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# Effect of Baking and Frying on the in Vivo Toxicity to Rats of Cornmeal Containing Fumonisins

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Fumonisins are mycotoxins produced by *Fusarium verticillioides* (=*F. moniliforme*) and other *Fusarium* species. They are found in corn and corn-based foods. Cooking decreases fumonisin concentrations in food products under some conditions; however, little is known about how cooking effects biological activity. Baked cornbread, pan-fried corncakes, and deep-fried fritters were made from cornmeal that was spiked with 5% w/w *F. verticillioides* culture material (CM). The cooked materials and the uncooked CM-spiked cornmeal were fed to male rats ($n = 5$/group) for 2 weeks at high (20% w/w spiked cornmeal equivalents) or low (2% w/w spiked cornmeal equivalents) doses. A control group was fed a diet containing 20% w/w unspiked cornmeal. Toxic response to the uncooked CM-spiked cornmeal and the cooked products included decreased body weight gain (high-dose only), decreased kidney weight, and microscopic kidney and liver lesions of the type caused by fumonisins. Fumonisin concentration, as determined by HPLC analysis, in the 20% w/w pan-fried corncake diet [92.2 ppm of fumonisin $B_1$ ($FB_1$)] was slightly, but not statistically significantly, lower than those of the 20% w/w baked cornbread (132.2 ppm of $FB_1$), deep-fried fritter (120.2 ppm of $FB_1$) and CM-spiked cornmeal (130.5 of ppm $FB_1$) diets. Therefore, baking and frying had no significant effect on the biological activity or concentration of fumonisins in these corn-based products, and the results provided no evidence for the formation of novel toxins or "hidden" fumonisins during cooking.

**KEYWORDS: Fumonisins; bioassay; cooking; food safety**

## INTRODUCTION

Fumonisins are mycotoxins produced by *Fusarium* species, primarily *F. verticillioides* (=*F. moniliforme*) and *F. proliferatum* (*1*). They exert a spectrum of toxic effects in animals including leukoencephalomalacia in horses (*2*, *3*), pulmonary edema in swine (*4*, *5*), and hepatopathy and nephropathy in rodents and other species (*6*). Fumonisin $B_1$ ($FB_1$), the most common and thoroughly studied analogue, is hepato- and nephrocarcinogenic to rats and mice (*7*−*9*). The impact of fumonisins on human health is unclear. However, dietary exposure to corn highly contaminated with fumonisin-producing fungi or fumonisins has been correlated with high rates of esophageal cancer in southern Africa (*10*−*12*) and China (*13*−*15*). A relationship between fumonisin exposure and liver cancer (*16*), an outbreak of neural tube defects (*17*), and cardiovascular disease (*5*, *18*, *19*) has also been suggested.

Fumonisins are found in corn and corn-based foods worldwide (*20*−*23*). Whereas relatively high concentrations of fumonisins are found in home-grown corn for human consumption in areas of southern Africa, relatively low concentrations, <1 ppm, are generally found in commercial corn products from North America, Europe, and also southern Africa (*11*, *23*−*29*). In one survey, fumonisins were found in about two-thirds of commercial, corn-based food products in the United States, mostly at levels ≤0.25 ppm (*26*). Concentrations tended to be higher in cornmeal, muffin, and cornbread mixes than in snack foods or other more highly processed items, suggesting that processing or cooking might reduce fumonisin concentrations in the products.

Cooking and steeping whole kernel corn in alkaline water (nixtamalization) effectively removes fumonisins from the corn and decreases its concentration in the masa and masa products (*30*, *31*). Extrusion reduces fumonisins in the products to varying degrees depending on temperature, screw speed, and the presence of reducing sugars in the food matrix (*32*−*34*), and cooking corn porridge using a traditional South African method reduced $FB_1$ concentrations ∼23% (*35*). The effectiveness of baking and frying for reducing fumonisin concentrations in food is somewhat variable and depends on product type and cooking conditions (*33*, *36*−*38*). Factors tending to reduce fumonisins are longer cooking times, higher cooking temperatures, and higher moisture content of the dough (*28*, *33*, *36*). Despite the reductions in measured fumonisin concentrations achieved by various cooking methods, the chemical fate of fumonisins in food matrices is poorly understood, and stability and recovery

