The role of biomarkers in evaluating human health concerns from fungal contaminants in food

Paul C. Turner1*, Brenna Flannery2,3, Catherine Isitt4, Mariyam Ali4 and James Pestka2,3,5
1Maryland Institute for Applied Environmental Health, School of Public Health, University of Maryland, College Park, MD 20742, USA
2Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824, USA
3Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA
4School of Medicine, University of Leeds, Leeds LS2 9JT, UK
5Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824-1224, USA

Abstract
Mycotoxins are toxic secondary metabolites that globally contaminate an estimated 25% of cereal crops and thus exposure is frequent in many populations. Aflatoxins, fumonisins and deoxynivalenol are amongst those mycotoxins of particular concern from a human health perspective. A number of risks to health are suggested including cancer, growth faltering, immune suppression and neural tube defects; though only the demonstrated role for aflatoxin in the aetiology of liver cancer is widely recognised. The heterogeneous distribution of mycotoxins in food restricts the usefulness of food sampling and intake estimates; instead biomarkers provide better tools for informing epidemiological investigations. Validated exposure biomarkers for aflatoxin (urinary aflatoxin M1, aflatoxin–N7-guanine, serum aflatoxin–albumin) were established almost 20 years ago and were critical in confirming aflatoxins as potent liver carcinogens. Validation has included demonstration of assay robustness, intake biomarker level, and stability of stored samples. More recently, aflatoxin exposure biomarkers are revealing concerns of growth faltering and immune suppression; importantly, they are being used to assess the effectiveness of intervention strategies. For fumonisins and deoxynivalenol these steps of development and validation have significantly advanced in recent years. Such biomarkers should better inform epidemiological studies and thus improve our understanding of their potential risk to human health.

Key words: Aflatoxin: Deoxynivalenol: Fumonisin: Mycotoxin: Growth: Children: Cancer

Background
Fungi provide valuable contributions to the diet (mushrooms, cheeses), to medications (penicillins, statins), to food preservation (for example, citric acid production) and to fermentation, and even support complex chemical synthesis of novel compounds; however, some species frequently contaminate cereal crops, and under certain environmental conditions can produce potent toxins (mycotoxins) that can contaminate many dietary staples1,2. These mycotoxins contaminate up to 25% of the world’s cereal crops3. Whilst there are many hundreds of mycotoxins identified, only a few have received significant research activity. Those of major concern to human health include those toxins produced from Aspergillus and Penicillium, the aflatoxins and ochratoxins; and those produced from Fusarium, the fumonisins, trichothecenes (for example, deoxynivalenol (DON), nivalenol, T2-toxin) and zearalenone. Based on their current worldwide frequency of contamination, their established toxicity and our ability to understand exposure based on the use of existing and newly developed biomonitoring tools (also known as exposure biomarkers), the present review will focus on aflatoxins, fumonisins and DON. It is probable that the global distribution of mycotoxin contamination will change alongside anticipated climatic adjustments over the next century5, and that this change in distribution of mycotoxins may ultimately change the focus of this research area. The present review will highlight the known human health effects, the suspected health effects and the hypothesised mechanisms of toxicity, with a particular focus on children and growth, and their possible carcinogenic effects. The complex immune toxicologies of the mycotoxins are not reviewed.

Abbreviations: BW, body weight; COX-2, cyclo-oxygenase-2; DG, deoxynivalenol-glucuronide; DON, deoxynivalenol; FB, fumonisin B; fD, ‘free’ deoxynivalenol; HBV, hepatitis B virus; IGF, insulin-like growth factor; NTD, neural tube defect; SCCO, squamous-cell carcinomas of the oesophagus.

*Corresponding author: Dr Paul C. Turner, email pturner3@umd.edu

Downloaded from https://www.cambridge.org/core. IP address: 54.70.40.11, on 25 Feb 2019 at 13:14:46, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
Epidemiologists focused on understanding disparate disease aetiologies should remember that exposure to mycotoxins is almost unavoidable in cereal-consuming populations and, as a result, they have the capacity to negatively affect human health and modify human disease susceptibility.

Mycotoxins are typically resistant to processing and are stable in many cooking processes; thus complete avoidance without major dietary restriction is not feasible \( ^{(1,2)} \). The specific type of mycotoxin produced is dependent in part on climatic conditions; aflatoxins and fumonisins are more prevalent in tropical and semi-tropical conditions whilst DON occurs in temperate climates \( ^{(1,2)} \). The frequency and the amount of mycotoxin exposure are influenced by wealth \( ^{(1-6)} \). In developed countries, wealth affords access to greater food choices and the freedom to avoid contaminated foods at the individual level and prevent it from entering commerce at the regulatory level. The poorest regions of the world have neither the infrastructure nor the luxury to allow for such decisions. Additionally, the wealthier countries can restrict imports of contaminated crops, resulting in an additional burden on developing countries \( ^{(3,5,6)} \). Despite the differences in contamination levels, exposure to mycotoxins is apparent on all continents, but the impact on health in most instances remains poorly examined in all regions and requires significant research effort.

Whilst the consequences of individual mycotoxins are more typically examined, in reality typical exposures will include mixtures of mycotoxins, on a background of a wide range of other susceptibility factors that affect human health including additional chemical and biological agents, genetic susceptibility, varied nutrition and immune status. Thus, there is an increasing recognition of the need to examine multiple exposures to understand the health effects from mycotoxin exposures.

**Aspergillus and Fusarium mycotoxins**

Of the about 200 species of *Aspergillus*, 10% of these are harmful to man through a variety of mechanisms \( ^{(1,2)} \). *Aspergillus flavus* and *A. parasiticus* produce a potent family of liver toxins, known as aflatoxins, whilst *A. ochraceus* produces the nephrotoxic ochratoxin A.

Aflatoxins predominantly occur in hot and humid regions of the world where an estimated 4.5 billion individuals are at risk of exposure \( ^{(5)} \). Aflatoxins contaminate dietary staples including maize and groundnuts; thus, populations highly reliant on these staples, and with limited agricultural capacity and storage facilities, are most frequently exposed though diet \( ^{(5,6)} \). Biomarker-derived exposure data (see below) reveal that some of the world’s poorest populations experience chronic exposure to aflatoxins throughout life, often at high levels.

*Fusarium verticillioides* (Sacc.) Nirenberg (formerly known as *F. moniliforme* Sheldon) and *F. proliferatum* (Matsushima) Nirenberg are important fungi that contaminate maize and produce fumonisins \( ^{(1,2,7)} \). Fumonisins predominantly contaminate maize in hot and humid climates, and co-contamination of maize with aflatoxins and fumonisins are reported \( ^{(6-10)} \). Chronic high levels of exposure are predicted in parts of South Africa, Central America and Asia. *Fusarium graminearum* (Gibberella zeae) and *F. culmorum* infection of wheat and maize in more temperate regions (Europe, North and South America and parts of Asia) causes significant economic loss in the form of head blight and contamination by DON and other related trichothecene mycotoxins \( ^{(1,2,19)} \). A survey of >45,000 food items from countries within the European Union revealed that 57% of cereals tested were contaminated with DON, demonstrating the frequent contamination of this mycotoxin \( ^{(20)} \).

**Biomarkers of exposure**

The presence of an accurately quantified amount of a toxin, and/or its metabolite(s), alone, is insufficient for a bio-measure to be classified as a biomarker. In the present review, an exposure biomarker is a biological measure which is correlated with the quantity of the xenobiotic ingested, resulting in improved exposure classification over approaches that are more traditional. The development and validation of aflatoxin exposure biomarkers occurred over 20 years ago \( ^{(6,21,22)} \); they have been extensively reviewed elsewhere \( ^{(6,21-23)} \) and so will not be discussed here in detail. The development of biomarkers for fumonisins and DON is more novel and the present review will highlight this.

