SPV 462) showed maximal AF (total) production on day 6 after infection. In another study (Kushal *et al.* 1995) AFs were estimated in two sorghum seed varieties (CSH-9 and AJ 140) collected in India and stored at three different temperature and humidity levels which showed 20°C and 73.5% relative humidity (RH) to be safe storage conditions. A maximum AF level was observed at 31°C and 81.0% RH. Though the fungus grew well at 40°C and all the humidity levels tested AF production was comparatively lower but reached hazardous level after 5 months of storage. Though all four aflatoxins were detected in both the seed samples, AFB_1 and AFG_1 were most predominant. Reports of AFs and zearalenone in grain sorghum (*Sorghum bicolor* L.) and their deleterious effects on swine prompted an investigation of grain sorghum grown in North Carolina (Hagler *et al.* 1987). The Studies were conducted from 1981 to 1985 to determine the effects of location, cultivar, grain moisture at harvest, and rainfall pattern on the presence of AFs, zearalenone, and deoxynivalenol at harvest. AF levels were very low in all studies. Significant location effects were detected for zearalenone in 1981, 1982, 1984, and 1985, and for deoxynivalenol in 1981, 1984, and 1985. The location effect may be a function of rainfall wherein heavy rainfall during anthesis and early grain fill predisposes the crop to *Fusarium* mycotoxin contamination. In general, sorghum is not considered as a good substrate for the production of AF compared to maize, groundnut (*Arachis hypogaea* L.), and other oil-rich seeds.

AFs IN CORN

AF contamination of preharvest corn (*Zea mays* L.) has been reported in several countries (Widstrom 1996). In the United States, it is a chronic problem in the southern states, and appears sporadically elsewhere (Wilson *et al.* 1994). It may be widespread in developing countries of the tropics and subtropics in which temperature conditions are likely to favor infection of corn by *Aspergillus* spp. (Widstrom 1990). AFs are not automatically produced whenever grain becomes moldy, the risk of AF contamination is greater in damaged, moldy corn than in corn with little mold. It is well known that environmental conditions strongly influence dispersion of fungal spores (Widstrom *et al.* 1990), penetration and establishment of hyphae in plants and on the production of AFs; also, cultural and agronomic conditions influence the synthesis of AF in corn (Payne 1986). High temperatures and drought conditions are conducive to heavy AF contamination (Widstrom *et al.* 1990). The interrelationship between soil type and level of AF contamination in corn requires further research; certainly the soil is important as an inoculum source (Lillehoj 1980), and altering edaphic factors by fertilization, irrigation or cultivation may affect spore numbers in soil (Widstrom 1996). However, Payne *et al.* (1986) demonstrated that deep ploughing in North Carolina reduced AF contamination. In Tamaulipas State, northeast Mexico, early sowing and proper irrigation decreased AF contamination from 246 to 6 mg kg^{-1}. On the other hand, corn stored in Tamaulipas, 1985 to 1988, revealed mainly a 2% incidence of *A. flavus* with unknown toxigenic activity and low levels of a AFB1 (Guzmán-de-Peña 1989). Mexico has one of the highest rates of human consumption of corn in the world (120 kg/year/per capita) (Figueroa 1999) and also represents a mosaic of environmental conditions in which corn is produced and/or stored for various periods of time. Yet information on AF contamination of corn in the main producing regions is scarce. In central Mexico, environmental conditions, particularly drought seem to be favorable to AF synthesis in the field. Furthermore mycotoxicosis in pigs associated to ingestion of contaminated feeds is frequently reported for this region. A study was therefore undertaken to investigate if the contamination of corn commonly observed in stored conditions in this part of Mexico known as "El Bajío" is related to infection by *Aspergillus* under field conditions. The results showed that corn ears artificially inoculated in the field with a toxigenic strain of *Aspergillus parasiticus* presented a low content of AF ranging from 13.6 to 24.7 μg Kg^{-1}. No significant differences were observed between the corn hybrids tested. The authors' data suggested that the outbreak of AF contamination of corn in this part of Mexico is not related to infection occurring during the crops growing period but most probably to poor storage conditions of corn (Bucio-Villalobos *et*

Table 8 FDA guidelines for acceptable aflatoxin level in corn based on intended use (Munkvold 2003)

Intended use	Aflatoxin level (ppb)
Milk	None detected
Corn of unknown destination	< 20
Corn for young animals	< 20
Corn for dairy cattle	< 20
Corn for breeding meat, swine and mature poultry	< 100
Corn for finishing swine	< 200
Corn for finishing cattle	< 300

al. 2001). The co-occurence of AFs and other mycotoxins such as fumonisins, ochratoxin, trichothecenes, zearalenone and also with fusaproliferin and beauvericin in pre-harvest maize (Yoshizawa *et al.* 1996; Ritieni *et al.* 1997; Ali *et al.* 1998; Scudamore *et al.* 1998) have been observed. This co-occurrence with moniliformin has also been observed in a shipment of South African corn exported to Taiwan (Rheeder *et al.* 1994). Other studies have reported similar observations in pearl millet, dried figs and spices (Patel *et al.* 1996; Resnik *et al.* 1996).

Removing or even reducing AFs contamination in crops is a high priority for food and feed safety. Studies showed that severe infestation by AF-producing fungi diminished the food quality of the southern US corn (maize) crop in 1998. Commercial corn hybrids (21 in 1998; 29 in 1999; and 15 in 2001; later years included some hybrids evaluated in 1998) were evaluated for resistance to mycotoxin contamination when naturally infected with *Fusarium* spp. and *Aspergillus* spp. At harvest, samples were evaluated for the presence of AFs and fumonisins. In 1998, kernel corn samples from all hybrids exceeded 20 ppb AF (mean 21 to 699 ppb) and 2 ppm fumonisins (mean 23 to 79 ppm). Samples from hybrids planted in the same and other locations in Arkansas in 1999 and 2001 were shown by similar methods to contain AF levels ranging from none detected to 255.3 ppb The AF levels were very low, ranging from <5 in most hybrids to 131 ppb. These authors suggested that monitoring of the corn crop for mycotoxins is more important under conditions of plant stress and that alleviation of stress during the growing season might help reduce toxin contamination (Hamed *et al.* 2005). Because AF is extremely poisonous to warm-blooded animals even at relatively low levels, grain handling facilities often check for the presence of the toxin before purchasing corn. AF-contaminated grain may be used locally by livestock producers for animal feed if the AF content is below 200-300 ppb (**Table 8**).

INTERVENTION STRATEGIES FOR PREVENTING AF EXPOSURE DURING FOOD PRODUCTION

An intervention to reduce exposure to AFs can occur at various stages of food production and preparation (**Table 9**). Before crops are planted, efforts can be made to reduce the future burden of AFs. Interventions can also occur before harvest, during harvest, and after harvest. The appropriate intervention or combination of interventions may differ depending on the crop and the country. Therefore further evaluation is needed with consideration towards the sustainability, cultural acceptability, economic feasibility, ethical implication, and overall effectiveness of potential interventions.

MINIMISING AF CONTAMINATION OF CROPS

Use of resistant cultivars resistant to AF-producing fungi

The use of cultivars resistant to seed invasion by AF-producing fungi or to AF production as a possible means of reducing AF contamination of crops will be of great value to the farmers in both developed and developing countries as there is no cost input. Therefore, breeding for resistance to *A. flavus* and *A. parasiticus* and/or AF production can play a significant role in preventing AF contamination and consequently associated economic losses and health hazards.

Attempts have been made to provide a genetic solution to the problem of AF contamination. For instance in groundnuts, Mehan (1989) identified the Shulamit and Darou IV genes for resistance to pod infection; PI 337394 F, PI 337409, GFA 1, GFA 2, UF 71513, Ah 7223, J 11, Var 27, U 4-47-7, Faizpur, and Monir 240-30 genes for resistance to *in vitro* seed colonization by *A. flavus* (IVSCAF); and U 4-7-5 and VRR 245 for resistance to AF production. Resistance to pod infection has been reported to be highly variable and of a low level. Similarly, IVSCAF-resistance is not absolute and even the best sources show up to 15% seed colonization (Rao *et al*. 1989). Peanuts genotypes with resistance to IVSCAF, field seed colonization (FSCAF) and preharvest aflatoxin contamination (PAC) have been reported, but no germplasm highly resistant to aflatoxin production has been found in cultivated peanut (Xue 2004). However these efforts have not resulted in complete eradication of AF contamination. A challenge attributed probably to the inability of researchers to locate germplasm lines which show complete resistance to fungi at the pod-wall, seed-coat, and cotyledon levels. It is expected that the problem of AF contamination could be overcome to a large extent by pyramiding resistance genes from different and diverse sources and by combining the three different kinds of resistance in one genetic background. The recourse to biotechnology, through modification of the AF biosynthesis pathway or the use of variants of hydrolytic enzymes to provide transgenic protection to crops vulnerable to infection by AF-producing fungi may help in obtaining such crops free from AF. Genetic resistance will have to be complimented with good crop husbandry and postharvest practices to eliminate the problem of AF contamination of food crops.

USE OF NON-AFLATOXIGENIC STRAIN OF *ASPERGILLUS FLAVUS*

AF contamination of agricultural commodities both pre- and postharvest is a serious food safety issue and a significant economic concern. Recently, biological control technology has been developed that prevents much of the contamination that might otherwise occur. Biocontrol is based on competitive exclusion whereby a dominant population of a non-toxigenic strain of *A. flavus* is established in the soil before peanuts are subjected to conditions favouring contamination (Dorner 2008). The applied strain competes with toxigenic strains for infection sites, resulting in significantly

Table 9 Measures for preventing or reducing aflatoxin exposure during food production.

Stage in food production	Intervention	References
Pre harvest	Timing of planting; crop planted, genotype of seed planted; irrigation, insecticides, competitive exclusion; time of harvest	Cotty and Bhatnager 1994; Wilson and Payne 1994; Dorner *et al*. 1999; Brown *et al.* 2001; Chen *et al.* 2001; Cleveland *et al*. 2003; Munkvold 2003
Post harvest: drying and storage	Hand sorting, drying on mats; sun drying; storing bags on wooden pallets or elevated off ground; insecticides; rodent control	Hell *et al.* 2000; Ono *et al*. 2002; Munkvold 2003; Fandohan *et al*. 2005a; Hawkins *et al*. 2005; Turner *et al.* 2005
Post harvest: food preparation	Hand sorting, winnowing; washing; crushing and dehulling; nixtamalization; acidification; chemoprotectant; enterosorption	Price and Jorgensen 1985; Elias-Orozco *et al*. 2002; Munkvold 2003; Kensler *et al.* 2004; Mendez-Albores *et al.* 2004; Castells *et al*. 2005; Fandohan *et al.* 2005b; Mendez-Albores *et al.* 2005; Wang *et al.* 2005

reduced concentrations of AFs in peanuts. Application of the technology showed that AFs were reduced by an average of 85% in farmers' stock peanuts and by as much as 98% in shelled, edible grade peanuts (Dorner 2008).

