Mycotoxins and human disease: a largely ignored global health issue

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Abstract

Aflatoxins, identified in the early 1960s, are secondary metabolites of Aspergillus flavus and Aspergillus parasiticus and contaminate a variety of staple foods, particularly maize and groundnuts, in low-income countries (6). Williams et al. (7) have estimated that 4.5 billion of the world’s population is exposed to aflatoxins. The aflatoxins occur mostly in tropical regions with high humidity and temperature and they accumulate post-harvest when food commodities are stored under conditions that promote fungal growth. The naturally occurring aflatoxins are AFB1, AFB2, AFG1 and AFG2, with AFB1 the most abundant, toxic and carcinogenic (6). AFM1 and AFM2, the hydroxylated products of AFB1 and AFB2, respectively, are found in milk and milk products. Since their identification, aflatoxins have been extensively studied in relation to liver cancer. However, in agriculture, other adverse effects, including toxicity, growth and immune impairment, have been widely reported and these end points are rightly of increasing focus in studies of exposed people (4,5,7). This review focuses on carcinogenesis but briefly reports on the related toxic effects in human populations.

Introduction

Mycotoxins contaminate the diet of a large proportion of the world’s population (1). In many low-income countries mycotoxins affect staple foods, including groundnuts (peanuts), maize (corn), other cereals and nuts, such that exposure is continuous and often at high levels. It is in these same regions that agricultural practices and regulation to control human exposure to mycotoxins are the least adapted to do so. Despite occasional high profile incidents such as acute poisoning outbreaks (2) or the presence of mycotoxins in nutritional supplements (3), mycotoxins have not been widely prioritized from a public health perspective in low-income countries. Where attention has been paid, it has been largely driven by the need to meet stringent import regulations to control exposure are largely non-existent or practically unenforceable. Aflatoxins are hepatocarcinogenic in humans, particularly in conjunction with chronic hepatitis B virus infection, and cause aflatoxicosis in episodic poisoning outbreaks. In animals, these toxins also impair growth and are immuno-suppressive; the latter effects are of increasing interest in human populations. FB have been reported to induce liver and kidney tumours in rodents and are classified as Group 2B ‘possibly carcinogenic to humans’, with ecological studies implying a possible link to increased oesophageal cancer. Recent studies also suggest that the FB may cause neural tube defects in some maize-consuming populations. There is a plausible mechanism for this effect via a disruption of ceramide synthase and sphingolipid biosynthesis. Notwithstanding the need for a better evidence-base on mycotoxins and human health, supported by better biomarkers of exposure and effect in epidemiological studies, the existing data are sufficient to prioritize exposure reduction in vulnerable populations. For both toxins, there are a number of practical primary and secondary prevention strategies which could be beneficial if the political will and financial investment can be applied to what remains a largely and rather shamefully ignored global health issue.

Abbreviations: AFB1-N7-Gua, 8,9-dihydro-8-(N7-guanyl)-9-hydroxy AFB1; CYP, cytochrome P450; FAPY, formamidopyrimidine; FB, fumonisins; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBx, hepatitis B x; HCC, hepatocellular carcinoma; LC-MS, liquid chromatography–mass spectrometry; NTD, neural tube defect; OC, oesophageal cancer; OR, odds ratio.
some of the toxic effects of AFB1 (10). Subsequent nuclear magnetic resonance studies showed that the form of AFB1–FAPY normally present in duplex DNA is mutagenic while the dominant species in single-stranded DNA is a block to replication (11).

AFB1 is more mutagenic and carcinogenic than AFG1, reflecting the fact that the AFB1 8,9-exo-epoxide intercalates more readily into DNA, yielding higher levels of adducts for a given dose. AFB2 and AFG2 are generally considered to be far less biologically active due to the absence of an 8,9 double bond and consequently 8,9-epoxide formation (8). Little is known about the importance of the reactive epoxide in relation to the non-mutagenic effects of aflatoxins.

Metabolism

The major human cytochrome P450 (CYP) enzymes involved in aflatoxin metabolism are CYP3A4, 3A5, 3A7 and 1A2 and the predominant site of metabolism is the liver (8,12). The overall contribution of these enzymes to AFB1 metabolism in vivo will depend on affinity and expression; CYP3A4 appears to be the most important, with the relative contribution of CYP3A5 varying by individual (12). Polymorphisms identified in the CYP3A5 promoter region were associated with different levels of aflatoxin biomarkers, suggesting this inter-individual variation could influence susceptibility to aflatoxin (13). Given the fact that aflatoxin is known to cross the placenta, it is also of interest that CYP3A7, a major CYP in human foetal liver, has the capacity to activate AFB1 to 8,9-epoxide (12,14).

Detoxification of the aflatoxin exo- and endo-epoxides is mainly through glutathione S-transferase-mediated conjugation with reduced glutathione (15). The exo- and endo-epoxides can also undergo rapid non-enzymatic hydrolysis to AFB1-8,9-dihydrodiol that in turn is subject to slow, base-catalysed ring opening to a dialdehyde phenolate ion. The dihydrodiol can react with the ε-amino group of lysine in serum albumin resulting in aflatoxin–albumin adducts, used as biomarkers (see below). A further metabolic step involves aflatoxin aldehyde reductase catalysing the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction of the dialdehydic phenolate ion to a dialcohol (16).

