Fumonisin contamination of food: progress in development of biomarkers to better assess human health risks

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Abstract

Fumonisins, fungal toxins produced by Fusarium moniliforme, contaminate maize based foods and feeds throughout the world. They cause liver and kidney toxicity in animals in addition to leukoencephalomalacia in horses and pulmonary edema in pigs. Fumonisin B1 is carcinogenic in rats and mice. Ecological studies have linked consumption of fumonisin contaminated maize with oesophageal cancer in human populations in South Africa and China. This review discusses the potential health risks for people exposed to the fumonisins, and describes how mechanistic studies of toxicity in animal models have allowed the development of putative biomarkers of fumonisin exposure at the individual level. The requirements for an applicable biomarker include sample availability as well as a high specificity and sensitivity for the exposure of interest. Most environmental toxic insults involve complex exposures both to other toxins and to infections; these confounding factors need to be considered in assessing both the validity of the biomarker and the exposure-disease associations. Fumonisins can be detected in the urine of animals in feeding studies but the sensitivity of the current methodology means only highly exposed people could be monitored. Mechanistic studies indicate that ceramide synthase, an enzyme involved in sphingolipid synthesis, is one cellular target for fumonisin toxicity and carcinogenicity, and this disruption to sphingolipid metabolism increases the ratio of two sphingoid precursors, sphinganine and sphingosine. The altered ratio has been observed in tissues, serum and urine for a number of animal models suggesting it as a good candidate marker of fumonisin exposure. Despite development of analytical methods to measure this biomarker there have been no studies to date correlating it to fumonisin intake in people. Given the toxic effects of fumonisins in animals and the widespread human exposure, which has been calculated to reach 440 μg kg⁻¹ body weight day⁻¹ in a population consuming high quantities 460 g day⁻¹ of contaminated maize, then the development of biomarkers and their application in epidemiological studies should be a priority for research on these toxins. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Fungal growth and subsequent contamination of animal feeds and human foodstuffs by toxins...
(mycotoxins) presents both an economic and a health risk. The mycotoxins of major concern for human health are produced by three main genera of fungi (Aspergillus, Fusarium and Penicillium). Fungal growth and mycotoxin contamination can occur during crop growth, harvesting or storage. Temperature and humidity are important factors affecting fungal growth and mycotoxin production, however, the associated health risks are also related to the regulatory infrastructure and financial support to enforce suitable monitoring and regulation. These conditions, coupled to restricted nutrition and often compounded by additional infection and disease, have predominately restricted adverse impact from mycotoxin contamination to the developing countries of Asia and Africa.

The fumonisins, produced by Fusarium moniliforme and F. proliferatum, are one family of mycotoxins which contaminate feeds and foodstuffs, predominantly maize-based products, throughout the world. This manuscript will briefly review the occurrence of fumonisins, their toxicity and aspects of their biological mechanism of action. The mechanistic information is presented in the context of identifying metabolites or biological effects of fumonisins which could serve as biomarkers of human exposure to these toxins. A number of proposed biomarkers of fumonisin exposure are critically evaluated. It is our contention that the ability to accurately measure human exposure to fumonisins is a prerequisite to assessing the health risks associated with these common toxins.

Fumonisins are produced mainly by F. moniliforme [1–4], one of the most prevalent fungi of maize based crops [4–6]. Fumonisin B$_1$ (FB$_1$) and FB$_2$ were structurally identified in 1988 [2]. Other members of the fumonisin family have been structurally characterised including: FA$_1$ and FA$_2$ [1]; FB$_3$, FB$_4$ and FB’s hydrolysis products [7]; FC$_1$ and FC$_2$ [8]; and FP$_1$, FP$_2$ and FP$_3$ [9]; but of the currently identified fumonisins FB$_1$, FB$_2$ and FB$_3$ occur most abundantly in contaminated maize [6].

