

Risk of Exposure to and Mitigation of Effect of Aflatoxin on Human Health: A West African Example

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CONTENTS

ABSTRACT	218
I. INTRODUCTION.	219
II. FACTORS INVOLVED IN HUMAN EXPOSURE TO AFLATOXIN.	219
A. Global Distribution—Developed vs. Developing Countries	219
B. Regional Distribution—Agroecosystem Influence.	221
1. Temperate Zones.	222
2. Humid Tropics	223

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3. Mid-Altitudes	226
C. Distribution of <i>Aspergillus flavus</i> Strains	227
D. Social System and Food Basket	227
III. ASSOCIATION OF AFLATOXIN EXPOSURE WITH DISEASE AND DISORDERS	230
A. Human Disease and Nutritional Status.	230
B. Carcinogenicity.	230
C. Growth Suppression/Immunotoxicity	231
1. Growth and Immune Suppression in Animals	231
2. Growth Suppression in Humans	232
IV. RISK MITIGATION.	233
A. Efficacy of Sampling Protocols and Regulatory Action Levels.	233
B. Cancer Risk Reduction.	235
C. Agricultural Management.	237
V. CONCLUSIONS	239
REFERENCES	241

ABSTRACT

The purpose of this chapter is to examine the relative risk of exposure of different human populations to food-borne aflatoxins; the types of health impact that may be incurred by dietary exposure to aflatoxins; and possible strategies likely to mitigate risks to human health. Risk of exposure is examined in a global context comparing risk of toxin exposure by levels of national socioeconomic development. Then risk of exposure is reexamined in the context of agro-ecology, distribution of toxigenicity of *Aspergillus flavus*, and social factors that influence food management practices. The effects of aflatoxin exposure on human health are explored in three sections: human disease and nutritional status, carcinogenicity, and child growth and development. The section concerning mitigation of the effects of aflatoxin on human health contrasts efficacy of regulation, food basket modification, and production-side agriculture intervention. It is concluded that risk of hepatocellular carcinoma in developing countries, such as West Africa, may be addressed by vaccination for hepatitis B virus (HBV) and other public health options. Young children in West Africa who are chronically exposed to aflatoxin in foods and who consume nutritionally

deficient diets have been shown to be stunted and underweight, as measured by World Health Organization (WHO) Z-scores.

Key Words: Aflatoxin; West Africa; *Aspergillus flavus*; Hepatocellular carcinoma; Hepatitis B virus; Agro-ecology; Child growth development; Nutritional status.

I. INTRODUCTION

This chapter will examine the relative risks of exposure of different human populations to food-borne aflatoxins and possible strategies likely to mitigate risks to human health. Risk of exposure is examined in a global context comparing risk of toxin exposure by levels of national socio-economic development. Risk of exposure is re-examined in the context of agro-ecology, distribution of toxigenicity of *Aspergillus flavus*, and social factors that influence food management practices. Efficacy of regulations, food basket modification and production-side agriculture intervention will be considered. Risk of hepatocellular carcinoma in developing countries, such as West Africa, may be addressed by vaccination for hepatitis B virus and other public health options. Young children in West Africa who are chronically exposed to aflatoxin in foods and who consume nutritionally deficient diets are shown in this study to be stunted and underweight, as measured by WHO Z-scores.

II. FACTORS INVOLVED IN HUMAN EXPOSURE TO AFLATOXIN

A. Global Distribution—Developed vs. Developing Countries

In developed countries, infrastructure exists for monitoring contaminant levels in foods and feeds. In developing countries and in poor, rural agricultural communities, exposure to aflatoxins has been shown to occur, as shown in Table 1 (Gong et al., 2002; Hall and Wild, 1994). Developed countries tend to have diverse and abundant food supplies and can divert aflatoxin-contaminated food or feed to other uses; for example, aflatoxin-contaminated corn can be fed to beef cows within the limits set by the U.S. Food and Drug Administration (FDA) (Food and Drug Administration,

1996). Developing countries may not have the choice of diverting contaminated foods away from human consumption and the overall food-basket diversity tends to be low. When aflatoxin-vulnerable foods are the primary staple, the chance of chronic and deleterious exposure is much higher (Egal et al., 2003). Developed-country farmers have more options to adopt agronomic practices that reduce aflatoxin contamination in crops, including choice of crop variety, possible crop rotation, planting density, weed control, nitrogen fertilization, insect control, irrigation to minimize drought stress, use of mechanized drying as opposed to field-drying, and provision of dry, pest-free crop storage facilities (Bilgrami and Choudhary, 1998). Regardless of the economic status of a country, aflatoxin contamination varies markedly with seasons due to climatic conditions, and when ideal conditions occur, even advanced management techniques may not suffice to prevent contamination.

Estimation of population exposure to aflatoxins is difficult. Classical methods of measuring food consumption are short-term recall food frequency questionnaires, daily recording of food items consumed, and analysis of food intake by weighing food before consumption. These are then combined with laboratory analysis of levels of mycotoxins in food samples to ascertain relative exposure rates for individuals. These methods

Table 1. Estimated population exposures based on analysis of aflatoxins in food.

Country	Sampling period	Food source ^a	Range of estimated aflatoxin exposure (ng/kg/day) ^b	Reference
Kenya	1969–70	H	3.5–14.8	Peers and Linsell (1973)
Swaziland	1972–73	H	5.1–43.1	Linsell and Peers (1977)
	1982–83	H	11.4–159	Peers et al. (1987)
Mozambique	1969–74	P	38.6–183.7	Van Rensburg et al. (1985)
Transkei	1976–77	P	16.5	Van Rensburg et al. (1985)
The Gambia	1988	P	4–115	Wild et al. (1992)
China (southern Guangxi)	1978–84	M	6.5–53	Shank et al. (1972)
Thailand	1969–70	P	66.5–53	Shank et al. (1972)

^aH = Uncooked food samples from the home; P = samples of cooked food; M = samples from the market.

^bAFB₁, except Kenya (B1 and B2) and The Gambia (B1, B2, G1, G2).

are not easy to apply even in developed countries but are particularly difficult to use in poor, rural agricultural communities (Hall and Wild, 1994). In general, more data on human exposure to aflatoxins are available from developed than from developing countries. Hall and Wild (Henry and Bosch, 2001) summarized estimates of population exposure to aflatoxins based on analysis of food from several developing countries (Table 1).

In most developed countries, aflatoxin levels in human food are regulated at the low parts per billion range ($\mu\text{g kg}^{-1}$) because of concerns about the potent hepatocarcinogenicity of aflatoxin B1 (Kuiper-Goodman, 1994; Pestka and Bondy, 1994). In developing countries, although CODEX standards are statutory laws in most countries, mechanisms to effect compliance are not in place. Except in developing countries where public awareness programs are attempted, consumers may be unaware that they are being exposed to high levels of mycotoxins in their staple diets (Cardwell, 2000).