Biomarkers

Comprehension of aflatoxin metabolism in animals and humans provided a foundation for the development of biomarkers of exposure. The result is one of the best examples where exposure biomarkers have transformed understanding of the human cancer risk associated with an environmental carcinogen (17). However, it is noteworthy that the key epidemiological studies incorporating the biomarkers were only performed some 30 years after the structural identification of the aflatoxins. The need for fundamental knowledge of mechanisms to be rapidly translated into tools for exposure assessment would be a valuable lesson for other environmental exposures.

The validation of the biomarkers as measures of aflatoxin intake at the individual level in China and Africa was of critical importance (17). A number of biomarkers have been developed and applied, including urinary aflatoxins (AFM1, AFB1-N7-Gua, AFP1, AFQ1 and AFB1-mercapturic acid) and aflatoxin–albumin adducts. The latter biomarker can integrate exposure over a number of weeks, is applicable to small volumes of serum or plasma and is measurable by high throughput (17). It has thus found widespread application in epidemiological studies.

More recently, the association of aflatoxin exposure with specific mutations, notably a G to T transversion in the third nucleotide of codon 249 of the TP57 gene (codon 249ser mutation), has led to the application of this marker in population-based studies. It is still unclear as to the balance between AFB1 specifically targeting the third nucleotide of codon 249 and the codon 249ser mutation being selected in vivo due to a selective growth advantage for hepatocytes (18). In The Gambia, the 249ser mutation was detected in plasma DNA from apparently healthy subjects (19), with cirrhosis (~15%) and hepatocellular carcinoma (HCC) patients (~40%) (19).

Jackson et al. (20) similar found the mutant sequence in samples of plasma and HCC from patients from Qidong County, People’s Republic of China. The detection of the 249ser mutation in the plasma of non-cancer patients in these studies could reflect an early neoplastic event, chronic exposure to aflatoxin or a combination of both.

Exposure

The biomarkers mentioned above have revealed the extent and level of human aflatoxin exposure in a way that food analyses did not. Notably, in our own studies over the last 20 years using the aflatoxin–albumin adduct in different parts of West Africa in people of all ages, >95% of blood samples contained detectable adduct. High exposures have been seen elsewhere, notably in East Africa, China and parts of south-east Asia (see Table I). Nevertheless, despite nearly 50 years of research, the extent of the global exposure to this carcinogen is still poorly documented, hampering attempts to estimate the associated disease burden. Application of these biomarkers in a structured manner to characterize exposure regionally around the world would be of great value.

Aflatoxins are lipophilic, are able to cross the placental barrier and can be bioactivated in utero, as revealed by the presence of aflatoxin–albumin adducts in cord blood samples (24). In West Africa, this exposure has been shown to continue in infancy and once children are weaned they have a similar high prevalence and level of exposure as observed in adults (Table I). The period of breast-feeding is generally associated with lower levels of exposure (24) because the mother’s metabolism limits transfer of dietary aflatoxins into the milk. Overall, however, the observations leave no doubt children are chronically exposed to high levels of aflatoxins in areas where food contamination is endemic and that this pattern of exposure continues throughout life. Thus, in considering the burden of aflatoxin-related disease, one has to take account of the lifelong exposure. A variety of effects may be manifest at different times during the life course of an individual. Indeed, we have previously hypothesized that in utero AFB1 exposure could result in a clonal expansion of hepatocytes with a codon 249ser mutation, thus creating a mosaic liver, susceptible to further genetic modifications post-natally (27).

Hepatocellular carcinoma

Epidemiology

Estimates suggest >600 000 people die of liver cancer worldwide each year with a majority of cases occurring in China, south-east Asia and sub-Saharan Africa (33). Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV), representing >350 million (5% of the world population) and 170 million people, respectively, worldwide, are major risk factors; the fraction of HCC cases attributable to HBV and HCV has been estimated to be 23 and 20% in developed countries and 59 and 33% in developing countries (33).

International Agency for Research on Cancer classified naturally occurring aflatoxins as human carcinogens based on the evidence from animal studies, epidemiological studies in exposed populations and mechanistic data (6). In experimental animals, there was judged to be sufficient evidence for the carcinogenicity of AFB1, G1 and M1 and limited evidence for AFB2. Liver is the predominant tumour site in rats, mice, hamsters, trout, salmon, ducks, tree shrews and monkeys. Tumours at other sites, e.g. kidney, have been observed but are much less common. The carcinogenic potency of AFM1 is ~10-fold lower than that of AFB1.