**Aflatoxins**

The aflatoxins are a family of highly substituted coumarins containing a fused dihydrofurofuran moiety \( ^{(23)} \). Aflatoxins B1, B2, G1 and G2 all occur naturally, whilst aflatoxin B1 occurs most frequently and is the most toxic and carcinogenic (Fig. 1). In many developing countries, maize and groundnuts (peanuts) are the predominant contaminated food items, often at high levels. Aflatoxin metabolism (Fig. 2) gives rise to a variety of metabolites \( ^{(23,24)} \) including aflatoxin M1, a frequent metabolite in milk of exposed lactating animals, including human breast milk following maternal exposure to dietary aflatoxin B1 \( ^{(23-25)} \). The parent toxins, aflatoxins B1, G1, etc., also occur in breast milk \( ^{(25,26,27)} \). The consequences of breast milk exposures to aflatoxins in the developing infant remain poorly examined.

Aflatoxin B1 is metabolised by a number cytochrome P450s \( ^{(28,29)} \) and generates two reactive epoxide species, an exo-8,9-epoxide and endo-8,9-epoxide, in addition to several hydroxy metabolites, for example, aflatoxins M1, Q3 and P1 \( ^{(6,22,23)} \) (Fig. 2). The two epoxides are highly reactive and can cause cellular and macromolecule damage by covalently binding to proteins and nucleic
acids (6, 22, 23, 30). The exo-epoxide is toxic and mutagenic; the specific role of the endo-epoxide is less well examined, but it is predicted to cause a similar level of toxicity as the exo-epoxide. The exo-epoxide additionally forms a stable covalent adduct with the N7 moiety of guanine (6, 22, 23, 30). Depurination at this site releases 8,9-dihydro-8-(N7guanyl)-9-hydroxy aflatoxin B1 (AFB1–N7-Gua), which is observed, in addition to aflatoxin M1, in the urine of aflatoxin-dosed animals and individuals exposed to aflatoxin through diet (31–36). The urinary concentration of AFB1–N7-Gua in two separate studies ($r = 0.80$, $P < 0.0001$; and $r = 0.82$, $P < 0.0001$) strongly correlated with aflatoxin intake in chronically exposed individuals, and both serve as validated exposure biomarkers (33–36). Hydrolysis of both epoxides to aflatoxin B1-8,9-dihydrodiol leads to a slow base-catalysed ring opening resulting in a resonating dialdehyde phenolate ion, capable of forming adducts with protein amino groups, particularly lysine (37, 38) (Fig. 2). One such protein adduct known as aflatoxin–albumin is present in the sera of aflatoxin-dosed animals and individuals naturally exposed to aflatoxin through diet (37–61). The concentration of aflatoxin–albumin in sera was strongly correlated ($r = 0.69$, $P < 0.0001$) with aflatoxin intake and provides an additional validated exposure biomarker (39, 43). The reliability of multiple measuring techniques (including immunoassay, HPLC and LC-MS) on a single set of samples by selected laboratories, and the long-term stability in cryopreserved samples provide additional confidence in its use as a valuable biomarker (62–64). In high-risk regions of the world, greater than 95% of those individuals tested are positive for aflatoxin–albumin over a 3 log range, from

Fig. 1. Structures of the four naturally occurring aflatoxins: (a) aflatoxin B1; (b) aflatoxin B2; (c) aflatoxin G1; (d) aflatoxin G2. The 8,9 position is where the reactive epoxide can be readily formed across the double bond. Me, methyl.

Fig. 2. Aflatoxin (AF) B1 metabolism and biomarkers. Me, methyl; GST, glutathione S-transferase; SG, glutathione; MA, mercapturic acid; →, epoxide-related toxicity pathways; ·····, non-epoxide-related toxicity pathways; ⋯⋯, excretion or blood routes. Adapted from Wild & Turner (6). (A colour version of this figure can be found online at www.journals.cambridge.org/nrr).
approximately 3–5 pg/mg albumin to >1000 pg/mg\(^{(37–59)}\), whilst more developed regions rarely have detectable levels of the biomarker\(^{(44,65)}\). Given that aflatoxins are genotoxic carcinogens, there is no safe threshold for exposure\(^{(5,6,21–25)}\). Whilst unmetabolised aflatoxin B\(_1\) occurs in the urine of exposed individuals, there is no significant correlation with intake\(^{(59)}\), perhaps in part due to extensive metabolism of the parent toxin to various metabolites. Thus, urinary aflatoxin B\(_1\) itself is not a useful indicator of the amount of aflatoxin exposure\(^{(6,22,33–36,59)}\).

**Fumonisins**

Fumonisins are secondary metabolites produced by *F. verticillioides* (Sacc.) Nirenberg and *F. proliferatum* (Matsushima) Nirenberg, primarily in maize grown in hot and humid climates\(^{(1,2,23)}\). Whilst numerous fumonisin analogues are described, the fumonisin B (FB) series predominates, and within this series the occurrence frequency is FB\(_1\) > FB\(_2\) > FB\(_3\); FB\(_1\) (Fig. 3) is reported to represent on average about 70% of the fumonisins in naturally contaminated maize\(^{(66)}\). Fumonisins do not appear to undergo significant metabolism\(^{(67–73)}\), thus biomarker development has not followed the metabolite profile approach used for aflatoxins.

Fumonisins mimic naturally occurring sphingoid bases, and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Fumonisin modulation of and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Whilst numerous fumonisin analogues are described, the fumonisin B (FB) series predominates, and within this series the occurrence frequency is FB\(_1\) > FB\(_2\) > FB\(_3\); FB\(_1\) (Fig. 3) is reported to represent on average about 70% of the fumonisins in naturally contaminated maize\(^{(66)}\). Fumonisins do not appear to undergo significant metabolism\(^{(67–73)}\), thus biomarker development has not followed the metabolite profile approach used for aflatoxins.

Fumonisins mimic naturally occurring sphingoid bases, and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Fumonisin modulation of and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Whilst numerous fumonisin analogues are described, the fumonisin B (FB) series predominates, and within this series the occurrence frequency is FB\(_1\) > FB\(_2\) > FB\(_3\); FB\(_1\) (Fig. 3) is reported to represent on average about 70% of the fumonisins in naturally contaminated maize\(^{(66)}\). Fumonisins do not appear to undergo significant metabolism\(^{(67–73)}\), thus biomarker development has not followed the metabolite profile approach used for aflatoxins.

Fumonisins mimic naturally occurring sphingoid bases, and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Fumonisin modulation of and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Whilst numerous fumonisin analogues are described, the fumonisin B (FB) series predominates, and within this series the occurrence frequency is FB\(_1\) > FB\(_2\) > FB\(_3\); FB\(_1\) (Fig. 3) is reported to represent on average about 70% of the fumonisins in naturally contaminated maize\(^{(66)}\). Fumonisins do not appear to undergo significant metabolism\(^{(67–73)}\), thus biomarker development has not followed the metabolite profile approach used for aflatoxins.

Fumonisins mimic naturally occurring sphingoid bases, and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Fumonisin modulation of and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Whilst numerous fumonisin analogues are described, the fumonisin B (FB) series predominates, and within this series the occurrence frequency is FB\(_1\) > FB\(_2\) > FB\(_3\); FB\(_1\) (Fig. 3) is reported to represent on average about 70% of the fumonisins in naturally contaminated maize\(^{(66)}\). Fumonisins do not appear to undergo significant metabolism\(^{(67–73)}\), thus biomarker development has not followed the metabolite profile approach used for aflatoxins.

Fumonisins mimic naturally occurring sphingoid bases, and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Fumonisin modulation of and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Whilst numerous fumonisin analogues are described, the fumonisin B (FB) series predominates, and within this series the occurrence frequency is FB\(_1\) > FB\(_2\) > FB\(_3\); FB\(_1\) (Fig. 3) is reported to represent on average about 70% of the fumonisins in naturally contaminated maize\(^{(66)}\). Fumonisins do not appear to undergo significant metabolism\(^{(67–73)}\), thus biomarker development has not followed the metabolite profile approach used for aflatoxins.