B. Regional Distribution—Agroecosystem Influence

Climatic and edaphic factors, agricultural management systems, insect pest pressure, and food processing customs are all factors in risk of aflatoxin accumulation in foods. The most vulnerable crops are maize (*Zea mays*), groundnut (*Arachid hypogaea*), and tree nuts. The risk of aflatoxin contamination is a function of several factors, including:

- Presence of toxigenic fungus in the soil (Cardwell and Cotty, 2000; Cardwell et al., 2001; Cotty, 1997; Cotty and Cardwell, 1999; Cotty et al., 1994).
- Soil infertility (Hell et al., 2000a; Lopez-Garcia and Park, 1998; Udoh et al., 2000).
- Drought stress.
- *Striga*, a parasitic weed, crop diseases, particularly vascular disease (Lopez-Garcia and Park, 1998).
- Insect damage (Hell et al., 2000b; Lopez-Garcia and Park, 1998; Setamou et al., 1997, 1998).
- Excessive heat during kernel development (Odvody et al., 1997).
- Delayed harvest (Hell et al., 2000a; Udoh et al., 2000).
- Harvest and postharvest damage to kernel/grain (Lopez-Garcia and Park, 1998).
- Sanitation and management of harvested produce (Hell et al., 2000a; Udoh et al., 2000).
- Dry down and moisture in storage (Hell et al., 2000a,b).
- Postharvest insect feeding (Hell et al., 2000b).

In agro-ecosystems with one or more of the risks, crops are likely to contain aflatoxin, increasing the potential for human exposure.

1. Temperate Zones

In cool temperate zones, including Canada and much of Europe, the risk of aflatoxin contamination of indigenous food supplies is very low (Kuiper-Goodman, 1994). *Aspergillus flavus* grows with higher temperatures and less available water than most other fungi (Klich et al., 1992), making it more competitive in these conditions than other fungi (Lacey, 1994). In cooler climates and mid-altitudes, other fungi such as *Fusarium* spp. are more likely to occur than *Aspergillus* spp. (Ngoko et al., 2001). In warm, drought-prone zones of the U.S., such as the Southeast, Arkansas, Texas, and Oklahoma, problems of aflatoxin contamination sporadically occur in maize, groundnut, and pistachio nut (*Pistacio vera*), primarily in drought years (Table 2). Maize is particularly severely affected in the southern U.S. when nighttime temperatures are high and kernel integrity is breached (Odovsky et al., 1997). As may be seen in Table 2, which is based on FDA data, levels of aflatoxins in shelled maize designated for human consumption and grown in the Corn Belt or elsewhere in the U.S. generally are lower than levels in maize grown in the Southeast, Arkansas, Texas, or Oklahoma.

Africa typifies the risk factors for aflatoxin that occur in different agro-ecologies in the tropics (Fig. 1). Across all of sub-Saharan Africa there is a

Table 2. Aflatoxins in shelled maize designated for human consumption in differing geographic regions of the United States.

Area of U.S.	Year	Total no. products examined	% of products >ng g ⁻¹	% of products >20 ng g ⁻¹	Maximum ng g ⁻¹
Southeast ^a	1992	53	20.7	11.3	82
Corn belt ^b		114	12.3	0.0	17
AR-OK-TX		35	17.1	5.7	77
Rest of U.S.		37	2.7	2.7	34
Southeast	1995	23	8.7	0.0	4
Corn belt		113	0.9	0.0	8
AR-OK-TX		44	20.4	4.5	681
Rest of U.S.		18	0.0	0.0	0

Source: Wood (1989, 1992a,b), Wood and Trucksess (1998).

^aAL, FL, GA, LA, MS, NC, SC, TN.

^bIA, IL, IN, KS, MI, MN, MO, NE, OH, SD, WI.

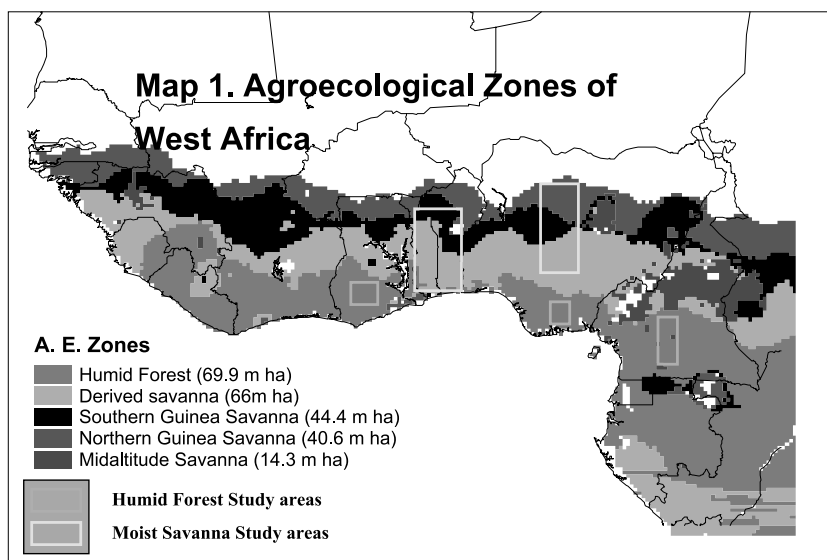


Figure 1. Agroecological zones of West Africa.

range of ecologies from desert to deep humid tropic, and cool mid-altitudes. West and central Africa have a gradient of agro-ecologies ranging from very humid forest and coastal savanna in the south to arid savannas bordering the Sahara desert in the north, with mid-altitude highlands regions of Nigeria and Cameroon (Hell et al., 2000a; Ngoko et al., 2001; Udoh et al., 2000). These agro-ecosystems have a range of climatic conditions, cropping systems, storage practices, and food consumption patterns (Cardwell, 2000; Egal et al., 2003; Gong et al., 2003; Hell et al., 2000a; Ngoko et al., 2001; Udoh et al., 2000). The risk of aflatoxin contamination and human exposure through consumption of contaminated foods is likely different in each of these agro-ecosystems. The conditions for risk of commodity contamination can be extrapolated to other tropical regions, while crop management practices, food processing, and consumption patterns may vary when taken on a larger geographic scale.