Experimental animal studies have linked fumonisins primarily to liver and kidney toxicity, and demonstrated hepatocarcinogenicity in rats [5]. In addition, a positive correlation in a number of ecological studies between dietary fumonisins and human oesophageal cancer rates has been reported in Africa and China [10–13]. Based on these animal and human data fumonisins are now considered a potential cancer risk to humans [14]. Many parts of the developing world rely on maize based foods as a major staple of their diet, and these populations can be chronically exposed to highly contaminated food. Animals probably present a minor route for human exposure via consumption of residues in meat, milk and eggs. The health implications of the known co-occurrence of fumonisins with the liver hepatocarcinogen, aflatoxin B$_1$ [12,15–20] is of concern, but has been little investigated in human populations. There is evidence of a synergistic effect on liver disease in pigs fed diets contaminated with aflatoxin and FB$_1$ [21], though no synergistic effects in the liver were observed for chickens [22]. Certainly, the fact that mycotoxins occur in mixtures in human foods implies that the toxicology is likely to be complex and cannot be ignored.

2. Toxicology of fumonisins

Toxicological studies on the fumonisins have concentrated on FB$_1$, the major fumonisin produced in culture and naturally in maize and maize-based foods and feeds. There are two diseases in animals for which fumonisins are clearly causal. Intravenous injection of horses with pure FB$_1$ was shown to induce equine leukoencephalomalacia (LEM) shortly after isolation of fumonisins was achieved [23]. LEM is a syndrome of acute illness and death in horses in which necropsy reveals softening or liquefaction of the white matter of the brain. FB$_1$ and FB$_2$ have been detected in maize samples associated with field outbreaks of LEM [24]. FB$_1$ administered to horses by stomach tube also induced equine LEM resulting in characteristic brain lesions [25]. Pulmonary edema (PE) was reproduced in pigs similarly following injection of pure FB$_1$ [26]. These and other confirmed associations, reviewed by Marasas [5], for fumonisins as causative agents in equine LEM and porcine PE indicate that fumonisin levels $>10$ and $>100$ mg kg$^{-1}$ in feed should be regarded as a risk for these conditions in horses and pigs, respectively.

Fumonisins also induce mild to fatal toxicity in liver, kidney and heart in horses, pigs, cattle, sheep, chickens, ducks, rabbits, rats and mice [27]. Nephro-
Toxicity has been reported in several species, and in rats and rabbits, the kidney appears to be the most sensitive target organ [28]. FB$_1$ does not cross the placenta, and observed foetal toxicity (dose-dependent foetal death and delayed foetal development) in Syrian hamsters [29] is possibly a secondary response to maternal toxicity [30] but this may depend on the species. FB$_2$ and FB$_3$ show similar toxicity in rat feeding studies, including hepatotoxicity and nephrotoxicity [31].

The fumonisins are structurally similar to sphingolipid precursors and cause inhibition of a key enzyme in sphingolipid biosynthesis (see below). Toxicity may be caused by increased levels of sphingolipid precursors (e.g., sphinganine (Sa) and sphingosine (So)) and decreased levels of complex sphingolipids, due to disruption of this biosynthetic pathway. However, the effects of the fumonisins consequent to the above actions are complex and there are also many alternative mechanisms of action under study; the critical action may differ with species and organ. Gelderblom et al. [32] have reviewed several potential mechanisms of fumonisin hepatotoxicity and carcinogenicity including:

- Interruption of sphingolipid biosynthesis
- Disruption of cellular lipids
- Fatty acid accumulation and cell proliferation
- Oxidative stress and lipid peroxidation
- Peroxisome proliferation

Of the above mechanisms of fumonisin toxicity, the disruption of sphingolipid biosynthesis, reviewed by Merrill et al. [33] has emerged as a useful mechanistic basis for biomarker development.

### 3. Fumonisins and sphingolipids

Sphingolipids are a class of membrane lipids that play an important role in cell regulation by controlling certain membrane protein functions. They maintain membrane and lipoprotein structure, cell–cell communication, interactions between cells and the extracellular matrix, regulation of growth factor receptors, and act as second messengers in signal transduction pathways, and thus they mediate cell growth, differentiation, and cell death [34]. The fumonisins, long-chain polyhydroxyl alkylamines with two propane acid moieties esterified to hydroxyls on adjacent carbons [2], are structural analogues of the sphingolipid precursor sphinganine. They are potent competitive inhibitors of ceramide synthase, the enzyme that catalyses the acylation of sphinganine in the de novo synthesis of sphingolipids [35]. In rat primary hepatocytes the tricarboxylic moiety is required for maximal inhibition of ceramide synthase. The presence of an amino group appears not to be a requisite for activity [33].

