An Overview of Rodent Toxicities: Liver and Kidney Effects of Fumonisins and *Fusarium moniliforme*

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Fumonisins are produced by *Fusarium moniliforme* (= *F. verticillioides*) and other *Fusarium* that grow on corn worldwide. They cause fatal toxicoses of horses and swine. Their effects in humans are unclear, but epidemiologic evidence suggests that consumption of fumonisin-contaminated corn contributes to human esophageal cancer in southern Africa and China. Much has been learned from rodent studies about fumonisin B_1 (FB_1), the most common homologue. FB_1 is poorly absorbed and rapidly eliminated in feces. Minor amounts are retained in liver and kidneys. Unlike other mycotoxins, fumonisins cause the same liver cancer promotion and subchronic (studies ≤ 90 days) liver and kidney effects as *F. moniliforme*. FB_1 induces apoptosis of hepatocytes and of proximal tubule epithelial cells. More advanced lesions in both organs are characterized by simultaneous cell loss (apoptosis and necrosis) and proliferation (mitosis). Microscopic and other findings suggest that an imbalance between cell loss and replacement develops, a condition favorable for carcinogenesis. On the molecular level, fumonisins inhibit ceramide synthase, and disrupt sphingolipid metabolism and, theoretically, sphingolipid-mediated regulatory processes that influence apoptosis and mitosis. Liver sphingolipid effects and toxicity are correlated, and ceramide synthase inhibition occurs in liver and kidney at doses below their respective no-observed-effect levels. FB_1 does not cross the placenta and is not teratogenic *in vivo* in rats, mice, or rabbits, but is embryotoxic at high, maternally toxic doses. These data have contributed to preliminary risk evaluation and to protocol development for carcinogenicity and chronic toxicity studies of FB_1 in rats and mice. *Key words:* developmental toxicology, fumonisins, *Fusarium moniliforme* (= *F. verticillioides*), hepatotoxicity, nephrotoxicity, sphingolipids. — *Environ Health Perspect* 109(suppl 2):259–266 (2001).
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Fumonisins are produced by *Fusarium moniliforme* Sheldon (= *F. verticillioides*), *F. proliferatum,* and other *Fusarium* species (*1–4*). They were discovered by Gelderblom et al. (*5*) in 1988, and their natural occurrence in corn was demonstrated soon thereafter (*6–10*). Fumonisin B_1 (FB_1) is the most common homologue (Figure 1); however, a growing number of other homologues and derivatives have been described. Fumonisins have worldwide distribution in corn and occur in corn-based feeds and foods. A comprehensive review of this subject by Dutton has been published (*11*). Equine leukoencephalomalacia (ELEM) and porcine pulmonary edema, fatal toxicoses associated with the consumption of (*F. moniliforme*) moldy feed by horses (*12,13*) and swine (*13*), respectively, have been experimentally reproduced using purified FB_1 (*14–18*).

The impact of fumonisins on human health remains unclear but is of concern. Consumption of *F. moniliforme*-molded, home-grown corn has been correlated with high esophageal cancer rates in areas of southern Africa (*19–21*) and central China (*22*), and comparatively high fumonisin concentrations are found in the corn from these high esophageal cancer areas (*4,23–28*). It has also been suggested that fumonisins are a risk factor for liver cancer (*29*), and FB_1, like some *F. moniliforme* isolates (*30,31*), was hepatocarcinogenic when fed (50 ppm) to male BD IX rats (*32*). Studies are limited, but hepatotoxicity and atherogenic serum lipid profiles, perhaps secondary to liver dysfunction, were found in nonhuman primates fed diets containing fumonisin (*13,33,34*). Finally, a possible link between fumonisin exposure and neural tube defects in humans has been proposed (*35*).

In vivo investigations using rodents and rabbits have contributed significantly to *F. moniliforme* and fumonisin research. Fumonisins were discovered using an *in vivo* liver bioassay (*5*). Data from subchronic toxicity studies have been used in preliminary risk evaluations (*36,37*), have been useful for developing protocols for chronic studies, and have otherwise increased our understanding of these compounds. An overview of toxicity and other important data obtained during *in vivo* investigations follows.

Absorption, Biodistribution, and Pharmacokinetics

Fumonisins are poorly absorbed and rapidly eliminated; small amounts accumulate in liver and kidneys (Table 1). Norred et al. (*38*) recovered 80% of the radiolabel from feces within 48 hr and ≤ 3% from urine within 96 hr after giving a single oral dose of $[^{14}C]FB_1$ (0.045 µCi) to rats. Small but relatively constant amounts of radiolabel were found in liver (about 0.4% of the dose) and kidney (about 0.1% of the dose) up to 96 hr postdosing. After administering the same dose for 3 consecutive days to rats, more than 75% of the $[^{14}C]$ was excreted in feces and about 4% in urine within 72 hr of the last dose. Liver- and kidney-specific activities peaked 24 hr after the last dose, but persisted for another 48 hr. Like FB_1, fumonisin B_2 (FB_2) is also rapidly cleared from plasma and excreted (82% within 72 hr, mostly during the first 24 hr), predominantly in the feces (*39*). Only about 1% of the dose was recovered in the urine.