2. Humid Tropics

The humid coastal savanna and humid forested areas of West and Central Africa are characterized by a bimodal rainfall distributed over 9 months and the possibility of multiple growing cycles in a year. Maize is the dominant crop and some groundnuts are produced. There are several factors in these ecologies that could lead to high toxin levels. However, in

several studies (Egal et al., 2003; Hell et al., 2000a; Ngoko et al., 2001; Udoh et al., 2000) this has not been the case. Very low amounts of aflatoxin were found in stored maize in Nigeria (Udoh et al., 2000), Benin (Hell et al., 2000a), and Cameroon (Ngoko et al., 2001). In Benin, this was confirmed in a study of blood albumin-aflatoxin adducts in young children. Some of the lowest exposure levels seen were in the humid coastal savannas of those countries (Gong et al., 2002; Wild et al., 1990, 1992). In the contiguous countries of Cameroon, Nigeria, Benin, Togo, and Ghana, humid zone maize is sown with the first rains (first season) and generally harvested early and eaten as “green” maize. What little mature maize is harvested, is eaten as quickly as possible because storing the crop safely is very difficult. It is rare for maize to be planted in the second cropping season because lepidopteran stem borers increase to the point where yield losses are too high to make maize worthwhile to plant (Bosque-Pérez and Schulthess, 1998; Udoh et al., 2000).

In most countries, groundnuts are not grown in coastal and forest areas, although some nongovernmental organization programs promote them as a source of dietary protein. In Benin and Togo, people in the humid coastal savannas eat groundnut on average 1.4 days/week (Egal et al., 2003). In these regions alternate foods, particularly plantain (*Plantago major* L.), and root and tuberous crops, are the locally produced primary staples. It is also possible that adequate rainfall in these zones makes the crop less susceptible to invasion by *A. flavus*, but more likely to be infected with *Fusarium* mycotoxins (Ngoko et al., 2001).

Southern Guinea Savanna (SGS) is moist grassland or derived forest, also with a 9-month rainy season. In the SGS, continuous maize planting is common, starting from the first rains in May until mid-September. Soil fertility levels are typically low and pre- and postharvest insect pressure is high (Hell et al., 2000b; Setamou et al., 1997). The first harvest often falls within a short, but unpredictable break in the rains in August. It is common for maize in this zone to be harvested with a 20 mg g⁻¹ grain moisture and then go into storage structures designed to facilitate grain drying (Hell et al., 2000b; Udoh et al., 2000). These are traditionally constructed of woven natural materials, on conical raised platforms, and covered with a thatched roof. They provide good natural aeration that allows the grain to dry to ca 14 mg g⁻¹ (Hell et al., 2000a). Insect feeding and concomitant rehumidification of the grain is common in these structures (Hell et al., 2000b; Setamou et al., 1998). Aflatoxin has been detected in up to 50% of grain samples drawn from these stores, with AFB1 concentrations ranging from 0 to 500 µg kg⁻¹ (Hell et al., 2000a). Egal et al. (2003) found that 93% of household maize samples in Benin and Togo were infested with an average of over 1000 colony-forming units of *A. flavus*. In that study,

aflatoxin-albumin blood levels in children in the SGS of Benin and Togo were the highest levels seen across the agro-ecological zones. Maize was consumed on average 6.6 days a week, while groundnut was consumed as a snack 4.5 days of the week (Egal et al., 2003). In this type of agro-ecology, the risk of chronic dietary exposure to aflatoxin is high.

Northern Guinea Savanna (NGS) from 9° latitude north, has rainfall starting in June and ending in November. Maize and groundnut are prevalent in the cropping systems. In Nigeria, this zone is the most developed for agriculture, where maize hybrid seed technology and fertilizer use is highest. Udoh, Ikotun, and Cardwell (Udoh et al., 2000) found the maize aflatoxin levels in this zone in Nigeria to be low. However, in Benin and Togo, where crop management systems are less developed, this zone was similar to the SGS in grain aflatoxin concentration (Cardwell et al., 2001; Hell et al., 2000a) and frequency and colony-forming unit (CFU) levels of *A. flavus* found in foods (Egal et al., 2003). In the NGS, farmers have the problem of fitting maize production into a 6-month rainy season. Most tropical lowland maize cultivars mature in 100–120 days, so multiple plantings are possible, though drying of early harvests is very difficult. It is common to find maize, groundnut, and cotton intercropped and rotated on the same land. This zone also has insect pressure in the field and in storage (Hell et al., 2000b; Setamou et al., 1997). Maize is consumed 5.6 days a week and groundnut 2.6. In this zone, it was found that wealthier households had higher blood aflatoxin albumin adduct levels. These were associated with more purchase and consumption of groundnut as a snack (Egal et al., 2003). Therefore, this type of agro-ecology also has conditions necessary for chronic exposure to food-borne aflatoxin.

Sudan savanna (SS) typifies tropical and subdesert savannas where maize is intercropped with sorghum (*Sorghum bicolor*) and millet (*Pennisetum glaucum*), and where the majority of groundnuts are grown. This zone is characterized by high temperatures and a short rainfall period lasting from July to September. Generally one crop a year can be grown, making storage and commerce of cereal and legume grains practical. Stored crops must last from one harvest to the next. Although the storage conditions are dry and with low insect pressure, growing conditions are often poor, with temperature and drought stress common. Drought stress is one of the most important factors in aflatoxin formation in both maize and groundnuts (Wicklow, 1994). The condition of the crop going into storage, and the general condition of the bin itself, are critical for maintaining good food quality (Hell et al., 2000a). In this zone in some countries, farmers shock maize and allow it to dry in the field. Hell et al. (2000a) and Udoh et al. (2000) found that field-dried maize had poorer quality, more insect damage, and a higher risk of aflatoxin contamination. A com-

mon practice in West Africa is to thresh maize in the field, thereby letting it come directly into contact with the soil. Weathered and exposed grain goes into clay bins covered by thatched roofs for up to 12 months. Even a slight wetting will result in mold growth and toxin production. High toxin levels can be found in foods in this zone (Cardwell, 2000; Cardwell et al., 2001). Maize is consumed 5.7 and groundnut 4.8 days/week (Egal et al., 2003).