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problems associated with the extraction and quantification of fumonisins in foods have long been recognized (*39*). The extent to which fumonisins are converted to novel compounds or become bound to food matrix components is a concern and, on the basis of the recent findings by Kim et al. (*40*), might be considerable. This is problematic not only for accurate measurement of fumonisins in foodstuffs but also for predicting the toxic potential of foods prepared from fumonisin-containing materials. Therefore, the purpose of this investigation was to determine the extent to which baking or frying fumonisin-contaminated cornmeal modifies its in vivo toxicity, using a rat feeding study as a bioassay.

## MATERIALS AND METHODS

**Ingredients and Cooking Procedures.** Ingredients and the formulated diets were stored frozen. Culture material (CM) was prepared using *F. verticillioides* strain MRC 826 (W. F. O. Marasas, MRC, Tygerburg, South Africa) (*41*). It was freeze-dried, ground, and then blended (5.4% w/w CM) with ground, sound seed corn (Reid's Yellow Dent) containing <0.05 ppm of fumonisins ($FB_1 + FB_2$) to make the spiked cornmeal.

*Baked Cornbread.* Six hundred grams of the spiked cornmeal was mixed with 600 mL of distilled water. This batter was baked at 190 °C (375 °F) for 1 h in a lightly oiled (Mazola, locally purchased) baking pan. After cooling, the cornbread was manually crumbled, freeze-dried to facilitate grinding, and ground using a Wiley mill.

*Pan-Fried Corncakes.* Batter (600 g of spiked cornmeal plus 1050 mL of distilled water) was poured onto a lightly oiled, preheated electric frying pan. The pancake style corncakes were fried at 218 °C (425 °F) for 10−12 min, at which time they appeared dry with dark brown, crispy edges. After cooling, they were blotted to remove excess oil, crumbled, freeze-dried, and ground.

*Deep-Fried Fritters.* The batter (600 g of spiked cornmeal plus 650 g of distilled water) was formed into fritters of approximately 6−8 cm × 4−5 cm in size. The fritters were dropped into preheated, 193 °C (380 °F), corn oil and deep-fried until brown and crisp on the outside (~10 min). They were then cooled, blotted, crumbled, freeze-dried, and ground.

**Diet Preparation and Analysis.** The formulated diets were prepared by mixing the ground cornbread, pan-fried corncakes, or deep-fried fritters with sound corn and the basal rodent feed (Teklad LM 485) using a Patterson Kelley V blender with stirring intensifier bar. All diets contained 80% w/w basal feed. The high-dose diets contained 20% w/w spiked cornmeal equivalents of the baked cornbread, pan-fried corncakes, or deep-fried fritters, and the low-dose diets contained 2% w/w spiked cornmeal equivalents. The negative control diet contained 20% w/w unspiked cornmeal (ground seed corn). Diets containing 20 or 2% w/w of the uncooked, spiked cornmeal served as positive controls.

Triplicate 5 g samples of each high-dose diet were extracted with 25 mL of acetonitrile/water (1:1, pH adjusted to 4.5 with 6 N HCl) for 30 min at room temperature with gentle agitation provided by a wrist-action shaker. After removal of the extract by filtration (Whatman no. 4 paper), the sample was extracted a second time. The first and second extracts were combined and cleaned up using Fumonitest immunoaffinity columns (Vicam, Watertown, MA), and the fumonisin concentration ($FB_1 + FB_2$) was determined by HPLC as previously described (*31*).

**Animals.** Male Sprague−Dawley rats (Harlan, Indianapolis, IN), 5 weeks of age at initiation of the study, were acclimated for 1 week and then randomly assigned to nine groups of five animals each. The rats were individually housed in stainless steel, wire-mesh cages in an environmentally controlled room (22−27 °C, 50−60% relative humidity, 12 h light/dark cycle). Diets and fresh tap water were provided ad libitum.

