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 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 control, decontamination, breeding for resistance as well as surveillance and awareness creation.

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

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 B₁ (AFB₁) has been implicated in the etiology of hepatocellular carcinoma (HCC) which is one of the most common cancers worldwide, causing nearly...
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 AFB1, 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 in 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.

**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. niger and A. tamarii 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 by base of their structures 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 M1 (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 AFB1, and inadequate evidence for the carcinogenicity of AFB1 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, AFI P1, AFB2 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). AFB1, AFB2, AFG1, AFG2 and M1 are found in milk or milk products obtained from livestock that has ingested contaminated feed. Aflatoxins were first detected 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 (Asuo
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, AFB$_1$ is one of the most potential environmental carcinogens, with toxic effects on humans through its direct consumption in food products or as metabolites in animal tissues.

TOXICOLOGY AND METABOLISM

Of the AFs, AFB$_1$ is the most prevalent, the most occurring in contaminated samples and AF$_1$ B$_2$, G$_1$ and G$_2$ are generally not reported in the absence of AFB$_1$. Dietary intake of AF$_1$s 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 AF$_1$s because of their high level of toxicity to humans and their potent carcinogenic effects in laboratory animals. AF$_1$s are toxic and carcinogenic to animals, including humans. Among these, AFB$_1$ is one of the most potential environmental carcinogens, with toxic effects on humans through its direct consumption in food products or as metabolites in animal tissues.

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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 is labor-intensive and expensive (Wild et al. 1990; Shea-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 screening methods showed a sensitivity and specificity of 98 and 91%, respec-
tively. This investigation demonstrates that rapid lateral flow immunoassays 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 it difficult to determine sensitive sampling schedules. Although biomarkers have been developed to circumvent this problem, including serum AF-alk 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-alk, 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-alk 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-alk was strongly associated with growth faltering, particularly stunting (Gong 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 AFM1 in cord sera has been reported from Thailand, Ghana, Nigeria and Gambia. Several studies have demonstrated the presence of AFM1 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 AFB1, 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; Park and Ueno (1977); for the first time developed ELISA for AF 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 AFB1 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 sensitivity of AF reflects the level of toxin found in food. In subsequent contaminated food. Also, the toxin ingested regularly, does not disappear rapidly, levels remaining significantly high due to release of toxin from tissue sources. Hence, it would be worthwhile to test this assay for AFB 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 (Truckssess 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 conventional paper filter (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 Affilast 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 VICAM Series 4 fluorometer set at 360 nm excitation and 450 nm emission.

**INTERACTION OF AF WITH THE HEPATITIS B VIRUS**

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 AFM1-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, the probability of damage to hepatitis B or C virus is 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
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 toxic 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 aflatoxin 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 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-

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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Low-risk countries:</th>
<th>High-risk countries:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Japan and Europe</td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td>Estimate Range</td>
<td>Estimate Range</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>&lt;15% 4-50% 20%</td>
<td>18-44% 60%</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>60% 12-64% 50%</td>
<td>40-80% 10%</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>Limited exposure</td>
<td>Limited exposure</td>
</tr>
<tr>
<td>Alcohol</td>
<td>&lt;15%&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;20%</td>
</tr>
<tr>
<td>Tobacco</td>
<td>&lt;12%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>40%</td>
</tr>
<tr>
<td>Oral contraceptive</td>
<td>10-50%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>NE</td>
</tr>
<tr>
<td>Other</td>
<td>&lt;5%</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<sup>1</sup> Attributable risk not quantified. One study suggested attributable fraction close to 50%.
<sup>2</sup> Restricted to liver cancer in women. Likely to increase in future generations.
<sup>3</sup> Unaspirated of hepatocellular carcinomas (notably HCC) are necessary co-factors.
<sup>4</sup> Not including double infections with HBV and HCCV. Very few studies available using second-generation assays.
<sup>5</sup> Estimates from the USA
<sup>6</sup> Estimates from three studies of LC in men

NE: not evaluated.
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 regulating monitoring of aflatoxin 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 processors 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 AFs in foodstuffs of 0.01 to 50 μg/kg, while those for toxic AF have been 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, vomiting, 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 Aspergillus 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 exposure 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 (Shepard 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 AF-alb adducts in children with low 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).