Fumonisins mimic naturally occurring sphingoid bases, and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Fumonisin modulation of and inhibit sphingolipid metabolism via competing with ceramide synthase\(^{(67,74,75)}\). Whilst numerous fumonisin analogues are described, the fumonisin B (FB) series predominates, and within this series the occurrence frequency is FB\(_1\) > FB\(_2\) > FB\(_3\); FB\(_1\) (Fig. 3) is reported to represent on average about 70% of the fumonisins in naturally contaminated maize\(^{(66)}\). Fumonisins do not appear to undergo significant metabolism\(^{(67–73)}\), thus biomarker development has not followed the metabolite profile approach used for aflatoxins.

There have been relatively few studies in human subjects reporting urinary FB\(_1\) measurements. In one study, a subset of Mexican (Morelos County) women were selected based on tortilla consumption (lowest \((n = 25\)) , medium \((n = 25\)) and highest \((n = 25\)) from a larger cohort \((n = 99\)) , and their urine analysed for FB\(_1\)\(^{(79)}\). Overall, fifty-six of seventy-five women (74.6%) had detectable urinary FB\(_1\) (>20 pg/ml) (range of non-detectable to 9312 pg/ml). Urinary FB\(_1\) was detected more frequently in the high (96%) compared with the medium (80%) and the low (45%) consumption groups, and the geometric means were associated \((P<0.001)\) with consumption of tortillas (geometric means and 95th percentiles were 147 (88, 248), 63 (37, 108) and 35 (19, 65) pg/ml, respectively). No food samples were collected from this survey; though based on the urinary measures, Gong *et al.*\(^{(79)}\) cautiously estimated FB\(_1\) intake in this region to range from non-detectable to 23 μg/kg body weight (BW) per d, using a number of assumptions, including estimated average transfer from animal models. The provisional maximum tolerable daily intake for FB\(_1\), FB\(_2\) and FB\(_3\) combined or individually is 2 μg/kg BW per d\(^{(80)}\) and, consequently, fumonisin exposure is a significant health concern in this region.

Urinary FB\(_1\) was also measured in the urine from Chinese adults from Huai'an County, Jiangsu Province \((n = 43)\) and Fusui County, Guangxi Zhuang Autonomous Region \((n = 34)\). The frequency of urinary FB\(_1\) detection was similar in both regions, with 84 and 83% of samples positive for FB\(_1\), respectively, though the mean concentrations were significantly higher in Huai'an county (13630 pg/mg creatinine: range non-detectable to 256 000 pg/mg; median 3910 pg/mg) as compared with Fusui county (720 pg/mg: range non-detectable to 3720 pg/mg; median 390 pg/mg). Moreover, the average estimated FB\(_1\) intakes were about three- to four-fold higher in the Huai'an region\(^{(81)}\). These data would suggest that about 1–2% of the ingested FB was transferred to urine. However, FFQ information and food measures of household items for FB\(_1\) were used to estimate typical FB\(_1\) intake. No significant correlation between urinary FB\(_1\) and estimated intake was found, an observation likely to reflect that the FFQ used measured typical intakes over weeks rather than recent intake (in d) at the time of urine collection. Regardless, in both regions, the mean predicted intake of fumonisin was similar to that in Mexico\(^{(79)}\) and South Africa\(^{(82)}\), and the provisional maximum tolerable daily intake will frequently be exceeded.

A more targeted validation approach to assess the relationship between urinary FB\(_1\) and FB\(_1\) ingestion was attempted in South Africa\(^{(82)}\). Here, urinary FB\(_1\) was measured on two consecutive days whilst FB\(_1\) intake was assessed using plate-ready food (a maize porridge) on the days immediately before urine collection. Here, the resulting urinary FB\(_1\) concentrations were roughly of the same order as those in both Mexico\(^{(79)}\) and Fusui County, China\(^{(81)}\), but significantly lower than those reported in Huai'an County, China\(^{(81)}\). In the South African
study the geometric mean daily estimated FB1 consumption over 2 d was 4·84 (95th percentiles 2·87, 8·14) μg/kg BW per d, and the geometric mean urinary FB1 concentration for subsequent mornings’ first void was 225 (95% CI 144, 350) pg/ml. Urinary FB1 data were also presented after adjustment for creatinine (geometric mean 470 (95% CI 295, 750) pg/mg). After data were natural log-transformed, a statistically significant, albeit moderate correlation ($r^2$ 0·31) was observed between estimated FB1 intake/kg BW per d and urinary FB1 adjusted for creatinine. Based on rough estimates (by authors of the present review) back-transformed data suggest that the highest estimated intake of FB1 was 90 μg/kg BW per d (forty-five times the recommended tolerable daily intake for FB1) and the highest urinary FB1 concentration was approximately 4900 pg/mg; these were not measures for the same individual. The reported correlation used natural log-transformed data, and whilst statistically appropriate, comparisons of the correlations or $r^2$ values in this survey and those used in part to validate other mycotoxin exposure markers are not straightforward. The authors of the South Africa study additionally conducted a successful hand-sorting and kernel-washing intervention on the maize to reduce FB contamination in it\textsuperscript{82}. Overall, a significant ($P<0·05$) 62% reduction in estimated FB1 intake was reported based on FB1 in plate-ready maize porridge before and following the intervention, whilst a borderline ($P=0·06$) 41% reduction in mean urinary FB1 (pg FB1/mg creatinine) was reported. Further, this paper presented an estimate of the transfer of FB1 to urine to be approximately 0·075 % of that ingested, a value comparable with, but slightly lower than that reported in animals\textsuperscript{68–72} and similar to values in a limited human kinetics study conducted in the USA\textsuperscript{83}. Based on this quantitative data, urinary FB1 was regarded as an exposure biomarker for fumonisin\textsuperscript{82}.

Urinary FB1 concentrations were more moderate in a recent report from Sri Lanka, though biomarker validation was not discussed\textsuperscript{84}. Further confirmatory work in using urinary fumonisin as an exposure biomarker in humans is anticipated, including stability studies, with additional interest in measurements of modified sphingoid bases, rather than the unsuccessful alternative putative markers of fumonisin exposure (at least to date) that utilised sphinganine and sphingosine bio-measures\textsuperscript{76,85}.

**Deoxynivalenol**

DON is a type B trichothecene mycotoxin (Fig. 4) which is predominantly associated with crop contamination with *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum* fungi. Both of these fungi are important plant pathogens that cause *Fusarium* head blight in wheat and *Gibberella* ear rot in maize\textsuperscript{11}. Also known as vomitoxin, DON is a common contaminant of cereals such as wheat, maize and barley\textsuperscript{19,86}. The potent gastrointestinal effects (and hence ‘vomitoxin’) in animals and suspected in humans have been reviewed in detail\textsuperscript{19,86} and not discussed further.

In resistant animal species, DON is readily detoxified by gut microbiota to a de-epoxy metabolite known as DOM-1 (de-epoxydeoxynivalenol)\textsuperscript{87–90}, though this pathway was not apparent in a small survey of human faecal metabolism\textsuperscript{91}. DON can also be metabolised in the liver to a glucuronide\textsuperscript{89,90}. Based on DON metabolism and toxicokinetics in the rat and a pilot survey in human subjects, the combined measure of unmetabolised DON or ‘free’ DON (FD) and DON-glucuronide (DG), subsequently referred to here as urinary fD + DG, was suggested as a putative urinary exposure biomarker by Meky et al.\textsuperscript{92}. Improved DG enzymic digestion conditions to release fD, and the use of [\textsuperscript{13}C15]DON as an internal standard have provided significant improvements in precision and accuracy in the urinary assay\textsuperscript{93}. The process of validating this putative exposure biomarker was subsequently undertaken using this modified assay.

In a survey of UK adults, urinary DON was measured in a subset of 300 individuals selected based on cereal consumption from the 2nd/3rd, the 5th/6th and the 9th deciles (100 individuals from each group) from a larger cohort of 1724 individuals. Urinary fD + DG was detected in 98·7 % of individuals (95% CI 82·, 9·7; range non-detectable–48·2) ng/mg)\textsuperscript{94}. A modest, but significant, positive association was observed between the urinary measure and cereal consumption ($P<0·001$; $r^2$ 0·23). Of the cereal items consumed, bread predominated in both frequency and mean level of consumption. In order to improve our understanding of the role of cereal in the diet and its relation to the biomarker concentration, a wheat restriction intervention was used to assess the effects of a 4 d avoidance of major sources of dietary DON on subsequent urinary fD + DG concentration. The intervention reduced wheat intake by >90 %, and the urinary concentration of fD + DG was also significantly
Table 1. Summary of the main studies of mycotoxin biomarker correlations with intake*  

<table>
<thead>
<tr>
<th>Biomarker Matrix</th>
<th>Location of study</th>
<th>Estimated relevant time frame</th>
<th>Approximate dose transferred (%)</th>
<th>Unadjusted $R^2$</th>
<th>Adjusted $R^2$</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin–N7-guanine Urine</td>
<td>Guangxi, China</td>
<td>24–48 h</td>
<td>1</td>
<td>0.80</td>
<td>0.82</td>
<td>0.69</td>
</tr>
<tr>
<td>Aflatoxin–N7-guanine Urine</td>
<td>The Gambia</td>
<td>24–48 h</td>
<td>1</td>
<td>0.82</td>
<td>0.82</td>
<td>0.69</td>
</tr>
<tr>
<td>Aflatoxin M1 Urine</td>
<td>Guangxi, China</td>
<td>24–48 h</td>
<td>1–3</td>
<td>0.82</td>
<td>0.82</td>
<td>0.69</td>
</tr>
<tr>
<td>Aflatoxin–albumin Sera</td>
<td>Guangxi</td>
<td>2–3 months</td>
<td>1–3</td>
<td>0.69</td>
<td>0.69</td>
<td>0.54</td>
</tr>
<tr>
<td>Ochratoxin A Sera</td>
<td>UK</td>
<td>?</td>
<td></td>
<td>0.24</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>Ochratoxin A Urine</td>
<td>UK</td>
<td>?</td>
<td></td>
<td>0.52</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>Fumonisin B1† Urine</td>
<td>South Africa</td>
<td>24–48 h</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Deoxynivalenol§ Urine</td>
<td>UK</td>
<td>24–48 h</td>
<td>75</td>
<td>0.74</td>
<td>0.74</td>
<td>0.74</td>
</tr>
</tbody>
</table>

*Only the fumonisin study used intake/kg body weight per d. The higher the correlation coefficient the stronger the relationship; thus, for example, urinary OTA would be regarded as the preferred exposure biomarker to assess the level of ochratoxin A exposure compared with serum ochratoxin A, and urinary aflatoxin–N7-guanine would be regarded as strong for aflatoxin exposure.

†Correlation used natural log-transformed data of both intake and the urinary measure; correlations for all other mycotoxin comparisons used unmodified data.

‡Adjusted for age.

§Deoxynivalenol and deoxynivalenol-glucuronide combined.

kAdjusted for age, body weight and sex.

(P<0.001) reduced (geometric mean pre-intervention 7.2 (95% CI 4.9, 10.5) ng/mg) by >90% (geometric mean post-intervention 0.6 (95% CI 0.4, 0.9) ng/mg). This intervention restricted the types of food consumed rather than attempting to reduce the contamination level within the consumed food; thus readers should avoid a direct comparison of this outcome with that of the FB intervention above. The intervention on DON was a more simple study to improve our understanding of the biomarker, whilst the FB intervention was a more practical study aimed at providing a solution to the exposure. These data on DON provided further support for the approach of Meky et al. using urinary fD + DG as an exposure biomarker.

Subsequently the concentration of urinary fD + DG was assessed over a longer period (6 d) during the consumption of the normal diet. The frequency of detection and range were similar to those of earlier studies (geometric mean 10·1 ng/mg creatinine; range non-detectable–70·7 ng/mg); again, the concentration of DON was significantly, but moderately, associated with cereal intake ($R^2$ 0.23; $P<0.001$). A 4 d dietary restriction was then initiated in which bread provided the only major source of cereals potentially contaminated with DON; consumption of alternative starch-based foods were suggested to maintain energy intake (for example, rice, potatoes). Participants provided a duplicate portion of every bread sample consumed for DON analysis, and they recorded the quantity consumed of each bread sample. The mean bread consumption was 155 (range 0–455) g/d. The mean contamination level of the bread was 74 (range 20–316) μg/kg, and the estimated average daily intake of DON was 10·6 (range 0–42·5) μg/kg. On a daily basis, urinary fD + DG was correlated with DON intake ($P<0.001$; $r^2$ 0.56, 0.49, 0.54, 0.64, for each day respectively), and a more integrated assessment of the 4 d combined revealed a highly significant correlation ($r^2$ 0.74; $P<0.001$). After adjustment for age, sex and BMI, all correlations remained significant ($P<0.001$; adjusted $r^2$ 0.63, 0.68, 0.65, 0.65 and 0.83, respectively), providing the first data of a strong quantitative relationship between exposure and the urinary biomarker.

Further studies have been initiated to assess the stability of urinary fD + DG during the collection phase when samples may be at ambient temperature for prolonged periods (hours), and during the cryopreservation period (years). No losses were observed in samples stored at ambient temperature (18°C) for up to 24 h, or at 4°C for 48 h. No reduction in biomarker levels were found in cryopreserved samples kept at −40°C for up to 3 years. In studies to date DG appears to be the major metabolite of DON in human urine, whilst the de-epoxy metabolite has rarely been observed. Urinary fD + DG represents best exposure in the previous 24–48 h, though average cereal intake over 7 d was significantly, but not as strongly, associated with the biomarker. Additionally,
colleagues in Austria are investigating a putative direct measure of urinary DG\(^{103}\). The combined measure of urinary fD + DG now serves as a validated exposure biomarker for DON intake\(^{95–97,100}\), at least in moderately exposed populations. To date, populations predicted to have the highest DON exposures remain unexamined with this assay\(^{95}\). Nevertheless, for all of the European studies to date, approximately 1–5% of the population of adults are predicted to exceed the recommended tolerable daily intake of 1 \(\mu g/kg\) BW per d\(^{104}\). An earlier and perhaps less robust version of this assay was used to analyse urinary fD + DG in a pilot survey in a high-risk region of China\(^{92}\). Data from that survey revealed a mean level of urinary fD + DG about three to four times greater than the mean reported in surveys of Europeans\(^{100}\). Urinary fD + DG has now been observed in studies from the UK, France, Sweden and China\(^{100}\), and independently in Spain and Italy\(^{105,106}\), whilst urinary DG was observed in Austrians\(^{103}\).

All of the mycotoxin biomarker approaches discussed here are summarised in Table 1; ochratoxin A is additionally included for completeness, as the only other mycotoxin for which biomarker development has been reported\(^{107}\). Summaries of aflatoxin biomarker surveys are in numerous reviews; here only aflatoxin–albumin biomarkers are presented from studies of West African infants and children (Table 2), as these relate to latter sections of this review. A summary of surveys on Fusarium mycotoxins is included (Tables 3 and 4).

It is also prudent to emphasise a significant difference in the urinary assays for aflatoxin, fumonisin and DON. Whilst the analytical sensitivity may be suggestive of greater, lesser or a similar sensitivity in the exposure assessment, the overall sensitivity of the bio-measurement is also related to the transfer kinetics of the toxins. If, for example, we compare urinary biomarkers for aflatoxin M\(_1\), FB\(_1\) and fD + DG, the analytical limits of detection are 5, 20 and 500 pg/ml, respectively, whilst the mean estimated amounts transferred to urine are about 2, 0.075 and 72%, respectively. Assuming average BW of about 65 kg and 1.5 litres of urine excreted, the limits of detection correspond to a mean intake of 0.006, 0.615 and 0.015 \(\mu g/kg\) BW per d, respectively. Thus, similar levels of these biomarkers in urine do not represent similar levels of exposure, and across a range of typical human exposures, urinary DON will be detected more frequently than FB\(_1\), for example, despite a greater analytical sensitivity for FB\(_1\) compared with DON.