3. Mid-Altitudes

Agro-ecological zones 800 meters and more above sea level tend to have very low aflatoxin contamination in maize (Ngoko et al., 2001; Udoh

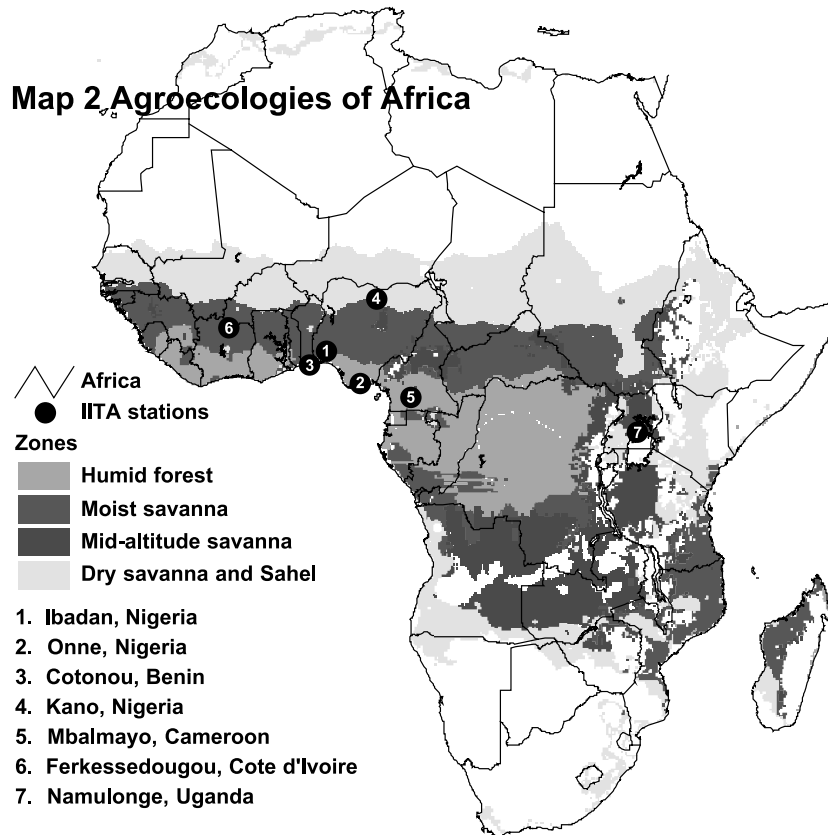


Figure 2. Agroecologies of Africa.

et al., 2000). This is presumably because of cooler climate and less stressful conditions during production and storage. However, *Fusarium* species and associated toxins have been shown to be prevalent under these conditions (Ngoko et al., 2001).

In summary, in parts of sub-Saharan Africa and similar agro-ecologies in other parts of the world where there are limited food basket options, the chances for chronic dietary exposure to unsafe levels of aflatoxin are high. The red and yellow zones shown in the map of Africa are areas of high risk of aflatoxin exposure, while in the green and blue zones more exposure to *Fusarium* toxins would be expected (Fig. 2).

C. Distribution of *Aspergillus flavus* Strains

Diversity in the population of *Aspergillus flavus* exists with respect to toxin production and sclerotial size (Bayman and Cotty, 1993; Cotty et al., 1994). S-strain (those producing small sclerotia) isolates in the U.S. produce high amounts of aflatoxin B only, while S-strains from Africa and Thailand produce both aflatoxins B and G consistently in high amounts (Cotty and Cardwell, 1999; Hesselstine et al., 1970). L-strains (with large sclerotia) on both continents produce a range of aflatoxin B from none to very high. In both the U.S. and West Africa, S-strain isolates are relatively more likely to be found in dryer ecologies (Cardwell and Cotty, 2002; Cotty, 1997).

In West Africa, as agro-ecology transitions from wetter to dryer from south to north, S-strain *A. flavus* became more prevalent relative to L-strain in field soils (Cardwell and Cotty, 2000, 2002) and in maize and groundnut (Egal et al., 2003). Although most white maize samples were infested with *A. flavus*, the percentage of samples containing *A. flavus* CFUs decreased significantly from north to south for both L- and S-strains. The average sample in the north contained more S-strain CFUs than in the south while samples in the center contained more L-strain CFUs than samples in the north and south (Cardwell and Cotty, 2002; Cotty, 1997).

D. Social System and Food Basket

Social factors such as education and access to disposable income determine food sanitation and the variety of foods in the household diet. One aspect of “developed” economies is the access to a broad array of consumer options. In these social systems, cheap, abundant, and safe food supply is considered a human right. Food safety is a public concern and is managed through regulation and consumer education. Monitoring of

vulnerable commodities in the U.S. shows that these foods are not highly contaminated; therefore, even frequent consumers are not exposed to high levels of aflatoxin (Table 3).

In developing countries, public sector management of food safety issues is not a given, leaving the majority of food-related issues to individual consumer and farmer awareness. Access to a broader array of foods is also lacking, leaving the majority of the caloric intake of the population from one or two principal staples. Presumably, protective food preparation would be a learned behavior and therefore might be expected to be practiced by people with more access to information and education. Also, in developing countries, it would be expected that households with

Table 3. U.S. peanut butter and tree nuts examined for aflatoxins.

Product	Year	Total no. products examined	% of products >1 $\mu\text{g kg}^{-1}$ aflatoxins	% of products >20 $\mu\text{g kg}^{-1}$ aflatoxins	Maximum $\mu\text{g kg}^{-1}$
Peanut butter	1992	82	22.0	0.0	9
	1995	70	41.0	0.0	18
Peanuts, shelled, roasted	1992	89	9.0	2.2	31
	1995	82	7.3	0.0	5
Peanuts, in-shell, roasted	1992	19	0	0.0	0
	1995	5	0.0	0.0	0
Almonds	1992	55	1.8	0.0	11
	1995	36	5.6	0.0	3
Pecan	1992	66	1.5	0.0	2
	1995	55	7.3	0.0	15
Pistachio	1992	18	5.5	0.0	2
	1995	11	9.0	0.0	5
Walnut	1992	51	5.9	2.0	87
	1995	44	15.9	6.8	44

Note: FDA action levels for total aflatoxins are 20 $\mu\text{g kg}^{-1}$ in all products, except milk designated for humans, and 0.5 AFM1 in milk and milk products. In animal feeds, the levels are 20 $\mu\text{g kg}^{-1}$ in corn and peanut products for immature animals and dairy cattle; 100 $\mu\text{g kg}^{-1}$ in corn and peanut products for breeding beef cattle, swine, and nature poultry; 200 $\mu\text{g kg}^{-1}$ in corn and peanut products for finishing swine; 300 $\mu\text{g kg}^{-1}$ in corn and peanut products for finishing beef cattle; 300 $\mu\text{g kg}^{-1}$ of cottonseed meal as a feed ingredient; and 20 $\mu\text{g kg}^{-1}$ for all other feedstuffs.

Source: Wood (1989, 1992a,b), Wood and Trucksess (1998).

higher economic status might have more access to a variety of foods and greater tolerance for removing and disposing of damaged grain, thereby lowering the overall aflatoxin exposure levels relative to less well-off neighbors. These hypotheses were tested in a series of villages in West Africa by Egal et al. (2003).

