Aflatoxin contaminated foods and health risk perspective for Pakistani population

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Abstract

Aflatoxin is a hepatotoxic, carcinogenic, immunosuppressive, antinutritional contaminant of many staple food commodities. Contamination may develop as a result of fungal action before, during and harvest and during storage. Conditions favorable for natural aflatoxin contamination of foods occur at latitudes between 40°N and 40°S of the equator. Human exposure is controlled in developed countries by regulations and capital-intensive, large-scale food production systems that make such regulations practical. But this is not the case in developing countries, wherein prevailing food production systems and economic conditions make management of aflatoxin contamination impractical. Cumulative aflatoxin exposure has been considered as one of the risks of liver cancer. Thus low exposure rates, particularly for the people in developing countries with HBV become significant. Aflatoxin contamination levels of various foods have been suggested to be associated with occurrence of liver cancer in Karachi. Animal studies show that aflatoxin also interferes with vitamins A and D, iron, selenium, and zinc nutrition. Risk of immunosuppression is well established in farm and laboratory animals, and immune system involvement of aflatoxin is confirmed for humans. To prevent future aflatoxicosis outbreaks, it is necessary to explore public health interventions that promote effective production, storage, and processing of homegrown and commercial grains. In addition, risks associated with aflatoxin-contaminated foods can be reduced through the use of specific processing and decontamination procedures. It is important to note that many herbs such as garlic extract and lemongrass extract etc. posses an inhibitory role against *Aspergillus flavus* and its aflatoxin production. Food preparation, which incorporates such commodities, may be blended with the fungal inhibitors at the starting point of their storage/processing to prevent from the aflatoxin contamination. Restricted human exposure is ensured by strict legislation.   .

Key words: Aflatoxin, Food spoilage, Aflatoxins and stored grains, Risk of cancer.

Fungal Spoilage of Food

Fungi are major plant and insect pathogens and frank growth of fungi on animal hosts produces the diseases collectively called mycoses. While dietary, respiratory, dermal, and other exposures to toxic fungal metabolites produce the diseases collectively called mycotoxicoses. Mycotoxins are fungal metabolites that are present in a large part of the world food supply and bear potential threat to food safety (De Koe, 1999; Park & Troxell, 2002; Bennett & Klich, 2003).

Molds are found in dry stored food and grains mostly as dormant structures, e.g., spores, mycelial fragments, or sclerotia. Fungal spoilage of foods involves; post-harvest diseases and losses of fruits and vegetables, deterioration of low water activity foods by xerophilic fungi, contamination of psychrotolerant or psychrophilic fungi on foodstuffs and processed foods during storage and distribution at low temperature and spoilage of heat processed foods and soft drinks by heat-resistant fungi. In accordance with global concern about food safety, mycotoxin contamination of foods has gained much attention in recent times owing to its potential health hazards (Udagawa, 2005).

The effects of mycotoxins are well known since antiquity. Modern trace analysis shows the wide prevalence of mycotoxins in the food chain. Aflatoxins (AFB1, AFB2, AFG1, AFG2), trichothecenes (deoxynivalenol, T-2 Toxin, HT-2 Toxin), zearalenone, fumonisins (FB1, FB2) and ochratoxin A are the most important mycotoxins worldwide. Foods of plant origin are normally contaminated more frequently and with higher concentrations than food of animal origin (Shapira *et al.,* 1996; Bauer, 2004).

Aflatoxins are potent carcinogenic, mutagenic, and teratogenic metabolites produced primarily by the fungal species *Aspergillus flavus* and *Aspergillus parasiticus.* Foods and feeds, especially in warm climates, are susceptible to invasion by aflatoxigenic *Aspergillus* species and the subsequent production of aflatoxins during preharvesting, processing, transportation and storage conditions (Cullen & Newberne, 1993; Eaton *et al.,* 1993; Shapira *et al.,* 1996).