### Table 2. Summary of some aflatoxin–albumin survey data in West African children*

<table>
<thead>
<tr>
<th>Country</th>
<th>Subjects (n)</th>
<th>Mean (pg/mg)</th>
<th>Range (pg/mg)</th>
<th>% Positive</th>
<th>Age group</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benin and Togo</td>
<td>479</td>
<td>33</td>
<td>nd–1064</td>
<td>99</td>
<td>Child</td>
<td>Gong et al.(^{48})</td>
</tr>
<tr>
<td>Benin†</td>
<td>200</td>
<td>37</td>
<td>nd–688</td>
<td>98</td>
<td>Child</td>
<td>Gong et al.(^{49})</td>
</tr>
<tr>
<td>Benin</td>
<td>200</td>
<td>39</td>
<td>nd–744</td>
<td>99</td>
<td>Child</td>
<td></td>
</tr>
<tr>
<td>Benin</td>
<td>200</td>
<td>88</td>
<td>5–1568</td>
<td>100</td>
<td>Child</td>
<td></td>
</tr>
<tr>
<td>Guinea</td>
<td>124</td>
<td>9</td>
<td>nd–262</td>
<td>96</td>
<td>Child</td>
<td>Turner et al.(^{51})</td>
</tr>
<tr>
<td>The Gambia</td>
<td>119</td>
<td>40</td>
<td>5–261</td>
<td>100</td>
<td>Pregnant</td>
<td>Turner et al.(^{52})</td>
</tr>
<tr>
<td>The Gambia</td>
<td>99</td>
<td>10</td>
<td>5–190</td>
<td>49</td>
<td>Cord</td>
<td></td>
</tr>
<tr>
<td>The Gambia</td>
<td>118</td>
<td>9</td>
<td>5–30</td>
<td>11</td>
<td>16 weeks</td>
<td></td>
</tr>
<tr>
<td>The Gambia</td>
<td>128</td>
<td>60</td>
<td>nd–391</td>
<td>92</td>
<td>52 weeks</td>
<td>Turner et al.(^{53})</td>
</tr>
<tr>
<td>The Gambia</td>
<td>466</td>
<td>24</td>
<td>nd–456</td>
<td>93</td>
<td>Child</td>
<td>Turner et al.(^{54})</td>
</tr>
<tr>
<td>The Gambia</td>
<td>444</td>
<td>41</td>
<td>3–459</td>
<td>100</td>
<td>Child</td>
<td>Turner et al.(^{55})</td>
</tr>
<tr>
<td>The Gambia</td>
<td>391</td>
<td>57</td>
<td>nd–720</td>
<td>83</td>
<td>Child</td>
<td>Allen et al.(^{48})</td>
</tr>
</tbody>
</table>

\(\text{nd, Non-detectable.}\)

\(*\text{Adapted from Turner et al.}^{107}\)

† A total of 200 children measured at three time points within an 8-month period.

### Mycotoxins and human disease

In many parts of the developing world, chronic exposure to aflatoxins at high levels remains a significant health burden\(^{5–7,21–23,108–110}\). About 20 years ago, aflatoxin B\(_1\) was classified as a potent liver carcinogen\(^{23}\); a conclusion in part defined using the above biomarkers, and mentioned only briefly here; readers are directed to an excellent recent review of the topic\(^{22,53}\) for further details. This section of the review will focus more on the recent observation between aflatoxin and growth faltering.

### Aflatoxins and liver cancer

In high-risk parts of Asia and Africa >95% of populations studied to date are chronically exposed to aflatoxins\(^{5,6,21–23}\). Aflatoxins are potent liver toxins and it is likely that a combination of non-specific liver damage/toxicity and specific DNA damage via aflatoxin exo-epoxide covalently binding to N7-guanine of DNA contribute to the carcinogenicity of aflatoxins\(^{5–7,21–23}\). It is notable that a synergistic interaction between aflatoxin and hepatitis B virus (HBV) occurs\(^{22,23,53}\), perhaps in part a reflection of modulation of aflatoxin metabolism (favouring activation over detoxification) and liver regeneration in HBV-infected individuals\(^{53}\); this effect may enrich the liver for mutations caused by aflatoxin–DNA damage. One important facet of
The global burden of liver cancer is about 550,000 to 600,000 cases annually, predominantly due to hepatitis infections and aflatoxins\(^{(25)}\). It was estimated that up to 28% of this burden might be due to aflatoxin alone\(^{(101)}\). Moreover, the onset of liver cancer in populations with both HBV and aflatoxin is significantly earlier than those with just HBV\(^{(5–7,21–23,108,109)}\), such that reductions in aflatoxin exposure may reduce not only the occurrence, but delay the onset of disease\(^{(22)}\). To improve our understanding of this potential mechanism, it would be of value to assess more detailed aflatoxin metabolite profiles in both HBV carriers and non-carriers. Such information may support intervention strategies that combine both reductions in exposure\(^{(111)}\) and dietary modulation of aflatoxin metabolism (for reviews, see Kensler\(^{(22)}\) and Wogan\(^{(109)}\)).

### Table 3. Exposure biomarker surveys for the *Fusarium* mycotoxin fumonisin B\(_1\)*

(Mean values and 95% confidence intervals)

<table>
<thead>
<tr>
<th></th>
<th>Detection rate</th>
<th>Fumonisin B(_1) (pg/ml)</th>
<th>Fumonisin B(_1) (pg/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>(n%)</td>
<td>Mean 95% CI</td>
</tr>
<tr>
<td>Mexico† (low consumption group)</td>
<td>25</td>
<td>11/25</td>
<td>44</td>
</tr>
<tr>
<td>Mexico† (medium consumption group)</td>
<td>25</td>
<td>20/25</td>
<td>80</td>
</tr>
<tr>
<td>Mexico† (high consumption group)</td>
<td>25</td>
<td>24/25</td>
<td>96</td>
</tr>
<tr>
<td>Centane, South Africa (baseline)</td>
<td>22</td>
<td>43/44</td>
<td>98</td>
</tr>
<tr>
<td>Centane, South Africa (intervention)</td>
<td>21</td>
<td>40/42†</td>
<td>95</td>
</tr>
<tr>
<td>Huaian, China</td>
<td>43</td>
<td>36/43</td>
<td>84</td>
</tr>
<tr>
<td>Fusai, China</td>
<td>34</td>
<td>28/34</td>
<td>82</td>
</tr>
</tbody>
</table>

*a* Geometric means are reported except for the study in China\(^{(116)}\).

† Overall, 56/75 were positive (> 20 pg/ml); mean 70 pg/ml; range non-detectable to 9312 pg/ml.

‡ Urinary data are the mean of 2 d data, overall range of data non-detectable to 3900 pg/ml (estimated by the authors of the present review).