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 et al. 2005).
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 developing 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 foods 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 food-stuffs in Argentina. In a preliminary data reported by Dalcer et al. (1997) on the occurrence of mycotoxa and aflatoxin B1, zearalenone and deoxynivalenol in poultry feeds in Argentina. Three hundred samples of poultry feeds from 5 factories of Rio Cuarto, Cordoba 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 observed in 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 AFB1 and AFB2, respectively; and one sample contained AFG1 (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).

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

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Commodity</th>
<th>Frequency of AF-positive samples</th>
<th>Contamination rate/concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botswana</td>
<td>Mphande et al. 2004</td>
<td>Raw peanuts</td>
<td>78%</td>
<td>12-329 μg/kg</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Bankole et al. 2004</td>
<td>Pre-harvest maize, dried yam chips, melon seeds</td>
<td>Total AFs in maize = 3-138 μg/kg in positive samples; Mean concentration of AFs in yam chips is about 27.1 ppb; AFB1 above 5 μg/kg in 32.2% of samples</td>
<td></td>
</tr>
<tr>
<td>Malaysia</td>
<td>Ali et al. 1999</td>
<td>Peanut</td>
<td>65%</td>
<td>50 μg/kg aflatoxal level</td>
</tr>
<tr>
<td>Senegal</td>
<td>Diop et al. 2000</td>
<td>Peanut oil</td>
<td>AFB1, in &gt;85% of samples</td>
<td>Mean contents about 40 ppb</td>
</tr>
<tr>
<td>Thailand</td>
<td>Waenor et al. 2002; Lipigorrison et al. 2003</td>
<td>Peanut, corn and milk</td>
<td>38.9% contained aflatoxin</td>
<td>73 μg/kg</td>
</tr>
<tr>
<td>South Africa</td>
<td>Odhay et al. 2002</td>
<td>Traditionally brewed beers</td>
<td>2/6 commercial beer samples</td>
<td>200 and 400 μg/l</td>
</tr>
<tr>
<td>Philippines</td>
<td>Ali et al. 1999</td>
<td>Peanut</td>
<td>40%</td>
<td>375 μg/kg aflatoxal level</td>
</tr>
<tr>
<td>Brazil</td>
<td>Freitas et al. 1998</td>
<td>Peanut</td>
<td>Corn</td>
<td>130 μg/kg aflatoxal level</td>
</tr>
<tr>
<td>China</td>
<td>Li et al. 2001</td>
<td>Corn and peanuts</td>
<td>Aflatoxins in 8-80%</td>
<td>2-16,862 μg/kg</td>
</tr>
<tr>
<td>Turkey</td>
<td>Dogan et al. 2006</td>
<td>Cacao</td>
<td>55% contained aflatoxins</td>
<td>0.065-25.753 ppb</td>
</tr>
</tbody>
</table>

AF: aflatoxin; R: range; F: frequency; C: concentration; K: kilogram(s); P: parts per billion; HPLC: high performance liquid chromatography; TLC: thin layer chromatography; DOI: deoxynivalenol; DON: diacetoxynivalenol; ZEA: zearalenone; AFB: aflatoxin B; AFG: aflatoxin G.

Examples of food commodities and aflatoxin (AF) contamination levels reported in the literature.
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 commodities 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 AF, the brain is also affected. Autopsy of Thai children who died due to encephalopathy showed AF in their specimens (Shank 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 contamination 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 (Coultet 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. In a study more than 15% of total sample. Adapted from Koirala et al. (2005).

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 showed AF in their specimens (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).

