FOOD SAFETY

doi: 10.14252/foodsafetyfscj.2015026

Review (Invited)

Aflatoxin Exposure and Associated Human Health Effects, a Review of Epidemiological Studies

Yun Yun Gong[a], Sinead Watson[a], and Michael N Routledge[b]

[a]Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, UK;
[b]Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, UK

Aflatoxins are fungal toxins that possess acute life threatening toxicity, carcinogenic properties and other potential chronic adverse effects. Dietary exposure to aflatoxins is considered a major public health concern, especially for subsistence farming communities in sub-Saharan Africa and South Asia, where dietary staple food crops such as groundnuts and maize are often highly contaminated with aflatoxin due to hot and humid climates and poor storage, together with low awareness of risk and lack of enforcement of regulatory limits. Biomarkers have been developed and applied in many epidemiological studies assessing aflatoxin exposure and the associated health effects in these high-risk population groups. This review discusses the recent epidemiological evidence for aflatoxin exposure, co-exposure with other mycotoxins and associated health effects in order to provide evidence on risk assessment, and highlight areas where further research is necessary. Aflatoxin exposure can occur at any stage of life and is a major risk factor for hepatocellular carcinoma, especially when hepatitis B infection is present. Recent evidence suggests that aflatoxin may be an underlying determinant of stunted child growth, and may lower cell-mediated immunity, thereby increasing disease susceptibility. However, a causal relationship between aflatoxin exposure and these latter adverse health outcomes has not been established, and the biological mechanisms for these have not been elucidated, prompting further research. Furthermore, there is a dearth of information regarding the health effects of co-exposure to aflatoxin with other mycotoxins. Recent developments of biomarkers provide opportunities for important future research in this area.

Key words: aflatoxin exposure; biomarker; child growth; developing countries; hepatocellular carcinoma; immune suppression

---

Received: 30 December 2015; Accepted: 23 February 2016; Published online: 30 March 2016
* Corresponding author: Michael N Routledge, Ph.D; Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, UK; E-mail: medmnr@leeds.ac.uk
The contents of this article reflect solely the view of the author(s).
Abbreviations: AF-alb, aflatoxin-albumin adduct; AFB1, aflatoxin B1; AFB2, aflatoxin B2; AFG1, aflatoxin G1; AFG2, aflatoxin G2; AFM1, aflatoxin M1; AFM2, aflatoxin M2; CI, confidence interval; CIT, citrinin; DNA, deoxyribonucleic acid; DON, deoxynivalenol; ELISA, enzyme-linked immunosorbent assay; FB1, fumonisin B1; GM, geometric mean; H(L)AZ, height (length)-for-age Z-score; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HPLC, high performance liquid chromatography; IGF, insulin-like growth factor; LOD, limit of detection; MS, mass spectrometry; ND, non detectable; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; OTA, ochratoxin A; PAR, population attributable risk; sIgA, secretory IgA; WAZ, weight-for-age Z-score; WHO, World Health Organisation; WHZ, weight-for-height Z-score; ZEN, zearalenone

1. Introduction

Aflatoxins are the secondary metabolites of the *Aspergillus flavus* and *A. parasiticus* fungi. They contaminate major cereal food crops including maize, tree nuts and groundnuts. They are highly prevalent in tropical regions, specifically sub-Saharan Africa and South East Asia, where they flourish under the hot and humid conditions that stimulate fungal growth[1]. The main types of aflatoxins are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2), which are found in food. Aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2), which are hydroxylated metabolites of AFB1 and AFB2, respectively, can be found in milk. AFB1, the most common type of the family, is also the most toxic. AFB1 has been recognised by the International Agency for Research on Cancer as a human carcinogen, and AFM1 is considered to be a 'possible carcinogen'[2].

Aflatoxin is a global food safety concern as recognised by the World Health Organisation (WHO)[3], with rural subsistence farming communities in developing countries being the populations most at risk of aflatoxin exposure. Staple foods that are susceptible to aflatoxin contamination, food insecurity, low aflatoxin awareness and lack of enforcement of regulatory limits are some of the contributors to the high level of aflatoxin exposure in these populations. Since it was first identified in the 1960s, knowledge of aflatoxin characterisation, metabolism, toxicity to human and animals, and prevention has made great advances. As a result of the development of biomarkers, it has been possible to measure aflatoxin exposure individually and study aflatoxin associated adverse health effects. A series of epidemiology studies have been conducted in the last three decades[4–6]. The recent epidemiological evidence for the role of aflatoxin in primary liver cancer, child growth impairment and immune suppression will be discussed in this review. Aflatoxin reduction interventions that are inexpensive, culturally acceptable and easy to implement at the population-level are required. To date, a number of agricultural and dietary interventions have been proposed[7]. Biomarkers are important tools to determine the true impact of the aflatoxin reduction interventions on human health. However, only one study has used biomarkers to evaluate the effectiveness of an intervention, which encompassed a package of postharvest aflatoxin reduction methods[8].

2. Exposure Measurement

Dietary exposure to aflatoxin can be estimated by analysis of food sample contamination levels combined with dietary intake surveys, such as 24-hour recalls and food frequency questionnaires. Although these methods can be effective in estimating aflatoxin exposure levels in large samples, they have many limitations. For instance, aflatoxin contamination in food typically has a heterogeneous distribution that can confound accurate measurement of contamination levels due to uneven sampling. Dietary intake surveys are subject to recall bias and social desirability issues, which additionally could lead to an under- or over-estimation of actual exposure.

Biomarkers are considered more accurate for measuring the degree of individual exposure, as they are objective indicators of exposure and are key determinants of internal dose and biologically effective dose. The biomarkers for aflatoxin exposure include the aflatoxin-N^7-guanine adducts excreted in urine, which reflect the previous day's exposure; AFM1, the hydroxylated metabolite of AFB1, which is found in breast milk and reflects exposure over the previous 24 hours; and the aflatoxin-albumin adduct (AF-alb) in plasma or serum, of a half-life of ~2 months, which permits the measurement of more chronic exposure to aflatoxin[6]. The AF-alb biomarker can be detected by an ELISA method and is expressed as pg AF-alb/mg albumin, or in some cases, pg AF-lysine equivalent/mg albumin[9]. This method has been well validated in many population-based studies, and the AF-alb biomarker shows a good correlation with aflatoxin intake from groundnut[4] and maize based diets[10]. Alternative methods for assessing aflatoxin exposure using the AF-alb adduct involve measuring the aflatoxin-lysine adduct (AFB1-lysine) in proteolytic digests of serum with HPLC-fluorescence or LC-MS/MS[11,12].

3. Worldwide Aflatoxin Exposure

The AF-alb biomarker has been used in a number of epidemiological studies to measure exposure and its associated health effects in many different populations groups. High risk populations are specifically those from rural subsistence farming communities in developing countries. In high-risk regions, AF-alb concentrations have previously been reported over a 2–3 log range from below the limit of detection (LOD) of 3 pg/mg to >1000 pg/mg[13]. East and West Africa

have the ideal climate of hot and humid conditions for *Aspergillus* growth and subsequent aflatoxin production, and intake of susceptible crops such as maize or groundnuts as staple foods is high. Reflecting this, it has previously been reported that over 95% of blood samples collected from different parts of West Africa, across different age groups, had detectable concentrations of AF-alb[1].

More recently, the AF-alb biomarker has revealed high aflatoxin exposure in East African countries including Kenya[14], where AF-alb was detected in 78% of 597 serum samples (non detectable (ND) − 211 pg/mg); in Uganda[15], where AF-alb was detected in 192/196 (98%) samples (range ND to 237.7 pg/mg) collected from adults and children; and in Tanzania[16], where AF-alb was detected in 67% to 99% of samples collected from young children (see **Table 1** for more details). In North and South Africa, where the climate is drier, the prevalence of aflatoxin exposure is lower than the levels observed in East and West Africa[17–19]. Turner et al[17] found AF-alb in 31/46 (67%) samples from Egypt (range ND–32.8 pg/mg). Piekkola et al[18] found AF-alb concentrations in 34/98 (35.6%) serum samples from pregnant Egyptian women in their third trimester, and Shephard et al[19] found no trace of AFM1 in urine samples collected from South African women (n = 53).