Liver and kidney accumulated relatively high amounts of $[^{14}C]FB_1$ following intraperitoneal (ip) or intravenous (iv) dosing to rats (Table 1). Up to 66% of the radiolabel appeared in feces, suggesting that FB_1 (or possibly a metabolite) is excreted in bile. This was confirmed by Shephard et al. (*40*) who, within 4 hr of giving 7.5 mg/kg body weight (bw) $[^{14}C]FB_1$ ip to cannulated rats, recovered about 67% of the dose as unchanged FB_1 in the bile. In contrast, only 0.2% of the radiolabel was recovered in bile following oral administration of the same dose (7.5 mg/kg bw $[^{14}C]FB_1$), further suggesting that gastrointestinal absorption of FB_1 by rats is low.

Little pharmacokinetic data is available. Shephard et al. calculated time of plasma maximum concentration (T_{max}) of about 20 min, peak plasma concentration (C_{max}) of 8.6 µg/mL, and a serum elimination half-life
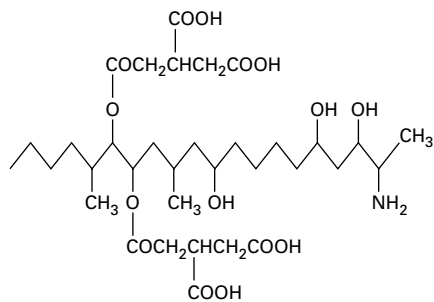
Figure 1. Chemical structure of FB$_1$.

($T_{1/2}$) of approximately 18 min for rats following a single ip injection of 7.5 mg/kg FB$_1$ (*41*). T_{max}, C_{max}, and $T_{1/2}$ of FB$_2$ are similar (Table 1). There have been no reports of fumonisin metabolism by the liver, kidney, or other tissues, although the intestinal flora of the rat, like that of the nonhuman primate (*42*), hydrolytically removes the tricarballylic acid groups from fumonisin's hydrocarbon backbone (*43*).

Toxicology: Subchronic Effects of Fumonisins in Rats

Fumonisins, *F. moniliforme,* and *in Vivo* Toxicity

Fumonisins are fungal products. Therefore, the relationship between fumonisin toxicity and toxicity of the fungi should be kept in perspective. Not all fungi identified as *F. moniliforme* are toxic. For example, culture material (0, 4, 8, or 16% w/w in the diet) of isolate MRC 826 caused significant dose-related toxicity and liver pathology (kidney was not examined) when fed to rats for 4 weeks, whereas isolate RRC 415 culture material was without effect (*44*). Similarly, only 3 of 11 *F. moniliforme* isolates induced γ-glutamyl transpeptidase (GGT)-positive liver foci in rats (*45*). MRC 826 culture material (0.5% in the diet), but not a 10-fold higher dietary concentration of isolate MRC 1069 culture material, caused hepatocarcinomas in rats (*30*). It has been shown that MRC 826 is a fumonisin producer (*46*), but that both MRC 1069 (*30*) and RRC 415 (*47*) produce predominantly fusarin C.

Additionally, *F. moniliforme* produces other biologically active, potentially toxic compounds including fusariocins (*48*), the mutagen fusarin C, other fusarins (*49,50*), and fusaric acid (*51*). None of these have reproduced the *in vivo* effects of toxic *F. moniliforme* isolates (*30,52–54*). Conversely, the link between *F. moniliforme* and fumonisin has been independently established by several research groups; that is, the *in vivo* toxicities of corn (involved in ELEM outbreaks) naturally contaminated with *F. moniliforme* (*9,55*), culture materials of (toxic) *F. moniliforme* isolates, polar culture material extracts, and purified FB$_1$ are qualitatively the same

Table 1. Selected studies on the bioavailability of fumonisins in rats following iv or ip dose administration.

Strain (sex)	Dosing	Findings	Reference
BD IX (male)	7.5 mg/kg [^{14}C]FB$_1$, single dose, ip	After 24 hr: 66% of dose recovered in feces; 32% recovered in urine; 1% found in liver; traces remained in kidney and blood cells.	(*120*)
Wistar (male), bile duct cannulated	7.5 mg/kg [^{14}C]FB$_1$, single dose, ip	After 24 hr: about 67% of dose recovered in bile, mostly (approximately 60% of dose) within the first 4 hr.	(*121*)
Sprague-Dawley (male)	0.0045 µCi [^{14}C]FB$_1$, single dose, iv	^{14}C appears in gastrointestinal tract (peaking at approximately 10% of dose after 1 hr); feces (approximately 35% of dose recovered after 96 hr); urine (approximately 10% recovered after 12–96 hr); liver (approximately 45 and 25% of dose found after 1 and 96 hr, respectively); and kidney (approximately 10% of dose from 10 min to 96 hr).	(*38*)
Sprague-Dawley (female), pregnant	0.145 µCi [^{14}C]FB$_1$, single dose, iv on GD 15	After 1 hr: about 45% of dose recovered in gastrointestinal tract, mostly in feces; liver and kidney contain about 14.5 and 4.0% of dose, respectively; only 1.2% of dose found in blood.	(*106*)
BD IX (males)	7.5 mg/kg FB$_2$, single dose, ip	T_{max} approximately 20 min; C_{max} = 3.5 µg/mL; serum $T_{1/2}$ of approximately 26 min. Concentration peaks in plasma 10 min after injection, followed by rapid elimination; approximately 1 and 84% of dose recovered from urine and feces, respectively, through 72 hr, mostly within first 24 hr.	(*39*)

(*5,32,45,56–61*). FB$_2$, fumonisin B$_3$ (FB$_3$), and probably also hydrolyzed FB$_1$ (HFB$_1$) exert the same *in vivo* effects (*62–64*).

Hepatotoxicity

Histopathologic effects in rats, which have been referred to by various terms such as hepatopathy, hepatosis, or toxic hepatitis, have been reported by several research groups. Their descriptions are consistent, differing only in detail and nomenclature (*5,56–58, 61,65–67*). The initial finding is small, rounded, eosinophilic hepatocytes that appear to have pulled away from neighboring cells. The chromatin of these cells is irregularly condensed and marginated or may be fragmented. Inflammatory response is absent to minimal. Although their appearance is consistent with apoptosis, these cells were commonly described as single-cell necrosis until their apoptotic nature was histochemically confirmed by Tolleson et al. (*61*) and Howard et al. (*68,69*). This does not mean, however, that necrosis (oncotic necrosis in the traditional sense, as opposed to programmed cell death or apoptosis) does not play a role in fumonisin-induced hepatotoxicity. Necrotic hepatocytes are also present early-on. Serum chemical indications of hepaocellular injury, including increased alanine and aspartate transaminase, alkaline phosphatase, and lactate dehydrogenase activities, as well as increased cholesterol and triglyceride concentrations (Figure 2), are routine, early findings. As tissue injury progresses, both apoptotic and necrotic cells increase in number, mitotic figures appear with increasing frequency, hepatocellular cytoplasm becomes increasingly vacuolated, and cytomegaly with variability in cell and nuclear size becomes obvious. Bile duct and oval cell proliferation,
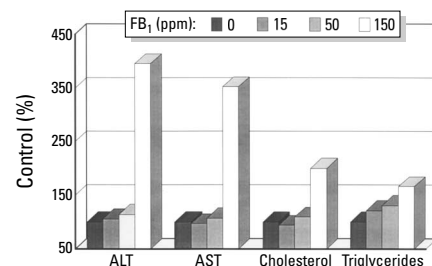

Figure 2. Selected serum chemical effects of FB$_1$. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase. Serum ALT and AST activities, international units/deciliter cholesterol, and milligrams per deciliter triglycerides were significantly increased ($p < 0.05$) in male Sprague-Dawley rats fed 150 ppm FB$_1$ for 4 weeks. Data from Voss et al. (*58*).

foci of cellular alteration, cholangiomatous lesions, and fibrosis occur in long-standing or advanced lesions, giving a picture of nodular regeneration or cirrhosis (*32*). Females are generally more sensitive than males (Table 2). Along with the aforementioned clinical chemical indicators, which continue to rise, serum GGT activity and bilirubin concentration increase as liver injury becomes more severe.

Fumonisins induced both GGT and the placental form of glutathione *S*-transferase (GSTP)-positive foci in BD IX and Fischer rats (*5,62,70,71*). Foci induction in diethylnitrosamine-pretreated Fischer rats was dose related, and GSTP was a more sensitive marker than GGT (Figure 3) (*70*). From these and other data, Gelderblom et al. (*72*) proposed that FB$_1$ was a tumor promoter at doses not causing significant liver pathology, but when given at overtly hepatotoxic doses, it was also a weak initiator. FB$_1$ (50 ppm) caused marked nonneoplastic changes (cirrhosis) and hepatocellular carcinomas when fed to BD IX males for 20 months or more (*32*). Relatively

Table 2. Dose response[a] in liver and kidney: subchronic feeding studies of FB_1 in rats.

Strain	Study duration	Doses (ppm FB_1)	Liver		Kidney		Reference
			Males	Females	Males	Females	
Sprague-Dawley	28 days	0, 15, 50, 150	50 < NOEL < 150	50 < NOEL < 150	NOEL < 15	15 < NOEL < 50	(*58*)
Fischer 344	90 days	0, 1, 3, 9, 27, 81	81 < NOEL	27 < NOEL < 81	3 < NOEL < 9	27 < NOEL < 81	(*57*)
Fischer 344	28 days	0, 99, 163, 234, 484	163 < NOEL < 234	99 < NOEL < 163	NOEL < 99	99 < NOEL < 163	(*61*)

[a]NOEL (no-observed-effect level) as defined by histopathology and other findings.
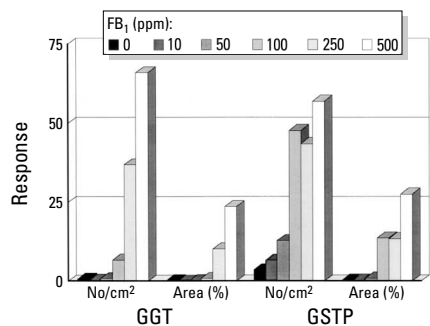

Figure 3. Dose-related induction of GGT and the placental form of GSTP-positive foci in liver of male Fischer 344 rats (*n* = 5/group). The animals were fed diets with up to 500 ppm FB_1 for 3 weeks, beginning 2 weeks after pretreatment with 200 mg/kg diethylnitrosamine (ip injection). The number of GGT and GSTP foci per square centimeter was significantly increased ($p < 0.05$) at ≥ 250 ppm and ≥ 100 ppm FB_1, respectively; the percent area involved per liver section was significantly increased ($p < 0.05$) at ≥ 100 ppm and ≥ 500 ppm FB_1, respectively. Figure adapted from the data of Gelderblom et al. (*70*).