### Table 4. Exposure biomarker surveys for the *Fusarium* mycotoxin deoxynivalenol

<table>
<thead>
<tr>
<th></th>
<th>Detection rate</th>
<th>Deoxynivalenol (ng/ml)</th>
<th>Deoxynivalenol (ng/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>(n%)</td>
<td>Mean</td>
</tr>
<tr>
<td>China (high risk)</td>
<td>9</td>
<td>9/9</td>
<td>100</td>
</tr>
<tr>
<td>China (moderate risk)</td>
<td>6</td>
<td>6/6</td>
<td>100</td>
</tr>
<tr>
<td>UK* (normal diet)</td>
<td>25</td>
<td>25/25</td>
<td>100</td>
</tr>
<tr>
<td>UK* (intervention diet)</td>
<td>25</td>
<td>17/25</td>
<td>68</td>
</tr>
<tr>
<td>UK* (2nd/3rd percentile group)</td>
<td>100</td>
<td>97/100</td>
<td>97</td>
</tr>
<tr>
<td>UK* (5th/6th percentile group)</td>
<td>100</td>
<td>99/100</td>
<td>99</td>
</tr>
<tr>
<td>UK* (9th percentile group)</td>
<td>100</td>
<td>100/100</td>
<td>100</td>
</tr>
<tr>
<td>UK normal diet</td>
<td>Day 1</td>
<td>35</td>
<td>35/35</td>
</tr>
<tr>
<td>Day 2</td>
<td>35</td>
<td>34/35</td>
<td>97</td>
</tr>
<tr>
<td>Day 3</td>
<td>35</td>
<td>31/35</td>
<td>86</td>
</tr>
<tr>
<td>Day 4</td>
<td>35</td>
<td>34/35</td>
<td>97</td>
</tr>
<tr>
<td>Day 5</td>
<td>35</td>
<td>32/35</td>
<td>91</td>
</tr>
<tr>
<td>Day 6</td>
<td>35</td>
<td>33/35</td>
<td>94</td>
</tr>
<tr>
<td>UK intervention diet</td>
<td>Day 1</td>
<td>10</td>
<td>6/10</td>
</tr>
<tr>
<td>Day 2</td>
<td>10</td>
<td>5/10</td>
<td>50</td>
</tr>
<tr>
<td>Day 3</td>
<td>10</td>
<td>3/10</td>
<td>30</td>
</tr>
<tr>
<td>Day 4</td>
<td>10</td>
<td>2/9</td>
<td>22</td>
</tr>
<tr>
<td>UK (pregnancy)</td>
<td>86</td>
<td>85/86</td>
<td>99</td>
</tr>
<tr>
<td>French male farmers</td>
<td>76</td>
<td>75/76</td>
<td>99</td>
</tr>
<tr>
<td>Swedish</td>
<td>29</td>
<td>28/29</td>
<td>97</td>
</tr>
</tbody>
</table>

nd, Non-detectable.

* Both interventions were voluntary restrictions of major sources of dietary wheat products. The two studies have completely independent data.
Aflatoxins and growth faltering

In animals, aflatoxins suppress the immune system and cause growth faltering\(^{112,113}\). Given the frequent chronic human exposure to this toxin, it is important to understand whether typical levels of ingestion in some high-risk regions restrict growth in children.

Aflatoxin–albumin exposure patterns in infants

Longitudinal biomarker data on aflatoxin exposure during the peri-natal and postnatal period are limited in the literature. One longitudinal study in The Gambia revealed a pattern of maternal, cord blood, week-16 infant and week-52 infant aflatoxin–albumin adducts at 100% (range 5–400 pg/mg), 49% (range non-detectable–50 pg/mg), 11% (non-detectable–50 pg/mg) and 92% (non-detectable–390 pg/mg) positive, respectively\(^{52,53}\). Whilst no data are available on infants from developed countries, a recent survey in North American adults revealed that less than 1% would exceed 3–5 pg/mg\(^{65}\), the limit of detection in the Gambian study\(^{52,53}\). Week-16 adduct positivity correlated with the introduction of weaning foods, and by 52 weeks, most infants (>95%) had started the weaning process. The week-16 and week-52 data reveal that aflatoxin from diet occurs early in life once weaning foods are introduced. The cord blood data reveal that both exposure and metabolism to reactive epoxides occurs in utero.

A cross-sectional survey of children aged 9–60 months from Benin revealed a 99% positive rate (mean adduct level 33 pg/mg; range non-detectable–1064 pg/mg), geometric mean aflatoxin biomarker levels were twice the level in 1- and 2-year-olds compared with children < 1 year old, and twice as high again in 2- and 3-year-olds\(^{48}\). No significant increases were apparent in older children up to 5 years old. In two separate studies of Gambian children the frequency of detection and range of aflatoxin–albumin adducts were similar between 3- and 4-year-olds (100% positive; range 2–459 pg/mg\(^{55}\) and 6- to 9-year-olds (93% positive; range non-detectable–456 pg/mg\(^{54}\) and such patterns of exposure reflect those observed in Gambian adults\(^{52,53}\). Exposure patterns between children (aged > 2 years) and adults in Guinea were similar also\(^{51,114–116}\). Thus, there is a rapid increase in aflatoxin–albumin biomarker frequency and level as infants from high-risk countries go through the weaning process.

Aflatoxin biomarker levels are associated with growth faltering

A cross-sectional survey in Benin demonstrated a strong association between aflatoxin biomarker level (aflatoxin–albumin) and growth of children aged ≤5 years\(^{48}\). Growth was assessed as height-for-age and weight-for-age Z scores. Both height-for-age and weight-for-age Z scores were inversely associated with the aflatoxin exposure biomarker, indicative of a relationship between aflatoxin and both stunting and being underweight (\(P<0.001\) for both).

A subsequent longitudinal study in infants from different villages, but from the same region, further support these cross-sectional observations\(^{49}\). These latter data suggested that across an exposure spectrum in which 16% of those infants measured exceeded an adduct burden of 100 pg/mg albumin, that 100 pg/mg difference in exposure approximates to about a 1 cm reduction in height over an 8-month period. In a separate study in slightly older Gambian children (aged 6–9 years), a more modest association between aflatoxin exposure and growth faltering was reported, though only 7% of samples in that study exceeded 100 pg/mg\(^{54}\). Thus, it remains unclear whether the strength of effect reflects differences by age, the adduct burden, or perhaps both.

In The Gambia, the average level of aflatoxin–albumin adducts in maternal blood during pregnancy (collected at two time points separated by >1 month) was strongly associated (\(P<0.001\)) with growth faltering of the infant during the first year of life\(^{52}\). The aflatoxin–albumin adduct level of the infant at 16 weeks significantly negatively correlated (\(P<0.05\)) with infant growth\(^{52}\). These studies are further supported by data in additional epidemiological studies\(^{117,118}\).

Aflatoxin and gastrointestinal toxicity

Growth faltering in West Africa does not seem to be fully explained by either lack of nutrition or by infectious episodes\(^{119–121}\), though these remain critically important contributors. Growth faltering and intestinal damage in this region are apparent following the introduction of weaning foods and independently strong associations between aflatoxin and growth faltering, as described above\(^{48,49,52}\) and elsewhere\(^{122,123}\) have been revealed. Human aflatoxin exposure is primarily through dietary contamination and, given the requisite metabolism to form the reactive epoxide, the intestine is a primary target for aflatoxin-induced damage. The intestinal epithelium separates the intestinal lumen from the underlying lamina propria. These epithelial cells are tightly bound by intercellular junctional complexes, which regulate the paracellular permeability, and thus maintain epithelial integrity. In confluent Caco-2 monolayers, a cell line that mimics the intestinal barrier, aflatoxin appears to affect the integrity of the monolayer by modulating paracellular transport\(^{124}\). Thus, aflatoxin may further exacerbate dietary restricted individuals, and perhaps prolong a cycle of poor nutrient retention, extended gastrointestinal infection, and enteropathy. Intestinal enteropathy, often described as ‘intestinal leakiness’, is associated with the level of growth faltering in Gambian infants\(^{319,120}\), though to date dose–response relationships between intestinal enteropathy and aflatoxin load remain to be demonstrated.
A variety of proteins are involved in the formation of intercellular functional complexes including tight junction complexes\(^{125}\). Tight junctions are in part regulated by specific phosphatases and kinases. Interactions between transmembrane proteins and the actomyosin ring are controlled by several signalling proteins, including protein kinase C, mitogen-activated protein kinases, myosin light chain kinase and the Rho family of small GTPases\(^{126}\). Phosphorylation of tight junction proteins controls epithelial barrier function\(^{126}\). One of the key toxic effects of aflatoxin is disruption of phosphorylation patterns of structural and enzymic proteins, due to steric hindrance caused by the epoxide binding\(^{127}\). It is therefore plausible that the intestinal cell enteropathy observed in vitro reflects aflatoxin-induced disruption of the phosphorylation of key structural proteins in tight junction formation. To improve our understanding of this potential mechanism of growth faltering it will be valuable to understand aflatoxin-induced changes in tight junction protein localisation and phosphorylation status.

**Aflatoxin and zinc**

Human Zn deficiency has been a recognised health concern for about 50 years; typical symptoms include growth retardation, skin abnormalities and mental lethargy\(^{128}\). Dietary deficiency is a particular problem in developing countries, and a number of studies support supplementation of children aged <5 years to improve linear growth and reduce growth stunting\(^{129}\). In animal models, aflatoxins have a clear effect on growth, and it is notable that piglets of aflatoxin-exposed sows exhibit growth faltering\(^{130}\). One interesting observation is a reduced plasma Zn level in piglets from aflatoxin-exposed sows compared with those from non-exposed sows\(^{131}\); this was related to a reduced Zn-carrying capacity due to thymulin–Zn complex formation in the offspring from exposed sows, rather than a lack of Zn in the diet\(^{131}\). Importantly, doses used in this animal study are in line with those observed in studies of maize for human consumption\(^{132}\). Thus, a second area of research into growth faltering in human subjects from regions with chronic and high aflatoxin exposure is to understand mechanisms that control the balance between inactive thymulin and active (Zn-bound) thymulin.

**Aflatoxin and insulin-like growth factor**

Aflatoxins can modulate gene expression in target organs including the liver. A number of growth factors are critical components in maintaining animal longitudinal growth. One such factor is liver-derived insulin-like growth factor (IGF-1), which supplies at least 75% of IGF-1 in circulation. Locally produced IGF-1 also affects linear bone growth, though both local and liver-derived sources are believed to be important\(^{132}\). Microarray data comparing livers of untreated and aflatoxin-treated chicks revealed down-regulation of genes responsible for fatty acid metabolism, oxidative phosphorylation, energy production, cell proliferation, immune response, metabolism, growth and development in treated animals\(^{133}\). Of particular interest regarding growth was the down-regulation of IGF-1, which could have contributed to the observed reduction in growth rates in this particular study. This type of growth faltering was consistent with earlier reports of the effect of aflatoxins on broiler chick growth\(^{134}\).

Studies in children suggest a link between aflatoxin exposure and kwashiorkor, a form of protein malnutrition\(^{122,123}\), though designs of this particular study were not ideal. Aflatoxin exposure was certainly occurring in those children; however, validated exposure biomarkers were not utilised, and thus dose–responses were not measurable. In one study, twenty-two children with protein–energy malnutrition (either kwashiorkor (n 9) or marasmus (n 13)) from Gabon aged <30 months were examined for their nutritional status with reference to the growth hormone–insulin-like growth factor axis\(^{135}\). Although aflatoxins were not measured, this region does experience chronic exposure to aflatoxins. In this study, IGF-1 was significantly lower in malnourished children compared with controls. These authors speculate that the change is related to the extent of malnutrition, as refeeding improved IGF-1 levels. However, no data were presented for a role for aflatoxin in this study, and IGF-1 levels may simply reflect poor growth, rather than exposure to aflatoxin; thus data on the temporal nature of aflatoxin exposure with this measure would be more informative. It has also been suggested that aflatoxin-induced up-regulation of IGF-2 may be important in hepatocarcinogenesis\(^{136}\), though IGF-2 does not play an important role in linear growth. At this point additional research is required to understand this hypothesised interaction between aflatoxin, the IGF axis and growth faltering.

**Fumonisin B, deoxynivalenol and growth faltering**

To date, there are no strong epidemiological data on links between *Fusarium* mycotoxins and child growth, though it is hoped that the use of biomarkers discussed above may allow improved exposure assessment to inform such studies. In cultured cells, prolonged exposure to FB\(_1\) has been demonstrated to reduce intestinal barrier integrity\(^{137}\), and to cause an increase in the ease of movement of macromolecules across the monolayer\(^{138}\). The mechanism(s) for such an effect remains unknown, though it is clearly established that fumonisins disrupt sphingolipid metabolism, key components in the integrity of the cell membranes\(^{67,78}\). A recent survey from Tanzania suggests an association between estimated FB consumption at age 6 months and the subsequent height and weight of the child at the age of 12 months\(^{139}\). The study included...
215 infants with maize consumption estimated for each child and the level of FB contamination in collected maize samples determined. Maize was consumed frequently (89% consumers) and 69% of the maize-consuming infants were exposed to detectable levels of fumonisin; the range of fumonisins in maize was 21–3201 μg/kg, and twenty-six infants were predicted to have exceeded the provisional maximum tolerable daily intake of 2 μg/kg BW (140). Infant height and weight were significantly negatively (P < 0.05) associated with estimated fumonisin intake. The authors identify a number of limitations in their study. First, there was no use of fumonisin exposure biomarkers. Second, exposure estimates were at 6 months but not 12 months. In that respect, it would also be having been interesting to have data on growth velocity. It is notable that the co-occurrence of both aflatoxin and fumonisin contamination had been reported in an earlier survey of maize in this region (146), and that aflatoxins and fumonisins also co-contaminate maize in Benin (10), where strong associations between aflatoxin and growth faltering were reported (48,49). This research arena eagerly awaits descriptive epidemiology on multiple mycotoxin biomarker measurements to understand the potential causes and mechanisms of dietary contaminants and growth faltering in high-risk regions.

In contrast to the aflatoxins and fumonisins, the trichothecenes occur most frequently in cooler, moist conditions (1,2). Their global distribution thus differs from aflatoxin and fumonisins, predominantly in Europe, North America and parts of South America, China, Japan and New Zealand. DON is one of the most frequently observed trichothecenes found globally and the mechanism of toxicity is associated with its ability to inhibit ribosomal protein synthesis by restricting elongation and termination of polypeptide chains (19). This ribosomal binding is responsible for DON’s effect on the immune system, termed the ‘ribotoxic stress response’, which causes increased apoptosis in leucocytes and increased cytokine production (141).

Most instances of DON-induced feed refusal occur in experimental animals exposed to dietary DON (142–144). However, pigs given an intraperitoneal infusion of DON also show reduced food intake of a DON-free diet (145), suggesting that DON may affect food intake via a neuroendocrine mechanism. Indeed, pigs given an intravenous infusion of DON shown increased levels of the serotonin metabolite 5-hydroxyindoleacetic acid in cerebral spinal fluid (146), although similar studies have found no changes in plasma serotonin levels (147). DON can also cross into brain tissue (148), and cause regional changes in the neurochemistry of pigs, turkeys and rats (149–151). Recent data from Girardet et al. (152) suggest that DON acts centrally to reduce food intake. Here, a 20 μg per mouse intracerebroventricular (ICV) injection of DON caused a significant reduction in food intake over a 12 h period. We point out that 2 μg DON per mouse (weight of 0.020–0.025 kg) is an approximate dose of 0.8–1.0 mg/kg BW and DON has been shown to cause acute anorexia when given via intraperitoneal injection at 1.0 mg/kg BW in female mice (153). Furthermore, Girardet et al. (152) determined that oral DON administration increased central protein expression of the transcription factor c-fos and mRNA expression of the pro-inflammatory mediators IL-1β, TNF-α, IL-6 and cyclo-oxygenase-2 (COX-2), suggesting that DON does indeed cause physiological changes within the brain.

Given DON’s capacity to elicit systemic pro-inflammatory cytokines and cause inflammation in vivo, it has been hypothesised that COX-2 may be important for DON’s ability to cause anorexia and subsequent growth suppression in vivo (154). COX-2, along with microsomal PGE synthase-1 (mPGES-1), are two enzymes responsible for converting arachidonic acid into PGE2 during an inflammatory response (155) and COX-2 has been shown to serve a function in anorexia caused by systemic inflammation (156,157). Indeed, DON induces COX-2 gene expression in vitro (RAW 264·7 murine macrophages) and in vivo (158,159), though the recent use of mPGES-1 knockout mice suggests that a PGE2-independent mechanism causes DON-induced anorexia (152).

Male and female mice fed 5 and 10 parts per million (ppm) DON over a 2-year period exhibit significant weight suppression (160), yet food intake was only significantly reduced in males fed 10 ppm DON, indicating that anorexia may not be the only mechanism by which DON reduces weight. Recently, DON exposure in mice was associated with decreased IGF acid-labile subunit (161), a binding protein of IGF-1 known to be critical for growth (162,163). DON-induced decreases in IGF acid-labile subunit may lead to alterations in the growth hormone system and subsequent growth suppression.

Taken together, growth suppression exhibited by experimental animals exposed to DON probably results from a combination of anorexia caused by alterations in neuroendocrine signalling and modifications of the growth hormone axis.

**Fumonisins and neural tube defects**

Fumonisins have been demonstrated to cause NTD in animals (164–166), and given the frequency and high levels of exposure in certain maize-consuming populations in South Africa, Central America and Asia, there are concerns of similar effects in humans. For example, women living in Cameron County, Texas–Mexico border had NTD incidence of 290 per 100 000, whilst Mexican Americans in general have NTD incidence of 90–160 per 100 000; this is a significantly higher frequency compared with white Caucasians (60 per 100 000) (167). Sphingolipids are important structural components in cell membranes, and fumonisin disruption of sphingolipid biosynthesis interferes with folate receptors, and thus folate bioavailability (164,168).
In a study conducted in pregnant mice, fumonisins given during early gestation caused NTD in 79% of exposed fetuses. Alterations in sphingolipid profiles for both maternal and embryonic tissues occurred, as did both the folate concentration and expression of the folate receptor. Of note in this study was that folate supplementation partially reduced the phenotype of NTD, although the dose level was relatively high, these observations raise some concern for humans chronically exposed to fumonisins.

An increased risk of NTD was significantly associated (OR 2.4; 95% CI 1.1, 5.3) with moderate compared with low consumption of tortillas in the first trimester of pregnancy for women on the Texas–Mexico border. Fumonisins are common contaminants of maize in this region, and maize consumption in the form of tortillas forms a major dietary staple. To better assess fumonisin exposure, maternal sphingolipid disruption (measured as the ratio of plasma sphinganine:sphingosine) and estimated FB consumption based on a limited food survey with FB measurements were determined. Both of these measures followed a similar pattern, with an increased risk of NTD between low and moderate groups, but not with the high exposure group. The authors suggest a threshold effect was possible, above which live births may not be occurring. It is important to note that the sphinganine:sphingosine measure was taken some time after birth (weeks), though the authors suggest that diets, and therefore exposure patterns, do not change much. More critical is the fact that sphinganine:sphingosine remains a putative but, to date, unvalidated exposure biomarker. NTD represents a significant health burden in maize-consuming populations where fumonisin exposure is frequent and at high levels. The animal and epidemiological data strongly support a role for this dietary toxin in increasing the risk of NTD through changes in folate receptor activity. Authors of the above studies highlight the desire for better exposure assessment tools to evaluate the temporal variation of fumonisins during critical exposure periods.

**Fusarium mycotoxins and cancer**

The incidence of squamous-cell carcinomas of the oesophagus (SCCO) is particularly high in parts of China, Iran and South Africa. China reports about 50% of all SCCO cases, with incidence rates in Lixian county as high as 151 and 115 per 100,000 for males and females, respectively. At-risk populations are typically maize consumers, and *Fusarium* mycotoxins, for example, fumonisins and trichotheccenes (DON, nivalenol), frequently contaminate maize in these regions. Ecological surveys examining the differences in the natural occurrence of *Fusarium* mycotoxins in high-risk regions of both China and South Africa are suggestive of a link with SCCO, and in *vitro and animal data provide additional support*, though no biomarker-driven epidemiology is available at this point. Fumonisins cause cancer in the liver and kidneys in rodents. Whilst FB₁ has been classified by the International Agency for Research on Cancer (IARC) as class 2B (possibly carcinogenic), data for DON are insufficient. Exposure biomarkers to *Fusarium* mycotoxins should support an improved understanding of their potential role in SCCO.

**Conclusions**

Mycotoxins are frequent contaminants of the diet in all locations of the world that consume cereals, and additional exposure from nuts predominates in some countries; in fact, 25% of the world’s cereal crops are predicted to be contaminated. In animals, toxicity is clearly established. Aflatoxins are carcinogenic, affect the immune systems and cause growth faltering. Moreover, aflatoxins cause immune suppression and increased susceptibility to infection. There is moderate evidence that they cause disruption to tight junctions and thus exposure may mimic the intestinal enteropathy observed in growth-faltering children. Fumonisins disrupt cell–cell communications and cell integrity via a mechanism linked to sphingolipid biosynthesis disruption; they also cause liver and kidney cancers in rodents, and in rodents cause NTD and intestinal toxicity. DON has potent effects on the immune system, causes gastroenteritis, anorexia and growth faltering in animals, though biomarker-driven epidemiology is lacking.

The present review has highlighted a number of areas including:

1. Established exposure biomarkers for the carcinogenic aflatoxins.
2. Biomarkers to assess the suggested role of aflatoxin in growth faltering.
4. Recent development and the need for *Fusarium* biomarkers for epidemiology.

Intervention strategies to restrict exposure to aflatoxins have been recently reviewed. Notable examples include intervention research in China that has focused on chemoprevention, whilst a Novacil clay that can be added in small quantities to food and act to bind and restrict the bioavailability of aflatoxin has been reported in clinical trials conducted in West Africa. In middle-income countries or regions where exposures may be more moderate, probiotic interventions have been suggested; these probiotics restrict aflatoxin bioavailability by binding and then shuttling the toxin through the gastrointestinal tract. Such an approach has been suggested as plausible in a region such as Egypt where sources of exposure are less well defined. In terms of growth, this approach was successful in a rodent model in that in the intervention arm, both the aflatoxin-driven growth
faltering was improved and the aflatoxin biomarker level was reduced\(^{(179)}\). Whilst effective but highly technical approaches are used in wealthier regions, the small-scale peasant farmers, who represent the majority of those at highest risk of exposure, still remain without established, affordable and sustainable methods to restrict exposures. A primary intervention in such populations successfully restricted contamination at the harvest/post-harvest stage by simple sorting and drying of the main crops\(^{(111)}\). The study focused on aflatoxin from the groundnut (peanut) crop in Guinea, and demonstrated over a 6-month period a significant \((P<0.001)\) reduction in aflatoxin biomarkers, by \(>50\%\), in the intervention villages. Such an approach is simple and relatively affordable. It is now important to demonstrate efficacy in different settings and with other high-risk crops such as maize. Dissemination and application of this and other intervention data, in an appropriate manner is then urgently required\(^{(180)}\), such that the research efforts are translated into the community settings where risk is greatest.

It is also important to note that we believe that whilst evidence for aflatoxin ‘contributing’ to growth faltering is strong, this occurs alongside a background of additional factors, including limited nutritional quantity and choice, and infection. Thus, one postulated mechanism is that aflatoxin exacerbates pathologies important in intestinal enteropathy-driven growth faltering. Other mechanisms related to aflatoxin toxicity discussed here remain poorly researched. The molecular epidemiology for other mycotoxins is lacking, but exposure biomarkers may better support current epidemiological approaches to assess the potential burden of mycotoxin-driven chronic disease.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All authors contributed to writing the manuscript. There are no conflicts of interest.

References

Biomarkers to evaluate mycotoxin exposure

Heterocyclic Aromatic Amines and Mycotoxins, vol. 56, IARC Monograph. Lyon: IARC.


93. Turner PC, Burley JV, Rothwell JA, et al. (2008) Dietary wheat reduction decreases the level of urinary...
Biomarkers to evaluate mycotoxin exposure


153. P. C. Turner et al.