Early ecological studies linking aflatoxin with liver cancer in sub-Saharan Africa and south-east Asia frequently did not take account of HBV infection or measure aflatoxin at the individual level. However, improvement of exposure assessment using biomarkers and the availability of prospective cohort studies in Asia revealed significant interactions between aflatoxins and chronic HBV infection in relation to HCC risk, with a more than multiplicative interaction being reported (34–36). Consistent with this, observations among hepatitis B surface antigen (HBsAg) carriers showed an increased HCC risk among those positive for aflatoxin biomarkers (37–40). However, in a more recent follow-up of the cohort in Taiwan with more HCC cases, Wu et al. (41)
Table I. Aflatoxin–albumin adducts in different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>No. subjects</th>
<th>AF–albumin (pg/mg); mean* (range)*</th>
<th>Frequency of positive samples (%)</th>
<th>Age group (years)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Gambia</td>
<td>2000</td>
<td>138</td>
<td>40 (5–261)*</td>
<td>100</td>
<td>Mother</td>
<td>(24)</td>
</tr>
<tr>
<td>Benin*</td>
<td>2001</td>
<td>200</td>
<td>37 (nd–688)*</td>
<td>98</td>
<td>1–3</td>
<td>(26)</td>
</tr>
<tr>
<td>Guinea</td>
<td>2002</td>
<td>124</td>
<td>9 (nd–66)</td>
<td>96</td>
<td>2–5</td>
<td>(27)</td>
</tr>
<tr>
<td>Guinea</td>
<td>2006–2007</td>
<td>300</td>
<td>31 (nd–780)</td>
<td>93</td>
<td>1–4</td>
<td>Gong et al.</td>
</tr>
<tr>
<td>Guinea</td>
<td>1995</td>
<td>75</td>
<td>58 (nd–385)</td>
<td>&gt;90</td>
<td>Adults</td>
<td>(28)</td>
</tr>
<tr>
<td>Kenya</td>
<td>2004</td>
<td>102</td>
<td>1000 (18–67000)</td>
<td>100</td>
<td>Adults</td>
<td>(29)</td>
</tr>
<tr>
<td>Egypt</td>
<td>1992</td>
<td>19</td>
<td>NA*</td>
<td>0</td>
<td>Adults</td>
<td>(30)</td>
</tr>
<tr>
<td>Thailand</td>
<td></td>
<td>160</td>
<td>12 (5–50)</td>
<td>11</td>
<td>Adults</td>
<td>(31)</td>
</tr>
<tr>
<td>Nepal</td>
<td></td>
<td>46</td>
<td>9 (5–18)</td>
<td>15</td>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>Guangxi, China</td>
<td></td>
<td>143</td>
<td>39 (5–437)</td>
<td>69</td>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>Shandong, China</td>
<td></td>
<td>69</td>
<td>NA*</td>
<td>0</td>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>France and Poland</td>
<td></td>
<td>74</td>
<td>NA*</td>
<td>0</td>
<td>Mixed</td>
<td></td>
</tr>
</tbody>
</table>

nd, non-detected.
*aGeometric mean.
*bmMatched samples from ‘the mother during pregnancy, **cord blood and ***infant aged between 0 and 16 weeks.
*Data from an 8 month longitudinal study with 200 children aged 1–3 years at recruitment, tested for AF–albumin in 1February, 2June and 3October of 2001.
*Data are from patients with acute aflatoxicosis.
*Data in bracket are 95% confidence interval. The overall range is from nd to >100 pg/mg. AF–albumin data are compared between populations in 1peri urban and2rural.
*AF, aflatoxin.
*NA, not applicable.

reported that the combined effect of AFB1 and HBV was more consistent with an additive model than the multiplicative one in the original report (35). The wide confidence intervals around the odds ratios (ORs) in each of these studies should be noted (Table II). No prospective cohort studies of aflatoxins, HBV and HCC have currently been published from other parts of the world than Taiwan and Shanghai.

A case–control study of HCC in The Gambia used the codon 249ser mutation as a biomarker of aflatoxin exposure. The presence of both the codon 249ser mutation and the HBV chronic infection was associated with an OR of 26.8 (8.7–82.1) in individuals chronically infected with HBV (43). In HCC from areas where aflatoxin exposure is high, up to 50% of tumours have been shown to harbour the codon 249ser mutation. The risk of HCC from aflatoxin in the absence of chronic HBV infection is difficult to determine in populations where HBV infection is endemic. In a summary of recent studies (42), the mutation prevalence is between 1.7 and 3.4. In the Taiwan cohort follow-up (41), aflatoxin biomarkers (urinary metabolites and albumin adducts, dichotomized to above and below mean) were associated with a statistically increased risk of HCC up to 3-fold.

In summary, aflatoxin appears to be a more potent carcinogen among HBV chronic carriers than among non-carriers. Nevertheless, the most valid statistical model of interaction remains debatable; the fact that the available studies are restricted to Asia is also noteworthy. Current evidence does suggest a significant increased risk of HCC in people exposed to aflatoxin in the absence of chronic HBV infection. In comparison with the study of aflatoxins and interaction with HBV, there has been little focus to date on the potential for interaction with HCV infection (44).

Molecular pathology

In HCC from areas where aflatoxin exposure is high, up to 50% of tumours have been shown to harbour the codon 249ser mutation. The risk of HCC from aflatoxin in the absence of chronic HBV infection is difficult to determine in populations where HBV infection is endemic. In a summary of recent studies (42), the OR in HBsAg-negative individuals exposed to aflatoxins was found to be between 1.7 and 3.4. In the Taiwan cohort follow-up (41), aflatoxin biomarkers (urinary metabolites and albumin adducts, dichotomized to above and below mean) were associated with a statistically increased risk of HCC up to 3-fold.

There is little information about aflatoxin and risk of liver cirrhosis. In The Gambia (43), lifetime groundnut intake was linked to a significant increased risk of cirrhosis, with the highest consumption associated with an OR of 2.8 (1.7–7.7). The presence of a codon 249ser mutation was associated with a similar magnitude of risk. There was an increased risk of ~8-fold with chronic HBV infection alone and an OR of 26.8 (8.7–82.1) in individuals chronically infected with HBV and having high groundnut intake.
described double mutant seen in the hepatitis B x (HBx) gene, may be associated with the 249ser mutation, opening new avenues for understanding the interaction between the two risk factors (47).