Aflasol (Ram 1975). In addition to these, the seasonal variations and the production system thereby leading to chances 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 3: Food and food products contaminated with aflatoxin

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

Figures in parentheses are percentages

Adapted from Tan 2007

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

<table>
<thead>
<tr>
<th>Food commodity</th>
<th>Proportion (%) of sample having aflatoxin &gt;30 ppb (number)</th>
<th>Range of aflatoxin B1 detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize grit and flour</td>
<td>19.7 (57)</td>
<td>64-859</td>
</tr>
<tr>
<td>Peanut</td>
<td>16.0 (32)</td>
<td>54-1806</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>19.8 (20)</td>
<td>64-1736</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornflakes</td>
<td>26.3 (15)</td>
<td>60-163</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>5.4 (27)</td>
<td>109-693</td>
</tr>
<tr>
<td>Total</td>
<td>151*</td>
<td>64-1736</td>
</tr>
</tbody>
</table>

* 18% of total sample. Adapted from Koirala et al. (2005)
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 showed 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 (Guzmán-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 (Méndez-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 (Guzmán-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 aduct 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). Given 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 toxics 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>
<thead>
<tr>
<th>Table 5 The occurrence and concentration of aflatoxins in all feed samples analyzed according to geographical regions.</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Asia</td>
</tr>
<tr>
<td>No. of Test</td>
</tr>
<tr>
<td>No. Positive</td>
</tr>
<tr>
<td>Percent positive</td>
</tr>
<tr>
<td>Median (μg/kg)</td>
</tr>
<tr>
<td>Average (μg/kg)</td>
</tr>
<tr>
<td>Maximum (μg/kg)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 6 Occurrence of aflatoxins in the feed samples according to the commodity type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
</tr>
<tr>
<td>Soybean meal</td>
</tr>
<tr>
<td>Wheat/bran</td>
</tr>
<tr>
<td>Corn gluten meal</td>
</tr>
<tr>
<td>Rice/bran</td>
</tr>
<tr>
<td>DDGS</td>
</tr>
<tr>
<td>Other feed ingredients</td>
</tr>
<tr>
<td>Finished feed</td>
</tr>
<tr>
<td>Stray/Hay</td>
</tr>
</tbody>
</table>

*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 US 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 spherella 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 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 unscurpulous 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 (food 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 B1 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.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Country</th>
<th>Month</th>
<th>Aflatoxin μg/kg B1</th>
<th>Aflatoxin μg/kg B2</th>
<th>Aflatoxin μg/kg G1</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Elsalvador</td>
<td>November</td>
<td>50</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>Nicaragua</td>
<td>January</td>
<td>30</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>Elsalvador</td>
<td>March</td>
<td>30</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>97</td>
<td>Guatemala</td>
<td>April</td>
<td>60</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*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 2.9×10^3 to 6.3×10^6 colony-forming units/g. AFB1 was found in 64.2% of samples with a mean of 25.5 ppb. AFB2, G1 and G2 were detected in 26.4, 11.3 and 2.8% of the samples with mean levels of 779, 72 and 4.8 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 85% relative humidity for 48 or 72 h. In all treatments, the 72-h samples contained more AFB1 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 uninfected ones. 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 r=0.426 respectively) with AF production, whereas the amylase 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 less susceptible to AF elaboration among the various genotypes tested. All four AFs (B1, B2, G1 and G2) were present in five genotypes in levels of 17.9, 13.7, 16.5 and 22.3 ppb 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 (CISH-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, AFB1 and AFG1 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 (Winn et al. 1987). The study was 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, north-east Mexico, early sowing and proper irrigation decreased AF contamination from 4.5×10^5 to 6×10^4 colony-forming units/g in 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. AFs are not produced in the harvested corn. However, differences 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 is significantly different from the levels observed in seeds grown 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 al. 1986).
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 ground-nuts, Mehan (1989) identified the Shulamit and Darou IV genotypes 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, Faipzur, 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). 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 complemented 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-toxicogenic strain of *A. flavus* is established in the soil before peanuts are subjected to conditions favouring contamination (Dorner 2008). The applied strain competes with toxicogenic strains for infection sites, resulting in significantly

