Fumonisin: toxicology, emerging issues, and prospects for control and detoxification

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Introduction

Fumonisin is produced by *Fusarium verticillioides* (formerly *F. moniliforme* Sheldon) and related *Fusarium* species^{1,2}. They occur in corn and in corn-based products worldwide and both fumonisins and fumonisin-producing fungi cause toxic effects in animals¹⁻³ (Table 1). Their risk to humans is unclear¹ but both are implicated as factors for esophageal cancer¹ and, more recently, neural tube defects^{1,4}. While much has been learned about fumonisins, research to further characterize their toxicity, their mode of action, as well as to determine their relevance to human health continue. Recent research on some issues of emerging importance is reviewed below.

Fumonisin toxicology and mechanism of action

The toxicology and mode of action of fumonisins have been extensively reviewed elsewhere and comprehensive reference lists of original research reports are available in these publications¹⁻³ (Table 1). Fumonisin's mechanism of action is multifaceted but evidence indicates that inhibition of ceramide synthase (fumonisins are structurally similar to the enzyme's sphingoid base substrates sphinganine (Sa) and sphingosine (So)) is the initial mechanistic event⁵. Consequently, sphingolipid metabolism is disrupted and Sa and So accumulate in cells and tissues, cell and tissue complex sphingolipids are depleted, and So (Sa)-1-phosphates and other sphingoid base metabolites accumulate. Consequently, critical sphingolipid-mediated cell signaling pathways or sphingolipid-dependent physiological functions are disrupted. For example, So-1-phosphate binds to G protein-linked Edg receptors and causes vasoconstriction of rat mesenteric and renal microvessels *in vitro*⁶ and *in vivo*⁷. It is therefore possible that

accumulated So-1-phosphate in the kidney of fumonisin-exposed rats might reduce blood flow and exacerbate apoptosis. Likewise, accumulated sphingoid bases in the serum or tissues might be the mechanistic trigger for cardiac insufficiency and, ultimately, pulmonary edema in swine⁸⁾.

Reproductive toxicity revisited

In previous studies, *F. verticillioides* and FB₁ were not teratogenic to rodents and rabbits and embryotoxicity, when present, occurred only at maternally toxic doses^{1,3)}. However, in several reports, decreased fetal birth weight, and/or increased fetal resorption were found following maternal FB₁ exposure. *Ex utero* exposure to FB₁ caused developmental defects in rat embryos (including exencephaly)^{1,3)} but, because ¹⁴C-FB₁ did not cross the placenta of pregnant rats (on Gestation Day 15) and increased Sa or Sa/So (biomarkers of fumonisin exposure^{1,5)}) was not found in fetuses of dams (rats, mice and rabbits) given FB₁, it was concluded that the placenta was an effective barrier to *in utero* exposure³⁾.

Fumonisin's potential as a risk factor for birth defects, specifically neural tube defects (cranial and spinal cord abnormalities ranging from spina bifida occulta to exencephaly or craniorhachischisis), has again become a concern. A spike in the incidence of neural tube defects within the Hispanic population near Brownsville, TX in 1990-91, a time when fumonisin levels in locally grown corn were coincidentally high, suggested a possible connection⁴⁾. In this regard, it is interesting to note that neural tube defect incidences are also high in regions of southern Africa, including the Transkei, and elsewhere where fumonisin exposure is likely to be high¹⁾.

Stevens and Tang⁹⁾ found that FB₁ inhibited carrier-mediated folate transport in Caco-2 cells. Folate deficiency during a critical phase of early pregnancy is associated with neural tube defects and the high-affinity placental folate transporter (folbp1 in mice and folate receptor *alpha* in man) is a sphingolipid-associated GPI-anchored protein. It was therefore hypothesized that fumonisins indirectly contribute to neural tube defects by depleting membrane sphingolipids that are critical for folate receptor function⁹⁾. Consistent with this hypothesis are the findings of Sadler *et al.*¹⁰⁾, that showed folinic acid supplementation could significantly reduce the incidence of FB₁-induced neural tube defects in murine embryo culture.

