

Significance of Aflatoxins in Rural and Global Health: Concern for Agricultural Workers

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Aflatoxins B₁, B₂, G₁, and G₂ are highly toxic and carcinogenic secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi. Aflatoxin B₁ (AFB₁) has been recognized as one of the most potent chemical carcinogens [1]. The acute toxicity, mutagenicity, and carcinogenicity of aflatoxins are associated with the presence of the 8,9 double bond and the terminal cyclopentanone ring (Figure 1). The order of the severity of acute and chronic toxicity is B₁ > G₁ > B₂ > G₂, reflecting the role of the 8,9 double bond and the greater potency associated with the cyclopentanone ring of the B series [2].

AFB₁ Contamination

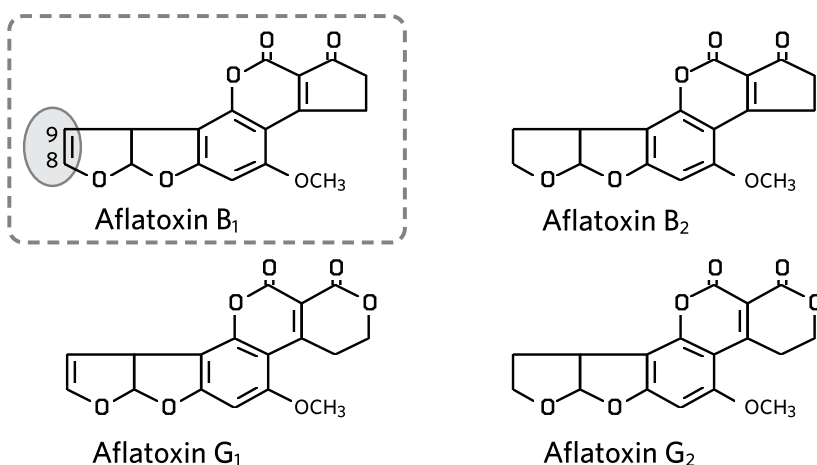
AFB₁-producing fungi (ie, *A. flavus* and *A. parasiticus*) are found naturally in soil and airborne dust. They invade many crops (eg, corn and groundnuts) and the crops' processed products in search of nutrition during fungal growth and maturity. Fungal invasion of corn, for example, takes place late in the growing season under hot (optimum temperature, 80°F-100°F) and humid conditions [3]. Increased fungal infestation is facilitated by plant stress due to poor agronomic methods, drought, and damage from strong winds, birds, and insects [4]. After harvest, the quantity of AFB₁ produced in contaminated corn usually continues to increase under unhygienic, hot, and humid storage conditions. Improper storage of contaminated grain may also result in fungal spread and increased production of AFB₁. Similarly, improper processing and storage of other agricultural commodities (eg, cereals, peanut, seeds, and nuts) may result in fungal infestation and aflatoxin production [5]. Worldwide, studies have shown that AFB₁ is commonly detected in dried fruits, vegetables, herbs, and medicinal plants, particularly in developing countries where proper processing and storage practices are not followed [6].

Dietary Exposure to AFB₁

Human exposure to AFB₁ occurs directly through consumption of contaminated foods (eg, cereals, nuts, dried fruits, and vegetables) and indirectly through consumption of animal products (eg, meat, milk, poultry, and eggs). The problem of human consumption of foods contaminated by AFB₁ is most serious in developing countries that lack proper processing procedures, storage facilities, and food-safety monitoring [7-9]. In addition, many of the rural populations in developing countries are dependent on stored grains for their daily diet and for feeding domestic birds or animals. Such practices constitute significant health risks through the propagation of hazardous levels of AFB₁ in the daily diet, owing to consumption of domestic birds and animals that have been fed contaminated grain.

Although consumption of significant levels of AFB₁ is commonly believed to be confined to countries lacking adequate storage facilities, the cumulative exposure to sources of AFB₁ among persons in developed countries could be significant. Recent evidence from Europe strongly suggests that most exposure to AFB₁ emanates from unidentified sources

Figure 1.
The Four Common Aflatoxins



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and is much higher than was previously suspected [10-12]. A study of cadaver tissues in England showed aflatoxin-DNA adduct levels nearly as high as those found in areas of Southeast Asia and Africa noted for high exposure to AFB₁.

AFB₁ and Liver Cancer

Acute toxic effects of aflatoxins have been fully documented for a large number of animal species and humans [1, 13-15]. AFB₁ has been recognized to produce cancer in the liver and other organs in a broad variety of animal species [16]. Epidemiological studies in Africa [17, 18] and Southeast Asia [19] have shown a strong correlation between the incidence of liver cancer among humans and the levels of AFB₁ contamination in their daily diet. An epidemiological study in Swaziland, a country with a food supply dependent on imported grains, showed an association between the incidence of liver cancer and the estimated levels of AFB₁ in the daily dietary intake [18].

Recent advances in molecular biology and concomitant understanding of the metabolism of AFB₁ have led to a better understanding of the interaction between AFB₁ and cellular DNA. AFB₁ reacts almost exclusively at the N⁷ position of guanine after activation to its reactive form, 8,9-exo-epoxide (AFB₁-8,9-exo-epoxide). The use of biomarkers has allowed the estimation of the amount of AFB₁ consumed at the individual level. In addition, specific assays have been developed and validated to measure aflatoxin-albumin adducts in serum [20], AFB₁ metabolites in urine [20, 21], and aflatoxin-N⁷ guanine adducts in urine [22]. Results of these studies indicate that the aflatoxin-N⁷ guanine adduct assay is well correlated with primary liver damage and accurately measures recent AFB₁ intake [23]. These assays, as well as the understanding of the pathways and metabolic products of AFB₁, provide additional evidence about the role of AFB₁ in the pathoetiologic process of hepatic and pulmonary carcinomas.