An open question concerns the role aflatoxins may play in inducing other types of genetic alterations in HCC (8). For example, AFB1 can induce mitotic recombination (48) and minisatellite rearrangements (49) providing alternative mechanisms of chromosomal instability. In comparisons of HCC from Qidong County and Beijing, China, the higher aflatoxin exposure and prevalence of codon 249ser mutation in the former region was paralleled by more frequent loss of heterozygosity at 4p11-q21, 16q22.1 and 16q22.24 loci (50). Wong et al. (51) detected double the number of genetic alterations in HCC from Shanghai (high prevalence of 249ser mutation) compared with Hong Kong (low prevalence 249ser mutation). More recently, a comparison of HCC from Qidong County and Beijing, China, the other types of genetic alterations in HCC (8). For example, AFB1 can be a significant increase in various indictors of HBV replication (61). Altered expression of functional HBsAg is required in the selection of a specific liver cell population (putative liver stem cells) containing the mutated TP53 gene. Chronic liver injury and regenerative hyperplasia are critical to the development of liver cancer (18). Therefore, aflatoxin-induced DNA adducts may be fixed as mutations consequent to an HBV-related increase in cell proliferation and hyperplasia. Inflammation and oxidative stress associated with chronic active hepatitis and aflatoxin exposure may also result directly in DNA damage and mutations (59). Alternatively, HBV could predispose hepatocytes to the carcinogenic action of aflatoxins. For example, human liver epithelial cells expressing wild-type TP53 and transfected with HBx gene were more sensitive to the cytotoxic action of AFB1-8,9-epoxide and to induction of mutations at codon 249 than were the parent cells (60). It is possible that HBx inhibits DNA excision repair thus leading to increased AFB1–DNA adduct persistence and mutation (18). HBV may also alter the hepatic expression of aflatoxin metabolizing enzymes and consequently the extent to which aflatoxins bind to DNA as observed in some HBV-transgenic lineages although there remains little work on these phenomenon in human liver (45).

Aflatoxins and HBV—models and mechanisms

Various animal models involving natural hepatitis infections have been used to examine the interaction with AFB1, but these have suffered from limitations, including the relevance of the pathology to humans and small study size (45). HBV transgenic and knockout mouse lineages expressing various HBV antigens (e.g. HBsAg, hepatitis B e antigen (HBeAg) and HBx) with some additionally engineered to express the HBx gene, AFB1 treatment induced significantly more liver tumours than in wild-type mice (56). Transgenic HBx expression in combination with AFB1 treatment resulted in doubling the number of induced GC to TA transversions compared with the non-transgenic lineage treated with AFB1 at the same dose (57).

Experiments in Hupki mice, in which exons 4-9 of the mouse TP53 were replaced by the corresponding human TP53 exons, revealed an increase in HCC after AFB1 treatment but no codon 249ser mutations (58). However, in this latter strain, unlike the studies of Sell et al., there was no expression of HBV antigens. It is conceivable that the presence of functional HBsAg is required in the selection of a specific liver cell population (putative liver stem cells) containing the mutated TP53 gene. Chronic liver injury and regenerative hyperplasia are critical to the development of liver cancer (18). Therefore, aflatoxin-induced DNA adducts may be fixed as mutations consequent to an HBV-related increase in cell proliferation and hyperplasia. Inflammation and oxidative stress associated with chronic active hepatitis and aflatoxin exposure may also result directly in DNA damage and mutations (59). Alternatively, HBV could predispose hepatocytes to the carcinogenic action of aflatoxins. For example, human liver epithelial cells expressing wild-type TP53 and transfected with HBx gene were more sensitive to the cytotoxic action of AFB1-8,9-epoxide and to induction of mutations at codon 249 than were the parent cells (60). It is possible that HBx inhibits DNA excision repair thus leading to increased AFB1–DNA adduct persistence and mutation (18). HBV may also alter the hepatic expression of aflatoxin metabolizing enzymes and consequently the extent to which aflatoxins bind to DNA as observed in some HBV-transgenic lineages although there remains little work on these phenomenon in human liver (45).

Aflatoxin could alter the pathogenicity of the hepatitis virus, perhaps affecting susceptibility to infection or viral replication. There is some evidence for this in ducklings where AFB1 treatment resulted in a significant increase in various indicators of HBV replication (61). Consistent with this, HepG2 cells transfected with recircularized
Aflatoxicosis

There have been sporadic historical reports of human poisoning with aflatoxins in India and Kenya but the studies were not definitive in assigning causation (63). A more recent outbreak affecting several hundred cases in Western Kenya was, however, better documented (2). A case–control study found aflatoxin levels in foods and AFB1–lysine adducts were associated with risk of aflatoxicosis; adduct levels were the highest ever reported in exposed people (2,30). It is of interest that acute aflatoxicosis has only been reported in relation to maize consumption and not, for example, in those consuming groundnuts. This may reflect the susceptibility of maize to aflatoxin contamination and the high daily intakes (300–500 g). In addition, the role of co-contaminating mycotoxins in maize, notably FB, has not been assessed and may add to the acute toxicity observed.

In the studies of aflatoxicosis in Kenya and India, staple foods contaminated with 5000 p.p.b. or above of aflatoxins were associated with fatality while daily consumption of foods with >1000 p.p.b. was linked to aflatoxicosis. The intake of total aflatoxins resulting in a risk of fatality can therefore be estimated to be >1 mg/day, or in excess of 20 µg/kg body wt/day in adults, based on the observed levels of contamination in the three main studies (Table III). The duration of consumption prior to aflatoxicosis and/or fatality is difficult to assess but is probably between 1 and 3 weeks. On this basis, it is informative to attempt to compare the intake values with the LD50 for animal species (Table IV). In Kenya (2), a 39% mortality rate was recorded. Assuming that the deaths occurred at the higher end of the estimated exposure range, i.e. ∼120 µg/kg/day total aflatoxins (60 µg AFB1/kg/day assuming AFB1 to be 50% of total aflatoxins), allowing between 7 and 21 days exposure prior to death, and adjusting to 50% mortality, then the total intake of AFB1 associated with half the exposed people dying would be between 0.54 and 1.62 mg/kg. This crude estimate is nevertheless similar to the LD50 value reported for rabbits, cats, dog, pigs and baboons but lower than for rodents. It is noteworthy that there were reports of dogs dying in the earlier Kenya and India aflatoxicosis incidents and the LD50 for dogs is 0.5–1.0 mg/kg. It is possible, therefore, that humans are sensitive to the acute toxicity of aflatoxins or that fractioned daily doses cause particular harm.

