Aflatoxins and Their Management

6

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Contents

Abstract

Contamination of foods with aflatoxins (AF) has received a great awareness during the last few decades. AF are highly substituted coumarins containing a fused dihydrofurofuran moiety which are produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi. AF contamination can occur in various

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commodities including cereals, nuts, dried fruits, cocoas, oil seeds, spices, and copras in the field and/or during storage. The toxicological consequences of AF in populations are quite varied due to a wide range of exposures leading to acute and chronic effects. They are known mutagenic, teratogenic, carcinogenic, and immunosuppressive toxins. In addition, contamination of foods with AF may create significant direct and indirect economic consequences both for producer and consumer countries. Therefore, many countries have set legislation with regard to AF in foods. Concerning management strategies for AF, a number of methods have been investigated to prevent AF contamination to remove AF from the contaminated foods and feeds, to detoxify AF in contaminated foods and feeds, or to prevent AF effects. One possible approach to the management of the risks associated with AF contamination is the use of the integrated system of Hazard Analysis and Critical Control Points (HACCP). This proposed control program for processed foods/feeds should be based on the HACCP approach and should involve strategies for prevention, control, good manufacturing practices, and quality control used at all stages of production from the field to the final consumer. In this chapter, various aspects of AF including producing fungi, occurrence, legislations, toxicokinetics, toxicology, and management strategies are reviewed.

Introduction

Mycotoxins are organic and complex secondary metabolites produced by various fungi species. They are mainly produced by fungal genera including *Aspergillus, Fusarium, Penicillium, Claviceps*, and *Alternaria* under proper conditions. Some of the factors that influence the growth of fungi on crops and their subsequent mycotoxin production include plant genetics; exposure to fungal spores; weather conditions and climate during planting, growing, and harvesting; insect damage; crop management; and use of fungicides. Mycotoxin-producing fungi are commonly subdivided into field fungi and storage fungi (Rodrigues and Naehrer 2012).

Just a few hundred mycotoxins out of the thousands of existing ones are associated with foodstuffs and only a handful present food safety challenges (Murphy et al. 2006). Aflatoxins, aflatoxin M1(AFM1), ochratoxin A (OTA), deoxynivalenol (DON), fumonisins (FM), zearalenone (ZEN), and patulin (PTN) are among the most important mycotoxins. Mycotoxin contamination can occur in all agricultural commodities in the field and/or during storage, if conditions are favorable to fungal growth. Mycotoxins may contaminate a wide range of agricultural products including cereals, nuts, dried fruits, coffees, cocoas, spices, oil seeds, fruits, etc. When mycotoxins present in foods in sufficiently high levels, they can produce toxic effects that range from acute to chronic (like cancer), mutagenic, and teratogenic effects (Murphy et al. 2006).

AF are considered to be the group of mycotoxins of greatest concern from a global perspective. They have become recognized as ubiquitous contaminants of the human foodstuff supply throughout the economically developing world (Kensler et al. 2011). Various food commodities including cereals, nuts, dried fruits, cocoas,

oil seeds, spices, and copras may be contaminated with AF (Murphy et al. 2006). The toxicological consequences of AF in populations are quite varied due to a wide range of exposures leading to acute and chronic effects. They are known mutagenic, teratogenic, carcinogenic, and immunosuppressive toxins (Kensler et al. 2011). In addition to health risks to populations, contamination of foods with AF may create significant direct and indirect economic consequences both for producer and consumer countries. In this chapter, different aspects of AF are discussed.

Historical Perspective

The AF were discovered in the late 1950s and early 1960s following the severe outbreak of turkey "X" disease which resulted in the deaths of numerous turkeys and other farm animals fed diets containing certain lots of peanut meal originating in South America. Experiments revealed that toxicity was associated with the presence of *Aspergillus flavus*, and when the fungus was inoculated into uncontaminated peanut meal, it produced toxins similar to those found in the contaminated meal. Therefore, the isolated toxins were named "aflatoxin" (*Aspergillus flavus* toxin) (Ayub and Sachan 1997; Kensler et al. 2011).

Producing Fungi and Production Conditions

AF are secondary fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi. AF production in innate substrates depends upon the various factors, that is, type and moisture content of substrate, physical damage of the kernels, fungal species, temperature, humidity, and minerals (Abrar et al. 2013). The producing fungi are ubiquitous and can affect many dietary staples of developing countries. Fungal invasion and contamination often start before harvest, and AF accumulate postharvest when food commodities are stored under conditions that promote fungal growth. AF occur mostly in tropical regions with high humidity and temperature (between 24 °C and 35 °C) (Wild and Gong 2010; Williams et al. 2004). Grains must be kept dry, free of insects, and free of damage. Grains stored at warm temperatures (>20 °C) under high moisture/humidity (>14 %) and/or inadequately dried can potentially become contaminated with AF. These conditions allow mold "hot spots" to occur in the stored grain (Richard 2007).