Metabolism and Biological Detoxification of AFB₁

The cytochrome p450 enzyme system is central to the metabolism of AFB₁. Because AFB₁ is relatively hydrophobic, it must be processed via enzyme-catalyzed reactions prior to excretion. As with the detoxification of many xenobiotics, the metabolism of AFB₁ involves activation and conjugation reactions. During activation, AFB₁ is biotransformed through hydrolysis, oxygen demethylation, or epoxidation reactions that yield one of several molecules [24].

Of the metabolic products created during phase I activation reactions, the most biologically important is the highly reactive electrophile AFB₁-8,9-epoxide. This product has been found to exist in 2 stereoisomers, of which the *exo* form is much less stable in water and approximately 500 times as mutagenic as the *endo* isomer. The increased mutagenicity of the *exo* isomer is attributed to its steric configuration that allows covalent bonding DNA, almost exclusively between

the N⁷ of guanine and the C⁸ of the 8,9-*exo*-epoxide [25-31]. Alternatively, the epoxide may be excreted as a glutathione-conjugated metabolite. Other cytotoxic effects of the epoxide include inhibition of liver protein synthesis and other protein interactions [32].

Association Between AFB₁ Exposure, Dietary-Protein Deficiency, and Liver Cancer

Previous studies from Africa and Asia provide evidence for an association between liver cancer, dietary-protein deficiency, and AFB₁ exposure in humans [33-37]. For example, in Africa, kwashiorkor, which is common among children with dietary-protein deficiency, has been linked to ingestion of aflatoxin because their tissue cannot metabolize or excrete the toxin [5, 38, 39]. These studies implicate the inability of tissue in such children to metabolize or excrete the toxin. In addition, the severity of AFB₁-related acute hepatotoxicity and hepatocarcinogenicity has been found to decrease after dietary supplementation with proteins that alter the distribution of AFB₁ and, thus, its toxicity and carcinogenicity [15, 40]. Castelli and colleagues [41] reported that administration of a diet containing 10.32% total protein plus 2 ppm AFB₁ prevented the development of liver tumors during the 300-day study period. Other studies have documented the effect of dietary protein in suppressing tumor development in animals exposed to cancer-causing agents such as nitrosamine and polychlorinated biphenyls [42-45].

No information is currently available about the mechanism by which proteins reduce or prevent the carcinogenic potential of AFB₁. The results in the studies cited above could be explained by a process in which the additional protein simply provides the necessary amino acids needed to bolster cellular biotransformation, a defense mechanism of protection. However, current knowledge regarding a molecular basis for the carcinogenic effects of AFB₁ (through the formation of the genotoxic AFB₁O and subsequent binding with DNA) and the potential formation of adducts between proteins and AFB₁ or transformation products suggest that a molecular mechanism may also be at work.

Occupational Exposure to AFB₁

Although dietary exposure to AFB₁ has been widely recognized, evidence has been accumulating to suggest potentially high risks of occupational exposure to AFB₁ through inhalation [46-52]. The presence of AFB₁ in corn and corn dust during years with relatively normal weather conditions and the increased risk of *A. flavus* infestation during drought conditions suggest that airborne exposure during agricultural activities should be of considerable concern. Twenty-four samples of airborne dust were collected from 8 farms in Iowa during harvest, 22 samples of airborne dust were collected from 9 farms during animal feeding, and 14 sets of Andersen samples were collected from 14 farms during bin cleaning. Fourteen samples of settled dust and 18 samples of bulk corn were also collected and analyzed. The airborne

concentration of AFB₁ detected in dust collected during harvest and grain unloading ranged from 0.04 to 92 ng/m³. Higher levels of AFB₁ were found in the airborne dust samples collected from enclosed buildings where animal feeding occurred (concentration, 5-421 ng/m³) and during bin cleaning (concentration, 124-4,849 ng/m³) [53]. AFB₁ levels of up to 5,100 ng/g were detected in settled dust collected from a confined environment in Iowa where animal feeding occurred.

The levels and distribution of AFB₁ in aerosolized grain dust were measured in samples collected from 11 Iowa farms during bin cleaning, which occurs infrequently but confers potential exposure to highly hazardous AFB₁ levels. The mass of each particle-size fraction was determined, and the average AFB₁ concentration for each fraction was calculated. Particles with diameters <2 µm contained, on average, approximately 10 times the concentration of AFB₁ than particles with diameters ≥2 µm, but the smaller particles only comprised one-fourth of the total measured airborne concentration of AFB₁. These data demonstrate that farmers and farm workers may be exposed to potentially hazardous concentrations of

AFB₁, particularly during bin cleaning and animal feeding in enclosed buildings.

The research described here is focused on 2 related areas that have implications for North Carolina: (1) investigating the health effects associated with farmers' exposure to airborne particulates and mixtures of hazardous chemicals (eg, mycotoxins, endotoxins, glucans, and microbial toxins) and (2) developing safe and effective biotechnological approaches for preventing the toxic and carcinogenic effects of AFB₁ in human food and animal feed. This research is based on current proteomic knowledge and the use of modern analytical techniques (eg, liquid chromatography quadrupole time-of-flight mass spectrometry) to separate and identify peptides and proteins of agricultural origin for effective detoxification of AFB₁. Findings of these investigations will also improve understanding of the interaction between small toxic molecules and large biomolecules that, in the future, may be used to detoxify other highly toxic chemicals. The goal is to prevent harmful exposures in the field and during food processing, as well as to understand the mechanisms by which these substances cause harm. NCMJ

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