**Feeding, Observations, and Histopathology.** The groups were fed the diets for 2 weeks ad libitum. The animals were observed daily and weighed twice weekly. Food consumption was determined concurrent with body weight measurements. The rats were then fasted overnight, euthanized with carbon dioxide gas, and examined by necropsy. The brain, heart, liver, lungs, kidneys, spleen, and testes were weighed and fixed in 10% neutral buffered formalin. Hematoxylin- and eosin-stained sections of liver and kidney, the two principal target organs of fumonisins in rats, were microscopically examined and lesions subjectively scored using established criteria (*42*).

**Statistics.** Statistical analyses generally followed the scheme of Gad and Weil (*43*) and were done using computerized software (SAS Institute or Instat GraphPad). Body weight, food consumption, and organ weight data were evaluated using one-way analysis of variance followed by Duncan's multiple-range test to identify differences among groups. Pathology scores were analyzed using the Kruskal−Wallis test and distribution free multiple comparisons. Fisher's least significant differences test was used to compare pathology incidences. Comparisons were two-tailed, and significance was judged at $p < 0.05$.

**Table 1.** Fumonisin $B_1$ ($FB_1$) and $B_2$ ($FB_2$) Concentrations of the High-Dose Diets Prepared from Uncooked Spiked Cornmeal, Baked Cornbread, Pan-Fried Corncakes, or Deep-Fried Fritters As Determined by HPLC

| | mean, ppm | | | % of amount in spiked cornmeal diet |
|---|---|---|---|---|
| diet | $FB_1$[a] | $FB_2$[b] | $FB_{1+2}$[c] | |
| spiked cornmeal | 87.0 (29.8) | 43.5 [37.5–49.5] | 130.5 | 100 |
| baked cornbread | 92.9 (19.1) | 39.3 [31.3–47.4] | 132.2 | 102 |
| pan-fried corncakes | 65.2 (19.4) | 27.0 [20.4–33.6] | 92.2 | 70.8 |
| deep-fried fritters | 87.0 (7.5) | 33.2 [31.5–34.8] | 120.2 | 92.3 |

[a] Values indicate mean of $n = 3$, with standard deviation given in parentheses. [b] Values indicate mean of $n = 2$, with range of results given in brackets. [c] Value is the sum of mean $FB_1$ plus mean $FB_2$.

## RESULTS

**Diet Analysis.** Baking and deep-frying had little to no effect on fumonisin concentrations (**Table 1**). The nominal $FB_1 + FB_2$ concentration (based on HPLC analysis of the CM) of the high-dose diets was 155 ppm. Therefore, "recovery" of fumonisins from the high-dose spiked cornmeal diet was ~83%, and "recoveries" from the cooked diets ranged from 59 to 85%. The fumonisin concentration of the pan-fried corncake diet was 25−30% lower than the other diets, but the difference was not significant (ANOVA).

**Bioassay.** The appearance and behavior of the animals were unremarkable. The low-dose diets had no significant effect on body weight gain. Weight gains of rats fed the high-dose spiked cornmeal or high-dose deep-fried fritter diets were significantly less than those of the negative control and low-dose groups after 1 week (**Figure 1**). Weight gains of the other high-dose groups were also decreased but differed significantly only from the negative control group. Weight gains of all high-dose groups were significantly decreased after 2 weeks; mean weight gains of these groups were reduced 8−20% compared to the negative controls.

No differences in food consumption were found during week 1. Food consumptions of the negative control and the low-dose groups did not differ during week 2; mean values ranged from 139 ± 8.5 (SD) g for the low-dose deep-fried fritter group to 148 ± 5.5 g for the low-dose pan-fried corncake group. Food consumptions of all high-dose groups were significantly reduced during week 2. Their mean values ranged from 129 ± 4.5 g (spiked cornmeal) to 134 ± 6.8 g (pan-fried corncake), compared to 144 ± 8.6 g for the negative controls.