Parts of Asia also have high prevalence of aflatoxin exposure[1]; eg, in Malaysia, where 97% of 170 samples had detectable AFB1-lysine adduct levels (detected by HPLC-fluorescence), ranging between 0.20 to 23.26 pg/mg[20]. Furthermore, a study examining aflatoxin exposure in pregnant women in South Asia using isotope dilution mass spectrometry to measure AFB1-lysine[21], found detectable levels of the biomarker in 94% of blood samples collected from Nepalese pregnant women (n = 141), with levels ranging between 0.45 to 2939.30 pg/mg. In the same study, AFB1-lysine was detected in 63/63 (100%) samples collected from pregnant women in their first and third trimester from Bangladesh, as well as in 63/63 (100%) cord blood samples and in 63/63 (100%) infants who were born to the mothers exposed to aflatoxin during pregnancy. Rice is a dietary staple of Nepal and in Bangladesh; however, the occurrence of aflatoxin contamination in rice is typically low[22–24]. Other food commodities regularly consumed in Bangladesh such as betelnut, lentils and red chilli powder, however, have been shown to have high levels of aflatoxin[24]. Maize is also a dietary staple in Nepal, and research has shown high levels of aflatoxin contamination present in maize commodities from Nepal[23,25]

Aflatoxin exposure is not a major issue for developed regions, as there are strictly enforced regulatory limits in place and the diet is more diverse. AF-alb is rarely detected in blood samples from populations in these regions[1,26,27]. For instance, in a subset of individuals (n = 2051) that participated in the 1999–2000 National Health and Nutrition Examination Survey (NHANES), which is a representative cross-sectional survey of the US population[26,27], only 1% had detectable levels (≥0.02 μg/L) of AFB1-lysine in their blood.

4. Co-exposure with Other Mycotoxins

The majority of research to date has focused on the negative impact of single mycotoxins, predominantly aflatoxin, on health outcomes in human populations. However, several mycotoxins can occur simultaneously in food[28]. This is concerning, as regulations in place for food and feed products are based on single mycotoxins, failing to take into consideration possible combined toxic effects.

The scarcity of epidemiology studies measuring co-exposure to multiple mycotoxins and the potential adverse health outcomes, is partly attributable to the underdevelopment of valid biomarkers. The AF-alb biomarker was developed in the early 1990s[9], and has been used since to assess aflatoxin exposure and its associated health effects in many populations worldwide (see above). Whereas, individual biomarkers for other prevailing mycotoxins of public health concern such as fumonisin, predominantly found in maize, and deoxynivalenol (DON), typically found in wheat, were only developed in 2008[29,30]; thus application of these biomarkers into human exposure and health risk studies is still at an early stage.

Co-exposure to aflatoxin with other mycotoxins, using individual biomarkers, was recently investigated in Tanzania. Children, aged between 6 and 14 months, were recruited at a maize harvest season, and were followed up twice at 6 months intervals. The children were found chronically exposed to AFB1, fumonisin B1 (FB1) and DON[31,32]. Blood AF-alb level[31] and urinary DON level[32] steadily increased over the 12 months, which likely corresponds to increased food intake that typically occurs as the child gets older. A linear trend was not apparent for urinary FB1, as the mean level at 6 months was significantly lower than mean levels at recruitment and at 12 months[31]. Fumonisin contamination is predominantly a field issue, with levels only marginally increasing during storage. It was postulated that the lower exposure levels observed 6 months post-harvest could be reflective of dwindled maize stocks at this time point leading to lower maize consumption[31]. The relationship between aflatoxin and fumonisin exposure with impaired child growth was also

Table 1. The relationship between aflatoxin exposure and child growth

Study	Location/ study design/ population characteristics	Aflatoxin exposure	Prevalence of malnutrition (% <2 SD*)	Relationship between mycotoxin and child growth impairment
Gong et al[42)]	- Benin and Togo - Cross-sectional - 480 children aged 9 mo** to 5 y**	-AF-alb biomarker -Detected in 475/479 (99%) -GM (range): 32.8 (5–1064) pg/mg	29% WAZ <2 SD 33% HAZ <2 SD 6% WHZ <2 SD	- AF-alb was negatively correlated with WAZ ($P = 0.005$), HAZ ($P = 0.001$) and WHZ ($P = 0.047$).
Turner et al[44)]	- The Gambia - Cross-sectional study - 472 children aged 6–9 y	- AF-alb biomarker - Detected in 434/466 (93%) - GM (95% CI): 22.3 (20.3, 24.5) pg/mg	17.5% WAZ <2 SD 11.5% HAZ <2 SD 14.9% WHZ <2 SD	- AF-alb was negatively associated with WHZ score ($P = 0.028$). - After adjusting for month of sampling, sex, birth weight, and other factors, there was a decrease in WHZ score up to 21 pg/mg.
Gong et al[43)]	- Benin - Longitudinal (8 months) - 3 time points (February, June and October) - 200 children aged 16 to 37 mo	- AF-alb biomarker February: 98% detectable June: 99.5% detectable October: 100% detectable - GM February: 37.4 pg/mg June: 38.7 pg/mg October: 86.8 pg/mg	Not calculated	- AF-alb (measured at recruitment or the mean of 3 time points) was inversely associated with HAZ and WHZ scores (measured at the last time point). - After adjusting for age, height, sex and weaning status, socioeconomic status (SES) and village, a correlation remained between AF-alb measured at recruitment ($P = 0.009$) or the mean of 3 time points ($P < 0.0001$) and HAZ (measured at last time point). - Reduction in height was 1.7 cm over the 8 month period between the highest and lowest quartiles of aflatoxin exposure.
Okoth and Ohingo[66)]	- Kenya - Cross-sectional - 242 children aged 3 to 36 mo	- Aflatoxin contamination (B1, B2, G1 and G2) was measured in flour samples - Aflatoxin contamination was found in 70/242 (29%) flour samples. Levels ranged 2–82 μg/kg.	30% WAZ <2 SD 34% HAZ <2 SD 6% WHZ <2 SD	- 53.8% of the children classified as wasted were fed aflatoxin contaminated flour, compared to 27.7% of children not wasted ($P = 0.002$). - 60% of the children with severe protein energy malnutrition (PEM) were fed aflatoxin contaminated flour, compared to 27.4% of children without PEM ($P = 0.004$).
Turner et al[58)]	- The Gambia - Longitudinal (Birth to 1 y) - 138 children	- AF-alb biomarker - AF-alb detected n (%): Maternal samples: 119/119 (100) Cord blood samples: 48/99 (48.5) Week 16 children: 13/118 (11.0) - AF-alb GM (95% CI) pg/mg: Maternal blood samples: 40.4 (4.8, 260.8) Cord blood samples: 10.1 (5.0, 89.6) Week 16 children: 8.7 (5.0, 30.2)	No prevalence rates calculated	- Maternal blood samples with higher AF-alb concentrations compared to the samples with lower concentrations were associated with lower HAZ ($P = 0.044$) and WAZ ($P = 0.012$) scores in children. - Reducing AF-alb concentrations in maternal blood samples from 110 pg/mg to 10 pg/mg would lead to an increase of 0.8 kg in child weight and an increase of 2 cm in child height. - AF-alb concentrations measured in week 16 blood samples from the children were negatively associated with HAZ scores ($P = 0.002$).
Mahdavi et al[63)]	- Iran - Cross-sectional - 182 children aged 90–120 days - 182 lactating mothers	- AFM1 measured in breast milk samples - Detected in 20/91 (22%) samples - AFM1 mean (SD): 6.96 (0.94) pg/mL	Prevalence rates not calculated	- After adjusting for maternal BMI, maternal energy intake and maternal height, AFM1 was inversely associated with infant HAZ scores ($P = 0.01$). - Infants consuming AFM1 contaminated breast milk compared to those not, had lower HAZ ($P = 0.005$) and WAZ ($P = 0.01$) scores.

Table 1. (continue)

Study	Location/ study design/ population characteristics	Aflatoxin exposure	Prevalence of malnutrition (% <2 SD*)	Relationship between mycotoxin and child growth impairment
Shouman et al[67)]	- Egypt - Cross-sectional - 46 children aged 1 mo to 4.5 y. - 46 mothers	AFB1 serum samples - Children 17/46 (36.96%) detectable - Mothers 17/46 (36.96%) detectable Median (IQ***) - Children 51.61 (30.57−62.80) ppm - Mothers 50.0 (35.59−84.93) ppm	Prevalence rates not calculated	- AF-alb positive children had lower HAZ sores compared to AF-alb negative children (P = 0.001). - AF-alb concentrations were inversely correlated with HAZ scores (P = 0.001) among the children who had detectable AF-alb concentrations.
Magoha et al[38)]	- Tanzania - Longitudinal (1st, 3rd and 5th month following birth) - 143 children aged under 6 mo - 143 lactating mothers	- AFM1 measured in breast milk samples - AFM1 detected: 1st month: 143/143 3rd month: 121/121 5th month: 118/118 - AFM1 median (IQ) ng/mL: 1st month: 0.07 (0.05–0.11) 3rd month: 0.08 (0.05–0.13) 5th month: 0.08 (0.06–0.12) - AFM1 mean (SD) exposure ng/kg bodyweight/day): 1st month: 11.08 (10.13) 3rd month: 11.94 (9.69) 5th month: 10.91 (6.82)	1st month 11% HAZ <2 SD 4% WAZ <2 SD 4% WHZ <2 SD 3rd month 13% HAZ <2 SD 9% WAZ <2 SD 1% WHZ <2 SD 5th month 17% HAZ <2 SD 10% WAZ <2 SD 3% WHZ <2 SD	- AFM1 was inversely associated with WAZ and HAZ scores (P < 0.05).
Shirima et al[31)]	- Tanzania - Longitudinal (recruited at maize harvest and followed up at 6, 12 months) - 166 children (n = 166) aged between 6 and 14 mo	- AF-alb biomarker - AF-alb detected n (%): Recruitment: 98 (67%) 6 months: 122 (84%) 12 months: 142 (99%) - AF-alb GM (95% CI) pg/mg: Recruitment: 4.7 (3.9, 5.6) 6 months: 12.9 (9.9, 16.7) 12 months: 23.5 (19.9, 27.7)	Recruitment 44% HAZ <2 SD 8% WAZ <2 SD 2% WHZ <2 SD 6 months 55% HAZ <2 SD 14% WAZ <2 SD 2% WHZ <2 SD 12 months 56% HAZ <2 SD 14% WAZ <2 SD 0.7% WHZ <2 SD	- There were no significant associations between mean AF-alb levels and growth parameters (WHZ, LAZ or WAZ score) at any time point.
Castelino et al[68)]	- Kenya - Cross-sectional - 199 children aged 6 to 17 y	- AF-alb biomarker - AF-alb GM (95% CI) pg/mg: 110.5 (95.4, 127.9)	No prevalence of malnutrition recorded	- Children with higher AF-alb levels (>198.5 pg/mg) had lower height measurements than children with lower levels (<74.5 pg/mg), after adjusting for age, sex, school, disease state and infection status (P < 0.001).

* SD: standard deviation; ** mo: month, y: year; *** IQ: interquartile

examined in this cohort of young children. Although there was no relationship observed between aflatoxin exposure and stunted child growth; increased fumonisin exposure was associated with reduced length-for-age Z-scores (LAZ)[31].

The first 1000 days of an infant's life, from conception to 24 months of age, is a critical time to ensure prospective growth and healthy development. Co-exposure to mycotoxins *in utero* has not been researched extensively. Only one study has been identified, which has measured co-exposure of aflatoxin and DON in pregnant women[18]. That study was conducted in Egypt, with samples taken in the women's third trimester. AF-alb were present in 36% of the blood samples; urinary AFM1 and DON were present in 47% and 68% of the urine samples. Under half (41%) the sample of pregnant women was concurrently exposed to both aflatoxin and DON. These prevalence rates and exposure levels are considerably lower than those observed in West African countries, especially for aflatoxin; where typically >95% of blood samples test positive for AF-alb[1]. Nevertheless, it is concerning that these pregnant Egyptian women are exposed to two mycotoxins simultaneously.

Within recent years, advances in multi-mycotoxin biomarker analysis using LC-MS/MS methods have emerged to allow mycotoxin co-exposure to be assessed in urine samples. For instance, a study[19] conducted in a South African population of women (n = 53), found 8 single or combined mycotoxins in urine samples including: DON, FB1, ochratoxin A (OTA) and zearalenone (ZEN). AFM1 was not detected in the urine; *Aspergillus flavus* does not typically infect maize in the region sampled. Another study conducted in Cameroon, Central Africa, detected 11 single or combined mycotoxins and their metabolites in 63% of 175 urine samples including AFM1, OTA and DON[33]. At least two or more types of mycotoxins were detected in 18% of the samples and one individual was positive for five mycotoxins. Similar results were found in a study of children, aged 1.5–4.5 years, also from Cameroon[34]. Ediage et al[34] additionally investigated the relationship between mycotoxin exposure and indicators of nutritional status in the same cohort of children. Although the high prevalence rates of underweight (37%), wasting (23%) and stunted growth (39%) coincided with the high prevalence of mycotoxin exposure in this population group, no significant relationships were identified. Ediage et al[34] concluded that the power of the study to detect significant relationships might have been compromised by the small sample size.

A pilot study[35] of 120 Nigerian children (n = 19), adolescents (n = 20) and adults (n = 81) from rural farming communities, found 8 mycotoxins and their metabolites, either singly or combined, in 61/120 (50.8%) urine samples. AFM1, FB1 and OTA were the mycotoxins most frequently detected. Owing to the wide age range of the participants recruited (1 to 80 years), this study provides evidence that co-exposure to aflatoxin with other mycotoxins can occur at any stage of life.

It is worth noting that these positive detection rates can also be influenced by the sensitivity of the analytical method. As detection methods improve more samples are likely to be recorded as being positive. However, it is also important to consider the quantitative levels of exposure. It is possible that low levels of co-exposure, whilst detectable, do not contribute significantly to risks associated with mycotoxin exposures. Whilst reporting the percentage of populations co-exposed to different mycotoxins highlights that potential additive effects should be taken into consideration, research is required to determine at what levels such exposures may contribute to risk. The occurrence of co-exposures in a high proportion of a population does not in itself indicate that all the mycotoxins detected are contributing to risk.

Aflatoxin exposure was not found in multi-mycotoxin exposure studies conducted in non-African countries. In a recent large study in Belgian adults and children[36] nine different mycotoxins were detected in urine samples but not AFM1. Citrinin (CIT), DON and OTA were the most frequently detected. This is in line with the rest of the literature, which suggests that aflatoxin exposure is not a major public health issue for European countries as it is for Africa and South Asia.

Multi-mycotoxin biomarker analysis is very much in its infancy, and the methods do require more validation, but the data described above shows the potential of this approach for assessing combined exposures. At present, due to their higher sensitivity, available single biomarkers, such as FB1, DON and AF-alb, are more frequently applied for studying the effects of exposure and co-exposure on human health.

5. Health Effects

The main route of exposure to aflatoxin is through the direct consumption of contaminated food. Aflatoxin exposure can occur throughout the life course, beginning *in utero* through transplacental exposure[37]. Breast milk is a pathway of exposure for young children at breastfeeding[38]; however, the AFM1 found in milk is less toxic than AFB1

found in food. In Africa, weaning foods are often cereal- and legume-based, both of which are susceptible to aflatoxin contamination and children's exposure increases during weaning[39].

High level exposure of aflatoxin that occurs over a relatively short period of time is recognised as causing acute aflatoxicosis. Although acute aflatoxicosis occurs on a case by case basis intermittently, large outbreaks have been reported in Africa[40,41]. For instance, in Eastern Kenya in 2004, 317 individuals were diagnosed with acute liver failure of which 125 (37%) subsequently died as a result of acute aflatoxicosis. The level of aflatoxin exposure (AF-alb adduct) was higher in patients than in healthy individuals[41]. A case-control study showed that the outbreak in Kenya may have been triggered by consuming aflatoxin contaminated home-grown maize[41]. Mean aflatoxin contamination levels in home-grown maize samples, collected from patients with aflatoxicosis, were 8 fold higher than those samples collected from those free from aflatoxicosis (354.53 ppb vs 44.14 ppb; $P = 0.04$). Chronic aflatoxicosis due to low dose aflatoxin exposure over a long period of time, is more prevalent than acute aflatoxicosis. The most well established health effect of chronic exposure is hepatocellular carcinoma (HCC). Other chronic toxic health effects include impaired child growth[42,43] and immune suppression[44–46], which will be discussed in detail below.

5.1 Hepatocellular Carcinoma

In 2012, HCC was recognised as the sixth most common cancer worldwide, with 83% of cases occurring in less developed regions[47]. The highest incidence rates are observed in Asian and African countries. Aflatoxin, owing to its mutagenic and carcinogenic properties, has been classified as a major risk factor, alongside the hepatitis B virus (HBV) and the hepatitis C virus (HCV)[48]. In fact it has been shown that aflatoxin and hepatitis B, which is also highly prevalent in Africa and South Asia, can synergistically interact, resulting in an increased risk of HCC[49,50]. The P53 gene hotspot mutation at codon 249 was associated with aflatoxin exposure[51]. Villar et al observed a seasonal variation in levels of the R249S mutation in circulating cell free DNA in the serum of subjects in Gambia that reflected seasonal variations in aflatoxin exposure and markers of HBV infection, suggesting an interaction between these risk factors for HCC[52].

A systematic review and meta-analysis[53] of 17 case-control and cohort studies carried out in sub-Saharan Africa, China and Taiwan, examined the population attributable risk (PAR) of aflatoxin-related HCC. PAR represents the number or proportion of patients in a population that would not occur if the risk factor were removed. It was found that the PAR of aflatoxin-related HCC was 17%, but was higher in HBV positive populations (21%) in comparison with HBV negative populations (8.8%). This highlights the potential reduction in HCC that could be achieved by significantly reducing aflatoxin exposure.

A recent study[54] considered the impact in China of agricultural reforms during the 1980s, which involved the change from a maize based diet to a rice based diet (typically lower in aflatoxin contamination) and the implementation of the mass HBV immunisation program in that country, on the prevention of primary liver cancer. With the use of The Qidong Cancer Registry data and the measurement of AF-alb concentrations in serum samples collected from 7 different cohorts between 1982 and 2012, a PAR for the reduction in primary liver cancer mortality as a result of these changes was estimated to be 65%. Mean AF-alb concentrations also declined between 1989 and 2012 from 19.3 pg/mg to undetectable (<0.5 pg/mg). This study highlights that a reduction in aflatoxin exposure by changing diet together with control of HBV can achieve a large reduction in liver cancer prevalence.

Aflatoxin has also been implicated in the aetiology of other liver diseases including cirrhosis[2] and hepatomegaly[55]. A study in Kenya by Gong et al[55] reported that the prevalence of hepatomegaly, a firm form of liver enlargement, increased in children with higher aflatoxin exposure. This is consistent with the fact that the liver is the key target organ for aflatoxin toxicity.

5.2 Impaired Child Growth

The first 1000 days of life, from conception to 24 months, is a critical period for healthy growth and development; hence, dietary intake during pregnancy plays a fundamental role in the child's future health status. In sub-Saharan Africa, malnutrition and child growth impairment are major public health burdens. The WHO[56] have defined stunting as a height-for-age Z-score (HAZ), of <-2, being underweight as a weight-for-age Z-score (WAZ), of <-2, and wasting as a weight-for-height Z-score (WHZ), of <-2. The impact of aflatoxin on growth impairment at different time points has been investigated.

5.2.1 *In utero* exposure

A number of studies have demonstrated that aflatoxin exposure can occur *in utero* through a transplacental pathway[37,57,58], and that higher exposure levels *in utero* have been associated with lower birth weights[59–61] and stunted

child growth[58]. It has been suggested that epigenetic changes, which may occur as a consequence of aflatoxin exposure *in utero*, is a potential mechanism to explain this relationship. A recent study by Hernandez-Vargas et al[62] examined the consequences of *in utero* exposure to aflatoxin on the white blood cell DNA global methylation level in children aged 2–8 months. Differential methylation of genes, including some growth and immune function related genes, was observed to be associated with AF-alb exposure. It is not yet known whether such changes are associated with impaired growth or other effects.

5.2.2 Exposure via breast milk

Although breast milk is full of nutritional and immunological components, it is the potential source of aflatoxin exposure for very young infants. AFM1, the hydroxylated metabolite of AFB1, is typically detected in breast milk 12 to 24 hours following ingestion of foods contaminated with AFB1. Only a few epidemiological studies have investigated the relationship between AFM1 in breast milk samples and impaired child growth (see **Table 1**). Mahdavi et al[63] reported that AFM1 concentrations measured in breast milk samples of lactating Iranian women, who were exclusively breast feeding their children, were negatively associated with their infants' HAZ scores (correlation coefficient $\beta = -0.31$, $P = 0.01$). The number of positive AFM1 samples in this study, however, was very small (20/182, 11%), and insufficient information is provided on how the data was used, which makes interpretation difficult.

Magoha et al[38] examined the relationship between AFM1, measured in breast milk samples of 143 lactating mothers, and growth impairment in their infants under 6 months of age in Northern Tanzania. Breast milk samples along with anthropometric data were collected at the first, third and fifth month following birth. All of the breast milk samples had detectable AFM1 concentrations ranging from 0.01 to 0.55 ng/mL. Mean AFM1 exposure concentrations of the infants at months 1, 3 and 5 were: 11.08±10.13, 11.94±9.69 and 10.91±6.82 ng/kg bodyweight/day, respectively. Significant inverse associations between AFM1 exposure and HAZ ($\beta = -0.013$, $P < 0.05$) and WAZ ($\beta = -0.009$, $P < 0.05$) but not with WHZ were reported. It is not possible to measure individual levels of breast milk consumption in such a study therefore AFM1 exposure estimates were based on age-specific average intakes stated by the United States Environmental Protection Agency (US-EPA). This study highlights the potential for exposure of AFM1 from breast milk contributing to child growth impairment.

Although significant inverse associations were found by the aforementioned studies, further research is necessary to draw reasonable conclusions regarding this complex relationship. Breast-feeding should, therefore, not be discouraged based on this limited evidence. The WHO recommendation of exclusive breast-feeding until 6 months of age should be encouraged owing to the high nutritional content and immunological properties of breast milk. Furthermore, infants who are exclusively breastfed appear to have lower AF-alb concentrations compared to those partially breastfed and fully weaned[39]. It must, therefore, be remembered that prolonged breastfeeding is protective to child health.

5.2.3 Exposure via weaning food

Children of weaning age in developing countries, especially sub-Saharan Africa, are considered a high-risk population group for aflatoxin exposure[39]. Maize and groundnuts, which are typical constituents of weaning foods, are highly susceptible to aflatoxin contamination[64]. Furthermore, exposure levels relative to body weight are higher for children than for adults and the rapid growth that occurs, and the additional nutrients required during this time period, mean that this is a critical time for the impact of aflatoxin on growth. It is evident that stunted growth is highly prevalent in parts of Africa and South Asia. For instance, in East and West Africa approximately 42% and 36%, respectively, of the children under the age of 5 years have stunted growth[65]. This is similar to the rates observed in South-central Asia, where approximately 36% of children aged under the age of 5 years have stunted growth[65].

There is evidence to suggest that aflatoxin exposure during this critical period of weaning may be an underlying determinant of impaired child growth (see **Table 1**). In a cross-sectional study of 480 children aged between 9 months and 5 years in Benin and Togo, aflatoxin exposure levels increased when children started on weaning food, peaking when children reached 3 years old. Multivariate regression analysis suggested that aflatoxin exposure was inversely correlated with HAZ, WAZ and WHZ after adjustment of confounding factors[42]. Okath and Ohingo[66], who recruited a sample of children (n = 242) aged between 3 and 36 months, found that the number of children who were wasted compared to those who were not wasted, were more likely to consume aflatoxin contaminated weaning flour (53.8% vs 27.7%, $P = 0.002$). In a more recent study, Egyptian children (n = 46) exposed to aflatoxin, aged 1 month to 4.5 years, had significantly lower HAZ scores compared to those who were not exposed to aflatoxin ($P = 0.001$)[67].

To date only two studies have investigated the temporal relationship between aflatoxin exposure and impaired child growth (see **Table 1**)[15,43]. One of the studies[43], which was conducted in Benin, West Africa over 8 months, found significant inverse associations between mean AF-alb measured at recruitment ($P = 0.009$) and mean of 3 time points

(February, June and October; $P < 0.0001$), and HAZ scores (measured at the last time point) in children (n = 200) aged between 16 and 37 months. The children in the highest quartile of aflatoxin exposure had a 1.7 cm reduced height gain over 8 months compared to those in the lowest quartile. This study provided stronger evidence than the cross sectional studies for the growth impairment effect of aflatoxin. The second study[15], which was conducted over 12 months in Tanzania, East Africa, where high fumonisin exposure has been shown to be frequent due to consumption of highly contaminated maize reported reduced growth associated with aflatoxin exposure in children aged between 6 and 14 months, although not reaching statistical significance, but found a significant inverse association between growth and urinary fumonisin B1. A probable explanation is that the AF-alb levels observed in the Tanzanian children were lower than those (see **Table 1**) observed in the Benin children, indicating that the relationship between aflatoxin exposure and impaired child growth might indeed be dose-dependent. Alternatively, it is of note that fumonisin exposure was not measured in the Benin study, so the confounding contribution of this mycotoxin cannot be ruled out.

The effects of aflatoxin on growth have also been investigated in older children. Turner et al[44] found a significant association between AF-alb and WHZ ($P = 0.028$), but not with HAZ or WAZ scores in Gambian children (n = 466). The children in that study were aged between 6 and 9 years. Another study in Kenyan children (n = 199) aged 6 to 17 years old found a borderline inverse correlation between aflatoxin exposure and child height ($P = 0.048$)[68]. Taken together these studies highlight that weaning is a critical period for the growth impact of aflatoxin exposure.

5.2.4 Potential mechanisms

It has been suggested that aflatoxin exposure may disrupt the insulin-like growth factors (IGF) pathway through liver toxicity. In the study in Kenya mentioned above[68] AF-alb concentrations were inversely associated with IGF1 levels ($P = 0.039$) and IGF binding protein 3 levels ($P = 0.046$). A path analysis showed that lower IGF1 levels explained about 16% of the effect of aflatoxin on child height. Other potential mechanisms for the aflatoxin child growth impairment include the immunosuppressive effect of aflatoxin exposure that may increase infection susceptibility, consequently impairing nutritional status through appetite suppression and reduced nutrient absorption[69]. Furthermore, in their review Smith et al[70] postulated that exposure to aflatoxin may promote intestinal damage through protein synthesis inhibition, consequently leading to a reduction in the absorption of essential nutrients and subsequent impaired growth.

Based on the evidence above, it is difficult to establish if the relationship between aflatoxin exposure and impaired child growth is in fact causal. The 1000 days is a critical period for healthy growth and development that can be impacted on by aflatoxin exposure. An intervention study targeted at aflatoxin reduction would confirm whether the relationship is causal.

5.3 Immune Suppression

The immunosuppressive effects of aflatoxin, which include reduced antibody production, increased susceptibility to infectious diseases and reduced cell-mediated immunity, have been thoroughly investigated in many animal species[71]. In humans the immunosuppressive effects of aflatoxin exposure, however, have not clearly been established, as only a limited number of studies have been carried out (see **Table 2**). A study conducted in the Gambia[44] found a reduction in the secretory IgA (sIgA) antibody in children (aged 6 to 9 years) with detectable AF-alb concentrations in their blood (n = 432) compared to those with non-detectable levels (n = 32). The sIgA is an important component of the mucosal barrier that protects against infectious diseases and uptake of harmful micro-organisms. This reduced level of sIgA driven by aflatoxin exposure could be a potential mechanism for the impaired child growth that was also observed in this cohort. An earlier study[72] conducted in the Gambia found an association between aflatoxin exposure and HB surface antigen carrier status in children aged 3 to 8 years. Aflatoxin exposure, hence, may increase the risk of HBV infection. This is concerning as individuals exposed to aflatoxin and HBV simultaneously, have a greater risk of hepatocellular carcinoma compared to individuals exposed only to aflatoxin[49,50].

There is evidence to show that aflatoxin exposure may alter the proportions of specific cell types involved in the immune response (see **Table 2**). A study of Ghanaian adults (n = 64)[45] found that those exposed to high levels of aflatoxin, measured by the AF-alb adduct biomarker, compared to those exposed to low levels of aflatoxin, had significantly lower percentages of CD3+CD69+ and CD19+CD69+ cells ($P = 0.002$), and lower percentages of CD8+ type T lymph cells that contained perforin or both perforin and granzyme A ($P = 0.012$). Furthermore, negative associations were observed between CD3+CD69+ ($P = 0.001$) and CD19+CD69+ ($P = 0.032$) cells and AF-alb concentrations after adjustment for age and other immune parameters. Reductions in these immunological parameters could consequently lead to impaired cell-mediated immunity increasing susceptibility to infectious diseases.

Table 2. The immune suppressive effects of aflatoxin examined in human epidemiological studies

Study	Location/ study design/ population characteristics	Aflatoxin exposure	Immune markers	Relationship between aflatoxin exposure and child growth impairment
Allen et al[72)]	- The Gambia - Cross-sectional - 391 children aged 3–8 y*	- AF-alb biomarker (n = 323) - Mean (log) 4.05 pg/mg	- Malaria − *Plasmodium falciparum* parasitaemia - Antibody titre − asexual stages of *P. falciparum* - HBV − HBsAg	- Mean AF-alb was significantly higher in children with *P. falciparum* parasitaemia compared with children no *P. falciparum* parasitaemia ($P = 0.01$). - Mean AF-alb levels were higher in children who were HbsAg +ve** than those who were HbsAg –ve**.
Turner et al[44)]	- The Gambia - Cross-sectional - 472 children aged 6–9 y	- AF-alb biomarker - Detected in 434/466 (93%) - GM (95% CI): 22.3 (20.3, 24.5) pg/mg	- Secretory IgA in saliva (sIgA) - Cell-mediated immunity (CMI) - Antibody responses to both rabies and pneumococcal polysaccharide vaccines	- Children with detectable levels of AF-alb had significantly lower sIgA compared to those with non-detectable AF-alb levels ($P < 0.0001$). - 1 of the pneumococcal antibody titers was weakly associated with AF-alb ($P = 0.05$).
Jiang et al[45)]	- Ghana - Cross-sectional - 64 adults aged 19 to 86 y	- AF-alb biomarker - Mean (SD***) AF-alb: 0.997 (0.40) pmol/mg	- Leukocyte immunophenotypes - Lymphoproliferative response of CD4+ T cells - Cytokine production by CD8+, CD4+ and CD3-CD56+ cells - Monocyte phagocytic function	- Participants with high levels of AF-alb, compared to those with low levels had lower percentages of CD3+CD69+ and CD19+CD69+ cells ($P = 0.002$), and lower percentages of CD8+ T cells that contained perforin or both perforin and granzyme A ($P = 0.012$). - AF-alb concentrations were negatively associated with CD3+CD69+ ($P = 0.001$) and CD19+CD69+ ($P = 0.032$) cells after adjustment for age and other immune parameters.
Jiang et al[46)]	- Ghana - Cross sectional - 116 HIV +ve adults: mean age (SD): 38.25 (9.44) y - 80 HIV –ve adults: mean age (SD): 40.77 (17.52) y	- AF-alb biomarker - HIV +ve mean (SD) AF-alb: 1.01 (0.53) pmol/mg - HIV –ve mean (SD) AF-alb: 1.01 (0.41) pmol/mg	- Leukocyte immunophenotypes - Cytokine production by CD8+, and CD3-CD56+ cells - Viral load	- HIV +ve participants with high aflatoxin exposure, had lower CD4+CD25+CD45RO+ regulatory T-cells ($P = 0.009$) and naïve CD4+CD45RA+CD62L+ T cells ($P = 0.029$), as well as lower percentages of B-cells ($P = 0.03$), compared to HIV +ve individuals with low aflatoxin exposure. - Among HIV +ve participants, AF-alb concentrations were inversely associated with perforin-expressing CD8+ T-cells ($P = 0.045$), T-regulatory cells ($P = 0.002$) and B-cells ($P = 0.012$).
Jolly et al[74)]	- Ghana - Cross-sectional - 155 HIV +ve adults - 159 HIV –ve adults	- AF-alb biomarker - HIV +ve mean (SD) AF-alb: 1.06 (0.60) pmol/mg - HIV –ve mean (SD) AF-alb: 0.91 (0.46) pmol/mg	- Viral load - CD4 count - Liver function parameters - HBV, HCV and malaria infection parameters	- HIV +ve participants with high AF-alb levels(>0.93 pmol/mg albumin based on group median) showed statistically significant increased odds of having higher HIV viral loads (OR, 2.84; 95% CI, 1.17−7.78) and higher direct bilirubin levels (OR, 5.47; 95% CI, 1.03−22.85) compared to the HIV+ group with lower AF-alb levels(<0.93 pmol/mg). - Higher levels of AF-alb were associated with lower levels of albumin ($P = 0.01$) as well as higher levels of total bilirubin ($P = 0.01$) and direct bilirubin ($P = 0.01$) in HIV +ve participants.
Keenan et al[75)]	- Ghana - Cross-sectional - 141 HIV positive adults	- AF-alb biomarker - Median AF-alb: 0.94 pmol/mg	- CD4+ T- cells - Viral load - Malaria - Tuberculosis - HBV - Pneumonia	- Participants in the highest AF-alb quartile had a higher risk of tuberculosis (HR, 3.39; 95% CI, 1.15−9.98; $P = 0.03$) compared to those in the lowest quartile.

* y: year; ** +ve: positive, –ve: negative; *** SD: standard deviation

The high prevalence of aflatoxin exposure coincides geographically with the high prevalence of the human immunodeficiency virus (HIV) in Africa. It has been suggested that the immunosuppressive effects of aflatoxin exposure may accelerate the progression of HIV[46,73]. There is evidence to show that HIV positive individuals with high aflatoxin exposure, have lower levels of immune markers such as CD4+ T regulatory cells ($P = 0.009$) and naive CD4 + T cells ($P = 0.029$), as well as lower percentages of type B lymph cells ($P = 0.03$), compared to HIV positive individuals with low aflatoxin exposure[46]. In the same study, negative relationships were observed between AF-alb and perforin-expressing CD8+ T cells ($P = 0.045$), T regulatory cells ($P = 0.002$) and B cells ($P = 0.012$) among HIV positive individuals. Furthermore, another study[74] conducted in Ghana showed that HIV positive individuals with high aflatoxin exposure, compared to those that were HIV positive but with low aflatoxin exposure, were more likely to have higher direct bilirubin levels, markers of liver disease and jaundice (odds ratio (OR), 5.47; 95% CI, 1.03–22.85), as well as higher HIV viral loads (OR, 2.84; 95% CI, 1.17–7.78). Keenan et al[75] also found in an HIV positive sample of adults from Ghana, that those with higher AF-alb levels had an increased risk of tuberculosis (hazard ratio (HR), 3.30; 95% CI, 1.34–8.11), compared to those with lower AF-alb levels.

The studies to date show that aflatoxin can influence some types of immune response, but more studies are needed, in particular with respect to the effect on antibody responses and in non-HIV populations, to determine whether aflatoxin plays a major role in suppressing immune status.

6. Conclusions and Suggestions for Future Research

Aflatoxin exposure as a result of contamination of staple cereal crops is a significant food safety issue for developing countries, especially within South Asia and sub-Saharan Africa. Very high exposure leads to acute toxicity that can be lethal. Chronic exposure to aflatoxin can occur at any age including *in utero*, and typically increases during weaning. Aflatoxin is an established risk factor for liver cancer, especially when HBV infection co-exists, and there is increasing evidence of other health impacts including child growth impairment and immune suppression.

Understanding the relationship between aflatoxin exposure and impaired child growth is complicated because aflatoxin exposure often coincides with poverty, hunger, poor food quality and infectious disease. To determine if the relationship between aflatoxin and impaired child growth is indeed causal, randomised controlled trials, focusing on aflatoxin reduction strategies in areas where there is high prevalence of aflatoxin exposure, will be necessary. It is also critical to further elucidate the mechanism behind the impaired growth effects of aflatoxin.

The immunosuppressive effects of aflatoxin exposure could increase susceptibility to infectious diseases, such as diarrhoea, reducing nutrient absorption leading to impaired child growth. Aflatoxin associated immunosuppression may also modify infection of HIV or progression to AIDS but more research is required to determine to what extent aflatoxin modifies immune response in healthy individuals, particularly in children.

Liver cancer risk has been reduced significantly in China over recent decades as a result of HBV vaccination and dietary changes that reduced aflatoxin exposure. In sub-Saharan Africa and South Asia HBV vaccination is also important to reduce liver cancer risk, but changes in dietary patterns among subsistence farming populations may not be feasible. Simple and low cost post-harvest interventions such as hand sorting, adequate drying and storing are perhaps more realistic methods in reducing aflatoxin exposure in these regions, and should be promoted.

Finally, the effects of co-exposure to aflatoxin with other mycotoxins are currently not well understood, but recent developments of biomarkers provide opportunities for important future research in this area. To enable human health studies in relation to co-exposures, sensitive, reliable and high throughput biomarker techniques are essential.

References

1. Wild CP, Gong YY. Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis. 2010; **31**: 71–82. [Medline]
2. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans; vol 56, Chemical agents and related occupations. IARC, Lyon. 2012; **100F**: 1–599.
3. WHO Foodborne diseases burden epidemiology reference group 2007–2015. WHO estimates of the global burden of foodborne diseases. Available at: http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/.
4. Wild CP, Hudson GJ, Sabbioni G, et al. Dietary intake of aflatoxins and the level of albumin-bound aflatoxin in peripheral blood in The Gambia, West Africa. Cancer Epidemiol Biomarkers Prev. 1992; **1**: 229–234. [Medline]

5. Groopman JD, Wild CP, Hasler J, Junshi C, Wogan GN, Kensler TW. Molecular epidemiology of aflatoxin exposures: validation of aflatoxin-N7-guanine levels in urine as a biomarker in experimental rat models and humans. Environ Health Perspect. 1993; **99**: 107–113. [Medline]
6. Routledge MN, Gong YY. Developing biomarkers of human exposure to mycotoxins. (Ed) S De Saeger, In *Determining mycotoxins and mycotoxigenic fungi in food and feed*, pp.225–244, Woodhead Publishing, Cambridge, UK, 2011.
7. Khlangwiset P, Wu F. Costs and efficacy of public health interventions to reduce aflatoxin-induced human disease. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2010; **27**: 998–1014. [Medline]
8. Turner PC, Sylla A, Gong YY, et al. Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in west Africa: a community-based intervention study. Lancet. 2005; **365**: 1950–1956. [Medline]
9. Chapot B, Wild CP. ELISA for quantification of aflatoxin-albumin adducts and their application to human exposure assessment. In: Techniques in Diagnostic Pathology, edited by Warhol, M., van Velzen, D., and Bullock, G.R. San Diego CA: Academic Press. 1991. p. 135–155.
10. Routledge MN, Kimanya ME, Shirima CP, Wild CP, Gong YY. Quantitative correlation of aflatoxin biomarker with dietary intake of aflatoxin in Tanzanian children. Biomarkers. 2014; **19**: 430–435. [Medline]
11. Sabbioni G, Ambs S, Wogan GN, Groopman JD. The aflatoxin-lysine adduct quantified by high-performance liquid chromatography from human serum albumin samples. Carcinogenesis. 1990; **11**: 2063–2066. [Medline]
12. Scholl PF, McCoy L, Kensler TW, Groopman JD. Quantitative analysis and chronic dosimetry of the aflatoxin B1 plasma albumin adduct Lys-AFB1 in rats by isotope dilution mass spectrometry. Chem Res Toxicol. 2006; **19**: 44–49. [Medline]
13. Turner PC, Flannery B, Isitt C, Ali M, Pestka J. The role of biomarkers in evaluating human health concerns from fungal contaminants in food. Nutr Res Rev. 2012; **25**: 162–179. [Medline]
14. Yard EE, Daniel JH, Lewis LS, et al. Human aflatoxin exposure in Kenya, 2007: a cross-sectional study. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2013; **30**: 1322–1331. [Medline]
15. Asiki G, Seeley J, Srey C, et al. A pilot study to evaluate aflatoxin exposure in a rural Ugandan population. Trop Med Int Health. 2014; **19**: 592–599. [Medline]
16. Shirima CP, Kimanya ME, Kinabo JL, et al. Dietary exposure to aflatoxin and fumonisin among Tanzanian children as determined using biomarkers of exposure. Mol Nutr Food Res. 2013; **57**: 1874–1881. [Medline]
17. Turner PC, Loffredo C, Kafrawy SE, et al. Pilot survey of aflatoxin-albumin adducts in sera from Egypt. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2008; **25**: 583–587. [Medline]
18. Piekkola S, Turner PC, Abdel-Hamid M, et al. Characterisation of aflatoxin and deoxynivalenol exposure among pregnant Egyptian women. Food Addi Contam Part A Chem Anal Control Expo Risk Assess. 2012; **29**: 962–971. [Medline]
19. Shephard GS, Burger HM, Gambacorta L, et al. Multiple mycotoxin exposure determined by urinary biomarkers in rural subsistence farmers in the former Transkei, South Africa. Food Chem Toxicol. 2013; **62**: 217–225. [Medline]
20. Leong YH, Rosma A, Latiff AA, Izzah AN. Associations of serum aflatoxin B1-lysine adduct level with socio-demographic factors and aflatoxins intake from nuts and related nut products in Malaysia. Int J Hyg Environ Health. 2012; **215**: 368–372. [Medline]
21. Groopman JD, Egner PA, Schulze KJ, et al. Aflatoxin exposure during the first 1000 days of life in rural South Asia assessed by aflatoxin B_1-lysine albumin biomarkers. Food Chem Toxicol. 2014; **74**: 184–189. [Medline]
22. Dawlatana M, Coker RD, Nagler MJ, Wild CP, Hassan MS, Blunden G. The occurrence of mycotoxins in key commodities in Bangladesh: surveillance results from 1993 to 1995. J Nat Toxins. 2002; **11**: 379–386. [Medline]
23. Koirala P, Kumar S, Yadav BK, Premarajan KC. Occurrence of aflatoxin in some of the food and feed in Nepal. Indian J Med Sci. 2005; **59**: 331–336. [Medline]
24. Roy M, Harris J, Afreen S, et al. Aflatoxin contamination in food commodities in Bangladesh. Food Addit Contam Part B. 2013; **6**: 17–23. [Medline]
25. Karki T, Sinha B. Mycotoxin contamination of foods and feeds in Nepal 1988. (eds) Semple RL, Frio AS. Hicks PA, Lozare JV. Mycotoxin prevention and control in food grains. Section 4. 1989.
26. Johnson NM, Qian G, Xu L, et al. Aflatoxin and PAH exposure biomarkers in a U.S. population with a high incidence of hepatocellular carcinoma. Sci Total Environ. 2010; **408**: 6027–6031. [Medline]
27. Schleicher RL, McCoy LF, Powers CD, Sternberg MR, Pfeiffer CM. Serum concentrations of an aflatoxin-albumin adduct in the National Health and Nutrition Examination Survey (NHANES) 1999-2000. Clin Chim Acta. 2013; **423**: 46–50. [Medline]
28. Speijers GJA, Speijers MHM. Combined toxic effects of mycotoxins. Toxicol Lett. 2004; **153**: 91–98. [Medline]
29. Gong YY, Torres-Sanchez L, Lopez-Carrillo L, et al. Association between tortilla consumption and human urinary fumonisin B1 levels in a Mexican population. Cancer Epidemiol Biomarkers Prev. 2008; **17**: 688–694. [Medline]
30. Turner PC, Burley VJ, Rothwell JA, White KL, Cade JE, Wild CP. Dietary wheat reduction decreases the level of urinary deoxynivalenol in UK adults. J Expo Sci Environ Epidemiol. 2008; **18**: 392–399. [Medline]
31. Shirima CP, Kimanya ME, Routledge MN, et al. A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. Environ Health Perspect. 2015; **123**: 173–178. [Medline]
32. Srey C, Kimanya ME, Routledge MN, Shirima CP, Gong YY. Deoxynivalenol exposure assessment in young children in Tanzania. Mol Nutr Food Res. 2014; **58**: 1574–1580. [Medline]
33. Abia WA, Warth B, Sulyok M, et al. Bio-monitoring of mycotoxin exposure in Cameroon using a urinary multi-biomarker approach. Food Chem Toxicol. 2013; **62**: 927–934. [Medline]
34. Njumbe Ediage E, Diana Di Mavungu J, Song S, Sioen I, De Saeger S. Multimycotoxin analysis in urines to assess infant exposure: a case study in Cameroon. Environ Int. 2013; **57-58**: 50–59. [Medline]