<table>
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<th>Stage in food production</th>
<th>Intervention</th>
<th>References</th>
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<tr>
<td>Pre harvest</td>
<td>Timing of planting; crop planted, genotype of seed planted; irrigation, insecticides, competitive exclusion; time of harvest</td>
<td>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</td>
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<tr>
<td>Post harvest: drying and storage</td>
<td>Hand sorting, drying on mats; sun drying; storing bags on wooden pallets or elevated off ground; insecticides; rodent control</td>
<td>Hell et al. 2000; Ono et al. 2002; Munkvold 2003; Fandohan et al. 2005a; Hawkins et al. 2005; Turner et al. 2005</td>
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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, Chlorophyllin) or dietary intervention (e.g. broccoli sprouts, green tea) 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; Kessler et al. 2004). The efficacy and 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 prostration and complete elimination of AF contamination might not be possible (Yates and Kessler 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 Kessler 2007). Many of these enzymes are regulated through Kelch ECH-associated protein 1 (Keap1)-NRF2-related factor (Nrf2)-antioxidant response element (ARE) signaling, making this pathway an important molecular target for chemoprevention (Lee and Surh 2005; Yates and Kessler 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 dihydrothiolenone 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 AFB1-8,9-epoxide with glutathione and enhancement of the activity of GST thereby reducing the formation of AF-N7-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 dihydrothienones 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) (Ferruzzi 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 Al 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 pathophysiologic biomarkers of AF3 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 chlorophyllidates and pheophorbidates. The use of CHL, in traditional medical applications is well documented including it application in wound 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; Ferruzzi 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 B1(1)

![Fig. 4 Structures of selected chemopreventive agents.](image_url)
exposure in a human population (Breinhol 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 AFB-N7-guanine, a DNA adduct biomarker derived from the ultimate carcinogenic metabolite of AFB1, AF-8,9-epoxide (Egner et al. 2003). Although the use of CHL in treating several diseases has been 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 AFB1-induced liver cancer (Lambert and Yang 2003).

Studies of Quin et al. (2000) in rats administered AFB1 and CCl4 as the initiator and promoter, respectively reveal that feeding of GT inhibits initiation and promotion steps of carcinogenesis including AFB1-induced liver cancer (Lambert and Yang 2003).

Molecular mechanisms underlying chemopreventive effects exerted by green tea and its components have been extensively investigated. EGCG is 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 isothiocyanate 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 sulphoraphane 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) AFB1-DNA adduct was reduced presumably due to induction of GST activity by sulphoraphane (Kensler et al. 2005). Thus AFB1 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 sized 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 casticated 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 kolavirnanone (*C. accuminata*) (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 hepatoprotective and chemoprotective 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. 2000) 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).

Mechanically, 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, uridylidiphosphoglucuronosyl transferase (UDPGT) (Farombi 2000; Farombi et al. 2005b) and NAPDH: 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...
ability 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 Nwaokafor 2005). Kolaviron scavenged H2O2, O2− and OH− in vitro (Farombi et al. 2002), suppressed in vivo lipid peroxidation (Farombi et al. 2003) and inhibited H2O2-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 in some edible parts, including young stems and inflorescence, which are used in traditional cooking (Murakami et al. 2003). ZER was identified as potent suppressor of TPA-induced Epstein-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 2004). ZER was found to be highly anti-mutagenic in phorbol ester-stimulated, differentiated HL-60 human leukaemia 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 (SOD1), glutathione peroxidase-1 (GPx-1), glutathione S-transferase-P1 (GST-P1) and NAD(P)H quinone oxidoreductase (NQO1) in the epithelium 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 aflatoxicosis 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 AF 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 nonexistent, 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.

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