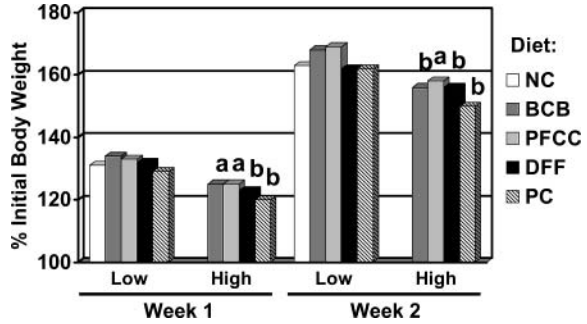


**Figure 1.** Group mean cumulative body weight gain of rats fed the negative control diet (NC), diets containing low doses (2% w/w spiked cornmeal equivalents), or diets containing high doses (20% w/w spiked cornmeal equivalents) of the baked cornbread (BCB), pan-fried corncakes (PFCC), or deep-fried fritters (DFF) prepared from cornmeal spiked with *F. verticillioides* CM. The groups fed diets containing 2 or 20% w/w of the uncooked spiked cornmeal served as the low-dose and high-dose positive controls (PC), respectively. Values are group means, $n = 5$; a = significantly different from the negative control group; b = significantly different from negative control and all low dose groups; significance was judged at $p < 0.05$.

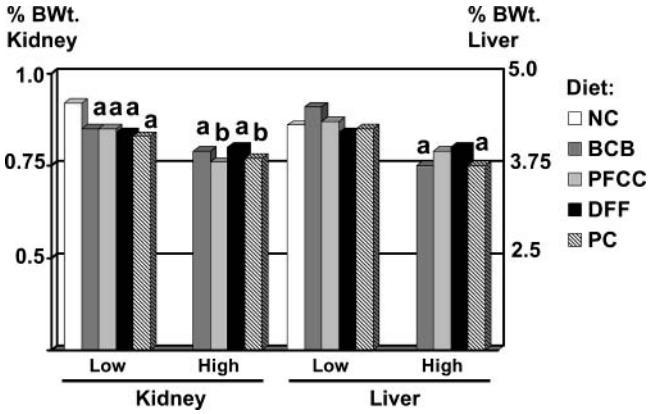


**Figure 2.** Group mean relative (percent body weight) kidney and liver weights of rats fed the negative control diet (NC) or diets containing low doses (2% w/w spiked cornmeal equivalents) or high doses (20% w/w spiked cornmeal equivalents) of the baked cornbread (BCB), pan-fried corncakes (PFCC), or deep-fried fritters (DFF) prepared from a batter containing *F. verticillioides* CM for 2 weeks. The groups fed the uncooked spiked cornmeal at concentrations of 2 or 20% (w/w) served as the low-dose and high-dose positive controls (PC), respectively. Values are group means, $n = 5$; a = significantly different from the negative control group; b = significantly different from the negative control and all low-dose groups; significance was judged at $p < 0.05$.

Relative kidney weights of all low-dose groups fed the baked cornbread, pan-fried corncake, deep-fried fritter, or spiked cornmeal diets were significantly decreased (**Figure 2**). Relative kidney weights of the high-dose groups were in turn less than those of their corresponding low-dose groups; however, the differences were statistically significant only for rats fed the pan-fried corncakes or the spiked cornmeal diets. Relative liver weights of the low-dose groups and the negative control group were similar. Relative liver weights of the high-dose groups tended to be slightly lower, but the difference was statistically significant for the baked cornbread and spiked cornmeal groups only. No other organ weight effects were found.

Noteworthy kidney and liver lesions were not found in rats from the negative control group. Baking, pan-frying, and deep-frying had no obvious effect on toxicity, as the type and severity of kidney and liver lesions in the groups fed the cooked products