Gelineau-van Waes and colleagues have studied maternal exposure and neural tube defects using the inbred LMBc mouse as an *in vivo* model¹¹⁾. In summary, they found dose-dependent increases in neural tube defects (exencephaly) in the litters of dams given intraperitoneal doses of FB₁ on gestation days 7.5 and 8.5; the incidence of affected fetuses ranged from 2 percent (dose = 5 mg FB₁/kg maternal BW) to 79 percent (20 mg/kg). Increased Sa concentration and Sa/So were found not only in the livers and

Table 1. A summary of selected toxic effects and diseases caused by fumonisins.¹

Species	Description and Comments
Horse	Equine Leukoencephalomalacia (ELEM): ELEM is characterized by hemorrhagic & liquifactive lesions of white matter. Its clinical onset and course are rapid and its neurological signs include lethargy, weakness, recumbency and facial paralysis. ELEM is unique to <i>Equidae</i> . Liver involvement similar to that in other species also occurs.
Swine	Porcine Pulmonary Edema (PPE): PPE is characterized by rapidly developing accumulations of fluid in alveolar membranes and spaces. Animals subsequently die of respiratory failure ⁸⁾ . PPE might be the result of compromised cardiac contractility. PPE is unique to swine, but the liver is also a target organ.
Rat	Liver and kidney are the main target organs. Apoptosis is an early finding and with prolonged exposure, necrosis, regenerative and other lesions develop. Serum chemistry findings support the pathology. FB ₁ promotes enzyme (GGT and GST) positive liver foci and is a liver and kidney carcinogen in rats (50 mg/kg in diet), although differences in target organs occur by sex (male vs. female F344/Nnctr rats) and rat strain (F344/Nnctr males vs BD IX males). Other effects including thymic apoptosis, inhibition or enhancement of immune or host-resistance responses, changes in hepatic gene expression, or indications of oxidative injury have been reported.
Mouse	The liver is the primary target organ in mice. Serum chemistry and pathology findings are consistent with those found in rats and, as in rats, apoptosis and mitosis/regeneration likely play a key role in pathogenesis. The kidney is also a target, but the mouse is not as susceptible as Sprague-Dawley and F344 rats to fumonisin nephrotoxicity. FB ₁ (50 mg/kg in the diet) was hepatocarcinogenic to female B6C3F ₁ mice. Gene knockout strains have been useful for studying how cytokines and various receptors mediate fumonisin toxigenesis.
Rabbit	The liver and kidney are target organs. Lesions and other findings are similar to those found in rodents. Pregnant rabbits are especially sensitive to FB ₁ .
Nonhuman Primates	The liver is a target organ based on pathology (apoptosis, perilobular fibrosis, nodular hyperplasia and other lesions in liver) and serum chemistry endpoints in vervet monkeys chronically fed <i>F. verticillioides</i> culture material.

*¹ Extensive citation lists of original research reports by the numerous groups that contributed information to this table is given in the review of fumonisins published by the FAO/WHO Joint Expert Committee on Food Additives¹⁾ and elsewhere³⁾.

kidneys of the treated dams, but also in their placentas embryos and biodistribution studies confirmed that ^{14}C -FB₁ crossed the placenta on gestation day 10.5. Why these findings contrast with earlier results³⁾ is not known, however, the LMBc studies were done much earlier in gestation, at a time when the placenta is less developed and less likely to provide an effective barrier against mycotoxin exposure. The FB₁ dose, the route and timing of dosing, or species/strain-related differences in placental physiology, sphingolipid metabolism, and/or genetic susceptibility to neural tube defects are also possible contributing factors.

In any event, folate plays a role in the LMBc mouse model. Fetal folate uptake was decreased by FB₁ and daily oral administration of folinic acid to the high-dose dams (n=10) beginning on the first day of gestation reduced FB₁-induced neural tube defect incidence from 79 to 43 percent. Administering the complex sphingolipid ganglioside G_{M1} on gestation days 6.5-9.5 (10 mg/kg/day ip) more effectively counteracted neural tube defect induction by FB₁, as the incidence of neural tube defects in the fetuses of dams (n=11) treated with the high-dose of FB₁ plus G_{M1} was only five percent. Immunohistochemical expression of the folate receptor and ganglioside G_{M1} in the yolk sac membrane and neuroepithelium of the embryos diminished following FB₁ exposure, also suggesting a connection between sphingolipid depletion and decreased folate transport. These investigations have established the LMBc mouse as a useful model for investigating how fumonisins, sphingolipids, and folate interact to affect neural tube closure. However, given the species/strain-dependent differences in response to fumonisins that occur, more studies in LMBc mice and other mouse strains and species are needed to place these findings in perspective. These include: determining dose-response and the no observed effect level in LMBc dams exposed to FB₁ via the diet; determining the influence of maternal toxicity on litter neural tube defect incidence; investigating transplacental transport of folate and fumonisins as a function of time (gestation day); establishing if folate transport in LMBc dams is uniquely sensitive to FB₁; defining more fully the molecular and pathophysiological mechanisms underlying induction of neural tube defects in LMBc mice and, determining the extent to which these mechanistic findings apply to other mouse strains and species.