In West Africa within eco-zones the consumption frequency of maize was stable across socioeconomic lines, consumed at a comparable frequency by the rich, poor, educated, and illiterate. The association between maize consumption and aflatoxin exposure in this study by Egal et al. (2003) was especially strong. Aflatoxin exposure was assessed by measuring aflatoxin-albumin (AF-alb) adducts in peripheral blood; these adducts reflect consumption of toxin over the preceding 2–3 months. AF-alb blood adducts increased in children as parts per billion of aflatoxin ($\mu\text{g kg}^{-1}$) and CFU levels of *A. flavus* in the household stores of maize and groundnuts rose. An increasing frequency of consumption correlated to increased AF-alb adduct levels in children under 5. In Benin, the average child consumed maize 6.2 days/week preceding the survey. Children in the southern half of Benin and Togo (CS and SGS) had a higher consumption frequency than children in the north (NGS and SS). The traditional diet in the SS includes millet and sorghum as cereal staples, with maize being produced for market, but maize is the most important crop under cultivation in the other zones. In the SS, where maize consumption frequency was low relative to the other zones, children of educated mothers consumed less maize in this region, perhaps because other foods might be more preferred by well-educated mothers.

In this study by Egal et al. (2003), the importance of groundnut consumption as a source of aflatoxin exposure was less obvious. There was great variation in frequency of consumption of groundnut. Groundnuts were consumed fresh, as roasted nuts, cookies, and groundnut oil that is used to prepare food. In the week preceding the survey, the average child consumed groundnut 3.4 days out of 7. Regional differences were considerable; the average consumption frequency in the SS and SGS was three-fold higher than in the CS where climate is unfavorable for groundnut production (respectively, 4.7 and 1.4 days out of 7). With the exception of the SS, the frequency of consumption increased as household and maternal economic status went up. The bulk of production of groundnuts takes place in the SS; in this area, it appears that disposable income is not as important a factor in influencing consumption. In the SGS, maternal education was another factor causing variation in frequency of intake. Maternal education was associated with more frequent consumption of groundnut among children in this region where nongovernmental organizations have promoted groundnut

consumption as a cheap and effective way to reduce Protein Energy Malnutrition (Kwashiorkor). Possibly, educated mothers have put this message into practice more so than uneducated mothers.

III. ASSOCIATION OF AFLATOXIN EXPOSURE WITH DISEASES AND DISORDERS

A. Human Disease and Nutritional Status

There have been extensive reviews of the effects of aflatoxin exposure on human health (Wild and Hall, 1996). Maize consumption has already been identified as a health hazard in earlier studies focusing on the outbreak of hepatitis associated with consumption of high levels of aflatoxin in India and Kenya (Krishnamachari et al., 1975; Nagindu et al., 1982); numerous studies have linked consumption of contaminated groundnut to liver cancer (IARC, 1993).

B. Carcinogenicity

Aflatoxins are among the most potent mutagenic and carcinogenic substances known in sensitive species. Acute toxicity of aflatoxin B₁, the most toxic of the aflatoxins, varies from a LD₅₀ of 0.34 $\mu\text{g kg}^{-1}$ in 1 day-old Pekin ducklings to $>150 \text{ mg kg}^{-1}$ in the CFW Swiss mouse. Chronic exposure to aflatoxins is an efficient method of inducing hepatocellular cancer (HCC); in the sensitive species F344 rats, two cycles each of 5 days duration with 25 $\mu\text{g AFB}_1$ per day to young (approximately 100 g) rats produced preneoplastic foci at 2–3 months postdosing. Hepatic cancers arose after about 1 year and a small incidence (about 12%) by 23 months postdosing (Roebuck and Maxuitenko, 1994).

Because the metabolism and toxicokinetics of aflatoxins are not clearly understood, sensitivity differences between species to the effects of aflatoxins cannot be fully explained. Differences in P450 isoform activities, due either to genetic polymorphisms or to environmental alteration in expression, may be important contributors. Glutathione-S-transferase detoxification is a crucial factor in susceptibility to AFB₁ toxicity (Massey et al., 1995).

Human hepatocarcinomas (HCCs) are common in some developing countries, particularly China, Southeast Asia, sub-Saharan Africa, and relatively common in Japan and some Mediterranean Basin countries. In the Americas and Northern Europe, HCCs are rare. Pockets of high-risk

Table 4. Liver cancer etiology: Attributable fractions in Europe–U.S. and in Africa–Asia.

Risk factor	Europe and U.S.	Africa and Asia
Hepatitis B	<15% (4–50%)	60% (40–90%)
Hepatitis C	60% (12–64%)	<10%
Aflatoxin	Limited or none	Not quantified
Tobacco	<15%	Not estimated
Alcohol	<12%	29% (one study)
Oral contraceptives	(10–50%)	Not estimated
Others including hemochromatosis	<5%	<5%

Source: JECFA (1998).

populations have been described in the Amazonian basin, among Eskimos, and in special populations such as renal transplant patients. The incidence of HCC is 2–3 times higher in men than in women (Henry and Bosch, 2001; JECFA, 1998).

A growing body of evidence points to a synergistic relationship between aflatoxins and hepatitis B (and possibly C) virus in the etiology of human HCC (Table 4). Most of the available epidemiological studies, including data on aflatoxin exposure, have been done in high-risk countries, where both HBV and aflatoxin are prevalent. Because the nature of the interaction between low doses of aflatoxin and HBV is not understood, extrapolation of human HCC risks from these areas to those where both HBV and aflatoxin exposure are less common, is very difficult (Henry and Bosch, 2001; JECFA, 1998). Although Table 4 may be regarded as a simplification of complex epidemiological data, it is clear that risk factors for human liver cancer vary between Europe–U.S. and Africa–Asia. Hence, considerations of risks of liver cancer must necessarily be different between Europe–U.S. and Africa–Asia, as will be discussed later.

C. Growth Suppression/Immunotoxicity

1. Growth and Immune Suppression in Animals

Several studies have shown aflatoxins to be immunomodulatory in domestic and laboratory animals at doses in the mg/kg range (Bondy and Pestka, 2000; Hall and Wild, 1994). Cell-mediated immunity (CMI) is suppressed and delayed-type hypersensitivity is impaired. In addition, nonspecific humoral substances are suppressed, antibody formation is

reduced, allograft rejection is suppressed, phagocytic activity is decreased, and blastogenic response to mitogens is decreased (Miller and Wilson, 1994). Maternal dietary exposure to AFB1 during gestation and lactation affected the immune system of developing pigs (Silvotti et al., 1997). Since aflatoxins impair both cellular and humoral immune systems, sensitive animals are more susceptible to bacterial, viral, fungal, and parasitic diseases (Miller and Wilson, 1994). Studies in poultry have indicated that immune competence is compromised when feeds contain about $100 \mu\text{g kg}^{-1}$ aflatoxin (Coulombe). By comparison, the levels shown to be affecting the immune system in poultry are similar to those levels in the food of 30% of Gambian children (Turner et al., 2000).