**Table 2.** Summarized Kidney and Liver Histopathology Findings in Male Rats Fed the Negative Control Diet or the Spiked Cornmeal, Baked Cornbread, Pan-Fried Corncake, or Deep-Fried Fritter Diets

| | kidney | | liver | |
|---|---|---|---|---|
| diet and group | incidence[a] | score[b] | incidence | score |
| negative control | 0 d | 0 d | 0 d | 0 d |
| low dose[c] | | | | |
| spiked cornmeal | 5 e | 2.6 ef [2–3] | 2 de | 0.4 d [0–1] |
| baked cornbread | 5 e | 1.8 e [1–2] | 1 d | 0.2 d [0–1] |
| pan-fried corncakes | 5 e | 1.8 e [1–2] | 2 de | 0.4 d [0–1] |
| deep-fried fritters | 5 e | 1.8 e [1–2] | 0 d | 0 d |
| high dose[c] | | | | |
| spiked cornmeal | 5 e | 3.0 f [3] | 5 e | 1.8 e [1–2] |
| baked cornbread | 5 e | 2.4 ef [2–3] | 5 e | 1.6 e [1–2] |
| pan-fried corncakes | 5 e | 2.8 f [2–3] | 5 e | 1.4 e [1–2] |
| deep-fried fritters | 5 e | 3.0 f [3] | 5 e | 1.6 e [1–2] |

[a] Number of rats per group with lesions, $n = 5$. Values in columns not sharing letters are significantly different, $p < 0.05$. [b] Values indicate the mean score; the range of scores for animals with lesions is indicated in brackets. Values in columns not sharing letters are significantly different, $p < 0.05$. [c] Low dose = 2% w/w spiked cornmeal equivalent weights; high dose = 20% w/w spiked cornmeal equivalent weights.

were essentially indistinguishable from those found in the spiked cornmeal group (**Table 2**). Apoptosis of tubule epithelium cells in the outer medulla, tubular basophilia, and other features of fumonisin exposure (*44*, *45*) were found in the kidneys of animals fed the spiked cornmeal diets or diets prepared from the cooked materials. The mean kidney pathology scores of the low-dose groups were significantly different from that of the negative controls. Although the mean kidney pathology score of the low-dose spiked cornmeal group was slightly higher (2.6) than those of the other low-dose groups (1.8), the difference was not significant. Kidney lesions in the four high-dose groups were of the same type but more severe than those found at the low-dose level. No significant differences in the mean pathology score were found when the high-dose groups were compared to one another.

Liver effects in the low-dose groups were minimal with $\leq 2$ rats per group exhibiting a few widely scattered apoptotic hepatocytes within an otherwise unremarkable parenchyma. Liver lesions found in the high-dose groups were, like kidney findings, consistent with the effects of fumonisin exposure. Lesions were mild to moderate in intensity, and the most common features were apoptotic hepatocytes, cytomegaly, and anisocytosis. No significant differences in mean pathology score for liver were found among the four low-dose or among the four high-dose groups.

## DISCUSSION

In vivo bioassays played a critical role in the discovery of fumonisins (*1*), in the elucidation of their organ-specific pathological effects (*1*, *7*−*9*, *44*, *45*), and in confirming their role as the toxic agent causing *F. verticillioides*-associated animal diseases (*2*, *3*, *46*). Rodent feeding studies utilizing diets spiked with *F. verticillioides* or *F. proliferatum* CM have been a particularly useful research tool for comparing the biological activity of the mycotoxin-contaminated diets before and after water extraction (*41*), alkaline hydrolysis (nixtamalization) (*47*, *48*), or ammoniation (*49*) as well as for comparing the toxicity of purified $FB_1$ and hydrolyzed $FB_1$ ($HFB_1$) (*50*) or $FB_1$ and $FB_1$-reducing sugar reaction products (*51*, *52*). We therefore used a 2-week rat feeding study to determine if baking, pan-frying, or deep-frying, using simulated "household" methods,