35. Ezekiel CN, Warth B, Ogara IM, et al. Mycotoxin exposure in rural residents in northern Nigeria: a pilot study using multi-urinary biomarkers. Environ Int. 2014; **66**: 138–145. [Medline]
36. Heyndrickx E, Sioen I, Huybrechts B, Callebaut A, De Henauw S, De Saeger S. Human biomonitoring of multiple mycotoxins in the Belgian population: Results of the BIOMYCO study. Environ Int. 2015; **84**: 82–89. [Medline]
37. Wild CP, Rasheed FN, Jawla MFB, Hall AJ, Jansen LAM, Montesano R. In-utero exposure to aflatoxin in west Africa. Lancet. 1991; **337**: 1602. [Medline]
38. Magoha H, Kimanya M, De Meulenaer B, Roberfroid D, Lachat C, Kolsteren P. Association between aflatoxin M1 exposure through breast milk and growth impairment in infants from Northern Tanzania. World Mycotoxin Journal. 2014; **7**: 277–284.
39. Gong YY, Egal S, Hounsa A, et al. Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning. Int J Epidemiol. 2003; **32**: 556–562. [Medline]
40. Ngindu A, Johnson BK, Kenya PR, et al. Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. Lancet. 1982; **319**: 1346–1348. [Medline]
41. Azziz-Baumgartner E, Lindblade K, Gieseker K, et al. Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ Health Perspect. 2005; **113**: 1779–1783. [Medline]
42. Gong YY, Cardwell K, Hounsa A, et al. Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. BMJ. 2002; **325**: 20–21. [Medline]
43. Gong Y, Hounsa A, Egal S, et al. Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. Environ Health Perspect. 2004; **112**: 1334–1338. [Medline]
44. Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. Modification of immune function through exposure to dietary aflatoxin in Gambian children. Environ Health Perspect. 2003; **111**: 217–220. [Medline]
45. Jiang Y, Jolly PE, Ellis WO, Wang JS, Phillips TD, Williams JH. Aflatoxin B1 albumin adduct levels and cellular immune status in Ghanaians. Int Immunol. 2005; **17**: 807–814. [Medline]
46. Jiang Y, Jolly PE, Preko P, et al. Aflatoxin-related immune dysfunction in health and in human immunodeficiency virus disease. Clin Dev Immunol. 2008; **2008**: 790309. [Medline]
47. Ferlay J, Soerjomataram I, Ervik M, et al. Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2014. Available from: http://globocan.iarc.fr, accessed on 19/11/2015.
48. Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. Best Pract Res Clin Gastroenterol. 2014; **28**: 753–770. [Medline]
49. Qian GS, Ross RK, Yu MC, et al. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epi Biom Prev. 1994; **3**: 3–10. [Medline]
50. Wu HC, Wang Q, Yang HI, et al. Aflatoxin B1 exposure, hepatitis B virus infection, and hepatocellular carcinoma in Taiwan. Cancer Epi Biom Prev. 2009; **18**: 846–853. [Medline]
51. Gouas D, Shi H, Hainaut P. The aflatoxin-induced TP53 mutation at codon 249 (R249S): biomarker of exposure, early detection and target for therapy. Cancer Lett. 2009; **286**: 29–37. [Medline]
52. Villar S, Le Roux-Goglin E, Gouas DA, et al. Seasonal variation in TP53 R249S-mutated serum DNA with aflatoxin exposure and hepatitis B virus infection. Environ Health Perspect. 2011; **119**: 1635–1640. [Medline]
53. Liu Y, Chang CC, Marsh GM, Wu F. Population attributable risk of aflatoxin-related liver cancer: systematic review and meta-analysis. Eur J Cancer. 2012; **48**: 2125–2136. [Medline]
54. Chen JG, Egner PA, Ng D, et al. Reduced aflatoxin exposure presages decline in liver cancer mortality in an endemic region of China. Cancer Prev Res (Phila). 2013; **6**: 1038–1045. [Medline]
55. Gong YY, Wilson S, Mwatha JK, et al. Aflatoxin exposure may contribute to chronic hepatomegaly in Kenyan school children. Environ Health Perspect. 2012; **120**: 893–896. [Medline]
56. WHO (2006) WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva: World Health Organization.
57. Denning DW, Allen R, Wilkinson AP, Morgan MRA. Transplacental transfer of aflatoxin in humans. Carcinogenesis. 1990; **11**: 1033–1035. [Medline]
58. Turner PC, Collinson AC, Cheung YB, et al. Aflatoxin exposure in utero causes growth faltering in Gambian infants. Int J Epidemiol. 2007; **36**: 1119–1125. [Medline]
59. De Vries HR, Maxwell SM, Hendrickse RG. Foetal and neonatal exposure to aflatoxins. Acta Paediatr Scand. 1989; **78**: 373–378. [Medline]
60. Abdulrazzaq YM, Osman N, Yousif ZM, Trad O. Morbidity in neonates of mothers who have ingested aflatoxins. Ann Trop Paediatr. 2004; **24**: 145–151. [Medline]
61. Shuaib FM, Jolly PE, Ehiri JE, et al. Association between birth outcomes and aflatoxin B1 biomarker blood levels in pregnant women in Kumasi, Ghana. Trop Med Int Health. 2010; **15**: 160–167. [Medline]
62. Hernandez-Vargas H, Castelino J, Silver MJ, et al. Exposure to aflatoxin B1 in utero is associated with DNA methylation in white blood cells of infants in The Gambia. Int J Epidemiol. 2015; **44**: 1238–1248. [Medline]
63. Mahdavi R, Nikniaz L, Arefhosseini SR, Vahed Jabbari M. Determination of aflatoxin M(1) in breast milk samples in Tabriz-Iran. Matern Child Health J. 2010; **14**: 141–145. [Medline]
64. Egal S, Hounsa A, Gong YY, et al. Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. Int J Food Microbiol. 2005; **104**: 215–224. [Medline]

65. UNICEF, WHO, World Bank Levels and trends in child malnutrition. Joint child malnutrition estimates. New York, NY: United Nations International Children's Fund; Geneva: World Health Organization; Washington DC: World Bank, 2012.
66. Okoth SA, Ohingo M. Dietary aflatoxin exposure and impaired growth in young children from Kisumu District, Kenya: Cross sectional study. Afr J Health Sci. 2004; **11**: 43–54. [Medline]
67. Shouman BO, El Morsi D, Shabaan S, Abdel-Hamid AH, Mehrim A. Aflatoxin B1 level in relation to child's feeding and growth. Indian J Pediatr. 2012; **79**: 56–61. [Medline]
68. Castelino JM, Routledge MN, Wilson S, et al. Aflatoxin exposure is inversely associated with IGF1 and IGFBP3 levels in vitro and in Kenyan schoolchildren. Mol Nutr Food Res. 2015; **59**: 574–581. [Medline]
69. Gong YY, Turner P. C, Hall AJ, Wild C.P. Aflatoxin exposure and impaired child growth in West Africa: An unexplored international public health burden? In: Leslie J.F., Banerjee R, Visconti A., editors. Mycotoxins Detection Methods, Management, Public Health and Agricultural Trade; 2008. p. 53–66.
70. Smith LE, Stoltzfus RJ, Prendergast A. Food chain mycotoxin exposure, gut health, and impaired growth: a conceptual framework. Adv Nutr. 2012; **3**: 526–531. [Medline]
71. Bondy GS, Pestka JJ. Immunomodulation by fungal toxins. J Toxicol Environ Health B Crit Rev. 2000; **3**: 109–143. [Medline]
72. Allen SJ, Wild CP, Wheeler JG, et al. Aflatoxin exposure, malaria and hepatitis B infection in rural Gambian children. Trans R Soc Trop Med Hyg. 1992; **86**: 426–430. [Medline]
73. Hendrickse RG, Maxwell SM, Young R. Aflatoxins and heroin. BMJ. 1989; **299**: 492–493. [Medline]
74. Jolly PE, Shuaib FM, Jiang Y, et al. Association of high viral load and abnormal liver function with high aflatoxin B1-albumin adduct levels in HIV-positive Ghanaians: preliminary observations. Food Addit Contam Part A Chemi Anal Control Expo Risk Assess. 2011; **28**: 1224–1234. [Medline]
75. Keenan J, Jolly P, Preko P, et al. Association between aflatoxin B1 albumin adduct levels and tuberculosis infection among HIV Ghanaians. Arch Clin Micro. 2011; 2.