2. Growth Suppression in Humans

In a cross-sectional study of 480 children in West Africa (Benin and Togo), Gong et al. (2002) showed a highly significant relationship between AF-alb serum adducts and growth faltering in children under 5. Aflatoxin was detected in 99% of the children sampled. Children with stunting or who were underweight had 30–40% higher mean AF-alb concentrations. The negative correlation between individual AF-alb concentration and each of the three growth parameters (height for age z score, weight for age z score, and weight for height z score) was highly significant. There was a clear dose-response relationship between AF-alb concentration and height for age and weight for age z scores. The effect was most acute at the time of weaning children from breast milk on to solid foods (primarily maize porridge) (Gong et al., 2003). It could not be determined from the cross-sectional design of the study whether the mechanism of interaction between aflatoxin exposure and impaired growth was the result of aflatoxin toxicity or reflected consumption of fungus-affected food of poor nutritional quality. Underweight children are more susceptible to chronic disease and are more likely to die early (Hunt, 2000).

In the Gambia, season of birth has been associated with altered morbidity and mortality (frequently infection-related) in adulthood (Moore et al., 1999). Aflatoxins are prevalent in the population's food supply, but there are seasonal variations in the level of food contamination and exposure to aflatoxin. These interactions were further studied by Turner et al. (2003).

In this study, 472 Gambian children aged 6–9 years born during a 5-year maternal dietary supplementation trial were the subjects. In rural Gambia, season can strongly influence both adult's and children's nutritional status. Weight loss occurs in pregnant and lactating women and birth weight is reduced 200–300 g during the annual wet season from July to November (the hungry season) relative to other seasons. Maternal

dietary supplementation can reduce this difference. Turner et al. (2003) studied levels of AF-alb, micronutrients levels, as well as a number of immune tests reflecting T-cell, B-cell, and mucosal secretion as measured by the CMI test, vaccine response, and the level of secreted Immunoglobulin A (IgA) in saliva in these children.

AF-alb level was strongly associated with the month of sampling. AF-alb was detected in 93% of the children. sIgA was markedly lower in children with detectable AF-alb levels compared with those with non-detectable levels. Antibody response to one of four pneumococcal serotypes, but not rabies vaccine, was weakly associated with high levels of AF-alb. There was no association between CMI response to test antigens and AF-alb. There were seasonal variations in a number of micronutrients, such as Vitamin C, alpha- and betacarotene and lycopene. These variations reflected periods in January–May when citrus fruits and mangos were available, and September–October when more green leafy vegetables were available. The only association with AF-alb was a negative correlation with Vitamin C. A weak association between adduct level and wasting was observed, but none for stunting or being underweight. These children were older than those observed in the Gong et al. (2002) study; younger children may be more sensitive to the growth-inhibitory effects of aflatoxin. Also, levels of AF-alb were higher in the children in Benin and Togo.

These studies emphasize the need to further investigate the relationship between growth, immunocompetence, and aflatoxin exposure, especially in highly aflatoxin-exposed populations of children.

IV. RISK MITIGATION

A. Efficacy of Sampling Protocols and Regulatory Action Levels

One method of reducing risk of exposure to food-borne aflatoxin is effective monitoring and exclusion of contaminated lots. However, sampling objectives differ; the level of accuracy and precision, and the risk of sampling error within a sampling protocol must be defined and understood. The highest precision is required if one is expecting to consistently detect low $\mu\text{g kg}^{-1}$ occurrence in a large lot of commodity for regulatory purposes. The degree of accuracy should not depend on the contamination level. The same accuracy can be maintained by sampling asymptotically more units from a sampling universe as the contamination level decreases. This is especially true for aggregated problems like *A. flavus* toxins. The distribution of aflatoxin in stored grains is not uniform,

and only a small portion of the grains may contain very high quantities of the toxin (Coker, 1998). The U.S. Department of Agriculture recommends 24 kg of peanut and 2- to 5-kg samples of maize, sorghum, and other grains to determine the average aflatoxin distribution in a lot (Whitaker et al., 1979). Coker (1998) compared various sampling procedures used in the U.S., United Kingdom, and the Netherlands. These plans are based upon probability of rejection of a lot/sample using operating curves predicated on the probability of positive detection with increased concentration of toxin per commodity. In the large-scale market operating paradigm, the use of statistics weighing the mean/variance ratio guides the size and number of samples required to minimize false positive and negative results.

In small-scale traditional agriculture systems, sampling procedures and/or sample analysis limitations for aflatoxin are problematic. To obtain a 10-pound sample from a village granary, small-scale marketer, or household food basket is often impossible in developing countries. Coker (1998) presents a sampling plan for “problematic” commodities based on a Weibull function with acceptable level of aflatoxin as the driving function variable. This procedure would still require the collection of six 1-kg samples, and three to six sample analyses per sample. In addition to the physical limitation on sample size, most developing-country laboratories have very limited operational budgets and number of sample analyses will be restricted. These limitations require consideration if the intent is to protect the consumer by monitoring vulnerable foods in dispersed, small-scale systems. This is where an honest assessment is required as to what levels of exposure are important, and are especially crucial if low contamination levels are important.

To assess risk of exposure in African villages, Egal et al. (2003) were able to collect 100-g samples of maize and groundnut from the family larder. Predictably, the detection of aflatoxin positive samples was low in these small samples. However, in this work, $\mu\text{g kg}^{-1}$ aflatoxin and CFUs of *A. flavus* in the food was highly correlated with blood toxin levels in children from the household where the sample was taken. Although detection of aflatoxin contamination of maize and groundnuts was infrequent (less than 10%), up to 90% of the maize and 58% of the groundnut samples were infested with *A. flavus*. Presence of the fungus may be a more reliable indicator for risk assessments of food-borne exposure. It also may be possible to turn the equations around so that an estimate of exposure could be calculated from limited sample size and number, based on likelihood of presence of toxin when the fungus is present. It would not be an exact measure, as is required for market regulation, but it would help to understand public health impact.

Another aspect of the sampling and standards question is illustrated by the work of Otsuki et al. (2001) at the World Bank. They attempted to quantify the impact of a European Union aflatoxin standard of $2 \mu\text{g kg}^{-1}$ on food exports from Africa. This case, as discussed below, is a good example of the trade-offs between acceptable levels of risk, relationships between international trading partners, and perspectives of developed vs. developing countries.

In 1998, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated that implementing a $10 \mu\text{g kg}^{-1}$ standard (total aflatoxin) would lead to a risk of 39 cancer deaths per year per billion people, with an uncertainty range between seven and 164 people. This estimate assumed a European population with 1% carriers of hepatitis B and an aflatoxin carcinogenic potency equal to 0.3 death per year per 100,000 carriers of HBV and 0.01 cancer death per year per 100,000 population among noncarriers.

The Middle East and Africa export most of their products to Western Europe. Hence, these areas are likely to be significantly affected by regulations set in European import markets. Based on the JECFA analysis, Otsuki et al. (2001) estimated that the proposed EU standard would impose a considerable loss of export revenue from cereals, edible nuts, and dried and preserved fruits in African countries. Maximum allowable aflatoxin levels in Europe range from $1\text{--}20 \mu\text{g kg}^{-1}$, while in Africa the average is $44 \mu\text{g kg}^{-1}$. African export revenue was estimated to decrease by 59% for cereals and 47% for dried and preserved fruits and edible nuts. This is a difference of approximately US\$400 million. Otsuki et al. (2001) illustrated the burden of the cost of compliance with WTO obligations in the least developed countries by noting that these costs can exceed total government budgets for all expenditures.

A risk reduction of 2.3 deaths per billion per year would be achieved in the EU under the proposed EU $2 \mu\text{g kg}^{-1}$ aflatoxin standard, according to Otsuki et al. (2001) estimates. This estimated reduction of liver cancer is small compared to the total number of deaths of liver cancer in the European Union. The World Health Organization has estimated that about 33,000 people die from liver cancer every year in the EU (population of one-half billion). Epidemiological studies would not even be able to measure reliably this small reduction in liver cancer cases.

B. Cancer Risk Reduction

An overwhelming body of data across species has demonstrated the potency of AFB1 as a carcinogen and mutagen (IARC, 1993). The best

evidence indicating an interaction between hepatitis B virus (HBV) and aflatoxin in human liver cancer has come from a cohort study in Shanghai, China involving more than 18,000 men (Qian et al., 1994; Ross et al., 1992; Yuan et al., 1995). Assays for urinary AFB₁, its metabolites AFP₁ and AFM₁, DNA adducts, and hepatitis B surface antigen (HBsAg) status have been undertaken. Subjects with liver cancer were significantly more likely than controls to have detectable concentrations of the aflatoxin compounds. Positivity for HBsAg was strongly associated with liver cancer risk. Thus, aflatoxin exposure in the presence of a persistent HBV infection increases the risk of human liver cancer (Henry and Bosch, 2001).

Such factors as unknown genetic and host response interactions may play a role in the liver cancer/HBV/AFB interaction (Henry and Bosch, 2001; JECFA, 1998). Evans et al. (1998) compared three independent cohorts of male HBsAg carriers in Senegal, in Haimen City, China and among HBsAg carriers in the U.S. (largely Asian origin). The risk of liver cancer in China (878 per 100,000 person years) was dramatically higher than in Senegal (68 per 100,000 py) or among U.S. HBV carriers (330 per 100,000 py). The prevalence of HBsAg was only moderately higher in Senegal (20% vs. 16%) than in China and the level of aflatoxin exposure was expected to be higher in the African setting.

Vaccination for HBV has been shown to drastically reduce liver cancer risk in some populations. The JECFA (2002) has recommended that vaccination for HBV must take high priority in preventing HCC. There are many HBV carriers (approximately 360 million worldwide). In addition, about 110,000 cases of HCC yearly worldwide have been attributed to hepatitis C virus infection (HCV). Access to HBV vaccine is incomplete, especially in developing countries; there is no vaccine available for HCV as yet.

The experience of Korea in reducing adult liver cancer by vaccination for HBV is an excellent example of vaccination importance. The prevalence of HBV infection and HCC are high (about 21 per 100,000); aflatoxin contamination is relatively high. In 370,000 males followed for more than 3 years, HBV vaccination drastically reduced HCC (incidence of 215 cases/100,000 vs. eight cases/100,000) (Lee et al., 1998). This reduction in HCC cases was accomplished without any additional resources being expended on reduction of aflatoxins in the food supply and without any change in aflatoxin regulations.

In Taiwan, an area of hyperendemic infection and moderate to high aflatoxin exposure, the immunization program against HBV reduced the rate of liver cancer in children 6 to 14 years of age, from 0.7 per 100,000 in 1981–86 to 0.57 in 1986–89, and then to 0.36 in 1990–94 (JECFA, 1998).

Since the incidence of liver cancer peaks in the sixth decade of life in Taiwan, at least 40 years may be required to see an overall decrease in the rate of liver cancer as a result of the vaccination program.

Both these populations should be followed in future years to further elucidate the relationship between aflatoxin, liver cancer, and HBV. These studies lend support to the hypothesis that the carcinogenic potency of aflatoxin may be reduced in humans by vaccination for HBV, as pointed out by JECFA (1998). The possibility should be considered, in the case of liver cancer, that scarce public health resources in developing countries may better be used for HBV vaccination programs (and thereby reduce incidence of liver cancer) than to lower aflatoxin levels to those required in the EU. In this context, effects of chronic exposure to relatively high levels of aflatoxins on growth and development of children should also be considered.

C. Agricultural Management

The best way to avoid the negative effects of aflatoxin on public health and economies is to limit or reduce the contamination of foods in the first place. There are numerous intervention points in the process of food production, starting at preharvest, through harvest and storage, to food processing and preparation. Crop and harvest management have been extensively treated in many fora (Hell et al., 2000a,b; Lopez-Garcia and Park, 1998; Udoh et al., 2000). There are some effective management practices such as efficient drying, that can reduce toxic contamination of foods wherever deployed. There are other management practices that are more specific to small-scale production systems. For example, in northern Nigeria, maize is shocked, dried, and then thrashed to shell the ears in the field, bringing grain in direct contact with soils (Udoh et al., 2000). It was shown that this practice considerably increased chance of aflatoxin contamination. Changing this practice could lower the risk. However, there is no single practice that can reduce aflatoxin-contamination risk to zero. The extent to which effective management modifications can be implemented is determined by agro-ecology and economic scale of the operation. Technologies such as host plant resistance and biological control can be scale-neutral. The following will not be an exhaustive treatment of all possible intervention strategies, only possibilities of biological control and host plant resistance, technologies that have not been extensively deployed as yet.

In the U.S., biological control has been used to reduce aflatoxin contamination in various crops such as cotton (Cotty et al., 1994), groundnut (Cole and Dorner, 2000), and maize (Brown et al., 2001; Dorner et al.,

1999). This technique involves the application to soil of a nonaflatoxigenic biological control strain of *A. flavus* or *A. parasiticus*, resulting in a high population density that allows the biological control strain to effectively compete with the native aflatoxigenic strains during invasion under conditions favorable for aflatoxin contamination. Invasion of a seed in soil (e.g., groundnut) solely by the biological control agent would be expected because of its high density relative to the wild-type strain in the soil. This would result in less aflatoxin contamination. For maize and cottonseed, the high population of the nonaflatoxigenic biological control strain in soil produces abundant spores on the soil surface that become airborne to infect grains and seeds (Horn et al., 2001).