Sphingolipids have a complex role in cell function and fumonisin disruption of normal sphingolipid metabolism therefore affects a large number of processes. The effects could be mediated by depletion of complex sphingolipids, an increase in free sphinganine or a decrease in re-acylation of sphingosine. For example, accumulation of sphingoid bases can occur and these are both growth inhibitory and cytotoxic [36,37]. They may also inhibit protein kinase-C, activate phospholipase D, and activate or inhibit enzymes involved in lipid signalling pathways [33]. They inhibit the Na$^+$/K$^+$ ATPase, and induce de-phosphorylation of retinoblastoma protein (a protein involved in regulating the cell cycle transition from G to S phase) [38]. Fumonisins also cause apoptosis in the liver and kidney of rats following consumption of contaminated feed [39]. Hepatocyte apoptosis and subsequent proliferation to replace apoptotic cells could be responsible for the synergistic response observed for FB$_1$ and aflatoxins in hepatocellular disease [21].

Clearly, compounds such as the fumonisins which disrupt the range of processes described above can have potentially wide ranging cellular effects. The role of altered sphingolipid biosynthesis in mediating the toxic effects of fumonisins is supported by the relationship between the altered Sa:So ratio, reflecting this disruption of biosynthesis, and the disease endpoints studied. However the associations are not completely straightforward and alternative mechanisms may be important. In a study where measurement of the Sa:So ratio was used to monitor horses fed FB$_1$ contaminated feeds [40], although many tissues had altered Sa:So ratios, the brains of horses with and without pathological lesions (LEM) did not have altered ratios. Clearly, the role of altered sphingolipid metabolism in the pathogenesis of fumonisin induced equine toxicity needs further examination.
As mentioned in Section 1, the purpose of this review is to examine development of biomarkers in relation to what is known about the mechanism of action of fumonisins. This approach is clearly complicated by the fact that (1) the toxic and carcinogenic mechanisms of action are still to be defined, (2) could comprise a combination of effects, (3) could differ for different diseases, and (4) could be dose-dependent. However, this discussion also needs to consider what type of information is sought from the biomarker. For example, the presence of free fumonisins in body fluids, if correlated with dietary intakes (external dose) of fumonisins at the individual level, would represent a valid biomarker of internal dose, i.e., a measure of the amount of toxin ingested and absorbed. If altered sphingolipid biosynthesis is of mechanistic relevance to the disease process then the Sa:So ratio would be a biomarker of biologically effective dose, i.e., a measure of the biological effect of the toxin at the target site. This type of biomarker provides not only an objective measure of exposure but also a relevant measure with respect to the disease process. In this context an altered Sa:So ratio would in many ways parallel the measurement of cellular carcinogen-DNA adducts which correlate with external dose but may also have some predictive value with respect to risk of disease because the events are on the disease pathway [41]. Consider, however, the situation where fumonisins cause an altered Sa:So ratio but that the disease proceeds through another independent mechanistic pathway. In this case if there is a correlation between Sa:So ratio and the independent mechanism then the former marker will still be a valid surrogate measure of the latter. Alternatively, if there is no correlation between these two then the Sa:So ratio will have no relevance to biologically effective dose. Nevertheless, even in this scenario, if the ratio correlates with fumonisin intake it could still be a valid biomarker of exposure. Of course it must then be asked whether the biomarker is sufficiently sensitive, i.e., do all exposed individuals exhibit an altered ratio, and specific, i.e., is an altered ratio limited to exposed individuals.

In our current understanding of the complexity of the mechanisms of action of fumonisins it appears worthwhile to pursue a number of avenues for development of appropriate biomarkers. Certainly, altered sphingolipid biosynthesis is the mechanism of action which has been best studied to date for fumonisins and for this reason we place emphasis on the Sa:So ratio in this review. We recognise, however, that other markers may be established as knowledge of mechanisms develops.