modifies the toxicity of fumonisin-contaminated cornmeal. The use of well-defined, fumonisin-specific histopathological changes as the experimental endpoint has the advantage of being responsive to biologically active, bioavailable fumonisins in the cooked products. This includes fumonisins that are not detected using routine extraction, cleanup, and chromatographic protocols (*28*, *33*, *40*, *53*), such as unknown anologues or decomposition products, and fumonisins that are reversibly bound to the food matrix (and liberated in the gastrointestinal tract). The extent to which these "hidden" fumonisins occur in foods is unknown; however, there is evidence suggesting their existence. Cooking and steeping 800 g of a *F. verticillioides* CM containing 1.58 mmol of $FB_1$ ($FB_1$ concentration = 1420 ppm), but no $HFB_1$, in alkaline water according to the nixtamalization method of Hendrich et al. (*48*) yielded 608 g of a product that contained 2.29 mmol of $HFB_1$ ($HFB_1$ concentration = 1528 ppm) (*47*). Variable recoveries obtained after spiking corn, masa, and tortilla chips with $FB_1$ suggest that some matrices more efficiently bind fumonisins than others (*31*). Kim et al. (*40*) further found that recovery of $FB_1$ from spiked rice flours and cornmeal decreased rapidly, within hours, at room temperature, thereby providing further evidence that fumonisin food matrix interactions occur readily. They also noted that recovery was influenced by extraction conditions including solvent composition, its apparent pH, and temperature. This emphasizes the possibility that chemical analyses might also (in addition to missing "hidden" fumonisins) underestimate fumonisin concentrations unless care is taken to optimize the extraction method for each commodity or food matrix.

Baking, pan-frying, and deep-frying did not affect the toxicity of the uncooked, spiked cornmeal in this study. Body weight, organ weight, and microscopic pathology findings were dose-related and consistent with the known effects of fumonisins in rats (*44*, *45*). It is important that, at both dose levels, mean kidney and liver pathology scores of the rats fed the uncooked spiked cornmeal were equal to or slightly higher than those of the baked cornbread, pan-fried corncake, or deep-fried fritter groups. Thus, the bioassay indicated that cooking neither liberated hidden fumonisins in the cornmeal nor led to the formation of significant amounts of novel fumonisin-like toxins. The bioassay, on the other hand, did not provide evidence that baking or frying reduced toxicity. It should be considered, however, that the bioassay and chemical results reflect net changes in fumonisin bioavailability and concentration. Accordingly, the possibility that liberation of hidden fumonisins, formation of biologically active decomposition products, and binding of free fumonisins or fumonisin decomposition products to the food matrix occur simultaneously during baking and frying cannot be dismissed on the basis of our results.

In any case, the analytical results were consistent with the bioassay findings. Baking and deep-frying had essentially no effect on fumonisin concentrations as shown by HPLC analysis of the high-dose diets. This contrasts with reports that baking reduces fumonisin concentrations in muffins (*33*, *36*) and cornmeal (*39*). In the latter study *(39)*, recovery of fumonisins $FB_1$ and $FB_2$ from spiked (2500 ng of FB/g) cornmeal batter (1:1 with water) after baking at 190 °C for 1 h ranged from only 23% ($FB_1$) to 32% ($FB_2$). It is likely that differences in the fumonisin source (purified fumonisins vs CM) and concentration in the batters had a significant impact on the results of the two studies. To explore this possibility, we made cornbread from cornmeal containing 0.8 ppm of $FB_1$ (contaminated corn, rather than CM, served as the fumonisin source) using the same recipe as in this experiment (Voss et al., unpublished results). After baking, the $FB_1$ concentration was reduced 65%, a result that is in good agreement with the findings of Scott and Lawrence (*39*). Other considerations such as recipe and cooking conditions are also confounding factors that make comparison of the results of various experiments difficult.

Fumonisin concentration of the pan-fried corncake diet was modesty reduced, by 25−30%, in comparison to the other diets. The detailed analytical investigations needed to determine the fate of fumonisins in the pan-fried and other cooked materials were outside the aims of this study. It is of interest that others (*37*, *38*) have reported that frying reduced fumonisin concentrations, especially when longer cooking times and higher temperatures were used. This might have relevance to our results, as pan-frying cooking temperatures were higher (25 °C higher) and cooking times ~20% longer (12 vs 10 min) than for deep-frying. Despite the slight reduction in fumonisin concentration in the corncakes, it cannot be concluded that any hidden fumonisins or new toxins were formed during pan-frying because, given the dose−response for fumonisin-induced liver and kidney lesions in male rats *(6*, *42*, *44*, *45*), it is unlikely that a 25−30% reduction in dietary fumonisin concentration is sufficient to affect any toxicologically meaningful change in target organ pathology. Although the toxicological significance of such a reduction for low-level, chronic fumonisin exposure in animals or humans remains undetermined, it is likely to be minimal. In this regard, Shephard et al. (*35*) concluded that the 20−23% decrease in $FB_1$ concentrations achieved by making maize porridge was insufficient for reducing the fumonisin-related risk in southern African populations depending on contaminated, home-grown corn as a dietary staple.