The potential to reduce aflatoxin contamination in maize using the biological control tactics mentioned above has been evaluated in Benin, where 90% of the *Aspergilli* are *A. flavus* (Cardwell and Cotty, 2002). The nonaflatoxigenic strains of *A. flavus* (BN22 from Benin and AF36 from the U.S.) were tested against aflatoxigenic strains of *A. flavus* (BN40 from Benin and AF13 from the U.S.) and *A. parasiticus* (BN48) in vitro (Bandyopadhyay and Cardwell, 2003; Cardwell and Cotty, 2000). All nonaflatoxigenic isolates significantly reduced toxin production by the African *A. parasiticus* isolate BN48. In vitro, the American nonaflatoxigenic isolate AF36 was effective against the American aflatoxigenic isolate AF13, but not the aflatoxigenic African S-strain, BN40, suggesting that there may be specificity of action of some nonaflatoxigenic strains. The African nonaflatoxigenic L-strain BN30 was the only isolate that reduced toxin production by the highly aflatoxigenic African S-strain, BN40. BN30 was also very effective in reducing the amount of toxin produced in maize in the field when coinoculated with the highly toxic S-strain (Bandyopadhyay and Cardwell, 2003). Selected nonaflatoxigenic strains specific for different agro-ecozones need to be identified and tested in large areas.

This technology has a potential to remove aflatoxin from foods grown in high-risk environments. The additional benefit of this technology is that it would reduce aflatoxin levels in maize, groundnut, and cotton, where they are intercropped and rotated. It would not eliminate the causal fungi from the commodities, but only reduce direct impact by toxic contamination. Additionally, the logistics of deployment are a challenge, requiring a concerted effort by governments, industry, and the international development sector (Food and Drug Administration, 1996).

Host resistance and genetic engineering are the technologies that would give the most easily deployable solution if and when aflatoxin or fungal resistances are found. Plant breeders have been using traditional techniques for over 25 years to try to develop plant cultivars that are resistant to aflatoxin. Progress has been made in maize (Brown et al., 2001), but it is

open to discussion as to whether traditional plant breeding will ever provide sufficient resistance to be a solution in and of itself.

New genetic tools, however, may provide important new advances in plant genetic engineering for resistance. Engineering of maize and cotton to reduce lepidopteran damage and is expected to bring concomitant parallel benefits of reduced mycotoxin contamination (Munkvold, 2003). However, insect vectoring is not the only mechanism of ingress of the fungus, and therefore insect control is only one factor in reducing contamination levels (Cardwell et al., 2000; Payne, 1998).

It may eventually be possible to engineer plants for reduced fungal ingress and survival by inserting genes for antifungal compounds or aflatoxin blockers. A West African inbred maize line was found to support fungal infection, but with little aflatoxin formation (Brown et al., 2001). Other resistance mechanisms such as resistance to the infection process, and resistance to environmental stresses (Wicklowsky, 1994) may be available to build into maize to reduce aflatoxin development in the field.

In the case of developing countries where maize is a primary staple food, with little option for food basket diversification, it may be necessary to rethink the paradigm of how to breed for increased resistance. Often, to increase yield, breeders have selected for increased kernel size (Cardwell et al., 2000). This tends to increase susceptibility to silk-cut (Odyssey et al., 1997) and other types of pericarp and testa breaks facilitating ingress of *A. flavus*. An alternative strategy, particularly for tropical agro-ecologies, would be to increase yield by increasing kernel density and number, but not kernel size. The advantage of this would be improved postharvest process; reduced damage during shelling, quicker drying, increased postharvest insect resistance, etc. The importance of reducing susceptibility in food crops cannot be overstated.

V. CONCLUSIONS

The CODEX standards for aflatoxins in foods were put in place because of the possible carcinogenicity of aflatoxins as observed in animal models, and in geographical correlations of risk of liver cancer with probability of food-borne exposure. The most recent epidemiology has cast some doubt on the relative importance of aflatoxin as an independent causal factor in human liver cancer. Reducing aflatoxin levels in foods by regulation may not be the most effective means to reduce risk of human liver cancer. In a 1998 analysis of liver cancer, aflatoxin, and hepatitis B, JECFA stated, "The carcinogenic potency of aflatoxin in HbsAg-negative individuals is substantially higher than the potency in HBsAg positive

individuals. Thus, reduction of the intake of aflatoxin in populations with a high prevalence of HbsAg-negative individuals will have greater impact on reducing liver cancer rates than reduction in populations with a low prevalence of HBsAg negative individuals.” This study further indicated that “populations with a low prevalence of HbsAg-positive individuals and/or with a low mean intake of aflatoxin (less than $1 \text{ ng kg}^{-1} \text{ bw}$) are unlikely to exhibit detectable differences in population risks for standards in the range of $10\text{--}20 \text{ ug kg}^{-1}$.” Therefore, JECFA has recommended that developing countries could effectively lower liver cancer incidence by vaccinating for HBV. The development of a global alliance for vaccines and immunization is a hopeful beginning (Hall and Wild, 1994).

The macroeconomic consequence of high child morbidity and mortality on a country more than overshadows the costs of surveillance and crop management practices to reduce risk of exposure to aflatoxins. Child survivorship and developmental health is one of the cornerstones of stable population growth and economic development. The studies by (Gong et al. 2002, 2003) revealed a striking association between exposure to aflatoxin and both stunting and being underweight in children in West Africa. Given the immunotoxicity of aflatoxin in animal models (Pestka and Bondy, 1994), aflatoxin may have the potential to suppress immune system development in human children as well. In the West African study, 99% of the children tested were positive for serum-aflatoxin adducts. These observations emphasize the need to develop strategies to reduce exposure to aflatoxin, possibly involving interventions targeted at the postweaning period in African children (Gong et al., 2002, 2003).

The bottom line is that liver cancer may not be the only important public health effect of aflatoxin. Evidence is accumulating that aflatoxin effects on child growth in developing countries, such as West Africa, in populations chronically exposed to high levels of aflatoxin may be equally important to overall public health. Regulatory standards for aflatoxins are justified, and should be extended to countries not currently in compliance, but these must be accompanied by the research and development in the agricultural sector to assure a food supply that is not contaminated. The protection offered by the regulatory standards in developing countries has to be balanced against the loss of contaminated crops to human food and the loss of export income.

Current regulatory action levels for aflatoxins in developed countries are offering adequate protection from liver cancer as a well as protecting child development and growth. If current regulatory standards are lowered in developed countries, the impact of this action on developing countries should be considered in the context of global public health and the global economy.

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