Cooking and "hidden" fumonisins

There is evidence that "hidden" fumonisins (matrix-bound forms not detected using "routine" extraction and analysis methods) are present in some foods (Table 2). Fumoinisin-matrix interactions and reaction products in foods are not well characterized. Several have been detected (Table 2) and others are possible. When extracted (using a "routine" method) cornflake residues were re-extracted with sodium dodecyl sulfate and the second extract then base hydrolysed, a significant amount (2.6 times the amount

Table 2. Examples of Fumonisin Reaction Products and their Potential Biological Activity.
Refer to the cited reports for experimental details.

Product	Formation, Toxicology Data, and Analysis Methods
Hydrolyzed Fumonisin ¹ · (HFBs)	HFBs are formed by base hydrolysis of fumonisins during nixtamalization (cooking in alkaline water) of corn to make masa, the basic ingredient of tortillas. HFBs occur in masa products, although usually in lesser amounts than fumonisins. <i>In vitro</i> and <i>in vivo</i> bioassay results are variable but the weight of evidence suggests HFBs are no more and likely less toxic than fumonisins. HFBs can be measured by HPLC or mass spectroscopy ¹³⁻¹⁵ . HFBs substitute for sphingoid bases as substrates for ceramide synthase ²⁹ .
Maillard reaction products ¹ .	<i>N</i> -(Carboxymethyl)- and <i>N</i> -(deoxy-D-fructos-1-yl)-fumonisins form when fumonisins are heated in the presence of reducing sugars such as glucose. The extent to which these products occur in foods is not well studied, but significant <i>N</i> -(carboxymethyl)- or <i>N</i> -(1-deoxy-D-fructos-1-yl)-fumonisins were not found in masa and tortilla chips. <i>N</i> -(Carboxymethyl)-FB ₁ was not toxic to mice ²⁵) and toxicity to rats of FB ₁ -glucose reaction product(s) was reduced compared to FB ₁ ²⁴) possibly due to limited bioavailability of the fumonisin-sugar compound, lack of a free amino function on the reaction product, or both. These compounds are measured using mass spectroscopy or other methods not dependent on derivatization of the amino group ^{16,27} .
3-keto fumonisins	3-keto FB ₁ was formed by ozonation (a candidate detoxification method) of an aqueous FB ₁ solution. 3-keto FB ₁ retains biological activity ²⁸) but its toxicity has not been tested <i>in vivo</i> in mammalian or avian species. 3-keto FB ₁ can be quantified by routine HPLC or mass spectrometry methods ²³).
Deaminated fumonisins	Deaminated FB ₁ is formed by reaction of FB ₁ with NaNO ₂ ²⁸). Deaminated FB ₁ was not toxic to <i>Hydra</i> and did not inhibit ceramide synthase <i>in vitro</i> . Bioavailability and toxicity has not been tested in mammals. The deaminated product can be quantified using mass spectrometry but the loss of the amino group precludes detection of the deamination product using "routine" HPLC methods for fumonisins.
Fumonisin-starch & protein conjugates	Heating FB ₁ , with sucrose, methyl α -D-glucosylpyranoside (a starch model), or <i>N</i> - α -acetyl-L-lysine methyl ester and BOC-L-cysteine methyl ester (protein surrogates), yielded conjugation products. In contrast to FB ₁ -reducing sugar products, the sugar (or amino acid) bound to the tricarballic acid function of FB ₁ . It is possible that conjugated fumonisins could become bioavailable by hydrolysis of the conjugated product during digestion. Analysis is difficult but can be indirectly accomplished by HPLC or mass spectrometric analysis of hydrolyzed fumonisins after base hydrolysis of the food or grain sample ¹⁶). It is possible that these might contribute to "hidden" fumonisins in some foods ¹²).

*¹ Fumonisin reaction products have been detected in foodstuffs.

measured initially) of "hidden" fumonisins was liberated¹². The toxicological importance of these "hidden" fumonisins in foods depends upon their concentration, bioavailability, and inherent toxicity.