In summary, baking, pan-frying, and deep-frying had no effect on the biological activity of fumonisin-contaminated cornmeal using a short-term rat feeding study as a bioassay. The results provided no evidence for the generation of hidden fumonisins or other novel toxins during cooking. Additional bioassays are needed to more thoroughly study how cooking conditions affect fumonisins and their biological activity.

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## LITERATURE CITED

(1) Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggaar, R.; Kriek, N. P. J. Fumonisins—novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *54*, 1806−1811.

(2) Kellerman, T. S.; Marasas, W. F. O.; Thiel, P. G.; Gelderblom, W. C. A.; Cawood, M.; Coetzer, J. A. W. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin $B_1$. *Onderstepoort J. Vet. Res.* **1990**, *57*, 269−275.

(3) Marasas, W. F. O.; Kellerman, T. S.; Gelderblom, W. C. A.; Coetzer, J. A. W.; Thiel, P. G.; van der Lugt, J. J. Leukoencephalomalacia in a horse induced by fumonisin $B_1$ isolated from *Fusarium moniliforme*. *Onderstepoort J. Vet. Res.* **1988**, *55*, 197−203.

(4) Constable, P. D.; Smith, G. W.; Rottinghaus, G. E.; Haschek, W. M. Ingestion of fumonisin $B_1$-containing culture material decreases cardiac contractility and mechanical efficiency in swine. *Toxicol. Appl. Pharmacol.* **2000**, *162*, 151−160.

(5) Smith, G. W.; Constable, P. D.; Tumbleson, M. E.; Rottinghaus, G. E.; Haschek, W. M. Sequence of cardiovascular changes leading to pulmonary edema in swine fed culture material containing fumonisin. *Am. J. Vet. Res.* **1999**, *60*, 1292−1300.

(6) Voss, K. A.; Riley, R. T.; Norred, W. P.; Bacon, C. W.; Meredith, F. I.; Howard, P. C.; Plattner, R. D.; Collins, T. F. X.; Hansen, D. K.; Porter, J. K. An overview of rodent toxicities: liver and kidney effects of fumonisins and *Fusarium moniliforme*. *Environ. Health Perspect.* **2001**, *109* (Suppl. 2), 259−266.

(7) Howard, P. C.; Eppley, R. M.; Stack, M. E.; Warbritton, A.; Voss, K. A.; Lorentzen, R. J.; Kovatch, R. M.; Bucci, T. J. Fumonisin $B_1$ carcinogenicity in a two-year feeding study using F344 rats and $B6C3F_1$ mice. *Environ. Health Perspect.* **2001**, *109 (Suppl.2)*, 277−282.

(8) Howard, P. C.; Warbritton, A.; Voss, K. A.; Lorentzen, R. J.; Thurman, J. D.; Kovatch, R. M.; Bucci, T. J. Compensatory regeneration as a mechanism for renal tubule carcinogenesis of fumonisin $B_1$ in the F344/N/Nctr BR rat. *Environ. Health Perspect.* **2001**, *109* (Suppl. 2), 309−314.

(9) Gelderblom, W. C. A.; Kriek, N. P. J.; Marasas, W. F. O.; Thiel, P. G. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin $B_1$ in rats. *Carcinogenesis* **1991**, *12*, 1247−1251.

(10) Marasas, W. F. O. Fumonisins: history, worldwide occurrence and impact. *Adv. Exp. Med. Biol.* **1996**, *392*, 1−17.

(11) Rheeder, J. P.; Marasas, W. F. O.; Thiel, P. G.; Sydenham, E. W.; Shephard, G. S.; van Schalkwyk, D. J. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* **1992**, *82*, 353−357.

(12) Marasas, W. F. O. Discovery and occurrence of the fumonisins: a historical perspective. *Environ. Health Perspect.* **2001**, *109* (Suppl. 2), 239−243.