4. Fumonisins and cancer—animal carcinogenicity

FB₁ is a cancer promoter in a short term cancer initiation promotion assay [42] and at high levels of chronic exposure cancer initiation occurs [43–45]. FB₁ has been reported to cause liver cancer in rats [46]. The final results of more extensive carcinogenicity studies in mice and rats from the USA National Toxicology Program—Food and Drug Administration at the National Centre for Toxicological Research have been completed and results will be published in 1999. These can be accessed on the NTP website from mid-April 1999. Briefly, these data reveal renal tubule neoplasms in male rats and hepatocellular neoplasms in female mice. Despite the evidence for carcinogenicity, the carcinogenic mechanisms of the fumonisins are unknown. FB₁ is non-mutagenic in the Salmonella mutagenicity test [47], does not induce unscheduled DNA synthesis in isolated rat hepatocytes [48], and is not genotoxic in the in vivo or in vitro DNA repair assays in rat primary hepatocytes [45,49,50]. However, FB₁ caused chromosomal aberrations in primary hepatocytes [51], and FB₁ may induce DNA damage indirectly by lipid peroxidation. Fumonisins have been shown to be mitogenic in cultured 3T3 cells [52], via the accumulation of sphingoid bases [39,53]. The ability of FB₁ to cause accumulation of sphingosine or sphinganine and arrest the cell cycle in some cells but not others may play an important role in carcinogenesis or other diseases [54].

There is considerable debate regarding the reported association of human oesophageal cancer, with exposure to fumonisins. The majority of animal toxicity studies suggest that fumonisins mainly target the liver and kidneys. One study has attempted however to test the effects of FB₁ on oesophageal carcinogenicity in rats. FB₁ (5 mg kg⁻¹ b.wt.) was administered, daily for 5 weeks by gavage, to male
BDVI rats and did not lead to the development of oesophageal papillomas, determined by examination at 45 weeks post dosing [55]. This level of dosing was also combined with treatment with a known oesophageal carcinogen N-methylbenzylnitrosamine (NMBN), and again no increase in oesophageal papillomas over the incidence seen with NMBN alone was observed. Interestingly, one study in male Sprague–Dawley rats [56] did show increased cell proliferation occurring in the oesophagus following a single i.v. dose of $\text{FB}_1$ (1.25 mg kg$^{-1}$ b.wt.). The route of administration in this study is important as it suggests that the oesophagus is a specific target for fumonisin toxicity.

The fumonisins are a family of compounds in which selection of animal models to relate to human risk is crucial and yet extremely difficult. This is exemplified by the diverse nature and potency of induction of specific toxicosis in a range of animal species. To obtain useful information with relevance to human risk assessment a variety of animal models and in vitro systems may be required.

5. Fumonisins and cancer—human carcinogenicity

The data linking fumonisin exposure to human cancer risk are mainly restricted to correlation studies of oesophageal cancer in South Africa and China (for reviews see Refs. [14,57]). These populations have among the highest oesophageal cancer rates in the world and yet the major risk factors responsible for this tumour in the western world, tobacco and alcohol, are not considered to be as significant here. It has however long been suggested that mycotoxins may be among alternative factors of importance [58]. It is noteworthy that several of the high risk populations worldwide have traditionally consumed maize as a dietary staple, e.g., in the northeast of Italy, parts of South America as well as southern Africa and China although the explanation normally given for this association has been that maize consumption is associated with vitamin deficiencies [59–62].

Initial epidemiological studies on the mycotoxin hypothesis focused on correlations between the prevalence of fungal contamination of foods, mainly maize, and oesophageal cancer. Marasas et al. [63] compared Fusarium contamination in maize samples from 12 households in high and low risk areas for oesophageal cancer in the Transkei, South Africa. The households in high-risk areas were selected based on the presence of cytological oesophageal abnormalities in an adult member of the family. Whilst $F. \text{graminearum}$ contamination was actually more prevalent in households from low risk areas, $F. \text{moniliforme}$ was elevated in both healthy and moldy maize from the high-risk area. Similarly, a study of five high-risk and three low-risk counties in China reported a significant association with contamination of cereals by fungi, including $F. \text{moniliforme}$ [64]. Levels of Fusarium toxins (tricothecenes) in maize and wheat was reported to be higher in high risk areas of Henan Province, China (Linxian) than low risk (Shangqui) [65]; this analysis was based on a total of 47 maize samples and 30 wheat samples collected from 27 families in Linxian having a family member with oesophageal cancer or from 20 families from Shangqui with no family member having the disease.