Of concern is the fact that the levels of aflatoxins inducing acute toxicity are only one to two orders of magnitude higher than occur on a regular basis in the staple foods of many populations worldwide. The wider occurrence of cases of jaundice and acute liver failure, unrecognized as aflatoxin poisoning, cannot be ruled out.

Immunomodulation

The immunomodulatory effects of aflatoxins have been considered predominantly in experimental studies in cell models and animals (7). Many studies in poultry, pigs and rodents showed that exposure to aflatoxin results in suppression of various aspects of the cell-mediated immune response. Some of these effects may be mediated through altered cytokine expression (66). Reduced humoral immunity has also been observed in aflatoxin-exposed animals as has increased susceptibility to infections or reduced response to vaccines (see ref. 7).

There are few studies of aflatoxins and immunity in human populations. A study in The Gambia found that children with malaria...
parasitaemia had significantly higher mean aflatoxin–albumin adducts but that there were no significant associations with experience of malaria infection, antibody titre to asexual stages of *Plasmodium falciparum* or lymphoproliferative responses (21). In a second study (23), there were no associations between aflatoxin–albumin adducts and either a test of cell-mediated immunity or antibody titres to vaccines but higher adduct levels were associated with lower salivary IgA. In Ghana (67), alterations were reported in different lymphocyte subgroups in relation to aflatoxin–albumin adduct level. In a second larger study (68), high aflatoxin–albumin adducts (more than median) was associated with alterations in some lymphocyte subsets.

Overall, the studies of immunomodulation in aflatoxin-exposed populations are inconclusive. The studies have been cross-sectional, including relatively few subjects and have not been repeated in different populations. Nevertheless, the data suggest that effects on immune parameters in populations exposed chronically to aflatoxins could occur and given the potential impact this certainly merits further investigation.

**Growth impairment**

Studies in different animal species indicate that aflatoxin exposure can severely affect growth (7). However, until recently the effects of aflatoxin on human growth have not been investigated. As with immune modulation, it is important to research into these effects, particularly given that exposure occurs from the perinatal period onwards (69).

In a cross-sectional study (25) of children aged 1–5 years in Benin and Togo, a striking inverse association was found between aflatoxin–albumin adducts and growth. Children who were stunted or under-weight had 30–40% higher mean aflatoxin–albumin levels. In a subsequent 8 months longitudinal study (26), there was a strong negative correlation between aflatoxin–albumin adducts and height increase over the 8 months follow-up. Most recently, these studies were extended to consider *in utero* exposure in a group of Gambian children (24) and again an association was found between exposure and impaired growth, on this occasion in the first year of life.

Growth faltering in West African children occurs at a time of introduction of solid foods when there is high exposure to aflatoxin. The dose–response relationship between level of aflatoxin biomarkers and growth effects is consistent with a causal effect. However, other confounding factors cannot be excluded. The mechanisms by which aflatoxin may exert an effect on growth are currently unknown, although the possibility of a compromised intestinal integrity, through altered barrier function as a consequence of endothelial cell toxicity or immune suppression, is a valid hypothesis to explore further (69).

**Fumonisins**

FB are a family of mycotoxins produced by the fungi *Fusarium verticillioides* (formerly *Fusarium moniliforme*) and related fungi that primarily contaminate maize, and it is from this source that the major health threats emerge, although other commodities may be contaminated (70–72). FB contamination of maize occurs in many parts of the world with reported levels >100 p.p.m. in some regions (6,70,73). The determinants of contamination include location, climate and susceptibility of the plants to fungal invasion, insect damage and crop stress (74).

FB were first isolated and their structure was identified in 1988 (75). FB consist of a long hydroxylated hydrocarbon chain with added tricarballylic acid, methyl and amino groups. FB1, FB2 and FB3 are the major naturally occurring FB. FB1 is by far the most prevalent in the human diet and was categorized as a Group 2B carcinogen by International Agency for Research on Cancer (6).

**Toxicity in animals**

FB1 causes equine leukoencephalomalacia, porcine pulmonary oedema and a variety of hepatotoxic and nephrotoxic effects in animals (71). FB induce hepatic injury in all species regardless of the route of administration although horses and pigs appear more sensitive than other species. The pattern of target organs does differ by species and sex with, for example, lung, liver and pancreas being affected in pigs, whereas in rats and mice, the liver and kidney are primary target organs (72).