(13) Chu, F. S.; Li, G. Y. Simultaneous occurrence of fumonisin $B_1$ and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. *Appl. Environ. Microbiol.* **1994**, *60*, 847−852.

(14) Yang, C. S. Research on esophageal cancer in China: a review. *Cancer Res.* **1980**, *40*, 2633−2644.

(15) Yoshizawa, T.; Yamashita, A.; Luo, Y. Fumonisin occurrence in corn from high- and low-risk areas for human esophageal cancer in China. *Appl. Environ. Microbiol.* **1994**, *60*, 1626−1629.

(16) Ueno, Y.; Iijima, K.; Wang, S.-D.; Sugiura, Y.; Sekijima, M.; Tanaka, T.; Chen, C.; Yu, S.-Z. Fumonisins as a possible contributory risk factor for primary liver cancer: A 3-year study of corn harvested in Haimen, China, by HPLC and ELISA. *Food Chem. Toxicol.* **1997**, *35*, 1143−1150.

(17) Hendricks, K. Fumonisins and neural tube defects in South Texas. *Epidemiology* **1999**, *10*, 198−200.

(18) Fincham, J. E.; Marasas, W. F. O.; Taljaard, J. J. F.; Kriek, N. P. J.; Badenhorst, C. J.; Gelderblom, W. C. A.; Seier, J. V.; Smuts, C. M.; Faber, M.; Weight, M. J.; Slazus, W.; Woodroof, C. W.; van Wyk, M. J.; Kruger, M.; Thiel, P. G. Atherogenic effects in a non-human primate of *Fusarium moniliforme* cultures added to a carbohydrate diet. *Atherosclerosis* **1992**, *94*, 13−25.

(19) Casteel, S. W.; Turk, J. R.; Rottinghaus, G. E. Chronic effects of dietary fumonisin on the heart and pulmonary vasculature of swine. *Fundam. Appl. Toxicol.* **1994**, *23*, 518−524.

(20) Dutton, M. F. Fumonisins, mycotoxins of increasing importance: their nature and their effects. *Pharmacol. Ther.* **1996**, *70*, 137−161.

(21) Bullerman, L. B. Occurrence of *Fusarium* and fumonisins in food grains and foods. *Adv. Exp. Med. Biol.* **1996**, *392*, 27−38.

(22) International Programme on Chemical Safety. *Fumonisin $B_1$*, *Environmental Health Criteria 219*; World Health Organization: Geneva, Switzerland, 2000.

(23) Shephard, G. S.; Thiel, P. G.; Stockenström, S.; Sydenham, E. W. Worldwide survey of fumonisin contamination of corn and corn-based products. *J. AOAC Int.* **1996**, *79*, 671−687.

(24) Scott, P. M.; Lawrence, G. A. Analysis of beer for fumonisins. *J. Food Prot.* **1995**, *58*, 1379−1382.

(25) Scott, P. M.; Lawrence, G. A. Determination of hydrolyzed fumonisin $B_1$ in alkali-processed corn foods. *Food Addit. Contam.* **1996**, *13*, 823−832.

(26) Pohland, A. E. Occurrence of fumonisins in the U.S. food supply. *Adv. Exp. Med. Biol.* **1996**, *392*, 19−26.

(27) Hopmans, E. C.; Murphy, P. A. Detection of fumonisins $B_1$, $B_2$ and $B_3$ and hydrolyzed fumonisin $B_1$ in corn-containing foods. *J. Agric. Food Chem.* **1993**, *41*, 1655−1658.

(28) Murphy, P. A.; Hendrich, S.; Hopmans, E. C.; Hauck, C. C.; Lu, Z.; Buseman, G.; Munkvold, G. Effect of processing on fumonisin content of corn. *Adv. Exp. Med. Biol.* **1996**, *392*, 323−334.

(29) Pittet, A.; Parisod, V.; Schellenberg, M. Occurrence of fumonisins $B_1$ and $B_2$ in corn-based products from the Swiss market. *J. Agric. Food Chem.* **1992**, *40*, 1352−1354.

(30) