

Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions¹⁻³

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ABSTRACT

Aflatoxins are well recognized as a cause of liver cancer, but they have additional important toxic effects. In farm and laboratory animals, chronic exposure to aflatoxins compromises immunity and interferes with protein metabolism and multiple micronutrients that are critical to health. These effects have not been widely studied in humans, but the available information indicates that at least some of the effects observed in animals also occur in humans. The prevalence and level of human exposure to aflatoxins on a global scale have been reviewed, and the resulting conclusion was that ≈ 4.5 billion persons 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 aflatoxin exposure results in changes in nutrition and immunity. The aflatoxin exposure and the toxic affects of aflatoxins 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 aflatoxins impractical in developing-country settings, but the strategy of using food additives to protect farm animals from the toxin may also provide effective and economical new approaches to protecting human populations. *Am J Clin Nutr* 2004;80:1106–22.

KEY WORDS Aflatoxin, chronic exposure, health risks, infectious diseases, iron, zinc, selenium, vitamin, protein, nutrition, immunity, HIV, developing country, prevention strategies, food additives

INTRODUCTION

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. The FDA's goal has been to minimize contamination; this goal was realized by implementing regulations that required special attention to the management of the problem. However, the methods used to ensure minimal contamination in developed countries cannot realistically be used in developing countries, because of the characteristics of the food systems and the technological infrastructure in those countries; therefore, aflatoxins are uncontrolled in these situations. The result is a "divide" in the prevalence of aflatoxicosis exposure between people living in developed and developing countries.

The World Health Organization (WHO) does not recognize aflatoxins as a high-priority problem because of their analysis of factors contributing to the burden of disease across the world (1). For developing countries where a short lifespan is prevalent, the priority issues identified by the WHO and their importance as factors contributing to the burden of disease [as measured by disability-adjusted life years (DALYs)] are presented in **Table 1**, and aflatoxin is not identified as a high-priority risk by the WHO. By application of the logic (derived from the current medical focus on the carcinogenicity of aflatoxin) that aflatoxin is reflected in the incidence of liver cancer alone, the priorities defined in Table 1 are justified. However, our review of literature shows that, because of the immunologic and nutritional affects of aflatoxin, there is a reasonable probability that the 6 top WHO risk factors (which account for 43.6% of the DALYs in countries where short lifespan is prevalent), as well as the risks of liver cancer, are modulated by aflatoxin.

This review does not focus on aflatoxin as a risk factor for cancer, because that sector of aflatoxin-related pathology is well documented and reviewed. Rather, it concentrates on the immunologic and nutritional effects and the associated modulation of infectious diseases. It also examines the scale, levels, and broad consequences of human exposure and new approaches to the management of the problem.

BACKGROUND

Aflatoxins were first isolated some 40 y ago after outbreaks of disease and death in turkeys (2) and of cancer in rainbow trout (3, 4) fed on rations formulated from peanut and cottonseed meals. The toxins are produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parasiticus* fungi when the temperatures are between 24 and 35 °C, and they will form within many commodities whenever the moisture content exceeds 7% (10% with

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² Supported by USAID grant LAG-G-00-96-90013-00 to the Peanut Collaborative Research Program.

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Received December 9, 2003.

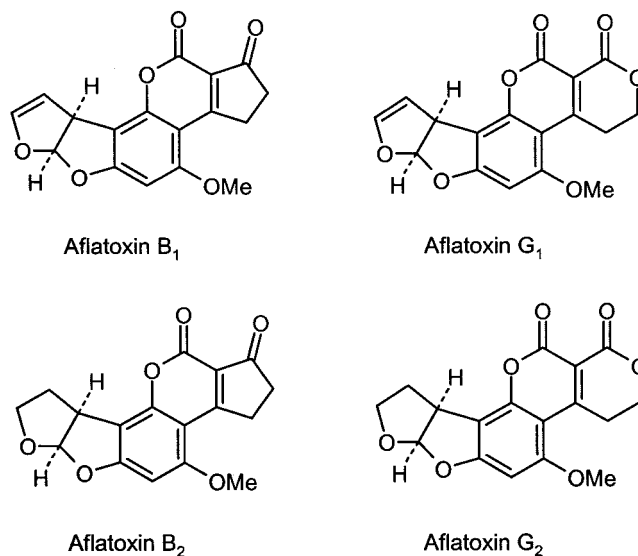
Accepted for publication April 15, 2004.

TABLE 1

World Health Organization priority health risks and associated burden of disease in disability-adjusted life years (DALYs) for developing countries where short lifespan is prevalent¹

Risk factor	Percentage of DALYs
	%
Underweight	14.9
Unsafe sex	10.2
Unsafe water	5.5
Indoor smoke from solid fuels	3.7
Zinc deficiency	3.2
Iron deficiency	3.1
Vitamin A deficiency	3.0
Blood pressure	2.5
Tobacco use	2.0
Cholesterol	1.9

¹ From reference 1.

**FIGURE 1.** Chemical structures of aflatoxins.

ventilation). The fungi responsible are ubiquitous and can affect many of the developing-country dietary staples of rice, corn, cassava, nuts, peanuts, chilies, and spices. The result is that, at latitudes between 40°N and 40°S of the equator, contamination of stored, inadequately dried produce is possible. Fungal invasion and contamination often begin before harvest and can be promoted by production and harvest conditions. Genotypes (5), drought (6), soil types (7), and insect activity (8) are important in determining the likelihood of preharvest contamination (9). Timely harvest and rapid and adequate drying before storage are also important (5). Even commodities dried to a satisfactory degree before storage can develop local pockets favorable to aflatoxin growth as a result of moisture generated by insect respiration and local condensation. Farmers of aflatoxicosis-prone crops in the United States exploit all of these facts to manage the problem, but contamination is not easily prevented without significant investment in production, drying, and storage facilities. These investments currently add significantly to the cost of delivering “safe” food to the people of the United States and the European Union. Despite the investment in these facilities, sizable losses still occur regularly in the United States when farmers are unable to meet even the more relaxed standards allowed for animal feeds (10).

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 aflatoxin, depending on the country involved (11, 12). As a consequence of the successful regulation of aflatoxin in developed countries, the human medical research literature is clearly focused on the carcinogenic aspects of aflatoxin, which reflects the concerns of North Americans and Europeans about the consequences of long-term cumulative exposure, which is the only concern at the low concentrations of aflatoxins that their food systems achieve. This literature is well reviewed (13) and is therefore covered only briefly here. In contrast, grains for animal feed in the United States are allowed 300 ppb aflatoxin (14), 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. In these animal feeding situations, the long-term risk of cancer is not a concern, except for the most susceptible species. Consequently, veterinary research has examined higher levels of exposure but for shorter time periods. This research provides

most of the information on the toxicities of aflatoxin at intermediate rates of exposure (100–500 ppb) and is the most potentially relevant information that is appropriate for the human situation in developing countries where no control of aflatoxin is exercised. However, the differences between species in response to aflatoxin introduce a measure of speculation into the extension of farm animal–derived information to the human situation.

TOXICOLOGY

Aflatoxins are a group of closely related compounds with small differences in chemical composition (Figure 1). Aflatoxin B₁ (AFB₁) is the most prevalent form and also the most potent of these toxins (15). In animals the toxin is processed through a number of competing pathways. These pathways have been well reviewed (16) and are summarized in Figure 2. The differences in susceptibility 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

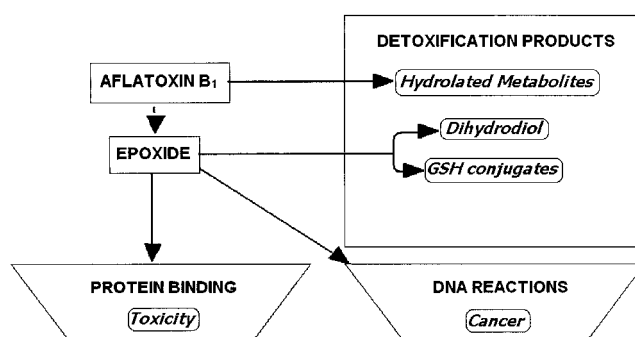


FIGURE 2. Pathways and consequences for aflatoxin in animal metabolism. Hydroxylated metabolites include aflatoxin M₁. GSH, glutathione. Reprinted from Eaton D, Ramsdell HS, Neal G. Biotransformation of aflatoxins. In: Eaton D, Groopman JD, eds. The toxicology of aflatoxins: human health, veterinary, and agricultural significance. London: Academic Press, 1993:45–72, with permission from Elsevier.

the fractions that follow the different possible pathways are influenced by dosage, perhaps because of the saturation of the most chemically competitive processes (16). Susceptibility to aflatoxin is greatest in the young, and there are very significant differences between species, persons of the same species (according to their differing abilities to detoxify aflatoxin by biochemical processes), and the sexes (according to the concentrations of testosterone). The toxicity of aflatoxin also varies according to many nutritional factors (17), and recovery from protein malnutrition is delayed by exposure to aflatoxin (18, 19).