Importance of the matter can be sensed by the fact that at least 77 countries have framed specific regulations for mycotoxins, 13 countries are known to have no specific regulations, whereas no data is available for about 50 countries, many of whom are situated in continent Africa (Van-Egmond, 2002). Recently, Udagawa (2005) has stressed that in addition to hazard assessment, data on the natural occurrence of mycotoxins in various commodities and processed food are needed to enable exposure assessment. Thus risk assessment of mycotoxins is, in fact, the product of hazard and exposure assessments.
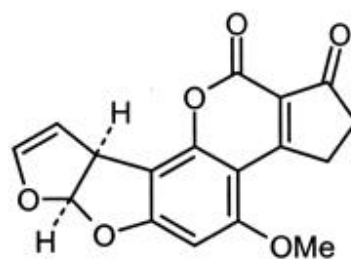
Chemical Nature of Aflatoxins

Aflatoxins are secondary metabolites produced by species of Aspergilli, specifically *Aspergillus flavus* and *Aspergillus parasiticus*. These molds are ubiquitous in nature and grow on a variety of substrates, thereby producing aflatoxins. Aflatoxins are of great concern due to their biochemical and biological effects on living organisms (Ellis *et al.,* 1991; Cullen & Newberne, 1993; Eaton *et al.*, 1993; Shapira *et al.,* 1996). Aflatoxins are a group of closely related compounds with small differences in chemical composition. Based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography the four major aflatoxins are called B1, B2, G1, and G2 (Ellis *et al.*, 1991; Shapira *et al.*, 1996; Bennett & Klich, 2003). Chemical structures of the four types of aflatoxins are shown in figure, 1.

Aflatoxin B1 is the most potent natural carcinogen and is usually the major aflatoxin produced by toxigenic strains. Aflatoxins are difuranocoumarin derivatives produced by a polyketide pathway by many strains of *Aspergillus parasiticus* and *Aspergillus flavus.* The latter is a common contaminant in agriculture (Cullen & Newberne, 1993; Eaton *et al.,* 1993; Bennett & Klich, 2003).

Economic pressures have necessitated the existence of double standards for allowable contamination of commodities destined for human and animal consumptions. Human foods containing 4 to 30 ppb of aflatoxins are considered acceptable with differing specific ranges within the limit by
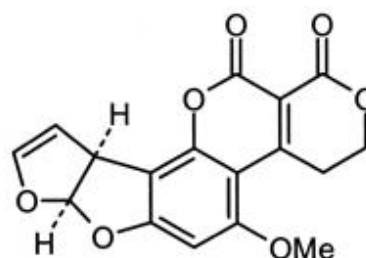
different countries while, grains for animal feed in the United States containing up to 300 ppb aflatoxin
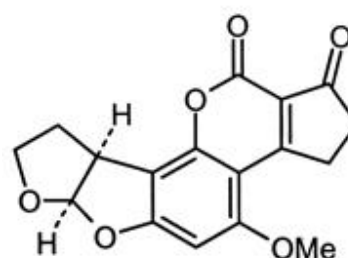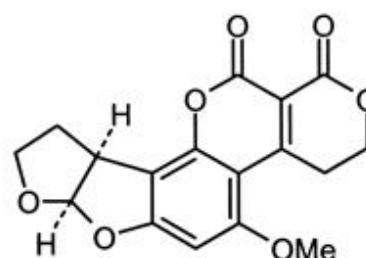

Figure 1: Chemical structures of aflatoxins (Williams *et al.*, 2004).

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are allowed, because this concentration not only provides protection against acute aflatoxicosis but also is low enough to allow most of the grain produced to be traded (Williams *et al.,* 2004).

Liquid chromatographic method is the first action for the determination of aflatoxins B1, B2, G1 and G2 in peanut butter and corn at concentrations greater than or equal to 13 ng/g. Other methods include PCR and HPLC detection (Park *et al.,* 1990; Shapira *et al*., 1996; Scudamore & Patel, 2000)

Genes involved in the aflatoxin biosynthetic pathway may form the basis for an accurate, sensitive, and specific detection system, using PCR, for aflatoxigenic strains in grains and foods. It is revealed that the PCR technique is efficient in distinguishing *A. parasiticus* and *A. flavus* from other molds commonly inhabiting stored grains (Shapira *et al.*, 1996).

Production of Aflatoxins

Many substrates support growth and aflatoxin production by aflatoxigenic molds. Cereals, figs, oilseeds, nuts, tobacco and a long list of other commodities are commonly attacked by aflatoxigenic molds (Van-Egmond, 2002; Bennett & Klich, 2003). Aflatoxin is a common contaminant of foods, particularly in the staple diets of many developing countries. This toxin is produced by fungal action during production, harvest, storage, and food processing and it is considered by the US Food and Drug Administration (FDA) to be an unavoidable contaminant of foods (Williams *et al*., 2004).

The fungi responsible are ubiquitous and can affect many of the developing-country dietary staples of rice, corn, cassava, nuts, peanuts, chilies, and spices. Williams *et al.* (2004) concluded that at latitudes between 40°N and 40°S of the equator, contamination of stored, inadequately dried produce is responsible for fungal invasion and that the contamination often begins before harvest and can be promoted by production and harvest conditions.

Production of aflatoxins is related to the highly variable relative humidity of the area, which influences moisture content of grains. Average relative humidity can be used to predict AF production (Dabbert & Oberheu, 2001; Williams *et al*., 2004). The toxins are produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parasiticus* fungi when the temperatures is between 24 and 35°C, and the moisture content exceeds 7% (10% with ventilation) (L'vova *et al.,* 1984; Williams *et al*., 2004).

Aflatoxins in Various Stored Grains

A large number of workers have demonstrated varying concentrations of different aflatoxins in a variety of foods stored in different conditions including rice bran, corn, maize and barley (Jayaraman and Kalyanasundaram, 1990; Wang *et al*., 1995; Yoshizawa *et al*., 1996; Ali *et al*., 1998; Scudamore & Patel, 2000; Henry *et al*., 2002; Park *et al*., 2002). Drought conditions stress maize plants and render them susceptible to contamination by *Aspergillus* spp. and the warm environment inside the windowless homes and storage of maize on the dirt floor promote fungal growth in wet maize kernels (Azziz-Baumgartner *et al.,* 2005).

Naturally occurring toxicant contamination of foods with mycotoxins is unavoidable and unpredictable and poses a unique challenge to food safety. Primary commodities susceptible to aflatoxin contamination include corn, peanuts and cottonseed and animal-derived foods such as milk when the animal is fed aflatoxin-contaminated feed (Park, 2002). L'vova *et al*., (1984) had demonstrated formation of mycotoxins under the conditions of experimental storage of rice grain and reported that Aflatoxins B1 and G1 are accumulated in the rice grain with initial moisture of greater than or equal to 16%. They found the amount of aflatoxins to reach a maximum when the temperature of the grain mass was 35-45 °C. Pande *et al*., (1990) in a screening study revealed that quantity of aflatoxin was highest in rice samples in comparison to wheat and maize.

Commercial parboiling of rice in Sri Lanka and many south Asian countries provides ideal conditions for the occurrence of aflatoxins because the rice is steeped (allowing fermentation) thus providing ideal conditions for growth of toxigenic *Aspergillus* species (Bandara *et al.,* 1991). The frequency of incidence as well as concentration of aflatoxin B1 (AFB1) increased with storage time in bran from untreated or raw rice (Rr) and parboiled rice (Pbr) and it was discovered that the rate of increase as well as overall concentration of aflatoxin B1 were much higher in Rr bran. Thus raw rice bran is unsuitable for prolonged storage (Jayaraman & Kalyanasundaram, 1994).

The levels of aflatoxin in maize and in sorghum stored in Brazil have been detected as aflatoxin B1+G1 in concentrations of 12 to 906 micrograms/kg in the samples collected from silos. In samples collected directly from farm aflatoxin B1+G1 were detected in concentrations of 10 to 14 micrograms/kg (Hennigen & Dick, 1995; Da Silva *et al.,* 2000).

Freitas and Brigido (1998) analysed peanuts and their products marketed in the region of

Campinas, Brazil for aflatoxin B1, B2, G1, and G2 by thin-layer chromatography and levels of aflatoxin B1 (AFB1) reached the highest incidence and the highest upper limits compared with all the other aflatoxins.

Toxicology of Aflatoxins

Poisoning that results from ingesting aflatoxins is known as aflatoxicosis. Two forms of aflatoxicosis have been identified: the first is acute severe intoxication, which results in direct liver damage and subsequent illness or death, and the second is chronic sub symptomatic exposure. A large number of studies have reported cases of aflatoxicosis in farm animals as well as wild life species in laboratory condition (Smith *et al.,* 1976; Clarkson, 1980; Robens & Richard, 1992; Dabbert & Oberheu, 2001).

Vaid *et al.* (1981) studied milk cattle affected by chronic aflatoxicosis and reported clinical and necropsy observations on liver, which included proliferation of connective tissue along portal triads leaving small group of hepatocytes. Liver function tests showed liver damage in these cows. Osweiler and Trampel (1985) reported a large-scale outbreak of aflatoxicosis in cattle fed on aflatoxin-contaminated cottonseed or gin trash and suggested the need for careful quality control of feed products susceptible to aflatoxin contamination.

Cook *et al.* (1989) studied aflatoxicosis in Iowa swine and reported clinical signs as decreased feed consumption and weight loss but death often was preceded by a period of clinical disease. They also observed greater morbidity and mortality in swineherds that consumed greater concentrations of aflatoxin. Williams *et al.* (2004) reported that in farm and laboratory animals, chronic exposure to aflatoxins compromises immunity and interferes with protein metabolism and multiple micronutrients that are critical to health.

In animals the toxin is processed through a number of competing pathways. These pathways have been well reviewed by various authors and are summarized in fig 2.

The differences in suscepibility to aflatoxin across species and between persons depend largely on the fraction of the dose that is directed into the various possible pathways, with harmful "biological" exposure being the result of activation to the epoxide and the reaction of the epoxide with proteins and DNA. There is also evidence that the fractions that follow the different possible pathways are influenced by dosage, perhaps because of the saturation of chemically most competitive processes (Payne & Woloshuk, 1989; Cullen & Newberne, 1993; Eaton *et al.*, 1993).

Susceptibility to aflatoxin is greatest in the young, and there are very significant differences between species, individuals of the same species (according to their differing abilities to detoxify aflatoxin by biochemical processes), and the sexes (according to the concentrations of testosterone). Toxicity of aflatoxins also varies according to many nutritional factors as for instance recovery from protein malnutrition is delayed by exposure to aflatoxin (Rogers, 1993). The human gastrointestinal tract rapidly absorbs aflatoxins after consumption of contaminated food and the circulatory system transports the aflatoxins to the liver. One to 3% of ingested aflatoxins irreversibly bind to proteins and DNA bases to form adducts such as aflatoxin B1–lysine in albumin. Disruption of proteins and DNA bases in hepatocytes causes liver toxicity (Rogers, 1993; Azziz-Baumgartner *et al.,* 2005).

The dose and duration of exposure to aflatoxin clearly have a major effect on the toxicology and may cause a range of consequences such as

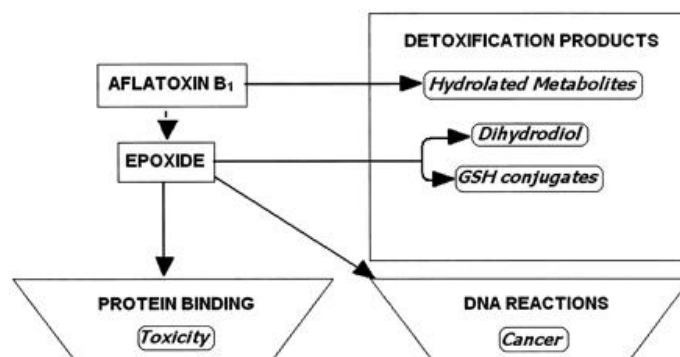a.   large doses may lead to acute illness and death, usually through liver cirrhosis

Figure 2: Pathways and consequences for aflatoxin in animal metabolism (Williams *et al.,* 2004).

b. chronic sublethal doses have nutritional and immunologic consequences

c. all doses have a cumulative effect on the risk of cancer.

The symptoms of severe aflatoxicosis include hemorrhagic necrosis of liver, bile duct proliferation, edema, and lethargy. Animal studies have shown two orders of magnitude difference in the median lethal dose for AFB_1. Susceptible species such as rabbits and ducks have a low (0.3 mg/kg) median lethal dose, whereas chickens and rats have greater (18 mg/kg) tolerance. Adult humans usually have a high tolerance of aflatoxin and in the reported acute poisonings, it is usually the children who die (Sutabhaha *et al.,* 1992; Eaton *et al.*, 1993; Henry *et al.,* 2002).

Synergic relation of aflatoxin toxicity and epidemics of hepatitis viruses is of great concern to public health. In many developing countries, epidemics of hepatitis B virus (HBV) and hepatitis C virus (HCV) affect ≲20% of the population. A strong synergy is observed between aflatoxin and these biological agents for liver cancer. In hepatitis B surface antigen-positive subjects, aflatoxin is ≈30 times more potent than in persons without the virus, and the relative risk of cancer for HBV patients increases from ≈5 with only HBV infection to ≈60 when HBV infection is accompanied with aflatoxin exposure. The suggested mechanism for this synergy is that aflatoxin suppresses DNA repair mechanisms that help limit the development of cancer from HBV, and HBV prevents detoxification. It is also possible that the immunotoxicity of aflatoxin interferes with the suppression of cancer (Groopman 1993; Henry *et al.*, 2002; Stewart & Kleihues, 2003).

It is very important to note that liver cancer cases in Pakistan have been associated with aflatoxin. For example, Nizami and Zuberi (1977) have described that long term ingestion of aflatoxin with food may have some effects on the existing patterns of liver cancer in Karachi. Similarly Qureshi *et al.* (1990) have documented aflatoxin contamination between 10% and 17% in various localities of Karachi and suggested an association of liver cancer with HBs Ag with the mycotoxins. In an earlier study (Munir *et al*., 1989) 6% of the samples namely maize and red chilies were found to be contaminated with aflatoxin B1 and B2, respectively. The percentage of samples contaminated among maize was 41.6 and the level of aflatoxin B1 ranged between 11.12 µg/Kg to 82.33 µg/Kg. In red chilies contamination was 25% and the level of aflatoxin B2 was found to be 41.67 µg/Kg.

Aflatoxins and other mycotoxins contaminate 25% of agricultural crops worldwide and are a source of morbidity and mortality throughout Africa, Asia, and Latin America. The

early symptoms of hepatotoxicity from aflatoxicosis can manifest as anorexia, malaise, and low-grade fever. Aflatoxicosis can progress to potentially lethal acute hepatitis with vomiting, abdominal pain, hepatitis and death. Because aflatoxin B_1–lysine adducts are not repaired, their half-life in human serum is approximately 20–60 days (Azziz-Baumgartner *et al.*, 2005).

Approximately 4.5 billion persons living in developing countries are chronically exposed to largely uncontrolled amounts of the aflatoxins, which result in changes in nutrition and immunity (Williams *et al.*, 2004). Evaluating the consequences of human exposure to aflatoxin requires the consideration of numerous facts. First, not all of the aflatoxin consumed is biologically significant a variable proportion of ingested aflatoxin is detoxified and the exposure may differentially affect various biological systems according to the fraction that is processed through each pathway. Whereas the relation between DNA-relevant exposure and cancer is understood well enough to calculate the consequences of varying concentrations of aflatoxins in food for the risk to people, effects on other metabolic processes in humans are not established (Robens & Richard, 1992; Henry *et al.*, 1999).

The lack of data on the temperature conditions needed for aflatoxin synthesis; the vulnerability of staple commodities to contamination; the systems for food production, storage, and marketing; and the regulation enforcement failures all indicate the risk of chronic aflatoxin exposure in developing countries.

Aflatoxins and Risk of Cancer

Aflatoxin has long been associated with both toxicity and carcinogenicity in human and animal populations. The diseases caused by aflatoxin consumption are loosely called aflatoxicoses. Acute aflatoxicosis results in death; chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions (Newberne & Butler, 1969; Shank *et al.*, 1972; Peers & Linsell, 1973).

Dragan and Pitot (1993) have described that aflatoxin is predominantly perceived as an agent promoting liver cancers in humans. The increased risk of hepatomas is caused by deletion mutations in the P53 tumor-suppressing gene and by activation of dominant oncogenes. Liver is the primary target organ of any toxic substance. Liver damage has been demonstrated in poultry, fish, rodents, and nonhuman primates fed with aflatoxin B_1. However, there are substantial differences in species susceptibility (Krishnamachari *et al.,* 1977; Robens & Richard, 1992; Bennett and Klich, 2003; Stewart & Kleihues, 2003). Aflatoxins are well recognized as a cause of liver cancer, but they have additional important toxic

effects. The cancer risk assessments and acute toxicity across species show that adult humans are relatively tolerant of aflatoxin but there is evidence that aflatoxin affects early growth and at least some aspects of human immunity and nutrition (Stewart & Kleihues, 2003; Williams *et al.,* 2004).

Underweight and nutrition-related epidemiology

Aflatoxin has been directly related to underweight status in children in Benin and Togo and to the condition of kwashiorkor. Autopsy evidence from children in Nigeria indicated presence of aflatoxin in tissues from most children examined post mortem, although the clinical cause of death was malnutrition and other diseases. Deaths of children in the Philippines, attributed to respiratory tract infections, also involved aflatoxin exposure. In Africa, the connection between infectious diseases and aflatoxin is also reported for malaria (Adhikari *et al.*, 1994; Denning *et al.*, 1995; Oyelami *et al.*, 1997; Gong *et al.,* 2003; Egal *et al.,* 2005).

Prevention of Aflatoxicosis

To prevent future aflatoxicosis incidence, it is necessary to explore public health interventions that promote effective production, storage and processing of homegrown and commercial grains. In addition, surveillance that monitors aflatoxin concentrations in food and incidence of acute jaundice in humans may prevent widespread outbreaks of acute aflatoxicosis. Risks associated with aflatoxin-contaminated foods can be reduced through the use of specific processing and decontamination procedures (Park, 2002).

In developed countries, where regulations allow higher aflatoxin concentrations in animals, the agricultural industries have developed alternative approaches [chemoprotection and enterosorption] to limit biologically effective exposure without the high cost of preventing contamination (Galvano *et al.,* 2001). Chemoprotection is based on manipulating the biochemical processing of aflatoxin to ensure detoxification rather than preventing biological exposure. Enterosorption is based on the approach of adding a binding agent to food to prevent the absorption of the toxin while the food is in the digestive tract; the combined toxin-sorbent is then excreted in the feces. This approach has been used extensively and with great success in the animal feeding industry (Rosa *et al.*, 2001). The traditional approach to prevent exposure to aflatoxin has been to ensure that foods consumed have the lowest practical aflatoxin concentrations. In developed countries, this has been achieved for humans largely by regulations that have required low concentrations of the toxin in traded foods (Williams *et al.,* 2004).

Hydrated sodium calcium aluminosilicate (HSCAS) can prevent aflatoxicosis in chickens and swine. The basic mechanism for this action appears to involve sequestration of aflatoxin in the gastrointestinal tract and chemisorption (i.e., tight binding) to HSCAS, which results in a reduction in aflatoxin bioavailability (L'vova *et al.*, 1984; Phillips *et al.*, 1990). Garlic extract is found to possess an inhibitory effect on growth of *Aspergillus flavus* and its aflatoxin production (Sutabhaha *et al.*, 1992). Currently, fungicides are not used to control fungal pests or mycotoxin production on stored rice. Citral a and b are the fungicidal constituents in lemongrass oil. And rice treated with the essential oil of lemongrass could be used to manage fungal pests as well as the insect pests in stored rice (Paranagama *et al.,* 2003).

In the context of afore mentioned facts it is alarming for the public health authorities of Pakistan to design strategies to make the public aware with seriousness of the aflatoxin issue. Due to improper storage and post-harvesting processes, grains and other commodities are highly prone to be contaminated with the toxigenic strains of *Aspergillus* sp. This situation coupled with poorly practiced food regulations and increasing incidence of the hepatitis epidemics in this country are suffices to open the eyes. Strong regulations and surveillance program and facilities for testing the food and feeds for the aflatoxin contamination are highly imperative for improving the increasingly decreasing health status of the public.

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