In an experiment to determine the effect of application of the nontoxigenic strains on preharvest AF contamination of corn, Dorner *et al*. (1999) inoculated soil in corn plots with nonaflatoxigenic strains of *A. flavus* and *A. parasiticus* during crop years 1994 to 1997. They observed that inclusion of a nonaflatoxigenic strain of *A. parasiticus* in a biological control formulation reduced AF contamination in corn. Using nonaflatoxigenic *A. flavus* isolates to competitively exclude toxigenic *A. flavus* isolates in agricultural fields has become an adopted approach to reduce AF contamination. From screening subgroups of nonaflatoxigenic *A. flavus*, Chang and Hua (2007) identified an *A. flavus* isolate, TX9-8, which competed well with three *A. flavus* isolates producing low, intermediate, and high levels of AFs, respectively. The competitive effect was found to be due to TX9-8 outgrowing toxigenic *A. flavus* isolates. This technology appears to be effective and can go a long way in preventing AF contamination and limit exposure to humans. However, the allergenic and human health aspects of the atoxigenic strain need to be evaluated.

Pre-harvest interventions

The presence and growth of *Aspergillus* on pre-harvested crops is dependent on the environment. Agricultural practices including proper irrigation and pest management can reduce AF contamination. Pre-harvest interventions include choosing crops with resistance to drought, disease, and pests and choosing strains of that crop which are genetically more resistant to the growth of the fungus and the production of AFs (Cotty and Bhatnagar 1994; Chen *et al*. 2001; Cleveland *et al*. 2003). A biopesticide, consisting of a nonaflatoxigenic strain of *Aspergillus*, may competitively exclude toxic strains from infecting the crop (Dorner *et al*. 1999; Cleveland *et al*. 2003) however; the allergenic and human health aspects of the atoxigenic strain need to be evaluated.

Post-harvest drying and storage

Before storage, crops should be properly dried to prevent the development of AFs. Sorting and disposing of visibly moldy or damaged kernels before storage has proven to be an effective method for reducing, but not eliminating, the development of AFs (Fandohan *et al*. 2005a; Turner *et al*. 2005). During storage, moisture, insect, and rodent control can prevent damage to the crop and reduce AF development. AF contamination of maize is influenced by the structure used for storage, the length of time in storage, and the form of maize stored (i.e. with husk, without husk, or as loose grain) (Hell *et al*. 2000). A community-based intervention trial in Guinea, West Africa focused on thorough drying and proper storage of groundnuts in subsistence farm villages and achieved a 60% reduction in mean AF levels in intervention villages (Turner *et al*. 2005).

Post-harvest food preparation

Interventions during food preparation or consumption involve removing contaminated portions of food, diluting contaminated food with uncontaminated food, neutralizing AFs present in food, or altering the bioavailability of the AFs consumed. AFs are not largely affected by routine cooking temperatures, but simple food preparation methods such as sorting, washing, crushing, and dehulling may reduce AF levels (Lopez-Garcia and Park 1998; Park 2002; Fandohan *et al*. 2005). Traditional methods of cooking food with alkaline compounds (i.e. nixtamalization) have been used to reduce AF exposure; however, the chemical reaction may involve temporary inactivation of AFs, a process that may reverse in the gastric acid of the stomach (Price and Jorgensen 1985; Elias-Orozco *et al*. 2002; Mendez-Albores *et al*. 2004). Additional strategies for reducing AFs, including enterosorption and chemoprotection, attempt to reduce the effects of AF exposure or the bioavailable portion of AFs in food. These strategies are expensive and therefore difficult to implement in poor communities. Enterosorption is the use of clay, such as NovaSil Plus, with a high affinity for AFs (Phillips 1999, 2002; Wang *et al*. 2005). Clay has been used as an anti-caking additive in animal feed and has been shown to protect animals from ingested AFs. Chemoprotection is the use of chemicals (e.g. Oltipraz, Chlorophylin) or dietary intervention (e.g., broccoli sprouts, greentea) to alter the susceptibility of humans to carcinogens and has been considered as a strategy to reduce the risk of HCC in populations with high exposures to AFs (Bolton *et al*. 1993; Wang *et al*. 1999; Kensler *et al*. 2004). The efficacy safety and acceptability of enterosorption and chemoprotection require further study.

CHEMOPREVENTIVE STRATEGIES

It has been suggested also that dietary change on the part of individuals and other strategies to reduce AF contamination in food stores could also assist in preventing HCC. However this may not be feasible since people prefer prescription to proscription and complete elimination of AF contamination might not be possible (Yates and Kensler 2007). Therefore HCC remains a disease for which alternative therapeutic modalities must be developed. In the developing world where the burden of liver cancer is highest, targeted chemoprevention strategies which alter AF disposition are rational and practical strategy to reduce the incidence of HCC in populations with high dietary AF exposure.

Mechanisms of chemoprevention

Chemopreventive agents can block carcinogens from reaching the target sites, undergoing metabolic activation or subsequently interacting with crucial cellular macromolecules such as DNA, RNA and proteins. In addition they can suppress the premalignant transformation and malignant formation of initiated cells during the stage of promotion and progression. Furthermore chemopreventive agents can alter AF disposition through induction of a set of detoxification phase 2 conjugating and cytoprotective antioxidant enzymes such as GST and NQO1. These inducers include phenolic antioxidants, dithiolethiones, isothiocyanates, chlorophyllin and triterpenoids (Yates and Kensler 2007). Many of these enzymes are regulated through Kelch ECH-associating protein 1 (Keap1)-NF-E2-related factor 2(Nrf2)-antioxidant response element (ARE) signaling, making this pathway an important molecular target for chemoprevention (Lee and Surh 2005; Yates and Kensler 2007).

CHEMOPREVENTIVE AGENTS IN ANIMAL MODELS AND HUMAN CLINICAL TRIALS

Dithiolethiones

Dithiolethiones are a well-known class of cancer chemopreventive agents. Amongst the Dithiolethiones that has been extensively investigated for cancer chemoprevention is oltipraz (**Fig. 4**) (Zhang and Munday 2008). Roebuk *et al*. (1991) demonstrated, in a clinical study with low levels of AF exposure, the efficacy of oltipraz against hyperplastic nodules and hepatocellular cancer compared to the placebo group. Another randomized, placebo-controlled, double-blind chemoprevention trial in China revealed the modulation of AF metabolism by inducing its major detoxification pathway. Weekly administration of high dose oltipraz for one month led to decrease in phase 1 metabolite AF M1 excreted in urine compared with administration of a placebo, while daily intervention with low dose oltipraz led to an increase in AF-mercapturic acid excretion suggesting that intermittent, high-dose oltipraz inhibited phase 1 activation

of AFs, and sustained low-dose oltipraz increased phase 2 conjugation of AF, yielding higher levels of AF-mercapturic acid. The modification of both phase 1 and 2 pathways by oltipraz demonstrated the ability of this dithiolethione to prevent AF carcinogenesis (Wang *et al*. 1999). Glintborg *et al*. (2006) demonstrated that oltipraz however may not affect biomarkers of oxidative damage such as DNA oxidation. In a double-blind, randomized, placebo-controlled trial performed on 233 healthy residents of Qidong, PRC, for 8 weeks treatment with a subsequent 8-week follow-up period, oltipraz had no major effect on oxidative DNA damage. When urinary Urine oxidized guanine derivatives were measured in the subjects. The results of this clinical trial indicate that mechanisms other than prevention of oxidative DNA damage may be of higher importance when oltipraz is used as a chemopreventive agent in humans (Glintborg *et al*. 2006).

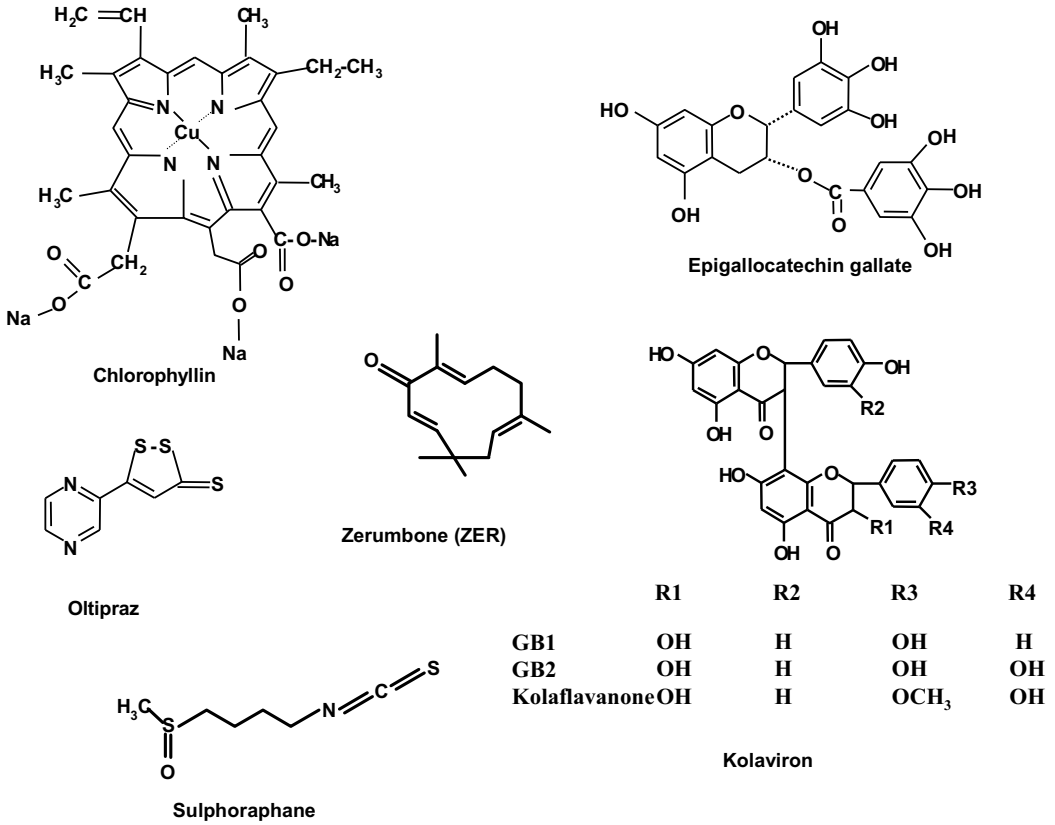
Bolton *et al*. (1993) reported the protective effect of oltipraz against the development of hepatic AF-DNA adducts in rodents by mechanisms involving conjugation of AFB_1-8,9-epoxide with glutathione and enhancement of the activity of GST thereby redcuing the formation of AF-N^7-guanine adducts. In addition other investigators have reported that oltipraz has inhibitory effect on certain Phase 1 enzyme such as CYP1A2 and CYP3A4 (Langouet *et al*. 1995, 2000). The animal model data have been corroborated by Sofowora *et al*. (2001) who reported a significant decrease in CYP1A2 activity in healthy human volunteers, administered orally with 125 mg oltipraz for eight days.

The key mechanism of action of dithiolethiones involves activation of Nrf2-dependent activation of antioxidant response elements (ARE) signaling and induction of phase II enzymes (Zhang and Munday 2008). In mechanistic terms oltipraz was shown to disrupt the interaction between Keap1 and Nrf2 thereby allowing Nrf2 to translocate to the nucleus where it forms heterodimers with small MAF-family protein associated with ARE to induce the expression of phase 2 antioxidant genes (Kwak *et al.* 2001a, 2001b; Petzer *et al*. 2003). Additional mechanistic studies demonstrate that Pyrrolopyrazine thione, a metabolite of oltipraz has been shown to modify cyt c function by increasing ROS in mitochondria, a mechanism with implications for the regulation of apoptotic cell death. This function has been suggested to contribute to the mechanism by which the parent compound, oltipraz, might trigger the cancer chemopreventive increase in transcription of phase 2 enzymes (Velayutham *et al.* 2007).

Chlorophyll and chlorophyllin

Dietary chlorophyll is predominantly composed of lipophilic derivatives including chlorophyll *a* and *b* (fresh fruits and vegetables), metal-free pheophytins and pyropheophytins (thermally processed fruits and vegetables), as well as Zn-pheophytins and Zn-pyropheophytins (thermally processed green vegetables) (Ferruzi and Blakeslee 2007). Dietary chlorophyll has been shown to be antimutagenic and a potent inducer of phase 2 detoxifying enzymes *in vitro* (Itoh *et al*. 1997). In the rainbow trout model of dietary carcinogenesis, hepatic DNA-adduct formation resulting from 200 ppm dibenzo αL pyrene exposure was reduced 66% by co-exposure to 3000 ppm chlorophyll in the diet (Chanas *et al*. 2002). Subsequently chlorophyll was shown to provide potent chemoprotection against early biochemical and late pathophysiological biomarkers of AFB_1 carcinogenesis in the rat liver and colon (Simonich *et al*. 2007).

Chlorophyllin (CHL) (**Fig. 4**) is a commercial-grade water-soluble derivative of chlorophyll. Other derivatives include chlorophyllides and pheophorbides. The use of CHL, in traditional medical applications is well documented including it application in would healing (Young and Bergei 1980), Biological activities attributed to chlorophyll derivatives consistent with cancer prevention include antioxidant and antimutagenic activity, mutagen trapping, modulation of xenobiotic metabolism, induction of apoptosis and formation molecular complexes with carcinogens, thereby blocking their bioavailability (Breinholt *et al*. 1995b; Ferruzi and Blakeslee 2007). CHL has been shown to be a potent antimutagen *in vitro*, an effective anti-carcinogen in several animal models, and was demonstrated to significantly reduce urinary biomarkers of aflatoxin B(1)



	R1	R2	R3	R4
GB1	OH	H	OH	H
GB2	OH	H	OH	OH
Kolaflavanone	OH	H	OCH_3	OH

Fig. 4 Structures of selected chemopreventive agents.

exposure in a human population (Breinholt *et al*. 1995a; Pratt *et al*. 2007).

CHL has been evaluated as a potential chemopreventive agent in a population at high risk for exposure to AF and subsequent development of HCC.Thus, administration of CHL to human volunteers in Quidong reduced the level of urinary excretion of AF-N^7-guanine, a DNA adduct biomarker derived from the ultimate carcinogenic metabolite of AFB_1, AF-8,9-epoxide (Egner *et al*. 2003). Although the use of CHL in treating several diseases has ben advanced, it is perhaps the potential of chlorophyll as a cancer preventative agent that has drawn significant attention very recently, Kensler and co-workers 2003 has therefore suggested that supplementation with green leafy vegetable foods rich in chlorophylls might be a more rational and pragmatic approach of administration of CHL.

Green tea

Teas are generally made from young leaves and leaf buds of the tea plant, *Camellia sinensis*, and are the world's second most consumed beverage (Shukla 2007). Teas have received a great deal of attention both from the general public and the scientific community because of the abundance of polyphenols which are strong antioxidants and free radical scavengers. Tea preparation has inhibitory activity against tumorigenesis and as such has been considered for the possibility of its use in cancer prevention. There are three main types of tea, all coming from the tea plant viz. black tea (fermented,) green tea (unfermented), or oolong tea (semi-fermented), classified based on the methods of brewing and processing.

Green tea polyphenols (GTP) represents secondary metabolite in tea plants and accounts for about 30-36% weight of the water extractable materials in tea leaves. The chemopreventive and chemoprotective activities of green tea have been attributed to the polyphenolic ingredient (-)-epigallocatechin-3-gallate (EGCG) (**Fig. 4**) (Graham 1992; Na *et al.* 2008).

GT and GTP have been considered as effective chemopreventive agents in various cell lines and experimental animal models for reduction of carcinogen-induced carcinogenesis including AFB_1-induced liver cancer (Lambert and Yang 2003).

Studies of Quin *et al*. (2000) in rats administered AFB_1 and CCl_4 as the initiator and promoter, respectively reveal that feeding of GT inhibits initiation and promotion steps of AFB_1 hepatocarcinogenesis and that the inhibition of cell proliferation is responsible for the suppression of promotion.

In humans, inverse relationships between the level of GT consumption and the risk of development of cancer have been observed (Nakachi *et al*. 2000; Fujiki *et al*. 2002). In human studies involving 124 healthy volunteers administered GTP, epigalocatechin (EGC) and epicatechin (EC) levels, components of GT showed significant and dose-dependent increases in urine of individuals administered with GT with concomitant decrease in the levels of 8-OHdG in both GTP-treated groups (Luo *et al*. 2005).

To evaluate the efficacy of GTP in modulating AFB1 biomarkers, serum and urine samples were collected from subjects treated for 3 months with 500 mg and 1000 mg GTP and placebo groups. Levels of AFB1-albumin adducts (AFB-AA) and aflatoxin M(1)(AFM(1)) were reduced whereas Significant elevations in AFB-NAC levels and the ratio of AFB-NAC:AFM(1) were found in both 500 and 1000 mg groups compared with the placebo group after 3 months of GTP intervention (Tang *et al.* 2008).

Molecular mechanisms underlying chemopreventive effects exerted by green tea and its components have been extensively investigated. EGCG a major green tea polyphenol, has been shown to induce expression of GST, glutathione peroxidase, glutamate cysteine ligase, hemeoxygenase-1, etc. that are involved in the elimination or inactivation of reactive oxygen species and electrophiles implicated in multi-stage carcinogenesis (Na and Surh 2008).

Molecular mechanisms of chemopreventive action of EGCG involve its ability to induce specific phase 2 enzymes via the activation of Nrf2. The induction of GSTM2 an isoform of GST by EGCG has been reported in rats (Chou *et al*. 2000). EGCG has been shown to modulate Nrf2-mediated cellular events (Shen *et al.* 2005; Xu *et al*. 2005; Na *et al.* 2008).

Broccoli sprouts

Glucosinolates are present abundantly in vegetables, especially broccoli sprout. Glucosinolates can be hydrolyzed by myrosinase, an enzyme which is released when the plant is chewed or in the intestinal microflora, to produce isothiocyanates. In animal models, sulphoraphane, a potent isothiocyanates from broccoli sprout has been shown to induce detoxification and cytoprotective phase 2 enzymes such as GST and NQO1 mediated by Nrf2-Keap1-ARE signaling cascade (Hu *et al.* 2006). Specifically sulphorapahane was demonstrated to directly interact with Keap1 (Zhang *et al*. 1994). A placebo-controlled, double blind, randomized phase 1 clinical study of broccoli sprout preparations containing either glucosinolate or isothiocyanate (Shapiro *et al*. 2006) and interventions using hot water infusions of broccoli sprout were evaluated in residents of Quidong, China (Kensler *et al*. 2005). In both studies, broccoli ingestion elicited no toxicities and it was well tolerated (Kensler *et al.* 2005; Shapiro *et al*. 2006; Yates and Kensler 2007) AFB_1-DNA adduct was reduced presumably due to induction of GST activity by sulphoraphane (Kensler *et al.* 2005). Thus AFB_1 metabolism and disposition can be altered by ingestion of glucosinolate-rich broccoli sprout preparation (Yates and Kensler 2007).

Kolaviron

Garcinia kola Heckel (Guttiferae) also known as bitter kola owing to its bitter taste is a medium sided tree usually found in moist forest and also cultivated in homesteads. It is distributed throughout west and central Africa and has been particularly located in Nigeria. The seeds are chewed as a refreshing past time as an alternative to true kolanuts (*Cola nitida* and *C. accuminata*). The masticated fruit pulp is used in folk medicine for its antiseptic action in the treatment of cuts and for the prevention of sore throat (Ainslie 1937). The seed is employed as general tonic and it is believed to have aphrodisiac properties.

Several chemical compounds have been isolated from *G. kola*. Kolaviron, a biflavonoid complex containing GB-1, GB-2 and kolaflavanone (**Fig. 4**) was isolated from *G. kola* seed (Iwu 1985).

A number of studies in various systems including experimental animal models have established Kolaviron as an effective hepatoprotctive and chemoprotetcive agent. Data from our laboratory have revealed the protective effects of kolaviron against hepatic oxidative damage and genotoxicity induced by several liver carcinogens including carbon tetrachloride (Farombi 2000), 2-acetyl amino fluorine (Farombi *et al*. 2000), aflatoxin B1 (Farombi *et al.* 2005) and dimethyl nitrosamine (Farombi *et al.* 2009). The ability of kolaviron to inhibit COX-2 and iNOS expression through down regulation of NF-κB and AP-1 DNA binding activities could be a mechanism to explain the hepatoprotective effect of kolaviron on drug-induced hepatotoxicity and possibly hepatocarcinogenesis (Farombi and Surh 2009).

Mechanistically, our studies showed that while kolaviron did not affect the activities of some representative phase I enzymes, it enhanced the activities of major phase II enzymes such as GST, uridyldiphosphoglucuronosyl transferase (UDPGT) (Farombi 2000; Farombi *et al*. 2005b) and NADPH: quinone oxidoreductase (Farombi *et al*. 2005b). The studies of Nwankwo *et al*. (2000) in Hep G2 cells also corroborated the induction of GST isozyme α-1 and α-2 by kolaviron. Thus kolaviron is a specific inducer of phase 2 enzymes and this property could therefore play a role in its

abilty to prevent against carcinogens.

The ability of phytochemicals to scavenge reactive oxygen species (ROS) has been suggested to contribute to overall mechanisms of chemoprevention. A number of studies have revealed kolaviron as potent antioxidant scavenger of ROS and metal chelator (Farombi *et al*. 2004a; Farombi and Nwaokeafor 2005). Kolaviron scavenged H_2O_2, $\cdot O_2^-$ and ·OH *in vitro* (Farombi *et al*. 2002), suppressed *in vivo* lipid peroxidation (Farombi 2000; Farombi *et al*. 2000) and inhibited H_2O_2-induced strand breaks and oxidative DNA damage human lymphocytes and rat liver cells (Farombi *et al*. 2004b).

Garcinia kolanut, from which Kolaviron is derived, is freely consumed in the West African sub-Saharan region with high prevalence of HCC. Considering also the prominent position the nut occupies in the social customs of the people in this region of the world, kolaviron may therefore merit further consideration as an edible phytochemical with a potential application in chemoprevention of liver cancer.

Zerumbone

Zerumbone is a sesquiterpenoid with very large amounts detected in rhizomes, which are used locally as anti-inflammatory medicines (Murakami *et al.* 2002, 2003; Farombi and Tanaka 2007) and is also present is some edible parts, including young stems and influorescence, which are used in traditional cooking (Murakami *et al.* 2003). ZER was identified as potent suppressor of TPA-induced Epsteine-Barr virus (EBV) activation in Raji cells (Murakami *et al*. 1999).

A number of biochemical and molecular pathways involving disruption of inflammatory signal transduction pathways have been proposed for ZER (Murakami and Ohigashi 2007). ZER was found to be highly anti-mutagenic in phorbol ester-stimulated, differentiated HL-60 human leukemia cells and LPS-stimulated RAW264.7 murine macrophages by mechanisms involving attenuation of iNOS mRNA expression (Murakami and Ohigashi 2006).

ZER possesses anti-growth and anti-inflammatory properties in several human cancer cell lines. ZER also down-regulates the cyclooxygenase-2 and inducible nitric oxide synthase expression via modulation of nuclear factor NF-kB activation in cell culture systems (Kim *et al.* 2009). In addition ZER was found to be an inducer of GSH-related phase 2 enzymes including GST. NrF2/ARE signalling has been implicated as mechanism responsible for its anti-tumor promoting properties (Murakami *et al*. 2004). Thus it was shown that ZER enhanced the mRNA expression of manganese superoxide dismutase (SOD), glutathione peroxidase-1 GPx-1), glutathione *S*-transferase-P1 (GST-P1) and NAD(P)H quinone oxidoreductase (NQO-1) in the epidermis of mouse treated with TPA (Murakami *et al*. 2004). Moreover, exposure of cultured rat liver epithelial cell line (RL34) to ZER resulted in the significant induction of GST (Nakamura *et al*. 2004). ZER was found to induce the nuclear localization of the Nrf2 leading to the expression of phase 2 antioxidant genes such as γ-glutamylcysteine synthetase, glutathione peroxidase, and HO-1 (Nakamura *et al*. 2004). α,β unsaturated double bond in zerumbone has been implicated in its ability to induce nuclear translocation of Nrf2 and consequently the expression of phase 2 antioxidant enzymes involved in the detoxification of toxic reactive intermediates from various carcinogens. Therefore, this compound may find relevance and expression in mitigating experimental aflatoxin carcinogenesis and possibly human liver cancer.

CONCLUSIONS

There is sufficient evidence from animal models and human epidemiological data to conclude that mycotoxins pose an important danger to human and animal health. The incidence of aflatoxicosis may be more common than suspected. The prevalence and level of human exposure to AFs on a global scale have been reviewed, and it has been estimated that about 4.5 billion people living in developing countries are chronically exposed to largely uncontrolled amounts of the toxin. A limited amount of information shows that, at least in those locations where it has been studied, the existing AF exposure results in changes in nutrition and immunity. The AF exposure and the toxic effects of AFs on immunity and nutrition combine to negatively affect health factors (including HIV infection) that account for >40% of the burden of disease in developing countries where a short lifespan is prevalent. Food systems and economics render developed-country approaches to the management of AFs impractical in developing-country settings, but the strategy of using food additives to protect farm animals from the toxin may provide effective and economical new approaches to protecting human populations. In addition, sufficient amounts of food combined with regulations that monitor AF levels in foods will protect human populations from significant AF ingestion in developing countries. However, in countries where populations are facing starvation or where regulations are either not enforced or non-existent, routine ingestion of AF will still continue to occur. Therefore chemoprevention may be a practical strategy to reduce the incidence of HCC. Studies have provided proof that AF disposition can be altered by the administration of dietary chemopreventive agents. Induction of Nrf2-keap1-ARE mediated signaling (**Fig. 5**) has proved to be a successful chemopreventive strategy in many models including human clinical trials and as such could be relevant in the prevention and treatment of human HCC.

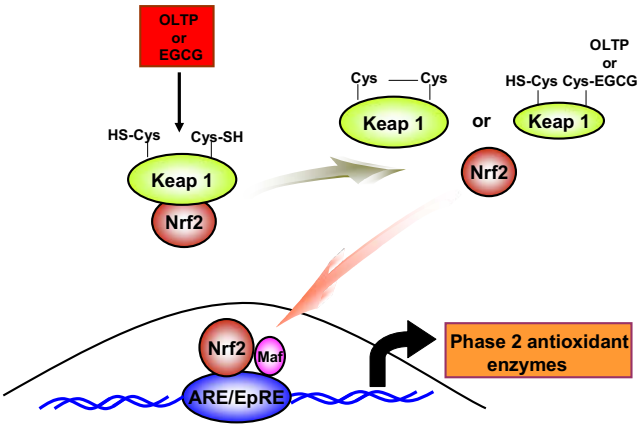


Fig. 5 Schematic representation of signaling cascades involving Nrf2. Nrf2 is kept in the cytoplasm by a Kelch-like-ECH-associated protein 1 (Keap 1). Phase 2 enzyme inducers can cause covalent modification of these cysteine residues which leads to the dissociation of Nrf2 from Keap 1. Nrf2 is subsequently translocated into the nucleus where it interacts with a small Maf protein, forming a heterodimer that binds to ARE which leads to the stimulation of ARE-driven expression of gene that encode phase-2 detoxifying enzymes such as glutathione *S*-transferase α2 (GSTA2), NADPH-quinone oxidoreductase (NQO1) and heme oxygenase (HO-1). Oltipraz (OLTP) and EGCG can facilitate the release of Nrf2 from Keap 1.

REFERENCES

Abarca ML, Bragulat MR, Sastella G, Cabanes FJ (1994) Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. *Applied Environmental Microbiology* **60**, 2650-2652

Abdulrazzaq YM, Osman N, Ibrahim A (2002) Fetal exposure to aflatoxin in United Arab Emirates. *Annals of Tropical Paediatric* **22**, 3-9

Abdulrazzaq YM, Osman N, Yousif ZM, Trad O (2004) Morbidity in neonates of mothers who have ingested aflatoxins. *Annals of Tropical Paediatric* **24**, 145-151

Akande KE, Abubakar MM, Adegbola TA, Bogoro SE (2006) Nutritional and health implications of mycotoxins in animal feeds: A review. *Pakistan Journal of Nutrition* **5**, 398-403

Akano DA, Atanda O (1989) The present level of aflatoxin in Nigerian groundnut cake ('kulikuli'). *Letters in Applied Microbiology* **10**, 187-189

Ali N, Hashim NH, Yoshizawa T (1999) Evaluation and application of a sim-

ple and rapid method for the analysis of aflatoxins in commercial foods from Malaysia and the Philippines. *Food Additives and Contaminants* **16**, 273-280

Ali NS, Yamashita A, Yoshizawa T (1998) Natural co-occurrence of aflatoxins and *Fusarium* mycotoxin (fumonisin, DON, NIV and zearalenone) in corn from Indonesia. *Food Additives and Contaminants* **15**, 377-384

Anonymous (1998) Respiratory health hazards in agriculture. *American Journal of Respiratory and Critical Care Medicine* **158**, S1-76

Asao T, Bucal G, Abdelkader MM, Chang SB, Wick EL, Wogan GN (1963) Aflatoxins B and G. *Journal of the American Chemical Society* **85**, 1706-1707

Azziz-Baumgartner E, Lindblade K, Gieseker K, Rogers HS, Kieszak S, Njapau H, Schleicher R, McCoy LF, Misore A, de Cock K, Rubin C, Slutsker L (2005) Case-control study of an acute aflatoxicosis outbreak-Kenya 2004. *Environmental Health Perspectives* **113**, 1779-1783

Bailey EA, Iyer RS, Stone MP, Harris TM, Essigmann JM (1996) Mutational properties of the primary aflatoxin B1-DNA adduct. *Proceedings of the National Academy of Sciences USA* **93**, 1535-1539

Bankole SA, Mabekoje OO (2004) Occurrence of aflatoxins and fumonisins in preharvest maize from south-western Nigeria. *Food Additives and Contaminants* **21**, 251-255

Bankole SA, Ogunsanwo BM, Eseigbe DA (2005) Aflatoxins in Nigerian dry-roasted groundnuts. *Food Chemistry* **89**, 503-506

Bannasch P, Khoshkhou NI, Hacker HJ, Radaeva S, Mrozek M, Zillmann U, Kopp-Schneider A, Haberkorn U, Elgas M, Tolle T (1995) Synergistic hepatocarcinogenic effect of hepadnaviral infection and dietary aflatoxin B1 in woodchucks. *Cancer Research* **55**, 3318-30

Beg MU, Al-Mutairi M, Beg KR, Al-Mazeedi HM, Ali LN, Saeed T (2006) Mycotoxins in Poultry Feed in Kuwait. *Archives of Environmental Contamination and Toxicology* **50**, 594-602

Bhat RJ, Shetty PH, Amruth RP, Sudersham RV (1997) A food-borne disease outbreak due to consumption of moldy sorghum and maize contaminating fumonisin mycotoxins. *Journal of Toxicology: Clinical Toxicology* **35**, 249-255

Biermann A, Terplan G (1980) Detection of AFB1 by means of ELISA. *Archives of Lebensmittelhygiene* **31**, 51-57

Blesa J, Soriano JM, Molto JC, Manes J (2004) Limited survey for the presence of aflatoxins in foods from local markets and supermarkets in Valencia, Spain. *Food Additives and Contaminants* **21**, 165-171

Bolton MG, Munoz A (1993) Transient intervention with oltipraz protects against aflatoxin-induced hepatic tumorigenesis. *Cancer Research* **53**, 3499-3504

Bosch FX (1995) HCV and liver cancer: the epidemiological evidence. In: Kobayashi K, Purcell R, Shimotohno K, Tabor E (Eds) *Hepatitis C Virus and its Involvement in the Development of Hepatocellular Carcinoma*, Princeton Scientific Publishers, N.J., pp 15-25

Bosch FX, Munoz N (1991) Hepatocellular carcinoma in the world: Epidemiologic questions. In: Tabor E, Di Bisceglie AM, Purcell RH (Eds) *Etiology, Pathology, and Treatment of Hepatocellular Carcinoma in North America* (Advances in Applied Biotechnology Series, Vol 13), Gulf Publishing Co., Houston, pp 35-54

Breinholt V, Hendricks J, Pereira C, Arbogast D, Bailey G (1995a) Dietary chlorophyllin is a potent inhibitor of aflatoxin B1 hepatocarcinogenesis in rainbow trout. *Cancer Research* **55**, 57-62

Breinholt V, Schimerlik M, Dashwood R, Bailey G (1995b) Mechanisms of chlorophyllin anticarcinogenesis against aflatoxin B1: complex formation with the carcinogen. *Chemical Research in Toxicology* **8**, 506-514

Brown RL, Chen ZY (2001) Resistance to aflatoxin accumulation in kernels of maize inbreds selected for ear rot resistance in West and Central Africa. *Journal of Food Protection* **64**, 396-400

Bucio-Villalobos CM, Guzmán-de-Peña D, Cabriales JP (2001) Aflatoxin synthesis in corn fields in Guanajuato, Mexico. *Revista Iberoamericana de Micología* **18**, 83-87

Bulatao-Jayme J, Almero EM, Castro CA, Jardeleza TR, Salamat LA (1982) A case-control dietary study of primary liver cancer risk from aflatoxin exposure. *International Journal of Epidemiology* **11**, 112-118

Campos Marit de, Santos CJ, Olszyna-Marzys AE (1980) Aflatoxin contamination in grains from the Pacific coast in Guatemala and the effect of storage upon contamination. *Bulletin of Environmental Contamination and Toxicology* **24**, 789-795

Castells M, Marin S (2005) Fate of mycotoxins in cereals during extrusion cooking: a review. *Food Additives and Contaminants* **22**, 150-157

Centers for Disease Control and Prevention (CDC) (2004) Outbreak of aflatoxin poisoning – eastern and central provinces, Kenya. *Morbidity Mortality Weekly Report* **53**, 790-793

Chanas SA, Jiang Q, McMahon M, McWalter GK, McLellan LI, Elcombe CR, Henderson CJ, Wolf CR, Moffat GJ, Itoh K, Yamamoto M, Hayes JD (2002) Loss of the Nrf2 transcription factor causes a marked reduction in constitutive and inducible expression of the glutathione S-transferase Gsta 1,Gsta2, Gstm2, Gstm4 genes in the livers of male and female mice. *Biochemical Journal* **365**, 405-416

Chang PK, Hua SS (2007) Nonaflatoxigenic *Aspergillus flavus* TX9-8 competitively prevents aflatoxin accumulation by *A. flavus* isolates of large and small sclerotial morphotypes. *International Journal of Food Microbiology* **114**, 275-279

Chen JG, Parkin DM, Chen QG, Lu JH, Shen QJ, Zhang BC (2003) Screening for liver cancer: results of a randomised controlled trial in Qidong, China. *Journal of Medical Screening* **10**, 204-209

Chen SY, Chen CJ, Chou SR, Hsieh LL, Wang LY, Tsai WY, Ahsan H, Santella RM (2001) Association of aflatoxin B(1)-albumin adduct levels with hepatitis B surface antigen status among adolescents in Taiwan. *Cancer Epidemiology Biomarkers and Prevention* **10**, 1223-1226

Chen ZY, Brown RL, Cleveland TE, Damann KE, Russin JS (2001) Comparison of constitutive and inducible maize kernel proteins of genotypes resistance or susceptible to aflatoxin production. *Journal of Food Protection* **64**, 1785-1792

Chou FP, Chu YC, Hsu JD, Chiang HC, Wang CJ (2000) Specific induction of glutathione *S*-transferase GSTM2 subunit expression by epigallocatechin gallate in rat liver. *Biochemical Pharmacology* **60**, 643-650

Chu FS, Ueno I (1977) Production of antibody againist AFB1. *Applied Environmental Microbiology* **33**, 1125-1128

Ciegler AM, Bennet JW (1980) Mycotoxins and mycotoxicoses. *Bioscience* **30**, 512-515

Cleveland TE, Dowd PF, Desjardins AE, Bhatnagar D, Cotty PJ (2003) United States Department of Agriculture-Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest Management Science* **59**, 629-642

Cotty PJ, Bhatnagar D (1994) Variability among atoxigenic *Aspergillus flavus* strains in ability to prevent aflatoxin contamination and production of aflatoxin biosynthetic pathway enzymes. *Applied and Environmental Microbiology* **60**, 2248-2251

Coulter JBS, Hendrickse RG, Lamplugh SM, Macforlane SBJ, Moody JB, Omar MLA, Suliman GL, Williams TE (1986) Aflatoxins and kwashiorkor: Clinical studies in Sudanese children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 945-951

Cullen JM, Newberne PM (1994) Acute hepatotoxicity of aflatoxins. In: Eaton DL, Groopman JD (Eds) *The Toxicology of Aflatoxins: Human health, Veterinary, and Agricultural Significance*, Academic Press, Inc., San Diego, CA, pp 3-26

Cullen JM, Ruebner BH, Hsieh LS, Hyde DM, Hsieh DP (1987) Carcinogenicity of dietary aflatoxin M1 in male Fischer rats compared to aflatoxin B1. *Cancer Research* **47**, 1913-1917

Dalcero A, Magnoli C, Chiacchiera S, Palacios G, Reynoso M (1997) Mycoflora and incidence of aflatoxin B1, zearalenone and deoxynivalenol in poultry feeds in Argentina. *Mycopathologia* **137**, 179-184

Dawlatana M, Coker RD, Nagler MJ, Wild CP, Hassan MS, Blunden G (2002) The occurrence of mycotoxins in key commodities in Bangladesh: surveillance results from 1993 to 1995. *Journal of Natural Toxins* **11**, 379-386

Denning DW, Allen R, Wilkinson AP, Morgan MRA (1991) Transplacental transfer of aflatoxin in humans. *Carcinogenesis* **11**, 1033-1035

Denning DW, Sykes JA, Wilkinson AP, Morgan MR (1990) High serum concentration of aflatoxin in Nepal as measured by enzyme-linked immunosorbent serum assay. *Human and Experimental Toxicology* **9**, 143-146

Desjardins AE, Manandhar G, Platter RD, Maragos CM, Shrestha K, McCormick SP (2000) Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and effect of traditional processing methods on mycotoxin level. *Journal of Agricultural Food Chemistry* **48**, 1337-1383

Diop Y, Ndiaye B, Diouf A, Fall M, Thiaw C, Thiam A, Barry O, Ciss M, Ba D (2000) Contamination by aflatoxins of local peanut oils prepared in Senegal. *Annales Pharmaceutical Francaises* **58**, 470-474

Diop YM, Ndiaye B, Fall M, Diouf A, Sall A, Ciss M, Ba D (2000) Aflatoxin contamination level in artisanal and industrial peanut butter food in Dakar-Senegal. *Dakar Medical* **45**, 134-137

Dogan M, Cemliman B, Sagdic O (2006) Total aflatoxin levels of instant foods in Turkey. *Archiv für Lebensmittelhygiene* **57**, 55-58

Dorner JW, Cole RJ (1989) Comparison of two ELISA screening tests with liquid chromatography for determination of aflatoxins in raw peanuts. *Journal of the Association of Official Analytical Chemists* **72**, 962-964

Dorner JW, Cole RJ, Wicklow DT (1999) Aflatoxin reduction in corn through field application of competitive fungi. *Journal of Food Protection* **62**, 650-656

Dorner JW (2008) Management and prevention of mycotoxins in peanuts. *Food Additives and Contaminants* **25**, 203-208

Egner PA, Munoz A, Kensler TW (2003) Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. *Mutation Research* **523-524**, 209-216

Elias-Orozco R, Castellanos-Nava A, Gaytan-Martinez M, Figueroa-Cardenas JD, Loarca-Pina G (2002) Comparison of nixtamalization and extrusion processes for a reduction in aflatoxin content. *Food Additives and Contaminants* **19**, 878-885

Essigman JM, Croy RG, Bennet RA, Wogan GN (1982) Metabolic activation of aflatoxin B_1: pattern of DNA adduct formation, removal and excretion in relation to carcinogenesis. *Drug Metabolism Review* **13**, 581-602

Fandohan P, Gnonlonfin B, Hell K, Marasas WF, Wingfield MJ (2005a) Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. *International Journal of*

Food Microbiology **99**, 173-183

Fandohan P, Zoumenou D, Hounhouigan DJ, Marasas WF, Wingfield MJ, Hell K (2005b) Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. *International Journal of Food Microbiology* **98**, 249-259

Farombi EO (2000) Mechanisms for the hepatoprotective action of kolaviron: studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. *Pharmacological Research* **42**, 75-80

Farombi EO (2006) Aflatoxin contamination of foods in the developing countries: Implications for hepatocellular carcinoma and chemopreventive strategies. *African Journal of Biotechnology* **5**, 1-14

Farombi EO, Tanaka T (2007) Cancer chemoprevention with Zingiberaceae and citrus-derived phytochemicals: Biochemical and molecular mechanisms. In*:* Tanaka T (Ed) *Cancer: Disease Progression and Chemoprevention*, Research Signpost Publishers, Kerala, India, pp 167-183

Farombi EO, Adepoju BF, Ola-Davies OE, Emerole GO (2005) Chemoprevention of aflatoxin B1-induced genotoxicity and hepatic oxidative damage in rats by kolaviron, a natural bioflavonoid of *Garcinia kola* seeds. *European Journal of Cancer Prevention* **14**, 207-214

Farombi EO, Akanni OO, Emerole GO (2002) Antioxidative and scavenging activities of kolaviron *in vitro*. *Pharmaceutical Biology* **40**, 107-116

Farombi EO, Hansen M, Ravn-Haren G, Moller P, Dragsted LO (2004a) Commonly consumed and naturally occurring dietary substances affect biomarkers of oxidative stress and DNA damage in healthy rats. *Food and Chemical Toxicology* **42**, 1315-1322

Farombi EO, Moller P, Dragsted LO (2004b) *Ex-vivo* and *in vitro* protective effects of kolaviron against oxygen-derived radical-induced DNA damage and oxidative stress in human lymphocytes and rat liver cells. *Cell Biology and Toxicology* **20**, 71-82

Farombi EO, Nwankwo JO, Emerole GO (2005a) The effect of modulation of glutathione levels on markers for aflatoxin B1-induced cell damage. *African Journal of Medical Sciences* **34**, 37-43

Farombi EO, Nwaokeafor IA (2005) Anti-oxidant mechanisms of kolaviron: studies on serum lipoprotein oxidation, metal chelation and oxidative membrane damage in rats. *Clinical Experimetal Pharmacology and Physiology* **32**, 667-674

Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO (2000) Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron – a *Garcinia kola* seed extract. *Food and Chemical, Toxicology* **38**, 535-541

Farombi EO, Shrotriya S, Surh YJ (2009) Kolaviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-κB and AP-1. *Life Science* **84**, 149-155

Ferrizzi MG, Blakeslee J (2007) Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutrition Research* **27**, 1-12

Figueroa JD (1999) La tortilla vitaminada. *Avance y Perspectiva* **18**, 149-158

Freitas VP, Brigido BM (1998) Occurrence of aflatoxins B1, B2, G1, and G2 in peanuts and their products marketed in the region of Campinas, Brazil in 1995 and 1996. *Food Additives and Contaminants* **15**, 807-811

Fujiki H, Suganuma M, Imai K, Nakachi K (2002) Green tea: cancer preventive beverage and/or drug. *Cancer Letters* **188**, 9-13

Fung F, Clark RF (2004) Health effects of mycotoxins: a toxicological review. *Journal of Toxicology: Clinical Toxicology* **42**, 217-234

Glintborg B, Weimann A, Kensler TW, Poulsen HE (2006) Oltipraz chemoprevention trial in Qidong, People's Republic of China: unaltered oxidative biomarkers. *Free Radicals in Biology and Medicine* **41**, 1010-1014

Gong Y, Hounsa A, Egal S, Turner PC, Sutcliffe AE, Hall AJ, Cardwell K, Wild CP (2004) Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. *Environmental Health Perspectives* **112**, 1334-1338

Gong YY, Cardwell K, Hounsa A, Egal S, Turner PC, Hall AJ, Wild CP (2002) Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *The British Medical Journal* **325**, 20-21

Gong YY, Hounsa A, Egal S, Turner PC, Hall AJ, Cardwell K, Wild CP (2003) Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: The critical role of weaning. *International Journal of Epidemiology* **32**, 556-562

Goto T, Peterson SW, Ito Y, Wiclow DT (1997) Mycotoxin producing ability of *A. tamarii. Mycotoxins* **44**, 17-20

Goto T, Wicklow DT, Ito Y (1996) Aflatoxin and cyclopiazonic acid production by a sclerotium-producing *Aspergillus tamarii* strain. *Applied Environmental Microbiology* **62**, 4036-4038

Graham HN (1992) Green tea composition, consumption, and polyphenol chemistry. *Preventive Medicine* **21**, 334-350

Groopman JD, Hall AJ, Whittle H, Hudson GJ, Wogan GN, Montesano R, Wild CP (1992) Molecular dosimetry of aflatoxin -N7-guanine in human urine obtained in The Gambia, West Africa. *Cancer Epidemiology Biomarkers and Prevention* **1**, 221-227

Groopman JD, Kensler TW (2005) Role of metabolism and viruses in aflatoxin-induced liver cancer. *Toxicology and Applied Pharmacology* **206**, 131-137

Groopman JD, Wogan GN, Roebuck BD, Kensler TW (1994) Molecular biomarkers for aflatoxins and their application to human cancer prevention. *Cancer Research* **54**, 1907s-1911s

Groopman JE, Donahue PR, Zhe J, Chen JS, Wogan GN (1985) Aflatoxin metabolism in humans: detection of metabolites and nucleic acid adducts in urine by affinity chromatography. *Proceedings of the National Academy of Sciences USA* **82**, 6492-6496

Guengerich FP (2001) Forging the links between metabolism and carcinogenesis. *Mutation Research* **488**, 195-209

Guengerich FP, Johnson WW, Shimada T, Ueng YF, Yamazaki H, Langouet S (1998) Activation and detoxication of aflatoxin B_1. *Mutation Research* **402**, 121-128

Guzmán-de-Peña D (1989) Micotoxinas en el Bajío Guanajuatense. *Avance y Perspectiva* **40**, 8-15

Guzmán-de-Peña D, Pena-Cabriales JJ (2005) Regulatory considerations of aflatoxin contamination of food in Mexico. *Revista Latinoamericana de Microbilogia* **47**, 160-164

Guzmán-de-Peña D, Trudel LT, Wogan GN (1995) Corn "nixtamalización" and the fate of radiolabelled aflatoxin B1 in the tortilla making process.. *Bulletin of Environmental Contamination and Toxicology* **55**, 858-864

Hagler WM Jr., Bowman DT, Babadoost M, Haney CA, Swanson SP (1987) Aflatoxin, zearalenone, and deoxynivalenol in North Carolina grain sorghum. *Crop Science* **27**, 1273-1278

Hall AJ, Wild CP (1994) Epidemiology of aflatoxin-related disease. In: Eaton DL, Groopman JD (Eds) *The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*, Academic Press, Inc., San Diego, CA, pp 233-258

Hamed A, Cartwright R, Xie W, Shier W (2005) Aflatoxin and fumonisin contamination of commercial corn (maize, *Zea mays*). *Crop Protection Journal* **25**, 1-9

Hatem NL, Hassab HM, Abd Al-Rahman EM, El-Deeb SA, El-Sayed Ahmed RL (2005) Prevalence of aflatoxins in blood and urine of Egyptian infants with protein-energy malnutrition. *The Food and Nutrition Bulletin* **26**, 49-56

Hawkins LK, Windham GL, Williams WP (2005) Effect of different postharvest drying temperatures on *Aspergillus flavus* survival and aflatoxin content in five maize hybrids. *Journal of Food Protection* **68**, 1521-1524

Hayes RB, Van Nieuwenhuize JP, Raatgever JW, Kate FJW (1984) Aflatoxin exposures in the industrial setting: an epidemiological study of mortality. *Food and Chemical Toxicology* **22**, 39-43

Hell K, Cardwell KF, Setamou M, Poehling HM (2000) The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. *Journal of Stored Products Research* **36,** 365-382

Hendrickse RG (1997) of sick turkeys, kwashiorkor, malaria, perinatal mortality, heroin addicts and food poisoning: research on the influence of aflatoxins on child health in the tropics. *Annals of Tropical Medicine and Parasitolology* **91**, 787-793

Henry SH, Bosch FX, Bowers JC (2002) Aflatoxin, hepatitis and worldwide liver cancer risks. *Advances in Experimental Medicine and Biology* **504**, 229-233

Henry SH, Bosch FX, Troxell TC, Bolger PM (1999) Reducing liver cancer – global control of aflatoxin. *Science* **286**, 2453-2454

Holmes RA, Boston RS, Payne GA (2008) Diverse inhibitors of aflatoxin biosynthesis. *Applied Microbiology and Biotechnology* **78**, 559-572

Hu X, Roberts J R, Apopa PL, Kan YW, Ma Q (2006) Accelerated ovarian failure induced by 4-vinyl cyclohexene diepoxide in Nrf2 null mice. *Molecular and Cellular Biology* **26**, 940-954

International Agency for Research on Cancer (IARC) (1993) Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. In: *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, IARC 56, Lyon, France, pp 23-87

International Agency for Research on Cancer (IARC) (2002) Traditional herbal medicines, some mycotoxins, napthalene, and styrene. *Monographs on the Evaluation of Carcinogenic Risks to Humans* **82**, 1-556

Ito Y, Peterson SW, Wiclow DT, Goto T (2001) *Aspergillus pseudotamarii*, a new aflatoxin producing sp. In Aspergillus section: *Flavin Mycolological Research* **105**, 233-239

Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y (1997) An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzymes genes through antioxidant response elements. *Biochemical Biophysics Research Communication* **236**, 313-322

Iwu MM (1985) Antihepatotoxic constituents of *Garcinia kola* seeds. *Experientia* **41**, 699-700

Jiang Y, Jolly PE, Ellis WO, Wang JS, Phillips TD, Williams JH (2005) Aflatoxin B1 albumin adducts levels and cellular immune status in Ghanaians. *International Immunology* **17**, 807-814

Johnson WW, Yamazaki H, Shimada T, Ueng YF, Guengerich FP (1997) Aflatoxin B1-8,9-epoxide hydrolysis in the presence of rat and human epoxide hydrolase. *Chemical Research in Toxicolology* **10**, 672-676

Kensler TW, Chen JG, Egner PA, Fahey JW, Jacobson LP, Stephenson KK, Ye L, Coady JL, Wang JB, Wu Y, Sun Y, Zhang QN, Zhang BC, Zhu YR, Qian GS, Carmella SG, Hecht SS, Benning L, Gange SJ, Groopman JD,

Talalay P (2005) Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxins-DNA adducts and phenanthrene tetraols in a randomized clinical trials in He Zuo township, Quidong, People's Republic of China. *Cancer Epidemiology and Biomarkers Prevention* **14**, 2605-2613

Kensler TW, Egner PA, Wang JB, Zhu YR, Zhang BC, Lu PX, Chen JG, Qian GS, Kuang SY, Jackson PE, Gange SJ, Jacobson LP, Muñoz A, Groopman JD (2004*)* Chemoprevention of hepatocellular carcinoma in aflatoxin endemic areas. *Gastroenterology* **127**, S310-318

Kensler TW, Qian GS, Chen JG, Groopman JD (2003) Translational strategies for cancer prevention in liver. *Nature Reviews Cancer* **3**, 321-329

Kim M, Miyamoto S, Yasui Y, Oyama T, Murakami A, Tanaka T (2009) Zerumbone, a tropical ginger sesquiterpene, inhibits colon and lung carcinogenesis in mice. *International Journal of Cancer* **124**, 264-71

Kitada M, Taneda M, Ohi H, Komori M, Itahashi K, Nagao M, Kamataki T (1989) Mutagenic activation of aflatoxin B1 by P-450 HFLa in human fetal livers. *Mutation Research* **227**, 53-58

Knight LP, Primiano T, Groopman JD, Kensler TW, Sutter TR (1999) cDNA cloning, expression and activity of a second human aflatoxin B1 metabolizing member of the aldo-keto reductase superfamily, AKR7A3. *Carcinogenesis* **20**, 1215-23

Koirala P, Kumari S, Yadav BK, Premarajani KC (2005) Occurrence of aflatoxin in some of the food and feed in Nepal. *Indian Journal of Medical Sciences* **59**, 331-336

Krishnamachari KA, Bhat RV, Nagarajan V, Tilak TBG (1975) Investigations into an outbreak of hepatitis in parts of western India. *Indian Journal of Medical Research* **63**, 1036-1049

Kusha M, Lakshminarasimhama V (1995) Aflatoxin contamination of sorghum seeds during storage under controlled conditions. *Zentralblatt für Bakteriologie* **282**, 237-243

Kwak MK, Egner PA, Dolan PM, Ramos-Gomez M, Groopman JD, Itoh K, Yamamoto M, Kensler TW (2001a) Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. *Mutation Research* **305**, 480- 481

Kwak MK, Itoh K, Yamamoto M, Sutter TR, Kensler TW (2001b) Role of transcription factor Nrf2 in the induction of hepatic phase 2 and antioxidative enzymes *in vivo* by the cancer chemoprotective agent, 3H-1,2-dimethiole-3-thione. *Molecular Medicine* **7**, 135-145

Lamplugh SM, Hendrickse RG, Ageagyei F, Mwanmut DD (1988) Aflatoxins in breast milk, neonatal cord blood and serum of pregnant women (short report). *British Medical Journal* **296**, 968-974

Langouët S, Coles B, Morel F, Becquemont L, Beaune P, Guengerich PF, Ketterer B, Guillouzo A (1995) Inhibition of CYP1A2 and CYP3A4 by Oltipraz results in reduction of aflatoxin B_1 metabolism in human hepatocytes in primary culture. *Cancer Research* **55**, 5574-5579

Langouet S, Furge LL, Kerriguy N, Nakamura K, Guillouzo A, Guengerich FP (2000) Inhibition of human cytochrome P450 enzymes by 1,2-dithiole-3-thione, oltipraz and its derivatives, and sulforaphane. *Chemical Research in Toxicolology* **13**, 245-252

Lee JS, Surh YJ (2005) Nrf2 as a novel molecular target for chemoprevention. *Cancer Letters* **224**, 171-184

Lewis L, Onsongo M, Njapau H, Schurz-Rogers H, Luber G, Kieszak S, Nyamongo J, Backer L, Dahiye AM, Misore A, DeCock K, Rubin C (2005) Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental Health Perspectives* **113**, 1763-1767

Li D, Cao Y, He L, Wang NJ, Gu JR (1993) Aberrations of p53 gene in human hepatocellular carcinoma from China. *Carcinogenesis* **14**, 169-173

Lillehoj EB, McMillan WW, Guthrie WD, Barry D (1980) Aflatoxin producing fungi in preharvest corn: Inoculum source in insects and soil. *Journal of Environmental Quality* **9**, 691-694

Li FQ, Yoshizawa T, Kawamura O, Luo XY, Li YW (2001) Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. *Journal of Agriculture and Food Chemistry* **49**, 4122-4126

Lipigorngoson S, Limtrakul P, Suttajit M, Yoshizawa T (2003) In-house direct cELISA for determining aflatoxin B1 in Thai corn and peanuts. *Food Additives and Contaminants* **20**, 838-845

Lopez-Garcia R, Park DL (1998) Effectiveness of post-harvest procedures in management of mycotoxin hazards. In: Bhatnagar D, Sinha S (Eds) *Mycotoxins in Agriculture and Food Safety*, Marcel Dekker, New York, pp 407-433

Lunn RM, Zhang YJ, Wang LY, Chen CJ, Lee PH, Lee CS, Tsai WY, Santella RM (1997) p53 mutations, chronic hepatitis B virus infection, and aflatoxin exposure in hepatocellular carcinoma in Taiwan. *Cancer Research* **57**, 3471-3477

Luo H, Tang L, Tang M, Billam M, Huang T, Yu J, Wei Z, Liang Y, Wang K, Zhang ZQ, Zhang L, Sun S, Martin C, Wang JS (2005) Phase IIa chemoprevention trial of green tea polyphenols in high-risk individuals of liver cancer: modulation of urinary excretion of green tea polyphenols and 8-hydroxydeoxyguanosine. *Carcinogenesis* **27**, 262-268

Lye MS, Ghazali AA, Mohan J, Alwin N, Mair RC (1995) An outbreak of acute hepatic encephalopathy due to severe aflatoxicosis in Malaysia*. American Journal of Tropical Medicine and Hygiene* **53**, 68-72

Mace K, Aguilar F, Wang JS, Vautravers P, Gomez-Lechon M, Gonzalez FJ, Groopman J, Harris CC, Pfeifer AM (1997) Aflatoxin B1-induced DNA adduct formation and p53 mutations in CYP450-expressing human liver cell lines. *Carcinogenesis* **18**, 1291-1297

McCoy LF, Scholl PF, Schleicher RL, Groopman JD, Powers CD, Pfeiffer CM (2005) Analysis of aflatoxin B1-lysine adduct in serum using isotope-dilution liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* **19**, 2203-2210

Mehan VK (1989) Screening groundnut for resistance to seed invasion by *Aspergillus flavus* and aflatoxin production. In: McDonald D, Mehan VK (Eds) *Aflatoxin Contamination of Groundnut: Proceedings of an International Workshop*, 6–9 Oct 1987, ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Center, Andhra Pradesh, India, pp 323-334

Méndez-Albores A, Arambula-Villa G, Loarca-Pina MG, Castaño-Tostado E, Moreno-Martínez E (2005) Safety and efficacy evaluation of aqueous citric acid to degrade B-aflatoxins in maize. *Food and Chemical Toxicology* **43**, 233-238

Méndez-Albores JA, Arambula VG, Vázquez BME, Mendoza EM, Preciado ORF, Moreno-Martínez E (2003) Effect of high moisture Maize storage on tortilla quality. *Journal of Food Science* **68**, 1878-1881

Méndez-Albores JA, Arambula-Villa G, Loarca-Piña MG, González-Hernández J, Castaño-Tostado E, Moreno-Martínez E (2004) Aflatoxins' fate during the nixtamalización of contaminated Maize by two tortilla-making processes. *Journal of Stored Products Research* **4**, 87-94

Mendez-Albores JA, Arambula-Villa G, Preciado-Ortiz RE, Moreno-Martinez E (2004) Aflatoxins in pozol, a nixtamalized, maize-based food. *International Journal of Food Microbiology* **94**, 211-215

Morgan MRA, Kang AS, Chan HWS (1986) Aflatoxin determination in peanut butter by ELISA. *Journal of Science and Food Agriculture* **37**, 908-914

Mphande FA, Siame BA, Taylor JE (2004) Fungi, aflatoxins and cyclopiazonic acid associated with peanut retailing in Botswana. *Journal of Food Protection* **67**, 96-102

Munkvold GP (2003) Cultural and genetic approaches to managing mycotoxins in maize. *Annual Review of Phytopathology* **41**, 99-116

Murakami A, Ohigashi H (2006) Cancer-preventive anti-oxidants that attenuate free radical generation by inflammatory cells. *Biological Chemistry* **387**, 387-92

Murakami A, Ohigashi H (2007) Targeting NOX, INOS and COX-2 in inflammatory cells: chemoprevention using food phytochemicals. *International Journal of Cancer* **121**, 2357-2363

Murakami A, Takahashi D, Kinoshita T, Koshimizu K, Kim HW, Yoshihiro A, Nakamura Y, Jiwajinda S, Terao J, Ohigashi H (2002) Zerumbone, a Southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, proinflammatory protein production, and cancer cell proliferation accompanied by apoptosis: the alpha,beta-unsaturated carbonyl group is a prerequisite. *Carcinogenesis* **23**, 795-802

Murakami A, Hayashi R, Tanaka T, Kwon KH, Ohigashi H, Safitri R (2003) Suppression of dextran sodium sulfate-induced colitis in mice by zerumbone, a subtropical ginger sesquiterpene, and nimesulide: separately and in combination. *Biochemical Pharmacology* **66**, 1253-1261

Murakami A, Tanaka T, Lee JY, Surh YJ, Kim HW, Kawabata K, Nakamura Y, Jiwajinda S, Ohigashi H (2004) Zerumbone, a sesquiterpene in subtropical ginger, suppresses skin tumor initiation and promotion stages in ICR mice. *International Journal of Cancer* **110**, 481-490

Na HK, Kim EH, Jung JH, Lee HH, Hyun JW, Surh YJ (2008) (-)-Epigallocatechin gallate induces Nrf2-mediated antioxidant enzyme expression via activation of PI3K and ERK in human mammary epithelial cells. *Archives in Biochemistry and Biophysics* **476**, 171-177

Nakamura Y, Yoshida C, Murakami A, Ohigashi H, Osawa T, Uchida K (2004) Zerumbone, a tropical ginger sesquiterpene, activates phase II drug metabolizing enzymes. *FEBS Letters* **572**, 245-250

Nesci A, Gsponer N, Etcheverry M (2007) Natural maize phenolic acids for control of aflatoxigenic fungi on maize. *Journal of Food Science* **72**, M180-185

Ngindu A, Johnson BK, Kenya PR, Ngira JA, Ocheng DM, Nandwa H, Omondi TN, Jansen AJ, Ngare W, Kaviti JN, Gatei D, Siongok TA (1982) Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *The Lancet* **1**, 1346-1348

Nwankwo JO, Tanhnteng JG, Emerole GO (2000) Inhibition of aflatoxin B1 genotoxicity in human liver-derived HepG2 cells by kolaviron biflavonoids and molecular mechanisms of action. *European Journal of Cancer Prevention* **9**, 351-361

Odhay B, Naicker V (2002) Mycotoxins in South African traditionally brewed beers. *Food Additives and Contaminants* **19**, 55-61

Omer RE, Kuijsten A, Kadaru AM, Kok FJ, Idris MO, Elkhidir IM, Vant VP (2004) Population-attributable risk of dietary aflatoxins and hepatitis B virus infection with respect to hepatocellular carcinoma. *Nutrition and Cancer* **48**, 15-21

Ono EYS, Sasaki EY, Hashimoto EH, Hara LN, Corrêa B, Itano EN, Sugiura UY, Hirooka EY (2002) Post-harvest storage of corn: effect of beginning moisture content on mycoflora and fumonisin contamination. *Food Additives and Contaminants* **19**, 1081-1090

Park DL (2002) Effect of processing on aflatoxin. *Advances in Experimental Medicine and Biology* **504**, 173-179

Park DL, Miller BM, Hart LP, Yang G, McVoy J, Page SW, Pestha J, Brown LH (1989) ELISA for screening AFB1 in cotton seed products and mixed feed: Collaborative study. *Journal of the Association of Official Analytical Chemists* **72**, 326-332

Patel S, Hazel CM, Winterton AGM, Mortby E (1996) Survey of ethnic foods for mycotoxins. *Food Additives and Contaminants* **13**, 833-841

Patten RC (1981) Aflatoxins and disease. *American Journal of Tropical Medicine and Hygiene* **30**, 422-425

Payne GA, Cassel DK, Adkins CR (1986) Reduction of aflatoxin contamination in corn by irrigation and tillage. *Phytopathology* **76**, 679-685

Petzer JP, Navamal M, Johnson JK, Kwak MK, Kensler TW, Fishbein JC (2003) Phase 2 enzyme induction by the major metabolite of oltipraz. *Chemical Research in Toxicology* **16**, 1463-1469

Phillips TD (1999) Dietary clay in the chemoprevention of aflatoxin-induced disease. *Toxicological Sciences* **52**, 118-261

Phillips TD, Lemke SL, Grant PG (2002) Characterization of clay-based enterosorbents for the prevention of aflatoxicosis. *Advances in Experimental Medicine and Biology* **504**, 157-171

Pratt MM, Reddy AP, Hendricks JD, Pereira C, Kensler TW, Bailey GS (2007) The importance of carcinogen dose in chemoprevention studies: quantitative interrelationships between, dibenzo[a,l]pyrene dose, chlorophyllin dose, target organ DNA adduct biomarkers and final tumor outcome. *Carcinogenesis* **28**, 611-624

Price RL, Jorgensen KV (1985) Effects of processing on aflatoxin levels and on mutagenic potential of tortillas made from naturally contaminated corn. *Journal of Food Science* **50**, 347-357

Qian GS, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, Wogan GN, Groopman JD (1994) A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiology Biomarkers and Prevention* **3**, 3-10

Qin G, Ning Y, Lotlikar PD (2000) Chemoprevention of aflatoxin B1-initiated and carbon tetrachloride-promoted hepatocarcinogenesis in the rat by green tea. *Nutrition and Cancer* **38**, 215-222

Ram BP, Hart P, Cole RJ, Pestka JJ (1986) Application of ELISA to retail survey of aflatoxin B1 in peanut butter. *Journal of Food Protection* **49**, 792-795

Rao MJV, Nigam SN, Mehan VK, McDonald D (1989) *Aspergillus flavus* resistance breeding in groundnut: progress made at ICRISAT Center. In: McDonald D, Mehan VK (Eds) *Aflatoxin Contamination of Groundnut: Proceedings of the International Workshop*, 6–9 Oct 1987, ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Center, Andhra Pradesh, India, pp 345-355

Ratnavathi V, Sashidharr B (2000) Changes in enzyme activities and aflatoxin elaboration in sorghum genotypes following *Aspergillus parasiticus* infestation. *Journal of the science of food and agriculture* **80**, 1713-1721

Resnik S, Neira S, Pacin A, Martinez E, Apro N, Latneite S (1996) A survey of the natural occurrence of aflatoxin and zearalenone in Argentine field maize: 1983-1994. *Food Additives and Contaminants* **13**, 115-120

Rheeder JP, Sydenham EW, Marasas WFO, Thiel PG, Shephard GS, Schlecter M, Stockenstrom S, Cronje DE, Viljoen JH (1994) Ear rot fungi and mycotoxins in S. African corn of the 1989 crop exported to Taiwan. *Mycopathologia* **127**, 35-41

Ritieni A, Moretti A, Bottalico A, Randazzo G, Monti SM, Ferracane R, Fogliano U (1997) Occurrence of fusaproliferin, fumonisin B1 and beauvericin in maize from Italy. *Journal of Agricultural and Food Chemistry* **45**, 4011-4016

Roebuck BD, Liu YL, Rogers AE, Groopman JD, Kensler TW (1991) Protection against aflatoxin B1-induced hepatocarcinogenesis in F344 rats by 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione (oltipraz): predictive role for short-term molecular dosimetry. *Cancer Research* **51**, 5501-5506

Rojas V, Martin MC, Quinzada M (2000) Aflatoxins in newly harvested corn in Panama. *Revista Medica de Panama* **25**, 4-7

Sabbioni G, Skipper PL, Tannenbaum SR (1987) Isolation and characterization of the major serum albumin adduct formed by aflatoxin B1 *in vivo* in rats. *Carcinogenesis* **8**, 819-824

Schneider S, De Leon R, García-Prendes M, Rolz C (1972) Determination of aflatoxin and aflatoxin-producing cultures in recently ginned cottonseed in Central America. *Journal of the American Oil Chemists' Society* **24**, 700-701

Scudamore KA, Nawaz S, Hetmanski MT (1998) Mycotoxin in ingredients of animal feeding stuffs. II Determination of mycotoxins in maize and maize products. *Food Additives and Contaminants* **15**, 30-55

Scussel VM (2004) Aflatoxin and food safety: Recent South American perspectives. *Journal of Toxicology-Toxin Reviews* **23**, 179-216

Sekhon CPS, Kapur I, Sodhi SS, Jand SK (1996) Detection of AFB1 by enzyme-linked immunosorbent assay. *Journal of Bioscience* **21**, 471-476

Shank RC, Bourgeois CH, Keschamras N, Chandavimol P (1971) Aflatoxin in autopsy specimens from Thai children with an acute disease of unknown etiology. *Food Cosmetics Toxicology* **9**, 501-507

Shapiro TA, Fahey JW, Dinkova-Kostova AT, Holtzclaw WD, Stephenson KK, Wade KL, Ye L, Talalay P (2006) Safety, tolerance and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase 1 study. *Nutrition and Cancer* **55**, 53-62

Shashidhar RB, Rao BSN (1988) Non-competitive ELISA for detection of AFB1. *Indian Journal of Experimental Biology* **26,** 984-989

Sheabar FZ, Groopman JD, Qian GS, Wogan GN (1993) Quantitative analysis of aflatoxin-albumin adducts. *Carcinogenesis* **14**, 1203-1208

Shen G, Xu C, Hu R, Jain MR, Nair S, Lin W, Yang CS, Chan JY, Kong AN (2005) Comparison of (-)-epigallocatechin-3-gallate elicited liver and small intestine gene expression profiles between C57BL/6J mice and C57BL/6J/Nrf2 (-/-) mice. *Pharmaceutical Research* **22**, 1805-1820

Shephard GS (2008) Risk assessment of aflatoxins in food in Africa. *Food Additives and Contaminants* **21**, 1-11

Simonich MT, Patricia AE, Roebuck BD, Orner GA, Jubert C, Pereira C, Groopman D, Kensler TW Roderick HD, Williams DE, Bailey GS (2007) Natural chlorophyll inhibits aflatoxin B_1-induced multi-organ carcinogenesis in the rat. *Carcinogenesis* **28**, 1294-1302

Sinha AK, Ranjan KS (1990) Incidence of aflatoxins in food rains during flood and post flood period at North Bihar. *Proceedings of the Indian National Academy of Science B* **56**, 295-298

Sofowora GG, Choo EF, Mayo G, Shyr Y, Wilkinson GR (2001) *In vivo* inhibition of human CYP1A2 activity by oltipraz. *Cancer Chemotherapy and Pharmacology* **47**, 505-510

Sorenson WG (1990) Mycotoxins as potential occupational hazards. *Development in Industrial Microbiology* **31**, 205-211

Sorenson WG, Jones W, Simpson J, Davidson JI (1984) Aflatoxin in respireble airborne peanut dust. *Journal of Toxicology and Environmental Health* **14**, 525-533

Speijers GJ, Speijers MH (2004) Combined toxic effects of mycotoxins. *Toxicology Letters* **153**, 91-98

Sudakin DL (2003) Dietary aflatoxin exposure and chemoprevention of cancer: a clinical review. *Journal of Toxicolology- Clinical Toxicology* **41**, 195-204

Tan L (2007) *Occurrence of Mycotoxins in Feed Samples from Asia*, IBiomin Mycotoxin Survey Program. Available online: www.engormix.com

Tanaka N, Chiba T, Matsuzaki Y, Osuga T, Aikawa T, Mitamura K (1993) High prevalence of hepatitis B and C viral markers in Japanese patients with hepatocellular carcinoma. *Journal of Gastroenterology and Hepatology* **28**, 547-553

Tang L, Tang M, Xu L, Luo H, Huang T, Yu J, Zhang L, Gao W, Cox SB, Wang JS (2008) Modulation of aflatoxin biomarkers in human blood and urine by green tea polyphenols intervention. *Carcinogenesis* **29**, 411-417

Thirumala-Devi K, Mayo A, Hall AJ, Craufurd PQ, Wheeler TR, Waliyar T, Subrahmanyam A, Reddy DV (2002) Development and application of an indirect competitive enzyme-linked immunoassay for aflatoxin M1 in milk and milk-based confectionery. *Agricultural and Food Chemistry* **50**, 933-937

Thomas DB (1991) Exogenous steroid hormones and hepatocellular carcinoma. In: Tabor E, Di Bisceglie AM, Purcell R (Eds) *Etiology, Pathology and Treatment of Hepatocellular Carcinoma*, PPC-Gulf Publishing Co., Houston, TX, pp 77-91

Trucksess M, Wood G (1994) Recent methods of analysis for aflatoxins in foods and feeds. In: Eaton DL, Groopman JD (Eds) *The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*, Academic Press Inc., San Diego, CA, pp 409-431

Trucksess MW, Dombrink-Kurtzman MA, Tournas VH, White KD (2002) Occurrence of aflatoxins and fumonisins in Incaparina from Guatemala. *Food Additives Contaminants* **19**, 71-75

Turner PC, Dingley KH, Coxhead J, Russell S, Garner CR (1998) Detectable levels of serum aflatoxin B1-albumin adducts in the United Kingdom population: implications for aflatoxin-B1 exposure in the United Kingdom. *Cancer Epidemiology Biomarkers and Prevention* **7**, 441-447

Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP (2003) Modification of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspectives* **111**, 217-220

Turner PC, Sylla A, Diallo MS, Castegnaro JJ, Hall AJ, Wild CP (2002) The role of aflatoxins and hepatitis viruses in the etiopathogenesis of hepatocellular carcinoma: A basis for primary prevention in Guinea-Conakry, West Africa. *Journal of Gastroenterology and Hepatology* **17**, S441-448

Turner PC, Sylla A, Gong YY, Diallo MS, Sutcliffe AE, Hall AJ, Wild CP (2005) Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in West Africa: a community-based intervention study. *The Lancet* **365**, 1950-1956

Upadhyaya HD, Nigam SN, Mehan VK, Reddy AGS Yellaiah N (2001) Registration of *Aspergillus flavus* seed infection resistant peanut germplasm ICGV 91278, ICGV 91283, and ICGV 91284. *Crop Science* **41**, 559-600

Vaamonde G, Patriarca A, Pinto VF, Comerio R, Degrossi C (2003) Variability of aflatoxin and cyclopiazonic acid production by *Aspergillus* section *flavi* from different substrates in Argentina. *International Journal of Food Microbiology* **88**, 79-84

Van Egmond HP (2002) worldwide regulations for mycotoxins. *Advances in Experimental Medicine and Biology* **504**, 257-69

Verma RJ (2004) Aflatoxins cause DNA damage. *International Journal of Human Genetics* **4**, 231-236

Verma RJ, Chaudhari SB (1997) Detection of aflatoxin in human urine. *Indian Journal Environmental Toxicology* **7**, 47-48

Waenlor W, Wiwanikit V (2002) Aflatoxin contamination of food and food products in Thailand: An overview. *Southeast Asian Journal of Tropical Medicine and Public Health* **34**, 184-190

Wagacha JM, **Muthomi JW** (2008) Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology* **124**, 1-12

Wang JS, Groopman JD (1999) DNA damage by mycotoxins. *Mutation Research* **424**, 167-181

Wang JS, Huang T, Su J, Liang F, Wei Z, Liang Y, Luo H, Kuang SY, Qian GS, Sun G, He X, Kensler TW, Groopman JD (2001) Hepatocellular carcinoma and aflatoxin exposure in Zhuqing Village, Fusui County, People's Republic of China. *Cancer Epidemiology Biomarkers and Prevention* **10**, 143-146

Wang JS, Luo H, Billam M, Wang Z, Guan H, Tang L, Goldston T, Afriyie-Gyawu E, Lovett C, Griswold J, Brattin B, Taylor RJ, Huebner HJ, Phillips TD (2005) Short-term safety evaluation of processed calcium montmorill montmorillonite clay (NovaSil) in humans. *Food Additives and Contaminants* **22**, 270-279

Wang JS, Shen X, He X, Zhu YR, Zhang BC, Wang JB, Qian GS, Kuang SY, Zarba A, Egner PA, Jacobson LP, Munoz A, Helzlsouer KJ, Groopman JD, Kensler TW (1999) Protective alterations in phase 1 and 2 metabolism of aflatoxin B1 by oltipraz in residents of Qidong, People's Republic of China. *Journal of National Cancer Institute* **91**, 347-354

Wang LY, Hatch M, Chen CJ, Levin B, You SL, Lu SN, Wu MH, Wu WP, Wang LW, Wang Q, Huang G T, Yang PM, Lee HS, Santella RM (1996) Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan. *International Journal of Cancer* **67**, 620-625

Whitten ME, Smith LL (1972) Effect of storage conditions on increase in aflatoxins in cottonseed: A small lot study. *Journal of the American Oil Chemists' Society* **49**, 596-597

Widstrom NW (1996) The aflatoxin problem with corn grain. *Advances in Agronomy* **56**, 216-280

Widstrom NW, McMillan WW, Beaver RW, Wilson DM (1990) Weather associated changes in aflatoxin contamination of preharvest maize. *Journal of Production Agriculture* **3**, 196-199

Wild CP, Hudson GJ (1992) Dietary intake of aflatoxins and the level of albumin-bound aflatoxin in peripheral blood in The Gambia, West Africa. *Cancer Epidemiology Biomarkers and Prevention* **1**, 229-234

Wild CP, Jiang YZ (1990) Evaluation of methods for quantitation of aflatoxin-albumin adducts and their application to human exposure assessment. *Cancer Research* **50**, 245-251

Wild CP, Turner PC (2001) Exposure biomarkers in chemoprevention studies of liver cancer. *IARC Scientific Publications* **154**, 215-222

Wild CP, Turner PC (2002) The toxicology of aflatoxins as a basis for public health decisions. *Mutagenesis* **17**, 471-481

Wild CP, Turner PC, Mendy M, Whittle H, Fortuin M, Hall AJ (2000) Hepatitis B infection and aflatoxin biomarker levels in Gambian children. *Tropical Medicine and International Health* **5**, 837-841

Wilkinson AP, Denning DW, Morgan MRA (1988) An ELISA method for the rapid and simple determination of aflatoxin in human serum. *Food Additives and Contaminants* **5**, 609-619

Wilkinson AP, Denning DW, Morgan MRA (1989) Immunoassay of aflatoxin in food and human tissue. *Journal of Toxicology: Toxin Reviews* **8**, 69-79

Williams JH, Phillips DT, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2004) Human Aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition* **80**, 1106-1122

Wilson DM, Payne GA (1994) Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination of crops. In: Eaton DL, Groopman JD (Eds) *The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*, Academic Press, Inc., San Diego, CA, pp 309-325

Winn RT, Lane GT (1978) Aflatoxin production on high moisture corn and sorghum with a limited incubation. *Journal of Dairy Science* **61**, 762-764

Xu C, Li CY, Kong AN (2005) Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Archives of Pharmacal Research* **28**, 249-268

Xue H (2004) Evaluation of peanut (*Arachis hypogaea* L.) germplasm for resistance to aflatoxin production by *Aspergillus flavus* Link ex Fries. PhD thesis, North Carolina State University Raleigh, 193 pp

Yates MS, Kensler TW (2007) Keap1 eye on the target: Chemoprevention of liver cancer. *Acta Pharmacologica Sinica* **28**, 1331-1342

Yoshizawa T, Yamashita A, Chokethaworu N (1996) Occurrence of fumonisin and aflatoxin in corn from Thailand. *Food Additives and Contaminants* **13**, 163-168

Young RW, Beregi JS Jr. (1980) Use of chlorophyllin in the care of geriatric patients. *Journal of the American Geriatrics Society* **28**, 46-47

Zarba A, Wild CP, Hall AJ, Montesano R, Hudson GJ, Groopman JD (1992) Aflatoxin M_1 in human breast milk from the Gambia, West Africa, quantified by combined monoclonal antibody immunoaffinity chromatography and HPLC. *Carcinogenesis* **13**, 891-894

Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P (1994) Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proceedings of the National Academy of Sciences USA* **91**, 3147-3150