Aflatoxicosis is the poisoning that results from ingesting aflatoxins. 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 subsymptomatic exposure. A review of the literature across all species provides clear evidence that the dose and duration of exposure to aflatoxin clearly have a major effect on the toxicology and may cause a range of consequences: 1) large doses lead to acute illness and death, usually through liver cirrhosis; 2) chronic sublethal doses have nutritional and immunologic consequences; and 3) all doses have a cumulative effect on the risk of cancer. This review focuses on the nutritional and immunologic consequences.

Acute illness and death

The symptoms of severe aflatoxicosis include hemorrhagic necrosis of the liver, bile duct proliferation, edema, and lethargy. Animal studies have found 2 orders of magnitude difference in the median lethal dose for AFB₁. Susceptible species such as rabbits and ducks have a low (0.3 mg/kg) median lethal dose, whereas chickens (18 mg/kg) and rats have greater tolerance. Adult humans usually have a high tolerance of aflatoxin, and, in the reported acute poisonings, it is usually the children who die (15).

Cancers

For humans, aflatoxin is predominantly perceived as an agent promoting liver cancers, although lung cancer is also a risk among workers handling contaminated grain (20). The increased risk of hepatomas is caused by deletion mutations in the P53 tumor-suppressing gene and by activation of dominant oncogenes (21). The risk of cancers due to exposure to the various forms of aflatoxin is well established (22) and is based on the cumulative lifetime dose. The International Cancer Research Institute identifies aflatoxin as a Class 1 carcinogen, resulting in the regulation of this toxin to very low concentrations in traded commodities [20 ppb in grains and 0.5 ppb in milk in the United States; 4 ppb in foods in some European countries (11)].

However, in many developing countries, epidemics of hepatitis B virus (HBV) and hepatitis C virus (HCV) affect $\leq 20\%$ 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 (23), and the relative risk of cancer for HBV patients increases from ≈ 5 with only HBV infection to ≈ 60 when HBV infection and aflatoxin exposure are combined (24). In some areas where aflatoxin contamination and HBV occur together, hepatomas are the predominant cancer (64% of cancers; 25), and they may be a predominant cause of death: $\approx 10\%$ of males in Gambia die of liver cancer (CP Wild,

personal communication, 1999), and in Qidong, China, 10% of all adult deaths were due to this cancer (26). Thus, to minimize the risk of liver cancer, it is critically important that exposure of HBV- and HCV-infected persons to aflatoxin is minimized.

A factor in this greater potency of aflatoxin in HBV-positive people is the finding that HBV positivity reduces the person's ability to detoxify aflatoxin (27). Whereas this synergy is recognized as an important factor for cancer, it is also of great potential importance for immunologic and nutritional toxicities, because it increases the level of biological exposure.

Immunologic suppression

As stated earlier, most of the information summarized below is derived from studies of farm animals or from animal models in which the exposure is chronic but not high enough to cause the symptoms usually associated with acute aflatoxicosis. Human exposure is likely to be more variable than that in these studies, because of the highly variable distribution of contamination within foods. Aflatoxin M₁ (AFM₁) in human urine reflects exposure over the previous 24 h and is usually found in approximately one-third of the members of a sample population, whereas aflatoxin-albumin adduct data, which reflect exposure over a longer period, are present in most ($>90\%$) of the same populations (24, 28, 29). This difference provides some uncertainty about the extension of animal data to humans, but some publications show that these animal responses are relevant to humans, at least in broad terms. The threshold dose for immunotoxic effects in humans is not known.

In animal experiments, AFB₁ has been shown to induce thymic aplasia (30), reduce T-lymphocyte function and number, suppress phagocytic activity, and reduce complement activity (30–32). Many studies conducted in poultry, pigs, and rats showed that exposure to aflatoxin in contaminated food results in suppression of the cell-mediated immune responses (17, 33–37). Thymic and bursal involution, suppression of lymphoblastogenesis, impairment of delayed cutaneous hypersensitivity (37, 38), and graft-versus-host reaction (39, 40) also occur in animals exposed to aflatoxin. Splenic CD4 (helper T) cell numbers and interleukin 2 (IL-2) production decreased significantly when mice were treated with AFB₁ at a dose of 0.75 mg/kg (41). Impairment of cellular function by aflatoxin seems to be due to its effects on such factors as the production of lymphokines and antigen processing by macrophages (42), as well as a decrease in or lack of the heat-stable serum factors involved in phagocytosis (43).

Macrophages play a major role in host defenses against infection. They present antigen to lymphocytes during the development of specific immunity and serve as supportive accessory cells to lymphocytes. Macrophages also increase their phagocytic activity and release various active products, such as cytokines and reactive intermediates, to carry out nonspecific immune responses (44). Several reports suggest that aflatoxin impairs the function of macrophages in animal species (45, 46). In addition to its reported effect in reducing phagocytic activity in rabbit alveolar macrophages (43), aflatoxin has more recently been shown in vitro to inhibit phagocytic cell function in normal human peripheral blood monocytes (46). AFB₁ at concentrations ≥ 100 pg/mL was cytotoxic to the monocytes, and concentrations of 0.5 to 1 pg/mL inhibited monocyte phagocytic activity and intracellular killing of *Candida albicans*; however, superoxide

production and the ability of the monocytes to destroy intracellular herpes simplex virus were not affected.

AFB₁ given orally at concentrations of 0.03–0.07 mg/kg suppressed natural killer (NK) cell-mediated cytolysis of YAC-1 target cells in BALB/c mice (47), but not in C57B1/6 mice at the same dose (41) or in rabbits fed 24 ppm aflatoxin in feed (48). Pigs that received 500 ppm AFB₁ in their feed had reduced total hemolytic serum complement activity (49), but complement activity was not affected in pigs fed 300 ppm or rabbits fed 95 ppm AFB₁. These differences serve to show that the species differences, noted for acute toxicity and carcinogenicity, also apply to immune responses.

Theumer et al (50) fed rats a diet containing 40 ppb AFB₁ for 90 d. The mitogenic response of spleen mononuclear cells (SMNCs) in vivo was higher in animals fed with AFB₁ than in those not so fed. The SMNCs of animals fed with AFB₁ produced lower concentrations of IL-2, higher concentrations of IL-4, and equal amounts of IL-10.

Marin et al (51) conducted studies to evaluate the humoral and cellular immune responses in weaning piglets ($\bar{x} \pm SD$ body weight: 11.42 \pm 0.11 kg) exposed to 140 and 280 ppb aflatoxin for 4 wk. Humoral and cellular immune functions were impaired, and aflatoxin reduced the primary and the secondary immune response. Antibody levels from immunization to *Mycoplasma agalactiae* (an infectious microorganism) were always lower in aflatoxin-fed animals than in control piglets.