Chemistry, Occurrence in Foods, and Legislations

Chemistry

Chemically, the AF are highly substituted coumarins containing a fused dihydrofurofuran moiety. The naturally occurring AF are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2), of which

AFB1 is the most abundant, carcinogenic, and toxic one (Wild and Gong 2010). The four major AF (AFB1, AFB2, AFG1, and AFG2) are called based on their relative chromatographic mobility during thin-layer chromatography and their fluorescence under ultraviolet irradiation (blue or green) (Bennett and Klich 2003). The blue fluorescent toxins (B) are characterized by fusion of a cyclopentenone ring to the lactone ring of the coumarin moiety, while the green fluorescent toxins (G) contain a fused lactone ring (Kensler et al. 2011). AFM1 and AFM2 are the hydroxylation products of AFB1 and AFB2, respectively, and found in milk and milk products (Wild and Gong 2010).

Occurrence in Foods

AF contamination can occur in various commodities in the field and/or during storage, if conditions are favorable to fungal growth. Many crops including cereals, nuts, dried fruits, cocoas, oil seeds, spices, and copras are contaminated with AF. Typical occurrence average ratio for AFB1 and AFB2 (mainly produced by *Aspergillus flavus*) is approximately 4:1. Typical average occurrence ratio for AFB1 and the sum of AFB2, AFG1, and AFG2 (the G toxins are mainly produced by *Aspergillus parasiticus*) is approximately 1:0.8, although variations do occur for both ratios (FAO 2004). Although contamination by the fungi may be universal, the levels or final concentrations of AF in the grain product can vary from less than 1 μg/kg to greater than 12,000 μg/kg. Indeed, in a recent outbreak of AF-induced death of people in Kenya, individual daily exposure of AFB1 was estimated to be 50 mg/day (Kensler et al. 2011).

A number of studies on the occurrence of AF in foodstuffs and feedstuffs have been published. Examples are given in Table 1 as well as in the following text.

The data show that AF are present in foodstuffs and feedstuffs and that maximum contamination levels exceeding the maximum levels or guidance values are likely to occur. AF production occurs mainly in regions with tropical or subtropical climates (Streit et al. 2012). Therefore, from a European perspective, the most common source of AF exposure is imported feed such as copra, palm kernel, peanut cake, and corn gluten meal (depending of origin). It has been stressed that as a consequence of rising average temperatures, patterns of mycotoxins occurrence in Europe are expected to change. For supporting this statement, Southern Europe was used as an example. It has been reported that while the importance of DON is about to decrease, *A. flavus* infection and AF contamination, which were uncommon in Europe, have become increasingly important. In 2003, in northern Italy, a hot and dry growing season resulted to severe corn infection with *A. flavus*. Analysis of AFB1 showed an incidence of 75 % with a mean contamination of 4.4 μg/kg. Using this corn as feedstuff for dairy cattle resulted in a widespread milk contamination with AFM1, and several thousand tons of milk exceeding the EU legal limit were discarded (Streit et al. 2012).

Rodrigues et al. (2011) analyzed various mycotoxins including AF in 324 grain, feed, and feed commodity samples, which were sourced directly at animal farms or

Table 1 Occurrence of AF in foodstuffs and feedstuffs

Food type	Country	Contaminated/ total	AF	Concentration (μg/kg)	Method	Reference
Rice	Canada	99/199	AFB1	<0.002–7.1	High-performance liquid chromatography (HPLC)	(Bansal et al. 2011)
Rice	IR Iran	59/71	Total AF (AFT)	2.097–10.94	HPLC	(Mazaheri 2009)
Wheat	Tunisia	10/46	AFT	0.15–18.6	HPLC	(Ghali et al. 2010)
Pistachio	IR Iran	3,699/10,068	AFB1	5.9 (Mean)	HPLC	(Cheraghali et al. 2007)
Hazelnut	Turkey	43/51	AFT	<0.625–10	Enzyme-linked immunosorbent assay (ELISA)	(Aycicek et al. 2005)
Infant milk food, milk-based cereal, weaning food, infant formula, and liquid milk	India	76/87	AFM1	0.063–1.012	ELISA	(Rastogi et al. 2004)
Rice	Vietnam	51/100	AFB1	3.31 (mean)	HPLC	(Nguyen et al. 2007)
Commodities, feeds, and feed ingredients	Egypt	3/16	AFB1	0.4 (mean)	HPLC	(Rodrigues et al. 2011)
Commodities, feeds, and feed ingredients	Jordan	9/20	AFB1	2 (mean)	HPLC	(Rodrigues, et al. 2011)
Commodities, feeds, and feed ingredients	South Africa	3/77	AFB1	0.3 (mean)	HPLC	(Rodrigues et al. 2011)
Peanuts and peanut products	Brazil	14/100	AFB1	Peanuts: 24.0–87.5 Peanut products: 22.0–84.6	Thin-layer chromatography (TLC)	(Hoeltz et al. 2012)

feed production sites in Middle East and Africa between February and October 2009. The incidence of AF in samples varied from 0 to 94 %. The mean level of AF contamination in total samples ranged from 0.2 μg/kg to 116 μg/kg. Warmer countries, such as Nigeria, Kenya, and Ghana, had a higher incidence of AF, while more temperate countries exhibited a totally different contamination pattern (Rodrigues et al. 2011). In a survey between January 2009 and December 2011, a total number of 7,049 corn, soybean/soybean meal, wheat, dried distillers grains with solubles, and finished feed samples from Americas, Europe, and Asia were analyzed for various mycotoxins including AF (Rodrigues and Naehrer 2012). The results showed that from 4,627 samples analyzed for AF, these toxins were present in 33 % of the samples with the average of 21 μg/kg. The maximum level of AF was found in corn samples at the level of 6,105 μg/kg (Rodrigues and Naehrer 2012).

To assess the incidence of mycotoxins in feed and feed raw materials, a 2-year survey program (from October 2003 to December 2005) was done by a feed additive producer (Binder et al. 2007). From North Asia, a total number of 3,420 samples were analyzed. The low proportion (0.03) of positive AFB1 samples was notable. From Southeast Asia, a total number of 2,040 analyses were undertaken. The incidence of AFB1 was 0.34 and the highest level found was 347 μg/kg. In South Asia, there was a clear indication of high AF occurrence (0.63). The average and median contamination levels were 52 μg/kg and 24 μg/kg, respectively (Binder et al. 2007).

Mycotoxin contamination of foodstuffs and feedstuffs in IR Iran has been reviewed (Yazdanpanah 2006). Pistachio nuts produced in IR Iran during March 2002 to February 2003 were analyzed for presence of AF (Cheraghali et al. 2007). In this regard, 3,356 pistachio nut samples were collected. After dividing samples to subsamples, 10,068 AF analyses were carried out. Among 10,068 samples analyzed, AFB1was detected in 3,699 samples (36.7 %), with the mean and median of 5.9 μg/kg and 0.1 μg/kg, respectively. The AFT was detected in 2,852 samples (28.3 %) with the mean and median of 7.3 μg/kg and 0.4 μg/kg, respectively (Cheraghali et al. 2007). In IR Iran, a survey of AFB1 was performed on 90 samples collected from Tehran retail market in June 2005 (Yazdanpanah et al. 2013). The results showed that none of the bread and wheat flour samples were contaminated with AFB1. The mean AFB1 levels in rice, puffed corn snack, and peanut samples were 4.17 μg/kg, 0.11 μg/kg, and 1.97 μg/kg, respectively. The level of AFB1 in 3 samples (one rice and two peanut samples) was found to be higher than 5 μg/kg (Yazdanpanah et al. 2013).

In IR Iran, 51 maize samples, intended for animal feed and human consumption, were collected from the four main maize production provinces and analyzed by HPLC for contamination by AF (Ghiasian et al. 2011). AFB1 was detected in 58.3 % and 80 % of the maize samples obtained from Kermanshah and Mazandaran provinces, respectively. The level of AFB1 in 15.68 % of the total samples was above the maximum tolerated limit (5 μg/kg) for AFB1 in maize in IR Iran. The mean contamination level of AFT (23.86 μg/kg) in the positive samples was higher than maximum tolerated limit for maize in IR Iran (20 μg/kg) intended for animal feed (Ghiasian et al. 2011).

Legislations

Widespread concern about the potential toxic effects of AF in humans and animals as well as possible transfer of residues into milk and edible animal tissues has demanded the need for establishment of control measures and limits by international authorities (Abrar et al. 2013; Kensler et al. 2011). Over the years, the number of countries which have set regulations for AF has markedly increased. The regulations for AF are often detailed and specific for various foodstuffs, feedstuffs, and dairy products (FAO 2004). The data published by FAO showed that the maximum tolerated levels for AFB1 in food have not changed dramatically in 2003 compared to the situation in 1995, although the range of limits has narrowed a little (1–20 μg/kg). In 2003, many countries regulated AFT sometimes in combination with a specific AFB1 limit.

Compared to 1995, the range of limits (0–35 μg/kg) has narrowed a little. AFM1 has been regulated in 60 countries in 2003, a more than threefold increase as compared to 1995. Many AF regulations exist for feedstuffs. Concerning AFB1 in feedstuffs for dairy cattle, many more countries (39) have set regulations in 2003, compared with those in 1995 (25 ones). In IR Iran, in 1997, Iranian National Standards Organization set maximum tolerated limits for mycotoxins in foods and feeds (FAO 2004).

Toxicokinetics

After absorption of AFB1 from the small intestine of broilers, it readily binds to plasma albumin, which serves as the major transporter of AFB1 in the blood. AFB1 is "procarcinogen," and enzymatic bioactivation is a prerequisite for its carcinogenic action. In the liver, AFB1 is oxidized by microsomal mixed function oxidase to several water-soluble metabolites (Abrar et al. 2013). Cytochrome P450 (CYP) enzymes are responsible for metabolism of AFB1 to the electrophilic, reactive, and major carcinogenic metabolite AFB1-8,9-epoxide (AFBE) or to the less mutagenic forms such as AFM1, aflatoxin Q1 (AFQ1), or aflatoxin P1 (AFP1) (Abrar et al. 2013; Bennett and Klich 2003; Murphy et al. 2006). In humans, epoxidation is catalyzed by CYP1A2 and CYP3A4 (Kensler et al. 2011). It is believed that AFBE formation and its subsequent covalent binding to DNA, RNA, and proteins play a critical role in both acute and chronic toxicity (Abrar et al. 2013). AFBE can take several pathways, one resulting in toxicity, another in cancer, and others in AFBE excretion (Murphy et al. 2006). The AFBE can react by interacting with DNA to produce a promutagenic AFB1-N^7-guanine adduct. In DNA, this adduct is unstable, rapidly undergoes depurination, and is excreted in the urine (Kensler et al. 2011). Formation of AFB1–DNA adducts (such as with N^7-guanine) leads to gene mutations and cancer (Murphy et al. 2006). A specific mutation of codon 249 is suspected to occur in the human p53 tumor suppression gene by AFB1–DNA adducts (Gomaa et al. 2008; Murphy et al. 2006). It has been shown that AFB1–DNA adducts can result in GC to TA transversions (Bennett and Klich 2003).

Among patients with hepatocellular carcinomas (HCC) in areas of high-risk AF exposure, this mutation was found with greater frequency (Murphy et al. 2006). AFB1 is more carcinogenic and mutagenic than AFG1 (Wild and Gong 2010). AFG2 and AFB2 are relatively nontoxic unless they are first oxidized metabolically to AFB1 and AFG1 in vivo (Kensler et al. 2011). Production of 8,9-dihydro-8,9-dihydroxy-AFB1 is the result of metabolic processing of the AFBE which causes injury of cell and eventual cell death (Caloni and Cortinovis 2011). AF toxicity may also arise through the intracellular reactive oxygen species generation during the metabolic processing of AFB1 by P450 system in the liver. These species may attack membranes as well as soluble cell compounds, eventually leading to the impairment of cell functioning and cytolysis (Abrar et al. 2013). In addition, AFB1 inhibits protein synthesis interfering with the formation of enzymes which are necessary for metabolism and energy and fat mobilization (Caloni and Cortinovis 2011).

Detoxification of the AF exo- and endo-epoxides is mainly through glutathione *S*-transferase-mediated conjugation with reduced glutathione (Wild and Gong 2010).

In humans, there are a number of urinary and serum biomarkers which were validated to accurately predict AFB1 cancer risk. AF-N^7-guanine in the urine serves as an elegant biomarker of biologically effective dose. Urinary measures of AFM1, the AF–albumin adduct, and AF-mercapturic acid are used as biomarkers of internal dose (Kensler et al. 2011). Serum AFB–albumin adducts which are positively associated with hepatocellular carcinoma in humans were found to be widely used in epidemiologic studies. Analysis of serum adducts indicates a positive correlation between dietary AFB1 exposure and serum AFB–albumin adducts (Rawal et al. 2010).

Toxic Effects of Aflatoxins on Animal and Human Health

AF are associated with both toxicity and carcinogenicity in human and animal populations (Bennett and Klich 2003). AF are immunosuppressive, carcinogenic, teratogenic, and mutagenic (Richard 2007). Among all known naturally occurring AF, AFB1 is the most toxic one. The degree of toxicity and mutagenic potency of AFB1 and its metabolites decrease as follows: AFB1 > aflatoxicol (AFL) > AFG1 > AFM1 > AFL-H1 > AFQ1 > AFB2 > AFP1 > AFG2 > AFB_{2k} > AFG_{2k} (Santacroce et al. 2008). Aflatoxicosis is the poisoning that results from ingesting AF (Williams et al. 2004). There are 2 forms of aflatoxicosis: (1) acute intoxication, which results in direct liver damage and subsequent illness or death, and (2) chronic subsymptomatic exposure. In all species, the dose and duration of exposure to AF clearly have a major effect on the toxicology and may cause the following consequences: (1) large doses of AF results into acute illness and death, usually through liver cirrhosis; (2) chronic sublethal doses of AF have immunologic and nutritional effects; and (3) all doses of AF have a cumulative effect on the risk of cancer (Williams et al. 2004).

AF were found to be moderately to highly toxic and carcinogenic in almost every animal species tested, including monkeys (Trucksess 2012). Concerning biological effects of AF, in most animal species, a wide variety effects such as toxicity, genotoxicity, carcinogenicity, teratogenicity, and impairment of immune and reproductive system were reported (Santacroce et al. 2008). The main factor in tolerance relates to the nature of the digestive system. Chickens, ducks, and ducklings are more sensitive, and ruminants are more tolerant. Breed variety, nutrition, sex, age, environmental stress, and presence of other disease agents are other factors contributing to differences in animal susceptibility to AF (Trucksess 2012).

Acute Toxicity

Exposure to large doses of mixed AF may cause acute toxicity with lethal effect. Animal species respond differently in their susceptibility to the acute and chronic toxicity of AF, and no animal species is resistant to the AF acute toxic effects. The AFB1 acute toxicity varies very much between animal species. For AF, LD50 value ranges from 0.5 to 10 mg/kg body weight for most species. AF effect is influenced by several factors including exposure level, duration of exposure, environmental factors, nutritional status, age, and health (Gnonlonfin et al. 2013). Exposure to small doses (a total of 2–6 mg) distributed over a prolonged period could lead to cancer, whereas exposure to estimated mixed total doses ingested with food of around 6,000 mg was reported to acute cause fatal toxicity in adult humans (Gnonlonfin et al. 2013). In humans, acute aflatoxicosis has been reported in several developing countries. Clinical manifestations of aflatoxicosis include vomiting, abdominal pain, pulmonary edema, disruption of blood clotting mechanism, reduced liver function, icterus, a decrease in essential serum proteins that are synthesized by the liver, necrosis of the liver, coma, convulsions, and death with cerebral edema and fatty involvement of the liver, kidney, and heart (Gnonlonfin et al. 2013; Kensler et al. 2011; Rawal et al. 2010). Other symptoms of acute to subacute aflatoxicosis include abdominal pain, vomiting, and edema of the lower extremities. Severe acute liver injury with high morbidity and mortality has been associated with high dose of AF exposure. Consumption of AF-contaminated food can result in outbreaks of sudden death within a population (Gnonlonfin et al. 2013). In the 1974, AF poisoning in India resulted from consumption of heavily contaminated maize. There were at least 97 fatalities (Kensler et al. 2011), and some adults may have eaten 2–6 mg of AF in a single day (Bennett and Klich 2003). In Kenya, in 2004 and 2005, acute aflatoxicosis caused more than 150 deaths (Kensler et al. 2011). In April 2004 in rural Kenya, one of the largest aflatoxicosis outbreaks occurred, resulting in 317 cases and 125 deaths. The source of the outbreak was AF-contaminated homegrown corn with an average concentration of 354 μg/kg (Trucksess 2012). It has been reported that despite eating similar quantities of maize as females, males were more likely to die from aflatoxicosis due to weaker male immune systems (Gnonlonfin et al. 2013).

Chronic Toxicity

Decreased milk or egg production, decrease in growth rate, and immune suppression are the symptoms of chronic exposure to AF in animal kingdom. In addition, liver damage is apparent due to the yellow color. AF affects all poultry species. Although relatively high AF levels are necessary to cause mortality, intake of low concentration of toxins over a long period of time leads to poor feed efficiency, poor growth, suboptimal production, and immunosuppression (Gnonlonfin et al. 2013). Regarding the carcinogenicity of AF, it has been shown that consumption of low levels for a prolonged period can result in primarily liver cancer in several animal species, including aquatic vertebrates (Santacroce et al. 2008). A wide variation exists in species susceptibility to AFB1 hepatocarcinogenesis. Fish and poultry responded to doses as low as 15–30 μg/kg. Rats responded at levels of 15–1,000 μg/kg, whereas mice showed no effects to levels as high as 150,000 μg/kg (Rawal et al. 2010).

A big part of the world population is chronically exposed to AF as evident from the presence of AFM1 in human breast milk as well as umbilical cord blood samples in several countries (Gnonlonfin et al. 2013). For humans, AFB1 is mainly considered as an agent promoting liver cancers, although lung cancer is also a risk among workers handling contaminated grain (Williams et al. 2004). AFB1 is a hepatocarcinogen and has been classified as group 1 human carcinogen (IARC 1993), but may be only part of the total answer to human liver cancer (Richard 2007). Hepatitis B can act synergistically with AF to increase the risk of HCC. According to the World Health Organization, in developing world including Asia and the Pacific Basin (excluding Japan, Australia, and New Zealand), sub-Saharan Africa, the Amazon Basin, parts of the Middle East, the Central Asian Republics, and some countries in Eastern Europe, chronic hepatitis B virus infection occurs more frequently (high infection >8 %). While in the rest of Europe, infection rates are below 1 % (EFSA 2007). Epidemiological studies of human populations exposed to diets naturally contaminated with AF revealed an association between the high incidence of liver cancer in Africa and elsewhere and dietary intake of AF (Turner et al. 2002). Often up to 1 in 10 of the population in sub-Saharan Africa is infected with hepatitis B and C, and AF intake raises the risk of liver cancer by more than tenfold compared to the exposure of both hepatitis alone (Gnonlonfin et al. 2013; Turner et al. 2003). Thus, AFB1 is an independent and possibly strongly potentiating factor for human HCC (Murphy et al. 2006). It has been reported that uncontrolled exposure to AF may cause 4.6–28.2 % of all liver cancer cases globally, with Southeast Asia, China, and sub-Saharan Africa bearing the brunt of the burden (Tillett 2010). In some countries, like Gambia and China, where hepatitis B virus and AF contamination occur together, hepatomas are the predominant cancer (64 % of cancers) and may be a predominant cause of death. It has been reported that 10 % of males' deaths or 10 % of all adults' deaths in Gambia or China (Qidong) were due to liver cancer, respectively. Greater potency of AF in hepatitis B virus-positive people is partly due to this finding that hepatitis B virus positivity reduces the person's ability to detoxify AF (Williams et al. 2004).

AF are immunotoxic to both livestock and humans. It has been reported that in animals, AFB1 induce thymic aplasia, suppress phagocytic activity, reduce T-lymphocyte function and number, and reduce complement activity. In poultry and rats, it has been shown that exposure to AF in contaminated food leads to suppression of the cell-mediated immune responses (Williams et al. 2004). Some of these effects may be mediated through altered cytokine expression (Wild and Gong 2010). In animals exposed to AF, suppression of lymphoblastogenesis, thymic and bursal involution, impairment of delayed cutaneous hypersensitivity, and graft-versus-host reaction also occurred (Williams et al. 2004). Reduced humoral immunity was shown in AF-exposed animals as was increased susceptibility to infections or reduced response to vaccines (Wild and Gong 2010). There are few studies regarding the immunologic suppression effect of AF in human populations. It has been estimated that 30 % of Gambian children are exposed to food with AF levels greater than 100 μg/kg. Previous studies in poultry have shown that when feeds contain similar levels of contamination, immune competence is compromised (Turner et al. 2003). Turner et al. (2003) reported that children are naturally exposed to AF through the diet at levels that compromise the immune system in other species and observed a highly significant association between AF exposure and reduced salivary secretory IgA. In another study in Gambia, children with malaria parasitemia had significantly higher mean AF–albumin adducts, but that there were no marked associations with experience of malaria infection and antibody titer to asexual stages of *Plasmodium falciparum* or lymphoproliferative responses (Wild and Gong 2010). In Ghana, in one study, alterations in different lymphocyte subgroups in relation to AF–albumin adduct level were reported. In another study, high AF–albumin adducts were associated with alterations in some lymphocyte subsets (Wild and Gong 2010). Totally, the studies of immunomodulation in AF-exposed populations are inconclusive. However, the data suggest that in populations exposed chronically to AF, effects on immune parameters could occur (Wild and Gong 2010).

Chronic AF exposure has major effects on nutritional status in animals. In animals exposed to AF, the efficiency of food use is consistently lower. In poultry, a 7–10 % drop in food conversion efficiency is observed, and decreased growth rates are a consistent sign of chronic AF exposure. In animals, it is well established that dietary AF reduces the rate of growth and other measures of productivity (Williams et al. 2004). Limited evidence suggests that growth suppression may also occur in humans. It has been reported that children in Togo and Benin who ate foods contaminated with high levels of AF were stunted and underweight, symptoms normally associated with malnutrition (Gnonlonfin et al. 2013). In Benin, the effects of AF exposure on growth were assessed in a longitudinal study over an 8-month period. There was a strong negative correlation between AF–albumin adducts and height increase over the 8-month follow-up. The highest quartile of biomarker was associated with a mean 1.7 cm reduction in growth over 8 months compared with the lowest quartile (Kensler et al. 2011). In Benin and Togo, a striking inverse association was found between AF–albumin adducts and growth in a cross-sectional study of children aged 1–5 years. Children who were stunted or

underweight had 30–40 % higher mean AF–albumin levels. In a subsequent 8-month longitudinal study, there was a strong negative correlation between AF–albumin adducts and height increase over the 8-month follow-up. These studies were extended to consider in utero exposure in a group of Gambian children and again an association was found between exposure and impaired growth, on this occasion in the first year of life. The mechanisms by which AF may exert an effect on growth are currently unknown, although the possibility of a compromised intestinal integrity, through altered barrier function as a consequence of endothelial cell toxicity or immune suppression, is a valid hypothesis to explore further (Wild and Gong 2010). In the blood, urine, and livers of children with symptoms of nutritional deficiencies (e.g., kwashiorkor), higher AF levels have been found in comparison with similar age-matched children. In comparison with AF-negative kwashiorkor children, AF-positive kwashiorkor children showed significantly greater severity of edema, increased number of infections, lower hemoglobin levels, and longer duration of hospital stay. It seems that protein deficiency reduces the capacity of the liver to detoxify AF; thus AF may be a contributory factor in increasing the morbidity of children suffering from other disease (Gnonlonfin et al. 2013).

There are few studies concerning the reproductive health effect of AF, and they have been reviewed by Shuaib et al. (2010). The available studies have largely focused on birth outcomes such as low birth weight and contamination of breast milk by AF. Six studies found marked associations or correlations between low birth weight and AF, while one study did not find any correlation. One study found maternal serum AF to be a risk factor for jaundice in infants. One study found a higher concentration of AF in the semen of infertile men. The findings showed a higher rate of AF contamination of maternal breast milk in developing countries, at levels beyond the acceptable limits. Totally, the reviewed studies were unable to draw definitive conclusions about the reproductive health effects of AF (Shuaib et al. 2010). However, considering the high contamination rate of breast milk by AF and the known toxic effects of AF on other organ systems, stakeholders in affected countries should take urgent steps to reduce exposure of vulnerable populations to the toxic effects of AF (Shuaib et al. 2010).

Aflatoxin Management Strategies

Many developing countries have found that reducing concentration of mycotoxins in foods will not only reduce financial burden on health care but also confer international trade advantages (Gnonlonfin et al. 2013). A great deal of research has been done for several years to find methods to reduce AF in contaminated agricultural produce. Recently, these AF management strategies have been reviewed by (Abrar et al. 2013; Gnonlonfin et al. 2013; Stoev 2013). A number of methods have been investigated to prevent AF contamination, to remove AF from the contaminated foods and feeds, to detoxify AF in contaminated foods and feeds, or to prevent AF effects. One possible approach to the management of the

risks associated with AF contamination is the use of the integrated system of HACCP. This proposed control program for processed foods/feeds should be based on the HACCP approach and should involve strategies for prevention, control, good manufacturing practices, and quality control used at all stages of production from the field to the final consumer (Stoev 2013). As an example, IR Iran, in the past decade, has implemented effective interventions to control AF in pistachio nuts. Implemented interventions such as establishing an efficient decision-making system; focusing on preventive methods; applying HACCP, good agricultural practice, and good storage practice guidelines; and using accurate and sensitive sampling and analytical methods proved to be effective. The statistics published by the European Commission (EC) regarding Rapid Alert System for Food and Feed (RASFF) for AF contamination in IR Iran pistachio nuts confirms a significant reduction in AF contamination in pistachio nuts exported from IR Iran. As a consequence, in regard to AF contamination, IR Iran experience to prevent and control AF contamination in pistachio nuts was fruitful (Cheraghali and Yazdanpanah 2010).

Preventive Measures of Aflatoxin Contamination of Foods/Feeds

The occurrence of fungi and mycotoxins can be decreased by application of a variety of preventative measures both preharvest and postharvest including appropriate control measures, timely harvesting, cleanup, drying and storage practices, management of insect infestation, crop rotation, creating of plant cultures resistant to fungi infestation, and others (Stoev 2013).

Biological strategies, such as toxigenic fungi, have been developed for prevention of AF contamination. In Nigeria, less toxigenic strain of *A. flavus* was isolated from soils. In the United States, such atoxigenic strains of *A. flavus* and *A. parasiticus* upon introduction to soil of developing crops have led to AF contamination in peanuts ranging from 74.3 % to 99.9 % of the original seen contamination. Postharvest (storage) AF contamination was reduced by 95.9 % through field application of non-toxigenic strains of *A. flavus* and *A. parasiticus* (Gnonlonfin et al. 2013).

Appropriate use of pesticides during the production process could help in reducing the fungal infection or insect infestation and subsequent mycotoxin contamination. Fungicides such as itraconazole and amphotericin B have been shown to effectively control the AF-producing *Aspergillus* species (Gnonlonfin et al. 2013).

Another tool is growing resistant varieties, which leads toward safety measure against AF contamination in field crops (Abrar et al. 2013). Rapid drying of agricultural products for lowering the moisture level is very critical. It has been shown that drying harvested maize to a moisture content of 15.5 % or lower within 24–48 h reduces the risk of fungal growth and subsequent AF biosynthesis (Gnonlonfin et al. 2013). In corn, several studies have shown that there is a positive correlation between AF contamination and insect damage. Therefore, for reduction

of mycotoxin contamination, proper management of insect pests through appropriate control strategy is needed (Gnonlonfin et al. 2013). The best method for controlling mycotoxin contamination is prevention through preharvest management. However, when mycotoxin contamination occurs, the hazards associated with various mycotoxins must be managed through postharvest procedures (Stoev 2013).

Physical Methods of Aflatoxin Decontamination of Foods/Feeds

Various physical methods including thermal inactivation, irradiation, cleaning, washing, segregation, mechanical sorting and separation, solvent extraction, etc., can be used to reduce or eliminate the risk of AF contamination in various types of foods. Regarding the effectiveness of cleaning for AF decontamination, an average reduction of about 40 % in concentration was usually reported (Stoev 2013). Significant amounts of AF can be removed from grains by immersing them in water and removing the upper floating fraction. It has been reported that sorting, winnowing, washing, and crushing combined with dehulling of maize grains were relatively effective in achieving a significant AF removal (Gnonlonfin et al. 2013).

Damaged or inadequately developed nuts highly contaminated with AF can be removed using automated sorting and segregation of peanuts. Fluorescence sorting is used mainly for screening and decontamination of corn, cottonseed, and dried figs, whereas electronic sorting is another method for peanuts decontamination, which is based on the color of roasted, blanched peanuts (Stoev 2013). In peanuts, it has been shown that a significant proportion (80 %) of the toxin is often associated with the small and shriveled seeds and moldy and stained peanuts, which can be removed by sorting (Gnonlonfin et al. 2013). AF can be eliminated from food commodities by utilization of various solvents, but the barriers for commercial exploitation of such methods are high prices and possible solvents residues (Abrar et al. 2013; Stoev 2013).

It has been shown that solar radiation, as a physical method of decontamination, is an inexpensive way of partial detoxification of AF in contaminated coconuts, peanuts, sesame, and corn (Stoev 2013). It has been shown that thermal inactivation could be a suitable method for AF decontamination in pistachio nuts (Yazdanpanah et al. 2005). In this regard, the effect of roasting on AF reduction in pistachio nuts was investigated. Although all treatment protocols showed some degree of AF degradation (ranging from 17 % to 63 %), roasting spiked samples at 120 °C for 120 min and 150 °C for 30–120 min caused substantial reduction of AF. Treatment of naturally contaminated whole pistachio kernels at 150 °C for 30 min significantly reduced level of AF contamination in samples (up to 81 % reduction in AFB1 level in pistachio nut with original contamination of 235 μg/kg). Degradation of AF was both time and temperature dependent (Yazdanpanah et al. 2005).

Chemical Methods of Aflatoxin Decontamination of Foods/Feeds

Numerous chemopreventives have been assessed for their effectiveness in AF decontamination. Various chemicals such as sodium bisulfite, ammoniation, hydrogen peroxide, ozone, propionic acid phosphine, fungicide, sodium bentonite, clay-based inorganic adsorbents, and limewater have been used to destroy or degrade AF effectively, but most of them are impractical or potentially unsafe to use due to the formation of toxic residues or the effect on nutrient content, flavor, odor, color, texture, and/or the functional properties of the product. Ammonization and reaction with sodium bisulfite are two techniques for detoxification of AF that have received considerable attention (Abrar et al. 2013).

Use of Different Compounds and Other Methods Preventing the Aflatoxin Effects

There is the possibility of addition of various chemicals or feed additives in order to fix and neutralize mycotoxins (Stoev 2013). Dietary strategies can prevent ingestion or absorption of mycotoxins in prepared foods and feeds. In this regard, food components (phenolic compounds, coumarin, chlorophyll and its derivatives, fructose, aspartame), antioxidant compounds (selenium, vitamins, provitamins), mineral and biological binding agents (hydrated sodium calcium aluminosilicate, bentonites, zeolites, activated carbons, bacteria, and yeast), and medicinal herbs and plant extracts can be used. Chlorophyllin and oltipraz and/or dietary intervention like broccoli sprouts and green tea was found to be effective in preventing the production of epoxide (that leads to chromosomal damage) or increasing detoxification processes. Enterosorption based on the use of certain clay minerals (such as Novasil) was found to be especially useful in binding mycotoxin from contaminated feedstuffs (Gnonlonfin et al. 2013). For example, hydrated sodium calcium aluminosilicate clay is very useful for preventing aflatoxicosis in farm animals and for reducing AF concentrations in milk (Stoev 2013). In a trial in Ghana, ingestion of capsules containing a clay compound resulted in a marked reduction of the biomarker of AF exposure (Gnonlonfin et al. 2013).

Food/Feed Processing as a Method of Aflatoxin Decontamination

Processing can be defined as any physical, chemical, or biological treatment that is applied to a raw material to produce the final consumer product and includes any procedure from dry and wet milling of grains, baking, extrusion, and steaming to feeding cereal-based complete feeds to animals to produce meat or milk. During processing, the stability of mycotoxins may be affected by biological or chemical reactions and factors such as temperature, moisture content, pressure, pH, buffering conditions, and the presence of other constituents and enzymes (Stoev 2013).

Stability of AF to heat in processes such as baking and extrusion depends on pH and temperature. For example, higher temperatures or alkaline processes (such as the use of leavening agents or tortilla production) can reduce AF levels. In the manufacture of tortillas, AF can be significantly decreased during the treatment of corn with limewater. AF are successfully eliminated during refining of oil. AF levels can be considerably decreased with addition of sodium chloride during the cooking of unshelled peanuts under pressure (Stoev 2013). In IR Iran, in Damghan city (Semnan Province), the pistachio nuts are roasted with lemon juice. In an investigation, the efficacy of lemon juice and/or citric acid in AFB1 degradation in pistachio nuts was evaluated (Amirahmadi et al. 2005). The results showed that roasting pistachio nuts with lemon juice at 90 °C or 120 °C for 30 min was not effective on AFB1 degradation. However, a synergistic effect on AF degradation was observed between heating pistachio at 120 °C for 1 h and adding lemon juice and citric acid. When pistachio nut samples (with AFB1 level: 268 μg/kg) are roasted with a mixture of lemon juice (15 ml), citric acid (2.25 g), water (30 ml), and sodium chloride (5 g) at 120 °C for 1 h or 150 °C for 30 min, AFB1 was degraded 58 % or 47 %, respectively. These roasting procedures improved taste, flavor, physical appearance, and acceptability of pistachio nuts (Amirahmadi et al. 2005).

In wet milling, a large percentage of AF are removed in the steep water. In dry milling, AF concentrate in the bran and offal fractions of wheat and germs (Stoev 2013).

Conclusions and Future Directions

From a global perspective, AF are considered to be the group of mycotoxins of greatest concern. They have become recognized as ubiquitous contaminants of the human foodstuff supply throughout the economically developing world. A big part of the world population is chronically exposed to AF which is associated with both toxicity and carcinogenicity in human populations. Due to unavoidable and unpredictable nature of AF, the contamination of foods with these fungal toxins presents a unique challenge to food safety. Many developing countries have found that reducing concentration of AF in foods will not only reduce financial burden on health care but also confer international trade advantages. Therefore, AF contamination must be managed through using proper management strategies. One possible approach to the management of the risks associated with AF contamination is the use of the integrated system of HACCP. This proposed control program for processed foods/feeds should be based on the HACCP approach and should involve strategies for prevention, control, good manufacturing practices, and quality control used at all stages of production from the field to the final consumer. AF reduction and control are dependent on the concerted efforts of all sectors involved in the food production chain. The key actions include AF awareness as a public health issue, strengthening laboratory and surveillance capacities, as well as establishing early warning system and training of farmers.

Cross-References

- ▶ Biotoxins and Food Safety
- ▶ Current Insights into Mycotoxins

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