With the suggested association with $F. \text{moniliforme}$ and the subsequent identification of fumonisins in the late 1980s correlation studies between exposure and oesophageal cancer could be refined to focus on these specific toxins. Using the original maize samples from the study by Marasas et al. [63] and new samples from the same areas in a more recent year, a positive correlation was found between the levels of both $\text{FB}_1$ and $\text{FB}_2$ in maize and high or low oesophageal cancer risk in Transkei [10,11]. Certain samples from the high-risk areas contained some of the highest levels of $\text{FB}_1$ (up to 117 mg kg$^{-1}$) and $\text{FB}_2$ (up to 23 mg kg$^{-1}$) yet recorded from naturally infected maize. It has been calculated that an average male adult (70 kg) in this area consuming 460 g of moldy maize per day has an exposure in the region of 440 $\mu$g kg$^{-1}$ b.wt. day$^{-1}$ [66]. Even higher levels of $\text{FB}_2$ contamination (up to 155 mg kg$^{-1}$) have been reported in home-grown maize from a high-incidence area of oesophageal cancer in China [12]. Yoshizawa et al. [13] re-analysed the maize samples collected for the study of tricothecenes in Henan Province [65]. Although the prevalence of fumonisin contaminated samples was slightly higher in Linxian (48%) than Shangqui (25%) the mean level and range of $\text{FB}_1$ and $\text{FB}_2$ did not
differ between the areas. This contrasted with the higher levels of trichothecenes in the high risk area.

Despite the above reports positively associating fumonisin exposure with oesophageal cancer rates in South Africa and China, no convincing evidence of causality has been presented to date. There is a clear need for analytical epidemiological studies but these would be far easier to conduct if accurate assessment of exposure to fumonisins at the individual level was feasible.

It is important not to omit reference to hepatocellular carcinoma when considering the carcinogenicity of fumonisins. Of the many aetiologic factors responsible for hepatocellular carcinoma in high risk areas of China and Africa, HBV and aflatoxins are the most important [67]. The co-occurrence of fumonisins and aflatoxins in maize based foods has been demonstrated [12,15–20] and a synergistic effect between these compounds in human disease could occur. A correlation study conducted over 3 years in Haimen (Jiangsu) and Penlai (Shandong Province) in China revealed a higher prevalence and level of fumonisins in the former area which has the higher mortality rate from hepatocellular carcinoma [19]. Aflatoxin levels were similar in maize from both regions. However, whilst this study indicates the need to consider fumonisins as a risk factor for this cancer there were a number of limitations which characterise the epidemiology of fumonisins to date. First, there was a lack of control for confounding by a major risk factor for this cancer, i.e., chronic HBV infection. Second, whilst the authors made attempts to control for confounding by mycotoxins other than fumonisins they only analysed maize whereas exposure could also come from rice, wheat and other cereals. Finally, the rapidly changing dietary habits and seasonal variations in mycotoxin levels referred to by the authors illustrate the difficulty in relating current dietary exposures to those occurring in the past and therefore presumably more relevant to disease development.

6. Contamination of feeds by fumonisins

Due to the prevalence of *F. moniliforme* in maize based crops, fumonisins will potentially occur wherever maize is grown, and could frequently contami-
reports of contamination will be cited to illustrate that exposure is not confined to developing countries. In the UK, 76 of 291 maize based retail products had a range of 11 to 2124 μg kg⁻¹ of FB₁ [71]; in the USA (MD, AZ and NE) 52 of 56 maize-based food products had a range of 200 to 7450 μg kg⁻¹ of FB₁ [72]. One recent study in the Czech Republic reports expected mean daily exposures to FBs from a variety of maize based foods at up to 0.05 μg kg⁻¹ b.wt. day⁻¹ [73]. Other examples are reviewed elsewhere in the literature [6,70].

There is an ongoing exposure assessment study of humans in the Netherlands to determine fumonisin contamination of foodstuffs. FB₁ was detected in 98% of 62 maize samples intended for food production. Of these 18% contained levels > 1000 μg kg⁻¹ [74]. Maize containing food products were purchased from local markets and 36% of these had detectable levels of FB₁ contamination (range 8–1430 μg kg⁻¹). In a study of 349 maize samples from 18 countries worldwide 323 contained FB₁, the average contamination level being 1,359 μg kg⁻¹ [75]. For people in the Netherlands in 1992 considered to be at most risk (gluten intolerant—e.g., people with celiac/Duhring’s disease), 37% (1% of whole population) were estimated to intake at least 10 μg FB₁ person⁻¹ day⁻¹ (0.14 μg FB₁ kg⁻¹ b.wt. day⁻¹ for a 70-kg person). More generally half of the whole population of the Netherlands would be exposed to at least 1 μg person⁻¹ day⁻¹ (0.014 μg kg⁻¹ day⁻¹) [75]. These examples indicate that there may be a significant human exposure, in developed countries, to fumonisins through the consumption of some maize-based foods although levels are orders of magnitude lower than estimates for people consuming large amounts of moldy corn [66].

An important question with respect to human exposure relates to the stability of fumonisins during food production and preparation. Fumonisins are not significantly destroyed when maize is either dried or thermally processed prior to consumption [76]. Fumonisin levels, 50–90%, remain following canning, no significant losses are observed after baking, although roasting (dry heat 218°C, 15 min) causes almost complete loss of fumonisins [77]. These methods simply identify whether fumonisins are still present, but the thermal products themselves may also be toxic. Thus, in general, decontamination by heat treatment (usually as part of normal cooking) and indeed alkali treatment or washing cannot be assumed to provide complete detoxification.

8. Exposure assessment

8.1. Food analysis

A major concern in controlling risk associated with mycotoxin contaminated foodstuffs is the reliability of assessing exposure levels, reviewed by Brera et al. [78]. Sensitive methods to detect fumonisins in foods involve extraction into organic solvents and enrichment using C-18 and/or ion exchange columns [97]. These are derivatised with a fluorescent label, usually α-phthalaldehyde (OPA), prior to quantitation by HPLC. The liquid chromatography method utilising OPA for the determination of FB₁, FB₂ and FB₃ in maize has been adopted by AOAC International [79]. Although a number of immunochemical methods for the analysis of fumonisins in food have been developed, a lack of sensitivity in many of the immunoassays, combined with poor correlations with physico-chemical methods (HPLC, GC-MS), generally restrict immunological methods to qualitative screening, or to immunoaffinity approaches to enrich, prior to quantitation [80].

Despite the existence of highly sophisticated analytical instrumentation that can be used for measuring fumonisins in foods, accurate exposure assessment is problematic, with study design and representative sampling being of importance in epidemiological studies [57,81].

8.2. Biomarkers of fumonisin exposure

A better estimate of human exposure at the individual level can be obtained by monitoring biological fluids and tissues [41,60]. For fumonisins this is a rapidly growing area of research, with methods being developed to determine both the parent compound and markers of biological effect. In the case of the latter approach, understanding the mechanisms of action of the fumonisins is important as discussed above (see Section 3). Studies on aflatoxin have
combined food analyses at the individual level with sampling of biological fluids at several time points in order to assess the relationship between exposure, biomarker level and associated health risk. All three are critical to the development of valid and worthwhile biomarkers. The practical difficulties raised by these types of studies need to be given serious consideration, particularly where the procedures are intrusive, e.g., plate food sampling, blood and urine collections. Biopsy material from target organs would be extremely informative in conducting risk assessment studies. The invasiveness of the procedure prohibits their use in most circumstances, and surrogate dose monitors in blood and urine are more commonly used. However for the oesophagus the cells of interest could be obtained directly.

For the fumonisins two markers of exposure are currently being investigated, namely fumonisins in urine and altered Sa:So ratio in urine and in blood. The majority of progress to date has concerned improvements in analytical methodology, but we are currently examining the correlation between fumonisin exposure, occurring through consumption of maize based foods, and biomarkers in a population in West Africa (see below).

8.2.1. Free fumonisin

The detection of free fumonisin in human blood is unlikely to provide a useful biomarker of exposure. $[^{14}C]FB_1$ distribution measured following oral and i.v. administration in the serum of non-human primates (vervet monkeys) indicated that $FB_1$ was cleared within a few hours of exposure [82]. The variable toxicokinetics for this type of measurement make dose/biomarker correlations problematic.

A better matrix for measuring free fumonisins is urine [83]. To date there are no data in humans comparing fumonisin contamination in maize and urinary fumonisin levels in a population consuming this maize. Recently a highly sensitive method for the measurement of $FB_1$ in human urine with a detection limit of 10 ng ml$^{-1}$ was reported [83]. This method could be used to monitor populations consuming $>28\ \mu g\ FB_1\ kg^{-1}\ b.wt.\ day^{-1}$, based on $\sim 0.5\%$ of ingested fumonisin being excreted unchanged in the urine [82], and a 70-kg adult producing 1 litre of urine day$^{-1}$. Based on probable daily intakes [32] populations consuming healthy maize or moldy maize in Transkei, South Africa (46.6 and 354.9 $\mu g\ kg^{-1}\ b.wt.\ day^{-1}$, respectively), and Linxian and Cinxian County, China (231.9 and 486.2 $\mu g\ kg^{-1}\ b.wt.\ day^{-1}$, respectively) would have measurable levels of urinary fumonisins. However, measurements of urinary fumonisins would not be possible with current methodologies in areas with lower levels of contamination or where populations consumed smaller daily quantities of maize, such as those in Europe discussed above.

8.2.2. Sphinganine / sphingosine ratio

As previously mentioned a cellular target for the fumonisins in cell culture and animal models is ceramide synthase [34], an enzyme involved in sphingolipid metabolism. The fumonisin induced disruption to sphingolipid metabolism causes an altered ratio of free sphingolipid precursors, Sa:So, in tissues as well as urine and in blood of vervet monkeys, ponies, chickens, rabbits and rats at least at relatively high doses [34,84,85]. Of interest was the observation that the altered ratio in ponies precedes markers of fumonisin associated toxicity, e.g., elevated serum transaminases as a marker of hepatotoxicity, such that the ratio may provide an early marker of exposure to fumonisins [86]. Feeding studies ($\sim 300$ and $800\ \mu g\ fumonisin\ kg^{-1}\ b.wt.\ day^{-1}$) on vervet monkeys gave significantly altered Sa:So ratios in serum [84]. The administered doses were comparable to those estimated for human populations consuming moldy corn [66]. For many populations potentially at risk due to consumption of contaminated maize (e.g., the Netherlands [75]), exposures are 100–1000 times lower than those in the above study. Urine may also provide a matrix for bio-monitoring of Sa:So ratio, though in the above study with vervet monkeys only a non-significant increase in the ratio was observed with fumonisin consumption. It may also be possible to measure the ratio in biopsies or other cellular material in some instances.

Methods to analyse sphingolipid bases [87,88] involve numerous steps and are extremely time consuming. A prerequisite for large scale biomarker studies is ease and rapidity of repeated measurements. Methodology for measuring the Sa:So ratio has been applied to rat urine, and urine and serum of
subjects in France and South Africa [89,90]. The method involves isolation of exfoliated cells from as little as 0.5 ml of rat urine or 2 ml of human urine, extraction of sphingolipid bases and derivatization with OPA prior to HPLC analysis with fluorescence detection. The method can be adapted to tissue analysis by partial digestion of the specimen to a cell pellet, then extraction of the sphingolipids as described above.

For human urine, measurements can be made in 2 ml of female urine, but in males much larger volumes are required due to the low levels of sphingolipid bases [89]. An alternative method has been proposed for the determination of Sa and So in human and animal urine which is based on the use of silica minicolumns for sample preparation prior to HPLC analysis essentially as described above for urine. This method was sensitive enough to detect high levels of exposure in Vervet monkeys [84] but in mice treated with 16.8 mg kg\(^{-1}\) FB\(_1\) three times a week for 68 weeks and rats treated with 1 mg kg\(^{-1}\) day\(^{-1}\) for 5 weeks only marginal changes in serum Sa:So ratios were observed [90]. The organ specific response in Sa:So ratio alteration [40] is also significant with regard to the sensitivity of this biomarker.