**Carcinogenesis in animals**

Initial studies demonstrated that culture material of *F. verticillioides* was hepatocarcinogenic in rats (70). FB1 was subsequently shown to be a liver cancer promoter in a diethylnitrosamine-initiated rat model (75). Furthermore, Gelderblom et al. (76) demonstrated liver cancer induction in male BD IX rats exposed to 50 mg/kg (estimated 1.6 mg/kg/day) of FB1 in the diet. In a 2 year feeding study (77) using 50 and 150 mg FB1/kg diet (2.2 and 6.6 mg/kg/day), kidney adenomas and carcinomas were induced in male Fischer 344/N/Nctr BR rats but no liver tumours, whereas no increase in tumours was observed in females. In the same study, female B6C3F1/N/Nctr BR mice showed an increased incidence of hepatocellular adenomas and carcinomas with dietary levels >50 mg FB1/kg diet (77). Gelderblom et al. (78) have highlighted the important modulatory role of dietary constituents in these various bioassays in terms of the outcome of the treatment regimens, particularly the targeting of effects to the kidney. Overall, however, FB1 has been shown to be a carcinogen in rodents exhibiting both cancer-initiating and -promoting effects.

**Mechanisms of action**

While the above studies have shown that FB1 is a carcinogen in animals, there are different potential mechanisms by which it may exert its effects (72,79). FB1 tested negative in several genotoxicity assays but did induce micronuclei and chromosomal aberrations in primary hepatocytes and Hep-G2 cells (6,80). Although the mechanism for DNA damage in these latter cases is unclear, an indirect effect through stimulation of oxidative damage and lipid peroxidation may play a key role (72). This is supported by experimental evidence that FB1 increases oxidative DNA damage, as measured by increased DNA strand breaks and malondialdehyde adducts, in rat liver and kidney (81) and lipid peroxidation (82) *in vivo*.

An alternative mechanism of action of FB1 involves the disruption of the *de novo* sphingolipid biosynthesis pathway by inhibition of the enzyme ceramide synthase (79). The inhibition of complex sphingolipid biosynthesis disrupts numerous cell functions and signalling pathways, including apoptosis and mitosis, thus potentially contributing to carcinogenesis through an altered balance of cell death and replication (72). Studies in knockout mice suggest that FB1 may exert an effect on apoptosis and cell division via perturbations of the tumor necrosis factor pathway (83).

Disruption of sphingolipid metabolism leads to changes in the sphinganine (Sa) to sphingosine (So) ratio (Sa:So), with increased Sa tissue concentrations (84). Such changes were demonstrated in rat liver and mouse kidney at carcinogenic doses of FB1 (85). This effect of FB has been used as a basis for biomarker development (see below).

In addition, the role of FB in immunomodulation has been highlighted (72). Thus, FB have been shown to alter levels of a range of cytokines *in vitro* while *in vivo* studies in pigs were associated with reduced antibody titres following vaccination against *Mycoplasma agalactiae* (86).

**FB biomarkers of exposure**

Population estimates of FB exposure have been based on food consumption patterns and FB contamination levels (87). However, for estimates of individual exposure such approaches are less useful, particularly in light of heterogeneity in contamination and variations in food processing and cooking. Valid biomarkers of FB exposure would offer an alternative and several approaches, involving alterations in sphingoid bases or detection of free FB, are briefly summarized below (see also refs 88,89).

The most extensively studied biomarkers are the sphingoid bases, particularly the accumulation of Sa in comparison with So, following...
the FB-mediated inhibition of ceramide synthase and the consequent increase in Sa:So ratio. Elevated ratios have been reported in tissues and body fluids from many species in a dose- and time-dependent manner (84,88–90). This led to the ratio being examined in human populations in France (91), South Africa and Kenya (85,92), Italy, Argentina, Brazil (93), China (94) and Burkina Faso (95). For the majority of studies there was little evidence of altered ratio with FB exposure, although a slight increase was reported in urinary Sa:So ratio in male volunteers in China eating home-grown maize (94). Apart from the problems of adequate sensitivity in responding to dietary FB levels, the Sa:So ratio also needs to be validated by comparison with FB intake at the individual level. Only the study in Burkina Faso addressed this question, but there were no reported alterations in ratio in relation to FB intake (95).

The overall conclusions from studies in exposed populations are that no clear differences in the Sa:So ratio in serum, urine or buccal cells occurred in relation to high and low FB exposure either within a country or between countries. While the Sa:So ratio may not be applicable to human population studies, other mechanism-based biomarkers, for example, the recently identified 1-deoxysphinagmine, offer promise (96).

Toxicokinetic studies from several species suggested the FB have a short half-life, low absorption and that the majority is excreted unmetabolized in urine and, predominantly, in the faeces (97). Nevertheless, the detection of free FB in body fluids is an alternative approach to development of an exposure biomarker. For example, FB has been detected in human faecal samples with differences between subjects from regions with high and low FB maize contamination (98). Hair has also been suggested as a source of samples for FB analysis (99).

Based on advances in LC-MS analysis, a method was developed recently for urinary FB1 (100). In a Mexican cohort, three groups of 25 women were formed based on consumption of maize-based tortillas (high, medium and low). Using the LC-MS method, 75% of the women were found to be positive for urinary FB1; the mean urinary FB1 level increased 3-fold from the low to the high consumption group (100). These findings indicated that urinary FB1 measured by LC-MS could be a suitable FB exposure biomarker but all the above methods remain to be validated by comparison with FB intake at the individual level.

Human cancer

Ecological studies in the former Transkei region of South Africa showed that both F. verticillioides and FB contamination were more prevalent in maize consumed by people in the southern part compared with the northern part of the region and that this was correlated with oesophageal cancer (OC) rates (6,70). Subsequently, other studies have reported high FB levels in maize from high OC incidence areas compared with low, for example, in China (101). Some reports have associated maize consumption per se with high OC incidence, but did not consider FB exposure specifically, e.g. in Italy (102). These studies on the whole are consistent with the hypothesis that FB exposure may be associated with OC risk but suffer from the inherent design limitations of ecological studies.