The immunosuppressive effects of aflatoxin were also shown to be transferred across the porcine placenta and to affect the unborn fetus (17). Pigs born to sows fed aflatoxin and sensitized with *Mycobacterium tuberculosis* had a smaller delayed cutaneous hypersensitivity reaction than did pigs who were not exposed to aflatoxin or sensitized with *M. tuberculosis*. Cell-mediated and phagocytic functions and, to a lesser extent, humoral immune function have also been shown to be reduced in the offspring of pigs and rats exposed to aflatoxin in their diets (17, 36, 52). Moreover, chick embryos exposed to a single 0.1-mg dose of AFB₁ had a depressed graft-versus-host response and a depressed cutaneous basophil hypersensitivity to injected phytohemagglutinin (39). In rainbow trout, long-term effects on immune system functions were shown to result from exposure of the ova to aflatoxin (53).

Given the effect of aflatoxin on the immune system, it is not surprising that there is evidence that the value of vaccination is modified by exposure to aflatoxin. Significantly, aflatoxin exposure was also shown to reduce the antibody response to vaccines. Studies conducted in poultry showed that daily dietary exposure through foods with aflatoxin concentrations of 200 ppb for ≤ 40 wk reduced antibody titers to vaccines for Newcastle disease, infectious bronchitis, and infectious bursal disease (54, 55). In rabbits, aflatoxin was also found to reduce antibody titers to *Mycobacterium bovis* (56), *Bordetella bronchiseptica* (57), and *Pasteurella multocida* (58). Decreased effectiveness of vaccination against swine fever (59), hemorrhagic septicemia (60), and foot and mouth disease (DK Singh, personal communication, 1997) in dairy cattle has also been observed.

As previously indicated, evidence for in vivo immunosuppression by aflatoxin in humans is limited, inconsistent, and uncertain. The available studies of human exposure usually measured

the aflatoxin-albumin adduct concentration, which reflects long-term exposure. Given the toxicology of aflatoxin and the recovery of cell processes within days of exposure (61), aflatoxin-albumin adduct may not always be an appropriate measure of exposure for immune function changes. Turner et al (62) did observe changes in immunity in Gambian children as a function of aflatoxin-albumin adducts, which were detected in 93% of the children (geometric \bar{x} : 22.3 pg/mg; range: 5–456 pg/mg). The aflatoxin-albumin adduct concentration was strongly influenced by the month of sampling. In a multivariable analysis, secretory immunoglobulin A (IgA) was markedly lower in children with detectable aflatoxin-albumin concentrations than in those with nondetectable concentrations. Furthermore, a weak antibody response to a pneumococcal challenge was observed, but the response to rabies vaccine and cell-mediated immunity responses to test antigens were not related to adduct status. In an earlier study, also in the Gambia, Allen et al (27) observed that there was no obvious relation between aflatoxin-albumin concentrations and malaria-specific antibody responses or in vitro lymphoproliferative responses. However, using the mouse-*Plasmodium berghei* model, Young et al (63) found that aflatoxin decreased morbidity because of a direct effect of aflatoxin on the parasite.

Our unpublished data (manuscript in preparation) show significant suppression of select cellular immune system components and functions when Ghanaian subjects with aflatoxin-albumin adduct concentrations above the median (0.80 pmol/mg) for the population were compared with those with concentrations below the median.

Nutritional interference

Chronic aflatoxin exposure has major effects on nutritional status in animals, but, as with the immunotoxicities, thresholds for these effects are not defined for any species. Covalent binding of aflatoxin to DNA and decreased protein synthesis occur rapidly after exposure and persist for ≤ 5 d (61). The efficiency of food use is consistently less in animals that are exposed to aflatoxin than in those that are not exposed. In poultry and pigs, a 7–10% drop in food conversion efficiency is observed (64), and decreased growth rates are a consistent sign of chronic aflatoxin exposure (65). Marin et al (51) observed a “dose-related” decrease in weight gain in weaning piglets exposed to 140 ppb and 280 ppb aflatoxin.

Thus, it is well established in animals that dietary aflatoxin reduces the rate of growth and other measures of productivity (64). Recent human research (66) also confirms that this effect applies to humans: a dose-response relation was seen between aflatoxin exposure and the degree of stunting and underweight in children < 5 y old in Benin and Togo, where all members of the study population had aflatoxin exposure (aflatoxin-albumin adducts between 5 and 1064 pg/mg albumin in 99% of the children). The toxin has also been shown, as a logical outcome of the effect of aflatoxin on protein synthesis, to be a factor modulating the rate of recovery from protein malnutrition (kwashiorkor) (19, 67) although it has not been shown to be responsible for the development of the condition (68).

It was established through feeding experiments that aflatoxin exposure modifies vitamin A nutrition in poultry (69, 70) and camels (71), approximately halving the serum retinol concentrations in those studies. The concentrations of aflatoxins in the liver and ruminal contents of the camels were 18.2 and 243.4 $\mu\text{g}/\text{kg}$,



respectively. Reddy et al (70) investigated the effects of 3 concentrations (0, 500, and 2000 ppb) of aflatoxin on female broiler chicks. They found that liver vitamin A decreased with increasing concentrations of aflatoxin. However, we found only one study that examined human vitamin A status as a function of aflatoxin-albumin status (62). In that report, although vitamin C was related to aflatoxin-albumin, no relation was observed for vitamin A; this discrepancy could result from species differences in the sensitivity of vitamin A metabolism to aflatoxin exposure, the pattern of exposure (uniform or intermittent), or the possible inappropriateness of aflatoxin-albumin as the measure for biological responses for this nutritional factor. If vitamin A status in humans is modulated by aflatoxin exposure in some manner, then preventing exposure to aflatoxin would be one means of greatly reducing the occurrence of vitamin A deficiency (VAD). This modulation could also result in increased responses to diets that include supplementation with this nutrient. Thus, it is obviously important to clarify the role of aflatoxin in relation to human vitamin A status.

In broiler chickens, the vitamin D concentration is affected by aflatoxin in the diet (72). Aflatoxin (1 ppm in the diet) reduced plasma 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)₂D] concentrations after 5 d of treatment. An effect from aflatoxin exposure via immune system competence is thereby possible. Like vitamin A, vitamin D is strongly involved in the maintenance of immune system competence.

The nutrition of both zinc and selenium is affected by aflatoxin in the diet (73, 74), and adequate concentrations of these minerals are essential for healthy immune systems. Mocchegianni et al (74) observed that, in pigs, maternal exposure to aflatoxin approximately halved the piglets' serum zinc content (56.0 $\mu\text{g}/\text{dL}$ compared with 119.2 $\mu\text{g}/\text{dL}$ in the control) but did not influence the maternal zinc concentrations. The outcome was decreased cellular immunity of the piglets due to less activated thymulin. A similar effect on serum zinc concentrations and the immune system has been noted in rats (75, 76).

In China, selenium concentrations in humans have been related to differences in aflatoxin-albumin adduct concentrations (77). The selenium concentrations were found to be significantly and inversely correlated to aflatoxin-albumin adduct concentrations in men. The mean plasma concentration of selenium was found to be $108 \pm 32.4 \mu\text{g}/\text{L}$ and the mean AFB₁-albumin adduct concentration was 20.95 fmol/mg. For chicks, a parallel effect of aflatoxin exposure on selenium was also observed in connection with lowered immunity (78). Clearly, more information, including identification of the appropriate indicators of exposure for relations between nutritional factors and aflatoxin exposure, is needed for the emergence of the full picture of when and how human nutrition is affected by aflatoxin.

HUMAN EXPOSURE TO AFLATOXIN

The nutritional and immunologic responses to aflatoxin reviewed above indicate the potential for aflatoxin to affect the immunity and nutritional status of chronically exposed persons as well as the need for much better information in these areas. The cancer risk assessments and acute toxicity across species show that adult humans are relatively tolerant of aflatoxin, but data reviewed in the earlier sections indicate that there is evidence that aflatoxin affects early growth and at least some aspects of human immunity and nutrition. Thus the really important questions are:

how contaminated are the diets in developing countries, how much of the ingested dose is significant to human health and nutrition, and what are the thresholds for effects on human immunology and nutritional health?

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 (Figure 2) according to the fraction that processes through each pathway. Whereas the relation between DNA-relevant exposure and cancer is understood well enough to calculate the consequences of changing the concentrations of aflatoxin in food for the risk to people (11), the effect of other metabolic processes in humans is not established. AFM₁ is a detoxification product that is rapidly excreted, but it may have significant immunologic and nutritional consequences in nursing young (74). Second, other aspects of the diet may have a significant role in determining the consequence of aflatoxin ingestion. In multiple animal species, the aflatoxin toxicities may be modified by the dietary intakes of antioxidant vitamins, such as vitamins A, C, and E (79–85). Third, the amount of biological exposure is conditioned by infection with HBV and HCV, and, whereas this phenomenon has been studied for its effect on cancer risk, it has not been evaluated for other known toxicities of aflatoxin.

Human food contamination

The characteristics of the food production system determine the feasibility of managing aflatoxin. Small-scale industries, subsistence production, and food insecurity usually make the economics and enforcement of regulations impractical. In developed countries, the food production systems are controlled by relatively small numbers of large-scale industrial processors, and the economies of scale allow the quality-control procedures to be effective and the regulations to be enforceable. Food security concerns of many of the people at risk mean that even knowing that food is contaminated would not help, because the people have no alternative sources of food; this was recognized in the report of the Third Joint FAO/WHO/UNEP International Conference on Mycotoxins (86).

Because of these issues, the amounts of aflatoxin allowed in foods by *Codex Alimentarius* have no relevance to most of the people in developing countries; their consumption of traded food items is small, and laboratories to test their foods are economically and financially inaccessible. The food consumed is usually food that families have produced, stored, and prepared without any consideration for the risks of aflatoxin. Where trade does occur, the least contaminated foods and feeds are exported, which may lead to enhanced exposure of the producers, because the more highly contaminated products are retained at home for consumption by a population that is already at the greatest risk of aflatoxin exposure (11).

There is no comprehensive data set from which to evaluate the extent and severity of biological exposure of humans in developing countries; direct measurements of human biological exposure to aflatoxin are available from only a small number of countries. However, these data, when combined with reports of contamination in foods sampled from markets and trade shipments, reports of acute poisoning incidences, postmortem reports in which the toxin has been measured in organs, allow the



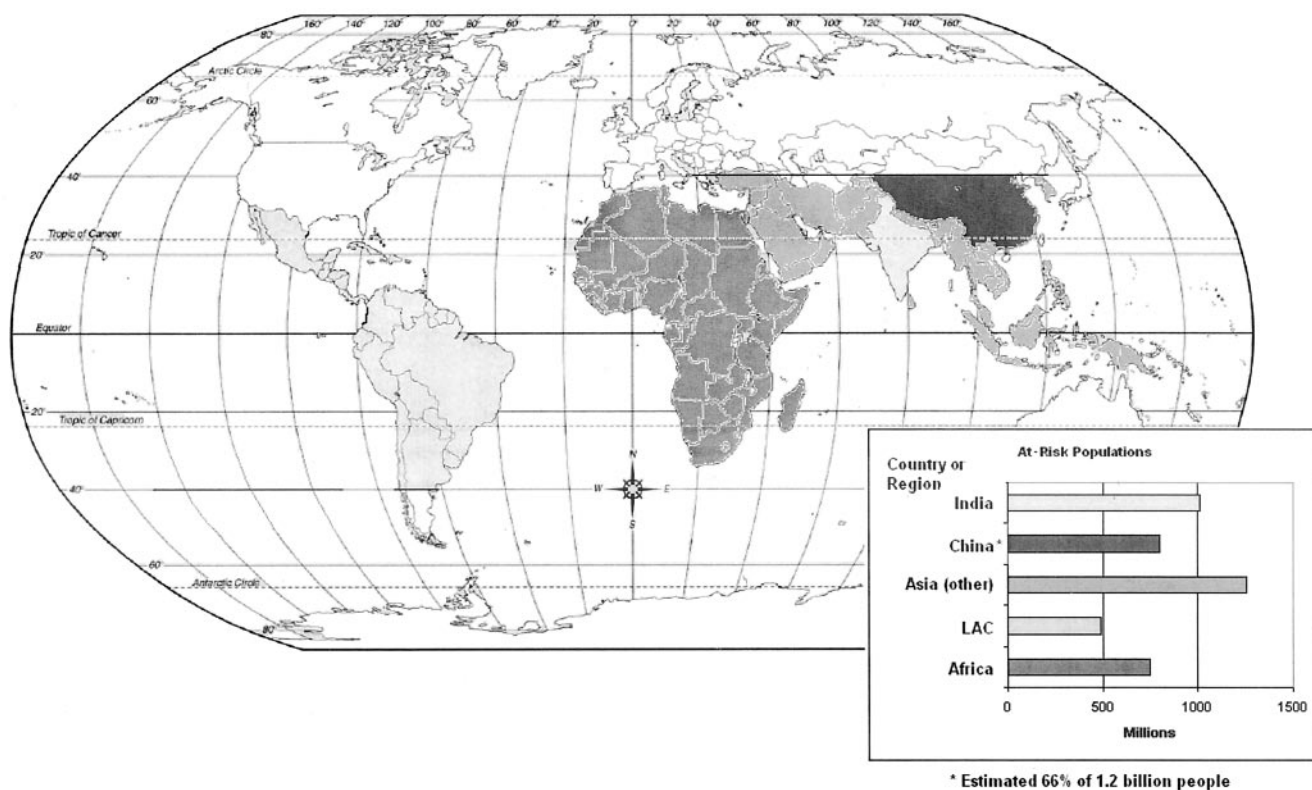


FIGURE 3. Areas and populations at risk of chronic exposure to uncontrolled aflatoxin. LAC, Latin America and the Caribbean.

formation of a picture of likely exposure. Exposure may also be inferred from liver cancer rates.

To evaluate both the prevalence and the amount of exposure, we have collected the data that we could find from this range of indicative sources and used them together with food system information to produce a picture of the situation based on informed judgment. The data available are described in the following sections.

Acute exposure to aflatoxins

Reported incidents of acute human aflatoxin poisoning serve to indicate that aflatoxin is a risk at the location of the incident, but because they are relatively infrequent, they also suggest that contamination is seldom serious enough to draw the attention of the medical and health system as a direct cause of illness or death. Acute poisonings, $\approx 25\%$ of which result in death, occur as a result of high levels of exposure (15). Up until the mid-1990s, reports of this nature were found in scientific journals, but subsequent reports are usually found only in the daily press. Reports of death and serious illness usually originate from developing countries (87–93) within the zone of risk (Figure 3).

The numbers of cases of acute poisoning are not large relative to the populations at risk, probably because people usually avoid obviously moldy foods, and humans are usually an aflatoxin-tolerant species. However, in times of food scarcity, or under conditions of poverty, people usually have no option but to use lower-priced, poorer-quality food, which commonly is contaminated.

Chronic exposure to aflatoxin

Two main approaches have been used to evaluate chronic exposure to aflatoxin in humans. The first approach involves

food samples. Food samples collected either from prepared meals and ingredients or from markets provide the most commonly available data. The most reliable sample source for a measure of exposure is through analysis of prepared meals, because people may sort grain and remove those kernels that are considered unfit to eat. However, market and trade samples provide information on the risk of exposure from various foods in the diet, particularly when local food processors undertake operations such as milling without any quality control. The second approach involves biological markers of exposure. In this approach, blood, milk, or urine samples are obtained from humans and analyzed for the presence of aflatoxin derivatives, each of which has a characteristic half-life in the body. This technology is relatively recent, and reports based on it are geographically restricted to a small fraction of developing countries.

Food, market, and trade samples

Food contamination data are available from many developing countries (Table 2). These data generally show that a wide range of commodities are contaminated and that a significant proportion of them are contaminated to a degree far above that allowed in the *Codex Alimentarius*. Examples of contamination for corn and peanut are provided below.

In samples from a Chinese area of high risk of liver cancer (99), AFB₁ in corn was the predominant toxin detected in terms of quantity and frequency: the concentration ranged between 9 and 2496 ppb and the incidence of contamination was 85%. Among the samples, 76% exceeded the Chinese regulation of 20 ppb for AFB₁ in corn and corn-based products intended for human consumption. The average daily intake of AFB₁ from corn in the high-risk area was 184.1 μg , and the probable daily intake is

TABLE 2
Examples of market sample contamination frequencies and concentrations

Country and commodity	Frequency of aflatoxin-	Contamination
	positive samples	rate
	%	ppb
Argentina		
Maize (94)	19.6	Positive ¹
Bangladesh		
Maize (95)	67	33.0 (mean)
Brazil		
Corn (96)	38.3	0.2–129.0
Peanut products (97)	67	43.0–1099.0
Peanuts (97)	27	43.0–1099.0
Sorghum (98)	12.8	7.0–33.0
China		
Corn (99)	76	>20.0
Costa Rica		
Maize (100)	80	>20.0
Cyprus		
Peanut butter (101)	56.7	>10.0
Egypt		
Hazelnut (102)	90	25.0–175.0
Peanut and watermelon seeds (103)	82	Positive ¹
Soybean (104)	35	5.0–35.0
Spices (103)	40	>0.250
Walnut (102)	75	15.0–25.0
Gambia		
Groundnut sauce (105)		162.0
Guatemala		
Incaparina (mixture of corn and cottonseed flour) (106)	100	3.0–214.0
Ghana		
Peanut (107, 108)	12.8–31.7	Positive ¹
India		
Chilies (109)	18	>30.0
	59	Positive ¹
Dry slices of quince (110)	23.14	96.0–8164.0
Groundnut (111)	21	>30.0
Maize (112, 113)	26	>30.0
Korea		
Barley food (114)	12	26.0 (mean)
Corn food (114)	19	74.0
Kuwait		
Milk (115)	6	>0.2
Portugal		
Yogurt (116)	18.8	19.0–98.0
Malaysia		
Wheat (117)	1.2	>25.62
Mexico		
Kerneled corn (118)	87.8	5.0–465.0
	58.5	>20.0
Nigeria		
Corn (119)	45	25.0–770.0
Maize-based gruels (120)	25	0.002–19.716
Qatar		
Pistachio (121)	8.7 to 33	>20.0
Senegal		
Peanut oil (122)	85	40.0 (mean)
Turkey		
Cheese (123)	12.28	Positive ¹
Hazelnut, pistachio (124)	Present ²	>4.0
Uganda		
Maize (125)	29	1–100
Peanut, cassava (126, 127)	12	>100

¹ The rate of contamination was not specified.

² The frequency of aflatoxin-positive samples was not specified.

estimated to be 3680 ng · kg body wt⁻¹ · d⁻¹. These corn samples were simultaneously contaminated with other mycotoxins, notably fumonisin.

According to several newspaper reports, concentrations of 27 163 ppb total aflatoxin and 16 505 ppb AFB₁ were reported in peanut butter given to schoolchildren in 2001 in the Eastern Cape area of South Africa. There, the maximum legal concentration of total aflatoxins in foodstuffs is 10 ppb, of which 5 ppb may be AFB₁ (128).

Variations in aflatoxin exposure exist between countries, largely as a function of diet. Data assembled by Hall and Wild (129) indicate that exposure to aflatoxin was 3.5–14.8 ng · kg⁻¹ · d⁻¹ in Kenya, 11.4–158.6 in Swaziland, 38.6–183.7 in Mozambique, 16.5 in Transkei (now South Africa), 4–115 in The Gambia, 11.7–2027 in southern Guangxi province of China, and 6.5–53 ng · kg⁻¹ · d⁻¹ in Thailand, whereas the exposure in the United States is 2.7 ng · kg⁻¹ · d⁻¹. The exposure in Ghana, as measured from peanut consumption alone, is estimated to be 9.9–99.2 ng · kg⁻¹ · d⁻¹ (130), but other commonly consumed corn-based foods (eg, kenkey) are also known to be contaminated (107,131), and thus the total exposure is likely to be higher.

However, it must be understood that these exposure rates are an average estimation based on annual consumption, as is appropriate for cancer risk, because of the cumulative nature of this response. The contamination and consumption patterns for various foods are often variable across seasons (132), and so doses in the shorter term may vary significantly from the average. Furthermore, because of the large variability associated with testing grain products for aflatoxin, the true lot concentration cannot be determined with complete certainty (133).

Biomarkers of exposure

The currently favored method of measuring human exposure consists of the analysis of body fluids for the presence of aflatoxin derivatives (28, 29). Each biochemical process results in derivatives that have a characteristic half-life within the body, and thus the exposure over a period of days, weeks, or months can be assessed. Recent exposure to aflatoxin is reflected in the urine as directly excreted AFM₁ and other detoxification products, but only a small fraction of the dose is excreted in this way. Measurements of aflatoxin and its byproducts in urine have been found to be highly variable from day to day, which reflects the wide variability in the contamination of food samples, and, for this reason, the measurement of AFM₁ on a single day may not be a reliable indicator of a person's chronic exposure (24, 28, 29). The aflatoxin-albumin adduct is measured in peripheral blood and has a half-life in the body of 30–60 d. Therefore, it is a measure that integrates the exposure over a longer period and hence is a more reliable indicator of a person's chronic exposure. However, it should be remembered that the fraction of the ingested aflatoxin processed into any particular metabolite is variable. A given concentration of any particular biomarker cannot be used to make assumptions about the total dose or the amounts directed into any other competing pathway.

Data relating to biological exposure (Table 3) are relatively rare, because the locations of such measurements are mainly in West Africa and China. These data show major variations in seasonal exposure (27), which reflects the natural development of contamination in storage. These biomarker data show that, regardless of food preparation practices, the human populations

TABLE 3

A compilation of data on biomarkers of exposure to aflatoxin in humans¹

Country	Exposure rate	Range or test type	Reference
	%		
Benin	99	5–1064 pg/mg ²	Gong et al (66)
China (Guangxi province)	89	0.9–3569 pg/24-h urine	Wang et al (26)
Gambia	95	0–720 pg/mg ²	Allen et al (27)
Guinea	90	0–385 pg/mg ²	Diallo et al (134)
Nigeria	40–90	Lung autopsies	Oyelami et al (135)
Sierra Leone	95–99	Aflatoxin in urine	Jonsyn-Ellis (136)
Sudan, Zimbabwe, Ghana, Liberia, Kenya, Transkei	32	Aflatoxin in urine	Hendrickse et al (137)
	99	Liver biopsy of kwashiorkors	
Thailand	93 ³	Liver DNA and AFB ₁	Hollstein et al (138)
	44	AFM ₁ in breast milk	El Nezami et al (139)

¹ AFB₁, aflatoxin B₁; AFM₁, aflatoxin M₁.² pmol aflatoxin-albumin/mg albumin.³ Hepatocellular cancer patients.

of these developing countries are widely and significantly exposed to aflatoxin. The parallel reports of food contamination suggest that biological exposure rates can be extrapolated (at least broadly) to those countries for which food contamination data are available but for which there are no biomarker data. Given that little is done to decontaminate foods in most developing countries, it is likely that the prevalence of chronic exposure in most countries is similar to that measured in these studies with the use of biomarkers.

HBV- or HCV-conditioned aflatoxin exposure

There is a broad ecologic association between areas of high aflatoxin exposure (such as China, Southeast Asia, and parts of Africa) and HBV, which is endemic in these areas; rates of HBV positivity in The Gambia, China, and Guinea are 15% (27), 14–20% (25), and 10% (134), respectively. The effect of HBV infection on the efficiency with which aflatoxin is detoxified leads to increased biological exposure to aflatoxin (23). Whereas the importance of this synergy to cancer is well recognized (23, 140), its importance to other health issues is largely unknown and clearly should be established.