Overall, it is unclear at present how sensitive the above approaches will prove with respect to detecting human exposure. Based on probable fumonisin daily intake [32] and the measurement of Sa:So ratio in vervet monkeys [84] it would appear that the sensitivity of this biomarker may be limited with the result that many exposed individuals would be classified as unexposed. It should be noted however that this assumption is based on data extrapolated from a limited number of data in a non-human primate. The use of the Sa:So ratio still awaits validation in human populations exposed to fumonisins.

As well as the biomarker being necessarily sensitive for fumonisin exposure, there is also a requirement for any biomarker to have high specificity for the exposure of interest. High doses (10 and 100 mM) of other mycotoxins (AFB\(_1\), cyclopiazonic acid, beauvericin, T-2 toxin, sterigmatocystin, luteoskyrin, verrucarin A, scirpentriol, and zearalenone) do not inhibit ceramide synthase in isolated rat liver slices [93] suggesting that among mycotoxins, the altered Sa:So ratio is likely to be specific for the fumonisins. An exception to this is the Alternaria alternata toxin which was demonstrated to disrupt sphingolipid metabolism by inhibition of ceramide synthase [36]. The effect of other environmental factors, e.g., diet or infections, on this ratio still require study in human populations. Nevertheless, the altered ratio potentially provides a highly specific biomarker of fumonisin exposure [86,94].

In summary, despite the advances in analytical methods to determine Sa and So there are several key points that need to be addressed for this biomarker [60]. As discussed above, the methodology has to be sensitive such that fumonisin exposure can be detected in populations naturally exposed at levels potentially hazardous to health. The specificity of the biomarker, both with respect to other mycotoxins, and additional confounding factors needs to be ascertained. Finally, the stability of the altered ratio needs to be determined in human populations to permit temporal associations between exposure, biomarker and disease to be established.

Biomarkers of fumonisin exposure are being currently validated by our group in a human population naturally exposed to fumonisins. To date maize and maize-based foods have been collected from two regions in Burkina Faso (Ouagadougou and Bobo-Dioulasso). Samples were analysed for FB\(_1\) by the method of Sydenham et al. [95]. From Ouagadougou, 8 out of 20, and from Bobo-Dioulasso, 7 out of 20, were contaminated by FB\(_1\) with levels of contamination ranging from 42 to 1121 \(\mu\)g kg\(^{-1}\). These preliminary results show that maize destined for human consumption is contaminated by fumonisins in this country. Fumonisin from plate food and urine, as well as serum and urinary Sa:So ratios will be measured following consumption of maize at harvest, after storage and when imported maize is consumed in order to validate these biomarkers at an
individual level in a population naturally exposed to fumonisins.

9. Summary

There is no direct established causal association between fumonisin exposure and disease in man, but there are several reasons for concern:
(a) Fumonisins contaminate maize grown throughout the world, and several populations rely on maize as a dietary staple.
(b) Animal exposure to FB₁ induces disease including equine LEM, porcine PE and cancer in rodents.
(c) Fumonisins have a broad range of biological effects, including disruption of sphingolipid biosynthesis.
(d) FB₁ intake is correlated with oesophageal cancer rates in South Africa [10,11], and China [12,13].

Clearly, the precise role and mechanisms of action of fumonisins in the context of human health risks still needs to be established. The fact that intake of fumonisins via the consumption of contaminated foods is not insignificant in many developed countries should spur these investigations. Advances in methods of food analysis and development of biomarkers (including elevation of the Sa:So ratio) based on mechanistic approaches should allow molecular epidemiological studies to aid the risk assessment process, a process which requires information on toxicology, exposure and disease associations. This combined approach will hopefully lead to a reduction in human and animal disease associated with fumonisins. Although an understanding of the toxicologic mechanisms of action of fumonisins is scientifically appealing and contributes to the risk assessment process, it is vital that emphasis is also placed on epidemiologic studies to determine risk in human populations. In the case of aflatoxins the structural identification of these potent hepatocarcinogens predated by approximately 30 years their association with liver cancer risk in a prospective study using biomarkers of exposure [96]. Just over a decade has passed since the structural identification of the fumonisins; the development of validated biomarkers of exposure and their application in epidemiologic studies should be one priority area in fumonisin research.

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