Abnet et al. (103) used the Sa:So ratio in serum as a FB exposure biomarker in a nested case–control study of OC in China, but saw no association with cancer risk. Nevertheless, subsequent biomarker research (see below) suggests that this approach is insufficiently sensitive to categorize human exposure to FB.

FB1 has been shown to interact with AFB1 in a short-term carcinogenesis model (104) and to promote AFB1 carcinogenicity in trout (6). Perhaps due to the focus on OC, the role of FB in terms of cancer risk in other organs has been largely unexplored. However, given that FB induce liver tumours in rodents, FB and aflatoxins frequently co-contaminate maize (6,105) and that both toxins occur in populations with high prevalence of HBV infection, a role of FB in HCC is plausible. High levels of FB in home-grown maize were reported in China in areas of high HCC incidence (105); (101). Ueno et al. (106) also reported FB contamination in maize samples from Hainan, a high HCC incidence area, with levels 10- to 50-fold higher than in Penlai, a low-risk area. However, to our knowledge, no case-control or cohort studies have so far reported on the role of FB and HCC.

Neural tube defects

Animal studies have demonstrated that FB exposure can cause neural tube defects (NTDs) (107) giving rise to further concerns that FB could cause similar effects in humans. There is a potential mechanism for this through the disruption of sphingolipid biosynthesis and consequently the structure and function of cell membranes. NTD is known to be associated with reduced folate levels and it is possible that FB-induced membrane disruption could lead to reduced folate absorption through damage to the folate receptors on the membrane (108).

A possible link between human NTD and FB consumption was made after a cluster of NTD was reported on the Texas–Mexico border in 1991, shortly after a severe outbreak of leukenocephalomalacia in this region (109). Exposure to FB in these regions can be elevated due to frequent contamination of corn. In a case–control study in the same region (110), moderate tortilla consumption compared with low in the first trimester of pregnancy was associated with an increased risk (OR 2.4; 95% confidence interval 1.1–5.3) of NTD but high consumption was not. A similar result was found using the estimates of FB intake from tortillas, whereas increasing Sa:So ratio was associated with increased risk apart from the highest category. The authors postulated that high FB levels may be lethal to the foetus, thus explaining the apparent threshold effect. The relation at the individual level between the Sa:So ratio and estimated FB exposure was not reported. Nevertheless, the epidemiological data coupled with a plausible mechanism make the subject of FB and NTD one that requires further study.

Human exposure and regulations

Regulations concerning FB contamination are in place in most developed countries. The US Food and Drug Administration regulation of FB levels on de-germed dry milled maize products for human consumption is 2 p.p.m. In the European Union, the tolerable daily intake for FB is set at 2 µg/kg body wt/day (98), whereas the maximum tolerable limit for FB in maize is 1 p.p.m. The joint FAO/WHO Expert Committee for Food Additives also set a provisional maximum tolerable daily intake of 2 µg/kg body wt/day for FB1, FB2 and FB3 alone or combined (87). This is based on a no-observable-adverse-effect level in male rat kidney and incorporates a 100-fold safety factor. In countries where intakes of maize are much higher, such as in South Africa, arguments may be made for a significantly lower maximum tolerable limit in order to keep tolerable daily intakes low (111). However, in many of the high-maize consumption areas of the world, regulation is either lacking or not enforced.

Estimated FB exposure levels in different parts of the world are listed in Table V. In some areas, e.g. parts of South Africa and Guatemala, the exposure far exceeds the WHO provisional maximum tolerable daily intake for a proportion of the population. It is also notable that considerable FB exposure can result in young children, around the time of introduction of maize-based solid foods (114). Ten to 26% of the infants in Tanzania, for example, are at a high risk of exposure to fumonisins levels, above the provisional maximum tolerable daily intake of 2 µg/kg body wt/day (114). The effects of this early life exposure to FB are to date unexplored.

Prevention of mycotoxin exposures

There are a number of ways to reduce human exposure to aflatoxins and FB. Fundamental to developing prevention strategies is an understanding of the interaction between the fungus and the host plant. Aspergillus spp. infect crops during cultivation but aflatoxins continue to accumulate post-harvest under poor storage conditions, which favour fungal growth and toxin production. Therefore, post-harvest interventions may contribute significantly to controlling aflatoxin (17,115). In contrast, Fusaria spp., infect the maize in the field and...
the majority of toxin is present at the time of harvest. Thus control of FB requires more attention to pre-harvest practices and to the subsequent effects of processing and preparation of foodstuffs (116,117). A number of approaches are discussed briefly below.

Under some circumstances, a shift in diet away from contaminated commodities can result in reduced exposure. For example, in China economic developments resulted in reduced maize consumption (118), formerly the major source of aflatoxin exposure in regions such as Qidong County. However, populations in some of the poorest countries facing the highest risk of mycotoxin exposure due to consumption of contaminated staple foods (119) are trapped by poverty and the lack of alternatives, making it virtually impossible to replace the contaminated food with that of good quality.