(marginally deficient) low dietary lipotrope levels may have contributed to the neoplastic response. Nonetheless, these results demonstrated carcinogenicity by FB_1 and the need for further studies. One such study, the recently completed chronic bioassay by the National Center for Toxicological Research of the U.S. Food and Drug Administration (*73*), showed that FB_1 was a kidney carcinogen in rats and a liver carcinogen in mice, respectively.

Nephrotoxicity

The kidney was the most sensitive target organ in Sprague-Dawley and Fischer 344 rats fed FB_1 for up to 90 days (Table 2) (*57,58,61,74*) or given FB_1 by gavage or ip injection for 4–11 days (*65–67,75*). Males were more sensitive than females. In contrast, Gelderblom et al. (*5,32*) described hydropic degeneration, occasional necrosis, and a few other renal abnormalities in their studies in BD IX rats, but did not refer to the kidney as a target organ. This suggests that significant differences in response to fumonisins may exist among various rat strains.

As in liver, apoptosis is the initial microscopic finding in kidney. Apoptotic cells are initially found almost exclusively in tubules of the outer medulla (designated "corticomedullary junction" in some publications). Many of the apoptotic cells appear rounded and detached from adjacent cells and the
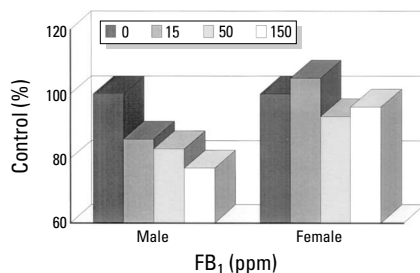

Figure 4. Relative (% bw) kidney weight was significantly ($p < 0.05$) decreased in male Sprague-Dawley rats (*n* = 5/group) fed 15, 50, or 150 ppm FB_1 for 4 weeks. Significant differences in relative kidney weight were not found in females; however, absolute kidney weight (g) of females fed 150 ppm was significantly lower than control values (not shown). Data from Voss et al. (*58*).

basement membrane. Mitotic figures appear, and the number of apoptotic cells increases in the tubule epithelium as injury progresses. Cytoplasmic vacuolation and basophilia, decreased cellular height, and alterations in nuclear size and staining become evident. At this point, lesions may extend deeper into the medulla or into the cortex, and epithelial cells are sloughed into the tubular lumina. Thus, there is simultaneous cell loss and replacement revealed on three levels: on the cellular level by apoptosis and mitosis, on the histologic level by tubular atrophy and hyperplasia (regeneration), and grossly by decreased kidney weight (Figure 4). The failure of regeneration to keep pace with cell loss may be quite important, as imbalances between cell loss and replacement in tissues may be a significant contributor to carcinogenesis (*76–78*).

Increased serum creatinine, decreased CO_2 and, less often, increased urea nitrogen occur in FB_1-treated rats (*57,58,79*). Increased activities of *N*-acetyl-β-glucosaminidase, GGT, and lactate dehydrogenase have been found in urine, giving further clinical evidence of tubular injury (*65,75*). Proteinuria may also occur and, because of the presence of high-molecular-weight proteins in the urine, it has been suggested that FB_1 may also cause glomerular injury (*75*). Alternatively, sloughed tubule cells, which can be numerous in urine, are a more likely cause of proteinuria (*80*).

There is little data on renal function in fumonisin-treated animals. Urinary output and water consumption were increased in male rats fed *F. moniliforme* culture material (*64*). These observations were consistent with

those of Bondy et al. (*65,67*) and Suzuki et al. (*75*), who studied renal function in rats given daily ip doses (7.5–10 mg/kg for 4 days) or oral doses (1–75 mg/kg for 11 days) of FB_1. Signs of renal dysfunction in these animals included increased output of hypoosmotic urine, increased urinary enzyme levels, proteinuria, increased serum Mg^{2+} and Ca^{2+} concentrations, and decreased anion (*p*-aminohippurate, up to 80% reduction) and cation (tetraethylammonium, up to 40% reduction) transport by renal cortex slices. Interestingly, some indications of renal dysfunction peaked on days 6–8 of the 11-day exposure period (*67*), suggesting that fumonisin-damaged kidneys have some functional adaptive capacity.

Other *in Vivo* Toxicologic Findings in Rats

There are indications that the immune system may be a target. Bondy et al. (*66*) found disseminated thymic necrosis with decreased thymic weight and increased serum IgM concentrations in FB_1-exposed rats. Others found that the immune responses to sheep red blood cells and to splenic clearance of *Listeria monocytogenes* were slightly decreased in rats given 15 (*L. monocytogenes*) or 25 (sheep red blood cells) mg/kg bw FB_1 for 14 days (*81*).