Cooking variably reduces measurable fumonisins in foods and consequently might reduce toxicity. For example, cooking in water significantly reduced both fumonisin concentration (ca. 90 %) and *in vivo* toxicity to rats of *F. verticillioides* molded corn¹³. Nixtamalization (cooking/steeping in alkaline water) extracts fumonisins from corn and reduces their amounts in masa and tortilla products by as much as 80 percent^{14, 15}. The fate of the remaining fumonisins, originally present in the corn, was not determined in these experiments but only trace amounts of Maillard reaction products (see explanation in Table 2) were detected in the masa¹⁵. In addition to Maillard reaction products, fumonisin-starch or -protein conjugates can be formed by reactions involving fumonisin's tricarballic acid groups¹⁶. This has potentially important toxicological implications because these compounds are not readily detected by routine analytical procedures and also because they might break down in the intestines, liberating free fumonisin¹⁶. The possible formation of unknown toxic fumonisin reaction products during cooking must also be considered.

To circumvent any analytical uncertainty, the effect of cooking on fumonisin toxicity was evaluated using an *in vivo* bioassay¹⁷. Briefly, pan-fried corncakes, deep-fried fritters, and baked cornbread were made from cornmeal that was spiked with *F. verticillioides* culture material. The cooked products, uncooked spiked cornmeal, or uncontaminated cornmeal (high dose only) were fed to rats at a low (2 % w/w mixed into rodent chow) or high (20 % w/w) doses for two weeks. The toxic effects induced by the cooked products and the uncooked spiked cornmeal, as determined by microscopic evaluation of liver and kidneys, were indistinguishable and typical of those caused by fumonisins. Measured fumonisin (FB₁ + FB₂; HPLC analysis) concentrations of the high-dose pan-fried corncake diet (92 mg/kg) were 30 percent less than that of the high-dose uncooked spiked cornmeal diet (131 mg/kg) but baking and deep-frying had essentially no effect on fumonisin concentration (120-132 mg/kg in the products). It could not be concluded that any "hidden" or unknown fumonisins formed during pan-frying because, at the dose levels used in the study, a 30 % reduction in fumonisin concentration is unlikely to affect dose-response. Importantly, however, the results indicated that baking and frying did not enhance toxicity. More investigations are needed to further elucidate how various cooking methods and recipes affect chemical fate and toxicity of fumonisins, especially in foods prepared from naturally contaminated corn having low (<5 mg/kg) fumonisin concentrations, and to optimize cooking conditions to reduce fumonisins in food products.

Prospects for detoxification

Strategies for reducing fumonisins in foods include controlling the growth of mycotoxigenic fungi in the field. In this regard, breeding programs to produce hybrid plants that do not accumulate fumonisins¹⁸⁾ or competitive exclusion using fumonisin non-producing fungi¹⁹⁾ or nontoxic bacteria²⁰⁾ might be effective. Developing transgenic plants that resist insect damage caused by corn borers and earworms²¹⁾ might also prove to be useful, however, none of these approaches have yet been developed into a management program proven to reduce *F. verticillioides* or fumonisins in corn under field conditions.

Chemical and physical treatments for detoxifying fumonisins have produced variable success (Table 2). Ammoniation using a procedure effective for reducing aflatoxin in corn was ineffective against fumonisins²²⁾. Ozone treatment²³⁾ was likewise ineffective against FB₁ as the reaction product, a 3-keto FB₁, was biologically active. On the other hand, FB₁-reducing sugar reaction (Maillard reaction) products were not toxic to rats²⁴⁾ or mice²⁵⁾. The addition of glucose to cornmeal or grits likewise decreased the amount of FB₁ measured in baked and extruded foods²⁶⁾ but the reaction products have not been characterized. Significant amounts of *N*-(carboxymethyl)-FB₁ were not detected and the presence or absence of *N*-(deoxy-D-fructos-1-yl)-FB₁ or fumonisin-sugar (protein) conjugates¹⁶⁾ was not reported.

Summary

Much has been learned about fumonisins since their discovery and investigations to further characterize their toxicity and mode of action continue. A recently developed mouse model for neural tube defects in fetuses of fumonisin-treated dams will be a valuable *in vivo* tool for elucidating the mechanistic relationships between fumonisin exposure, folate transport, and neural tube defects in this mouse model and for determining the relevance of the mouse model for humans. Determining how cooking and food processing affect fumonisins in food matrices and, using an approach combining bioassays and chemical analyses, their toxicity is another emerging research initiative. Ongoing research in these and other areas will contribute to better understanding of fumonisins and to the implementation of science-based mycotoxin management strategies.

Key words : fumonisins, toxicity, folate, review

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