Liver cancer–based estimates of aflatoxin exposure

The use of the incidence of cancer to indicate aflatoxin exposure is supported by the extensive studies on risk associated with exposure, but this approach provides only circumstantial evidence for exposure, and liver cancer is not caused solely by aflatoxicosis. Two major factors, aflatoxin and HBV, which commonly occur in the same populations, influence the risk of liver cancer. Independently, each factor significantly increases the relative risk of cancer, and most studies report them, together, to be synergistic (141), increasing the risk of cancer 25–30-fold; there is, however, one epidemiologic study that contradicts those findings (142).

The suggested mechanism for this synergy is that aflatoxin suppresses DNA repair mechanisms that help limit the development of cancer from HBV (143), and HBV prevents detoxification (23), but it is also possible that the immunotoxicity of aflatoxin interferes with the suppression of cancer. Although data on liver cancer are not routinely collected in the health statistics of developing countries, the occurrence is believed to be between 16 and 32 times the rates in the United States and European Union

(≈2.5/100 000; 11, 22); liver cancers were responsible for 450,000 deaths in 1990 (11). Liver cancers accounted for ≈8.8% (0.55 million) of the total cancer mortalities in 2000 (144).

The geographical distribution of chronic human aflatoxin exposure

The 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 that there is risk of chronic aflatoxin exposure between 40° N and S of the equator in developing countries. Population data from the FAO database indicate that ≈4.5 billion people live in this zone. The evidence of contamination in market and food samples and the human biomarker data are indicators that most of the population is likely to be exposed (Figure 3) but usually at a level less than that needed for direct acute illness and death. Having both the evidence from animals and humans that immunity and nutritional levels are affected by aflatoxin in “unmanaged” circumstances and the evidence of exposure, we continue our review by examining the evidence for consequences of this exposure on human health.

AFLATOXIN AS A FACTOR IN HEALTH RISKS

We have elected to use the WHO risk analysis for countries where short lifespan is prevalent (Table 1) as a basis on which to assemble and present the available evidence for the role of aflatoxin in world health. The possible role of aflatoxin in influencing human health has been researched mainly in relation to its role as a carcinogen; in this role, aflatoxin does not make the priority list. However, the pivotal role of the immune system with respect to the incidence, severity, and outcome of infectious diseases leads one to expect that, through its reported effect on the immune system and micronutrients, aflatoxin may also affect the epidemiology of many diseases and health risks in those countries where the toxin is uncontrolled. There is very clearly a great need to increase the information about possible effects of aflatoxin on health in developing countries. Throughout this section of this review, we point to a number of intriguing possibilities in the hope that we can stimulate research that will clarify the true picture.



Underweight and nutrition-related epidemiology

Underweight is the single most contributory risk factor to the burden of disease worldwide, contributing to 14.9% of DALYs. The WHO estimated that underweight caused 3.7 million deaths in 2000 and accounted for \approx 138 million DALYs. This risk factor operates through the effect of nutrition on the immune system and diseases. The conditions affected by underweight include child mortality and acute morbidity due to diarrhea, malaria, measles, pneumonia, and selected other infectious diseases. Perinatal conditions that are due to maternal underweight are also involved. Whereas the underweight status of people is strongly related to poverty, it is the poor who have the least opportunity to discriminate against poor-quality foods and who therefore have the greatest risk of aflatoxicosis—a result confirmed by our data (unpublished observations) from Ghana.

Aflatoxin has been directly related to underweight status in children in Benin and Togo (145) and to the condition of kwashiorkor (19, 146). Autopsy evidence from children in Nigeria found aflatoxin in tissues from most children examined post mortem, although the clinical cause of death was malnutrition and other diseases (135). Deaths of children in the Philippines, attributed to respiratory tract infections, also involved aflatoxin exposure (147). In Africa, the connection between infectious diseases and aflatoxin is also reported for malaria, and some interesting effects are noted.

Hendrickse et al (137) investigated the involvement of aflatoxin in malnutrition and noted that children with kwashiorkor were less prone to malaria; they also showed in a mouse model that aflatoxin provided some measure of protection against the parasite, because of the direct effects of aflatoxin on parasite replication. In The Gambia, children with malaria (*Plasmodium falciparum*) were found to have higher aflatoxin-albumin adduct concentrations, but there was no consistent relation between aflatoxin-albumin and malaria-specific antibody responses or morbidity due to malaria in the next fever season (27). Clearly there is a need to explore more completely the interaction of aflatoxin with underweight.

Unsafe sex

Unsafe sex (which is held responsible for 2.9 million deaths and 92 million DALYs) is identified as a risk factor largely because of the HIV epidemic. Whereas the risk is behavioral, the disease is viral, and the progress of the epidemic is determined by disease transmission, rate of disease progress, and opportunistic infections.

The disease of HIV is complicated, and the ways in which the virus interacts with another immunocompromising agent is also likely to be complicated. The animal data on immune suppression and nutritional interference (described earlier) show aflatoxicosis symptoms to be similar to HIV infection symptoms, differing mainly in that the removal of aflatoxin from the diet reverses the symptoms. The animal data on immune suppression suggest that the parameters of the epidemiologic model are likely to be modulated by aflatoxin at some level of exposure, either directly or indirectly through the known toxicities of aflatoxin. Nutrition is also a general area in which aflatoxin exposure can be expected to modulate HIV.

HIV transmission

The transmission of HIV may be between cohorts (horizontal) or between mother and child (vertical). Both types of transmission require the exchange of bodily fluids or cells with a viral load, and the size of this viral load is an established factor in the risk of transmission. Other factors that influence the probability of transmission include membrane integrity and the presence of other sexually transmitted diseases that could reflect the immune status of the uninfected participant.

The viral load is a function of the stage of disease and other factors, which may be connected to aflatoxin exposure. For example, while investigating the role of environmental toxins in HIV processes, Yao et al (148) found that aflatoxin, dioxin, and benzopyrene all changed an oxidative stress process involving P450 cytochrome. Using only the dioxin for further studies, they found that the replication rate of HIV was increased by this stress phenomenon. Oxidative stress also increases HIV replication (149) through increasing apoptosis of infected leukocytes.

Another possible manipulation of transmission rates by aflatoxin is suggested by the exposure of human fetuses to maternal aflatoxin (150, 151) and by the fact that aflatoxin is also secreted in human mothers' milk (139, 152-154). In animals, exposure of the fetus and via milk has been shown to have significant effects on the immunocompetence of progeny (36, 74), even when maternal immunity factors were not influenced by the exposure. Given that immunoglobulins in mothers' milk provide vital protection against infections (155), the decrease in immunoglobulins associated with aflatoxin exposure in humans (62) and calves (156) is also a concern with respect to HIV transmission to children and their ability to survive other infectious diseases.

The association of vitamins A and D, zinc, selenium, and other nutrients with HIV disease progress and vertical transmission has been well documented (157-161). However, although the results of supplementation suggest that vertical transmission rates are not manipulated by supplementation (162), it has not been established whether nutrient deficiencies created by a secondary antinutritional agent influence virus replication. The approximate halving of these nutrients observed in aflatoxin-exposed animals (70-72, 74) suggests that aflatoxin will generate more intense nutritional deficiencies and may therefore increase vertical transmission, although it is likely that this may happen indirectly through accelerated progression of the disease.

Rate of progression of HIV/AIDS

There is good evidence from animals that aflatoxin exposure results in increased aggressiveness of infectious diseases (163, 164). This is also observed for humans with HIV: when Hendrickse et al (165) investigated the reasons for the rapid progression of HIV and AIDS in heroin addicts in the Netherlands and Scotland, they found that street heroin was often contaminated with aflatoxin, and that aflatoxin derivatives were commonly found in the bodily fluids of the addicts. The rates and frequencies of AFM₁ in the urine of these addicts were comparable to those observed in West Africans exposed to aflatoxin through food products. Hendrickse et al speculated that the accelerated HIV progress was due to aflatoxin-related immune suppression, but they did not undertake further studies. This suggestion of synergy is also supported by the broad correlation between aflatoxin exposure estimated in this report and the commonly perceived faster rate of HIV progress in Africa than in Europe and



America—but it is recognized that many other environmental factors may influence differences in the rate of progression (with-out medication) between these locations. Certainly the HIV pandemic is critical enough for this possibility to be investigated as a matter of urgency.

Another factor that has been associated with the rate of HIV progression is the concentration of IL-2 (166), which reflects the role of CD4⁺ cells in IL-2 production. Aflatoxin has been shown to suppress IL-2 production through the down-regulation of transcription (167), and it has the potential to effectively accelerate HIV progression by making CD4⁺ cells less effective.

Studies of factors that influence the rate of progression from HIV infection to AIDS show very clearly that nutrition is of critical importance (160, 168-171), so it is at least likely that the interference of aflatoxin in nutrition accelerates the decline in nutritional status. At the macronutrient level, HIV-positive patients have a 50% greater protein requirement than do HIV-negative persons (172), whereas aflatoxin exposure decreases protein synthesis for ≤ 5 d (61). Thus, aflatoxin contamination of foods probably makes the satisfaction of the HIV-enhanced protein requirements more difficult.

Survival with AIDS

Survival after the onset of AIDS is determined largely by a person's ability to resist infection by secondary pathogens. The review of animal data related to the incidence of infectious diseases provides a good reason to believe that aflatoxin will also modulate this function of the disease's epidemic model. In children whose immune systems were compromised by malnutrition, Adhikari et al (19) found that the children with aflatoxin adducts were significantly more prone to infections than were those without. When both the virus and a second agent (aflatoxin) decrease the body's immune defenses, a significant reduction in survival time is likely, particularly because these factors also compromise the nutritional status of the infected person.

Unsafe water

The World Health Report of 2002 stated, "The vast majority of diarrheal disease occurrences in the world (88%) were attributable to unsafe water, sanitation, and hygiene. $\approx 3.1\%$ of deaths (1.7 million) and 3.7% of DALYs (54.2 million) worldwide are attributable to unsafe water, sanitation and hygiene" (1). Directly relevant animal experiments show that piglets with chronic aflatoxin exposure of 70 and 140 ppb are more prone to diarrheal disease, experience more severe symptoms, and are more likely to die (163) than are piglets without such exposure. This finding is likely to be applicable to humans, because it was established that, at existing levels of human exposure, secretory immunoglobulin A is decreased by aflatoxin (62), that immunoglobulin A is important to membrane barrier function, and that membrane barriers are important to gastric diseases.

Open fires in enclosed environments

In total, 2.7% of DALYs worldwide are attributed to the health effects of open fires in enclosed environments, and the evidence for aflatoxin as a modulating factor in this risk factor is the most tenuous of the risks discussed. The WHO estimated that indoor smoke from solid fuels causes $\approx 35.7\%$ of lower respiratory infections and may also be associated with tuberculosis. There is firm evidence that aflatoxin can be a cofactor in lower respiratory

diseases; postmortem examinations of children in Nigeria and Philippines diagnosed with pneumonia commonly identify aflatoxin in tissues (135, 147). The infectious disease components of this risk factor are partly due to the effects of chemicals (such as benzopyrenes) that create oxidative stresses, which contribute to suppression of the immune system (173). Thus aflatoxin, which creates the same oxidative stress as do benzopyrenes (148) and has immune system suppression, is likely to exacerbate the infectious disease component of this risk factor. The cancer risks attributed to this health risk could also be exacerbated directly by aflatoxin carcinogenicity, but no research on that possibility has yet been reported.

Zinc deficiency

Zinc influences health through its role in immunity, and it is also potentially important in economic development because of its effects on cognitive development and human capacity (174). Using dietary data, the WHO estimated that zinc deficiency (ZnD) affects about one-third of the world's population, with the caveat that severe ZnD is rare (1). The WHO estimated that ZnD is responsible worldwide for 16% of lower respiratory tract infections, 18% of malaria, and 10% of diarrheal disease, thus accounting for 0.8 million (1.4%) deaths and 28 million (2.9%) DALYs. The role of aflatoxin in ZnD in humans is unknown, but the evidence from animals described earlier suggests that aflatoxin may increase the extent of ZnD, particularly in early childhood, even when mothers do not themselves have ZnD (73, 74).

Iron deficiency

Iron deficiency (FeD) is one of the most prevalent nutrient deficiencies in the world, affecting an estimated 2 billion people: anemia, cognitive development, fitness and aerobic work capacity are affected. In total, 0.8 million (1.55%) deaths worldwide are attributable to FeD, as are ≈ 35 million DALYs (2.4% of the global total) (1). Although aflatoxin is known to affect nutritional concentrations of iron in animals (175-178), its potential effect on human iron concentrations is not known. Thus, research to examine the epidemiologic consequences of aflatoxin for FeD is definitely needed.

Vitamin A deficiency

The WHO risk analysis estimated that VAD caused ≈ 0.8 million (1.4%) deaths worldwide, and attributable DALYs were 1.8% of global disease burden. This deficiency increases the morbidity from malaria and diarrheal diseases. Aflatoxin has been shown to affect the retinol concentrations in serum of multiple animal species (69-71), but the effect in humans is still unclear, and doses needed to duplicate the animal results in humans are not established. Because of the role of vitamin A on the detoxification of aflatoxin, it is possible that VAD increases the biological exposure to aflatoxin.

POSSIBLE INTERVENTION STRATEGIES

Factors fundamental to a nation's ability to protect its population from aflatoxin include the following. First, a nation must have the political will to address the issue of aflatoxin exposure. Most nations are signatories to *Codex Alimentarius* (WHO/FAO documents that deal with food quality in traded commodities) and subscribe to the need to limit exposure of their populations to

aflatoxins. However, they also seek to minimize the economic consequences of achieving this health goal and to use the resources available for health on the highest-priority problems. Often they do not enforce these standards because liver cancers (the commonly perceived health risk) are relatively unimportant health risks for their populations, and aflatoxin does not feature on the list of WHO priority risks. Second, a nation must have the capability to test food for contamination, which determines whether regulations can be enforced. In developing countries, laboratories capable of analyzing foods for aflatoxin are very rare, if indeed they exist at all.

Preventing exposure to aflatoxin

The traditional approach to preventing 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. However, as discussed earlier, this approach has certain limitations and clearly has failed as a control measure for developing countries. In developed countries, where regulations allow higher aflatoxin concentrations in animals, the agricultural industries have developed alternative approaches [chemoprotection (179) and enterosorption] to limit biologically effective exposure without the high cost of preventing contamination. 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 (180-184).

The effective enforcement of regulations defining the concentrations of aflatoxins permitted in various foods in North America and Europe has turned aflatoxin into a problem with significant economic but minor human health consequences. To prevent the economic loss associated with failure to meet the regulations, a significant body of research has been published relating to 3 main points of leverage—production, storage, and processing.

Production

Although the initial focus of research was on the prevention of contamination in storage, it was established in about 1970 that contamination, or at least invasion by the causal fungi, could start in the field before harvest. For peanuts, environmental conditions such as drought during the grain growth stage (185), insect damage in the field (8), variety, and soil characteristics have proven to be determining factors in preharvest contamination (9). These conditions are now sufficiently well understood for computer simulation models to describe the risk of contamination of major crops (186). The result is that management can be used to minimize contamination, and the practice of inoculating the fields with nonaflatoxigenic strains of fungi (187) may shortly be a new tool in the battle to prevent economic loss. Because of the importance of drought as a factor predisposing crops to contamination, irrigation is a very important means of ensuring food quality (9). More recent developments have made use of biotechnology to introduce genes that either prevent the formation of aflatoxin as a result of fungal metabolism or prevent or decrease

fungal action. These approaches offer considerable long-term promise, but time and sizeable investment are still needed before the research can affect human health.

In developing countries, many of these preharvest opportunities to minimize contamination are not exploited by producers. Insect damage in the field is not controlled by pesticides or by cultural practices; drought is a common phenomenon, and most crops are produced without irrigation as an option. Harvesting is usually done without machinery, and drying is usually carried out very inefficiently and is dependent on the weather. Adverse weather at harvest results in slow and inadequate drying and brings attendant risks of contamination. However, models are available to aid in decisions affecting aflatoxin risks in production (186).