Testicular tubule epithelial degeneration (*59*), decreased heart weight (*54*), adrenal cortex hypertrophy, and cytoplasmic vacuolation (consistent with *Zona fasciculata* lipoidosis and probably a nonspecific stress response) (*64*), cytoplasmic vacuolation of myeloid precursor cells in bone marrow, and other hematologic findings (*66*) have been found in rats given fumonisins or *F. moniliforme* culture materials. Little toxicologic importance has yet been given to any of these observations.

Because of the human health implications, esophageal effects of fumonisins are of special interest. Marasas et al. (*31*) found basal cell hyperplasia of the esophagus in rats fed *F. moniliforme* culture material. Others have noted a transient increase in the 5-bromo-2′-deoxyuridine labeling index in esophageal epithelium 3 days following an iv injection of 1.25 mg/kg bw (*82*), which suggests that FB_1 may be mitogenic under some conditions. However, there is no evidence from subchronic (*5,57,58,61*) or chronic (*32,73*) feeding studies of purified FB_1 that the esophagus is a target organ. Furthermore, FB_1 (5 mg/kg bw/day for 5 weeks) had no effect on the

number of esophageal papillomas produced in rats concurrently given the esophageal carcinogen *N*-methylbenzylnitrosamine (*83*). Thus, there is no evidence from rodent studies that FB_1 is an esophageal carcinogen, and the possibility that *F. moniliforme* produces other potentially (esophageal) carcinogenic compounds should not be dismissed.

Hepatic and Renal Toxicity in Other Laboratory Species

Liver and kidney are also targets in mice (*74,84,85*). The pathology is similar to that seen in rats, and females are more sensitive to hepatotoxicity than males. Apoptosis, hepatocellular hyperplasia, bile canaliculi hyperplasia, and Kupffer cell hyperplasia were the principal findings in females ($B6C3F_1$) fed ≥ 99 ppm FB_1 and males fed 484 ppm FB_1 for 4 weeks (*68,73*). A hepatocellular cytoplasmic alteration described as reduced cytoplasm, basophilia, and loss of cytoplasmic vacuoles was found in both sexes fed ≥ 99 ppm. Histopathology findings (hepatopathy) in female $B6C3F_1$ mice fed 81 ppm FB_1 for 90 days (*57*) were characterized by apoptosis (= single-cell necrosis), cytomegaly, increased mitotic figures, scant inflammatory infiltrates, and pigmented macrophages. Serum chemical indications of hepatic injury of the same type found in rats also occurred.

Compared to rats, mice are resistant to nephrotoxicity. No kidney lesions were found in mice fed diets with 81 ppm FB_1 for 90 days (*57*) or diets with 484 ppm FB_1 for 28 days (*68*). However, when given at relatively high doses by oral or parenteral routes, FB_1 is nephrotoxic, as illustrated by the findings of Sharma et al. (*84*). They found terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL)-positive cells in the renal tubules of males (females were not examined) given subcutaneous injections of FB_1 for 5 consecutive days (Figure 5). Findings were corroborated by the dose-related increase in apoptotic cells, which was found during routine microscopic examinations (hematoxylin and eosin sections). Bondy et al. (*85*) found slight increases in single-cell necrosis (= apoptosis) in the renal tubules of female but not male $B6C3F_1$ mice given 15–75 mg/kg FB_1 by gavage for 14 days.

Rabbits are quite sensitive to FB_1 (*86*). As in male rats, the kidney is a more sensitive target organ than liver. Morphologic, serum chemical, and tissue sphingolipid findings were similar to those seen in rodents.

Fumonisins and Sphingolipids: Mechanistic Considerations

The preponderance of experimental evidence, including evaluations of unscheduled DNA synthesis, *Salmonella typhimurium* mutagenicity, SOS response in *Escherichia coli,* mitotic index, and micronucleus formation [reviewed by Howard (*87*)], suggests that fumonisins exert their effects through a nongenotoxic mode of action. A significant breakthrough in understanding these compounds occurred when Wang et al. (88) discovered that fumonisins inhibit the enzyme ceramide synthase [sphinganine (sphingosine)-*N*-acetyltransferase], leading to disruption of *de novo* sphingolipid biosynthesis. The immediate consequences thereof are accumulation of the sphingoid bases sphinganine (Sa) and sphingosine (So), an increase in the Sa to So ratio (Sa/So), and depletion of complex sphingolipids (CSLs) in tissues (Figure 6). Recognizing the importance of sphingolipids in cell regulatory processes, including those related to proliferation and apoptosis (*76,89–91*), investigators have proposed that ceramide synthase inhibition is the critical mechanistic step in fumonisin toxicity, starting a cascade of molecular events eventually leading to cytotoxicity or neoplasia.

Fumonisin exposure, sphingolipid effects, and toxicity are correlated *in vivo*. Liver and kidney Sa, So, and Sa/So were increased in rats fed 15–150 ppm FB_1 for 4 weeks (Figure 7) (*80*), and increases occurred at doses equal to or less than those causing microscopic lesions (apoptosis). Importantly, liver and kidney Sa/So increases were correlated with the severity of hepatopathy and nephropathy in rats fed *F. moniliforme* culture material (71 ppm FB_1), water-extracted culture material (11 ppm FB_1), or an alkali-treated (nixtamalized) culture material containing 58 ppm HFB_1 but no measureable FB_1 (*92,93*).

The role of sphingolipids as mediators of fumonisin toxicity has not yet been proven, and other mechanisms may come into play.

For example, several groups have presented data suggesting that fumonisins cause compositional or oxidative damage to cellular lipids, which in turn causes molecular events culminating in oxidative damage to DNA and other critical macromolecules (*94–99*). A more detailed discussion of molecular mechanism is beyond the purpose of this review but can be found elsewhere in this issue (*87,100–102*).

Reproduction and Teratology Studies

Javed et al. (*103*) found that FB_1 was embryotoxic and caused malformations when injected into chicken eggs. FB_1 and HFB_1 also inhibited growth of rat embryos exposed *in vitro* on gestation day (GD) 9.5 (*104*). Under similar conditions, 100 or 300 μM HFB_1 caused neural tube and other malformations (*105*). Although useful as screens, *in vitro* methods allow direct fetal exposure and avoid maternal gastrointestinal absorption, pharmacokinetics, placental transfer, and other potential barriers of *in utero* exposure.

To assess the reproductive effects of *F. moniliforme,* male and female Sprague-Dawley rats were fed culture material of isolate MRC 826 providing 0, 1, 10 or 55 ppm FB_1 (*106*). The culture material was minimally toxic to males (≥ 10 ppm) and females

Figure 6. Simplified depiction of the *de novo* synthesis of sphingolipids. Abbreviations: CSL, complex sphingolipids FB_x, fumonisins B; SM, sphingomyelin. FB_x inhibit incorporation of the sinto ceramide (Cer), thus increasinphingoid bases Sa and So g cellular Sa and So, depleting CSL, and otherwise disrupting sphingolipid metabolism. Data from Yoo et al. (*124*).
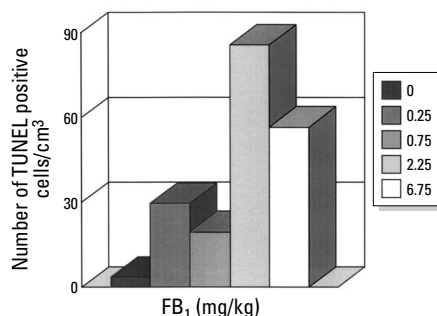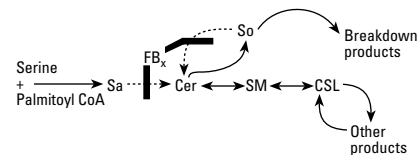

Figure 5. Daily subcutaneous injection of FB_1 for 5 consecutive days increased the number of renal tubular cells stained *in situ* by TUNEL, a technique marking apoptotic nuclei. Values indicate group mean; $n = 5$. The difference was statistically significant ($p < 0.05$) at 6.25 mg/kg FB_1 but, because of a large standard deviation, not at 2.25 mg/kg FB_1. Microscopic examination of hematoxylin and eosin-stained kidney specimens yielded similar results: apoptosis was found in all mice given 2.25 or 6.25 mg/kg FB_1, 2–3 mice/group given 0.25–0.75 mg/kg FB_1, and none of the controls. Figure adapted from the data of Sharma et al. (*84*).

Figure 7. Tissue Sa/So, a biomarker of fumonisin exposure, was increased in Sprague-Dawley rats ($n = 5$/group) fed 15, 50, or 150 ppm FB_1 for 4 weeks, primarily because of increased Sa. Differences in liver Sa/So were significant ($p < 0.05$) at 150 ppm in males and ≥ 50 ppm in females, and differences in kidney Sa/So were significant at ≥ 15 ppm in both sexes, doses that for both organs were equal to or less than the lowest dose causing microscopic lesions (arrows). Data from Voss et al. (*58*) and Riley et al. (*80*).

(55 ppm), as indicated by serum chemistry findings and kidney pathology. All reproductive end points in males, which included testicular morphology, sperm morphology, and sperm motility, were unaffected. One half of the mated females from each group were examined on GD 15, at which time no differences among groups were found in the number of corpora lutea, implantation sites, resorptions, dead fetuses, and live fetuses per dam. The remaining females gave birth and were observed, along with their litters, for 21 days postpartum. Other than a slight decrease in weight gain (but not absolute weight) of litters from groups given 10 and 55 ppm FB_1, no differences were found in maternal reproductive or offspring development variables. Although liver Sa/So of the dams fed 55 ppm FB_1 was significantly increased, no differences in the Sa/So of control and high-dose (55 ppm) fetuses were found on GD 15 (abdominal slices containing liver and kidney), indicating that fumonisins did not cross the placenta. This was corroborated in a second study in which no radiolabel (< 0.02% of the dose) was found in the fetuses following iv injection of $[^{14}C]FB_1$ to pregnant females on GD 15 (106).

Pregnant rats were given 1.875–15 mg/kg bw purified FB_1 on GD 3–16 (107) and examined on GD 17 and GD 20. FB_1 had no effect on maternal reproductive variables. The high dose (15 mg/kg FB_1) was maternally toxic, causing decreased weight gain on GD 17, decreased kidney weights, and increased Sa/So of liver, kidney, and serum. Sa/So was also increased in livers of dams given 7.5 mg/kg, kidneys of dams given ≥ 1.875 mg/kg, and serum of dams given 7.5 mg/kg FB_1. Decreased length and

weight of female fetuses were noted on GD 20. Otherwise, there was no evidence of fetal toxicity or teratogenicity. The study was repeated at doses ranging from 6.25 to 50 mg/kg FB_1 (108). Apoptosis and other microscopic findings typical of fumonisins were found in kidney (≥ 6.25 mg/kg) and liver (25 or 50 mg/kg). Increased Sa/So was found in maternal liver (≥ 25 mg/kg), kidney (≥ 6.25 mg/kg), and serum (≥ 25 mg/kg). Fetal deaths were increased at 25 and 50 mg/kg, and the number of viable fetuses/dam (12.0 ± 1.1 vs control value of 14.0 ± 0.5), fetal length, and fetal weight were decreased at 50 mg/kg. The incidence of hydrocephalic fetuses and skeletal anomalies such as wavy ribs and reduced ossification was increased somewhat at 50 mg/kg, but no teratogenic effects were found. Sa/So of fetal tissues were unchanged at any of the doses studied, suggesting that the fetal effects were indirect and secondary to maternal toxicity.

Results of other developmental toxicity studies (Table 3) generally agree and likewise suggest that FB_1 is not teratogenic but may be embryotoxic at maternally toxic doses (109,110). In contrast, Floss et al. (111,112) concluded that FB_1 was a developmental toxin in hamsters at dosages that were not maternally toxic. It is possible that there are species-related differences in maternal response. However, detailed serum chemical, histopathologic, or fetal and maternal tissue sphingolipid evaluations, which may have revealed maternal toxicity in the hamsters, were not undertaken. Collins et al. (107,108), LaBorde et al. (110), and Voss et al. (106) have shown significant organ weight, pathology, and sphingolipid effects in dams that otherwise appear unaffected by FB_1.

Applied Studies Using Culture Materials

FB_1 does not occur alone. Co-exposure undoubtedly occurs with other mycotoxins and mycotoxin products formed during grain handling or food preparation. As illustrated below, *F. moniliforme* culture material can be a useful, cost-effective tool for studying how co-exposure to other mycotoxins or mycotoxin products influences FB_1 toxicity. However, such experiments should be carefully designed and results interpreted with caution. Not all *F. moniliforme* strains are toxic, and culture materials are complex mixtures containing biologically active compounds (both known and unknown) that may confound results.

To determine the potential *in vivo* toxicity of FB_2 and FB_3, Voss et al. (64) studied three genetically related *F. moniliforme* isolates. Isolate M3125 produced FB_1, FB_2, and FB_3 in the approximate ratio of 1:0.35:0.15. Isolate 107-R-7 produced FB_2 (no detectable FB_1 or FB_3), and isolate 397-R-74 produced FB_3 (no FB_1 or FB_2). Low (4.6–6.9 ppm), mid (32–53 ppm), and high (219–303 ppm) levels of culture materials of each isolate were fed to rats for 3 weeks. All were toxic. Their effects were qualitatively indistinguishable, consisting of decreased weight gain, decreased kidney weights, increased serum chemical indications of hepatotoxicity, increased Sa/So, and apoptosis in the liver and kidneys. All findings were consistent with the effects of FB_1 (57,58), and elevated tissue Sa/So was correlated with various toxicologic end points. Thus, hepato- and nephrotoxicities can be induced by FB_1 nonproducing fungi, and toxicity studies of purified FB_2 and FB_3 are warranted.

Table 3. Summary of selected developmental toxicity studies of FB_1 in laboratory species.

Species	Dosing	Findings and comments	References
Fischer 344 rats	0, 30, 60 mg/kg FB_1; by gavage; GD 8–12	Fetal: decreased litter weight (approximately 20% reduction at high dose); hypoplasia (delayed or incomplete ossification) of sternebrae and vertebral bodies at 30 and 60 mg/kg. Maternal: no significant weight gain effects. Comment: other data relevant to maternal toxicity such as pathology, serum chemistry, or sphingolipid profiles not reported.	(122)
Syrian hamsters	0, 12, 18 mg/kg FB_1; by gavage; GD 8 and 9	Fetal: increased fetal death and resorption; decreased fetal weight; one litter had fetuses with hooked/curled tails; one litter had fetuses with ectodactaly. Maternal: no weight gain effects. No differences in serum AST and bilirubin. Reference to some hepatic and placental pathologic changes. Comment: dose response of liver and placental pathology not described. Kidney pathology or sphingolipid profiles (indicators of maternal toxicity) not reported.	(112)
New Zealand white rabbits	0, 0.1, 0.5, 1 mg/kg FB_1; by gavage; GD 3–19	Fetal: 13–16% decrease in body weight and decreased kidney and liver weight at 0.5 and 1 mg/kg. Otherwise, no significant findings. Fetal tissue Sa/So unaffected. Maternal: mortality increased at ≥ 0.5 mg/kg. Increased Sa/So of liver, kidney, serum, and urine. Comment: no evidence of teratology or significant developmental toxicity in presence of maternal toxicity.	(110)
CD-1 mice	0, 12.5, 25, 50, 100 mg/kg FB_1; by gavage; GD 7–15	Fetal: increased fetal death (resorptions), decreased fetal weight and increased incidence of hydrocephalus at ≥ 25 mg/kg. No increase in fetal hepatic Sa/So. Maternal: mortality at two highest doses. Significantly reduced maternal weight gain at 100 mg/kg. Significant hepatic pathology, increased serum ALT and increased liver Sa/So in dams given ≥ 25 mg/kg. Comment: fetal toxicity secondary to maternal toxicity; Sa/So indicates that FB_1 does not cross placenta. Corroborates results of study on developmental toxicity of *F. moniliforme* culture material extracts (106)	(109,123)

Abbreviations: AST, aspartate aminotransferase activity; ALT, alanine aminotransferase activity.

It has been suggested that fusaric acid, another mycotoxin commonly produced by *F. moniliforme* (*51,113*), exacerbates fumonisin toxicity (*114,115*). Bacon et al. (*116*) demonstrated synergistic embryotoxicity by simultaneous injection of FB$_1$ and fusaric acid *in ovo*. Diets containing *F. moniliforme* MRC 826 culture material providing low (3.4 ppm), slightly higher (18 ppm), or very high (437 ppm) amounts of FB$_1$ and, at each fumonisin level, 0, 20, 100, or 400 ppm fusaric acid were fed to rats for 4 weeks (*54*). Dose-related body weight, serum chemical, liver and kidney pathologies, and renal sphingolipid effects typical of fumonisins were caused by the culture material. No evidence of synergism was found. Fusaric acid alone up to 400 ppm in the diet was not toxic, and its presence did not modify the response of the animals to the culture material.

Masa flour is made from nixtamalized corn. During nixtamalization, corn is boiled under alkaline conditions sufficient to convert fumonisins to their hydrolyzed forms (*117,118*). A study by Hendrich et al. (*63*) showed that nixtamalization did not reduce hepatotoxicity or cancer-promoting activity of *F. proliferatum* culture material, even though FB$_1$ and FB$_2$ were converted to HFB$_1$ and HFB$_2$. However, others reported that although cytotoxic *in vitro*, purified HFB$_1$ had no effect *in vivo*, and they proposed that it was not gastrointestinally absorbed (*62*). To further study nixtamalization and *in vivo* toxicity, Voss (*92*) fed rats *F. moniliforme* culture material providing 71 ppm FB$_1$, water-extracted culture material providing about 11 ppm FB$_1$, or a nixtamalized culture material providing 58 ppm HFB$_1$, but no measurable FB$_1$. After 4 weeks the culture material and the nixtamalized culture material caused the hepatic and renal lesions typical of fumonisins, though the nixtamalized material was somewhat less potent. The water-extracted culture material elicited a noticeably lesser nephrotoxic response and was not hepatotoxic. Sa and Sa/So increases in liver and kidney were increased in all three groups, and the increases were correlated with the severity of liver and kidney injury (*93*). These results agree with the *in vitro* findings of Norred et al. (*119*), who reported that HFB$_1$ inhibited ceramide synthase in precision-cut rat liver slices, but less potently than FB$_1$. The consequences of chronic HFB$_1$ exposure remain unknown, and, given the popularity of masa-based food products, additional investigations on its occurrence in foods and its toxicity are needed.

Summary and Conclusions

In vivo studies of *F. moniliforme* and fumonisins in rodents have shown that FB$_1$ and probably FB$_2$, FB$_3$, and HFB$_1$ cause the toxic and pathologic effects of *F. moniliforme*. These studies have provided other important data including the following: *a*) Gastrointestinal absorption is low, absorbed fumonisins are rapidly eliminated, and only minor amounts are retained in liver and kidney. *b*) Liver and kidneys are the two major target organs, although differences in response occur between sexes, strains, and species. *c*) Fumonisins may have other, more subtle organ-specific effects; however, there is no compelling evidence that the esophagus is a target organ. *d*) Apoptosis is the initial and presumably critical event in the pathogenesis of liver and kidney lesions characterized by simultaneous cell loss and regeneration. *e*) A key molecular event in fumonisin cytotoxicity is inhibition of ceramide synthase, leading to disruption of sphingolipid metabolism and probably of sphingolipid regulatory function. *f*) FB$_1$ does not cross the placenta and is not teratogenic in laboratory species; however, fumonisins may be embryotoxic at maternally toxic doses. *g*) The use of culture materials provides a cost-effective means of studying *F. moniliforme* and fumonisins, as long as caution is exercised in study design and data interpretation. The relationships between *F. moniliforme*, fumonisins, and human health remain unresolved. Undoubtedly, *in vivo* investigations in rodents will continue to provide insight into the effects and modes of action of these important mycotoxins.

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