Pre-harvest mycotoxin controls include various good agricultural practices to reduce crop stress (e.g. improved irrigation, early sowing, low plant density, balanced fertilization, use of fungicides, pesticides and insecticides, use of strains resistant to fungal colonization, bio-

tolerable daily intake

Table V. Estimated FB intake in different regions: provisional maximum tolerated daily intake = 2 μg/kg/day

<table>
<thead>
<tr>
<th>Regions</th>
<th>FB intake (μg/kg/day); mean (range)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>State of Morelos, Mexico</td>
<td>0.4^a (0–23.2)</td>
<td>(100)</td>
</tr>
<tr>
<td>Texas–Mexico border</td>
<td>0.2 (0.7–9.4)^a</td>
<td>(110)</td>
</tr>
<tr>
<td>South Brazil/North Argentina</td>
<td>0.6</td>
<td>(93)</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>0.8 (0–2.4)</td>
<td>(112)</td>
</tr>
<tr>
<td>Bizana, South Africa (by age group in years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–9</td>
<td>6.6 (1.0–18.8)</td>
<td></td>
</tr>
<tr>
<td>10–17</td>
<td>4.0 (0.9–9.6)</td>
<td></td>
</tr>
<tr>
<td>18–65 (females)</td>
<td>3.0 (0.8–6.9)</td>
<td></td>
</tr>
<tr>
<td>18–65 (males)</td>
<td>3.8 (0.5–8.0)</td>
<td></td>
</tr>
<tr>
<td>Centane, South Africa (by age group in years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–9</td>
<td>14.1 (2.7–35.9)</td>
<td></td>
</tr>
<tr>
<td>10–17</td>
<td>8.3 (2.0–17.1)</td>
<td></td>
</tr>
<tr>
<td>18–65 (females)</td>
<td>8.2 (2.9–17.4)</td>
<td></td>
</tr>
<tr>
<td>18–65 (males)</td>
<td>9.2 (2.2–16.2)</td>
<td>(111)</td>
</tr>
<tr>
<td>Transkei, South Africa</td>
<td>3.8</td>
<td>(92)</td>
</tr>
<tr>
<td>KwaZulu-Natal, South Africa</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Boonet, Kenya</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>Guatemala</td>
<td>3.5 (urban) 15.6 (rural)</td>
<td>(113)</td>
</tr>
<tr>
<td>Lixinian, China</td>
<td>184.0 (0.4–740.0)</td>
<td>(94)</td>
</tr>
</tbody>
</table>

^aEstimated from urinary excretion.

^bUpper quartile.

Table VI. Summary of disease associations with exposure to aflatoxins and FB

<table>
<thead>
<tr>
<th>Aflatoxins</th>
<th>FB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predominantly contaminate</td>
<td>Cereals, including maize (corn), groundnuts (peanuts), tree nuts, cottonseed, some spices; AFM1 is also found in milk and milk products</td>
</tr>
<tr>
<td>International Agency for Research on Cancer classification</td>
<td>Naturally occurring mixtures of aflatoxins—Group 1; aflatoxin M1—Group 2B</td>
</tr>
<tr>
<td>Main tumour sites reported in animals</td>
<td>Liver; Hepatotoxicity; growth impairment; immune suppression; developmental toxicity</td>
</tr>
<tr>
<td>Other main adverse effects in animals</td>
<td>Liver; Equine leukoencephalomalacia; pulmonary oedema in pigs; hepatotoxicity and nephrotoxicity; developmental toxicity</td>
</tr>
<tr>
<td>Main tumour sites reported in humans</td>
<td>Liver; Aflatoxicosis; cirrhosis (?) growth impairment (?); immune suppression (?)</td>
</tr>
<tr>
<td>Other main adverse effects in humans</td>
<td>Liver and kidney; Oesophagus (?)^7^, liver (?)</td>
</tr>
</tbody>
</table>

^7(?) indicates that the relationship is not established.
achieved by the incorporation of clays into feeds and foods (7). This approach has been demonstrated in animals and recently extended to trials in exposed people (125) with reductions in both aflatoxin-albumin adducts and urinary AFM1 in Ghanaian subjects taking the clay-filled capsules over a 3 month period.

In terms of altered metabolism, a number of different compounds have been explored mainly in a series of elegant studies by Kensler and colleagues (17) in China. Chlorophyllin may act both to reduce absorption and to modify aflatoxin metabolism. In a chemoprevention trial in China, chlorophyllin resulted in a 55% reduction in urinary AFB1-N7-Gua compared with those taking placebo. Oltipraz is able to modify both bioactivation and detoxification of aflatoxins and led to an increase in the urinary excretion of the aflatoxin–mercapturic acid conjugate and a decrease in urinary AFM1 (17). Similar modulation of aflatoxin biomarkers was observed with green tea polyphenols (126). Finally, a chemoprevention trial using a broccoli sprout extract did not show reduction in urinary AFB1-N7-Gua excretion, probably due to the unexpected inter-individual variation in bioavailability of dihydrocarbamates from the broccoli. However, when a comparison was made at the individual level between bioavailable dihydrocarbamate and AFB1-N7-Gua, there was a strong inverse association (17).

Conclusions and perspective

There is no doubt that mycotoxins have adverse effects on human and animal health in many parts of the world (summarized in Table VI). The role of aflatoxins on HCC and acute aflatoxicosis is established while other effects on child growth and immunomodulation, because other effects on child growth and immunomodulation, because

in understanding decision-making processes (130). Much could be done if the value of the intervention is recognized (economically in agricultural terms and through improved health) and if the information is disseminated in an appropriate and accessible manner.

Reductions in exposure will surely serve to protect vulnerable populations while the full extent of the health burden is clarified. Notwithstanding the need for a better evidence-base on mycotoxins and health, given the existing experimental, human epidemiology and mechanistic data, a reduction in human exposure to these toxins is a priority now. When the economic benefit of less contaminated crops is coupled with improved health the reasons to act are compelling. The response, however, needs to be a concerted one, including international agencies, governments and non-governmental organizations, to address what remains a largely and rather shamefully ignored global health issue.

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