Storage

It is well understood that much of the contamination of commodities with aflatoxin occurs during storage. To preserve quality in storage, it is necessary to prevent biological activity through adequate drying (<10% moisture), elimination of insect activity that can increase moisture content through condensation of moisture resulting from respiration, low temperatures, and inert atmospheres (188). In other words, the conditions needed to prevent the development of contamination are known, but it is not always easy to produce them in storage systems in developing countries. One fact that makes storage such an important issue for these countries is the subsistence nature of most farming there. Most people in rural areas grow and store their own food; in consequence, most food is stored in small, traditional granaries, and there is little investment in the management of the conditions. Studies of grain quality in such storage structures show a steady increase in the aflatoxin content over time, which reflects the failure to maintain appropriate conditions (131). Achieving and preserving the conditions that prevent contamination is likely to prove a significant challenge for small-scale (household and farm level) storage and to be beyond the resources of most, even if they could be convinced of the value of making the effort.

Processing

Processing of commodities can be used to reduce the aflatoxin content and thereby prevent economic loss. Three main approaches exist: dilution, decontamination, and separation.

With regard to dilution, where regulations are enforced, the easiest means of satisfying the requirement is (unfortunately) to mix grain low in aflatoxin with grain exceeding the regulated limits. Thus, although the concentration is reduced, consumers are still exposed to the same overall aflatoxin burden. This approach fails when there is not enough “clean” grain to allow adequate dilution of the contaminated stock or when the infrastructure to hold stocks and achieve the desired mixing is lacking.

With regard to decontamination, considerable effort has been expended to develop methods by which contaminated commodities may be treated to denature the aflatoxin. Treatment with ammonia, alkaline substances (189), and ozone can denature aflatoxins, but whether this change is permanent is not clear. For instance, the processing of corn with caustic soda, as is used in traditional Mesoamerican cooking, has been shown to reduce the aflatoxin content, but there is some evidence both that the chemical change may be reversible and that, after consumption, the aflatoxin may be reformed in the acid conditions in the stomach (190).



With regard to separation, considerable success in reducing aflatoxin contamination can be achieved by separating contaminated grain from the bulk. This approach depends on the heavy contamination of only a small fraction of the seeds, so that removing those leaves a much lower overall contamination. Study of the distribution of aflatoxin in peanuts shows that a major portion (80%) of the toxin is often associated with the smaller and shriveled seed (191), and thus screening can lower the overall concentration in the bulk. Further removal of aflatoxin-contaminated seeds may be achieved by color sorting, which, in the case of peanuts, is most effective when the seeds are blanched. A consequence of this sorting approach to aflatoxin that is a serious concern is the fate of the now highly concentrated aflatoxin in the grain removed from the bulk. The poorest producers and laborers often consume those nuts, which should have been discarded, or they feed them to their animals.

Chemoprotection

Whereas it is highly desirable that feed is not contaminated, the reality is that much of the grain fed to animals is contaminated, even in the United States, and this condition results in substantial losses to producers if the feed is not treated (64). It is also clear that, in areas where regulations are not enforced, humans are commonly exposed to aflatoxins. In addition, aflatoxin has attracted attention as a chemical weapon, and there is military interest in protecting people from exposure either as a precursor to infectious biological weapons or as a "panic" weapon. Two major avenues of research have been developed to deal with these possibilities.

Chemoprotection against aflatoxins has been demonstrated with the use of a number of compounds that either increase an animal's detoxification processes (192) or prevent the production of the epoxide that leads to chromosomal damage (193). One technical solution is drug therapy, because several compounds, such as oltipraz and chlorophyll, are able to decrease the biologically effective dose (194, 195). However, sustained long-term therapy is expensive, may have side effects, and is not likely, given the health budgets of developing countries and their other pressing health problems.

For the animal feed industries, a major focus has been on developing food additives that provide protection from the toxins. One approach has been the use of esterified glucomanoses and other yeast extracts that provide chemoprotection by increasing the detoxification of aflatoxin (192).

Enterosorption

Another approach has followed the discovery that certain clay minerals can selectively adsorb aflatoxin tightly enough to prevent their absorption from the gastrointestinal tract (196). Whereas many toxins are adsorbed to surface-active compounds, such as activated charcoal, the bonding is not often effective in preventing uptake from the digestive system. Various sorbents have different affinities for aflatoxins and therefore differ in preventing the biological exposure of the animals consuming contaminated foods. There have been several claims for different adsorption agents, but their efficiency in preventing aflatoxicosis varies with the adsorbent (189).

With enterosorption, there is also a risk that nonspecific adsorbing agents may prevent the uptake of micronutrients from the food (197). *In vitro* tests of hydrated sodium calcium aluminosilicates (HSCAS) suggest that there is little other adsorption of

micronutrients, and Chung et al (198) confirmed this result. The use of HSCAS additives in contaminated feeds has proven effective in preventing aflatoxicosis in turkeys, chickens, lambs, cattle, pigs, goats, rats, and mice (182, 189, 196, 199–209). The use of radiolabeled aflatoxin shows that the addition of clay in a proportion of 0.5% of the volume to a contaminated feed reduced exposure in chicks by $\approx 95\%$ (189). Selected calcium montmorillonites have proven to be the most highly selective and effective of these enterosorbents. This approach is now widely used in animal production industries worldwide (180), and HSCAS is estimated by one manufacturer to be added to 10% of all animal feeds.

CONCLUSIONS

Aflatoxin is a hepatotoxic, carcinogenic, immunosuppressive, antinutritional contaminant of many staple food commodities. Contamination may develop as a result of fungal action before and during harvest and also 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 these regulations practical, but this is not the case in developing countries. There, the current food production systems and economic conditions make management of aflatoxin contamination impractical. Available data from market samples of staple foods, private granaries, prepared foods, and human biomarkers of exposure all suggest that chronic exposure is widespread between these latitudes in developing countries, where an estimated 4.5 billion people live.


The information available on the effects of aflatoxin exposure is influenced by the prevailing exposure risks for humans (low-level) and farm animals (natural) in developed countries. Studies have shown that exposure to aflatoxin has several established consequences and other likely consequences for human health, depending on levels of exposure:

- 1) The risk of cancer. This risk is a function of cumulative aflatoxin exposure, so low exposure rates still have significant health implications, particularly for the $\approx 20\%$ of people in developing countries with HBV.
- 2) Serious effects on childhood nutrition. At the levels of exposure in some developing countries, child nutrition and development are interfered with, as is selenium. Animal studies show that aflatoxin also interferes with vitamins A and D, iron, selenium, and zinc nutrition.
- 3) Immunosuppression. This risk is well established in farm and laboratory animals, and immune system involvement of aflatoxin is confirmed for humans in a few studies.
- 4) Modulation of infectious diseases and vaccination titers in animals. There is evidence suggesting that aflatoxin may well be a factor in the HIV epidemic and in malaria incidence.

Studies show that aflatoxin is a factor with established or potential influence on 6 of the 10 most important health risks identified by the WHO for developing countries where short lifespan is prevalent. This situation indicates that addressing the issues around aflatoxin contamination may be critical to improving world health.



In developing countries, the food systems and economic realities require a solution different from that which has been effective in developed countries. Some progress can be expected from adapting technologies currently used in developed countries, and potentially the most appropriate aflatoxin-minimizing technologies are the use of varieties with less risk of contamination, model-based risk-forecasting systems, improved postharvest crop processing and storage technologies, and separating out of contaminated produce. Detecting contamination in simple, affordable ways is of critical importance. A better understanding of the true cost of aflatoxin contamination, as outlined above, would potentially change policy and attitudes.

It is also important that ways of preventing biological exposure from unmanageable aflatoxin be exploited. To minimize biological exposure, 2 main possibilities exist. Chemoprotection through the use of drugs and dietary supplements that increase the fraction of aflatoxin that is detoxified is effective, but also relatively costly as a routine measure to provide protection against chronic exposure. Therefore, testing of enterosorptive food additives that bind the toxin to render aflatoxin biologically unavailable to humans is clearly warranted. 

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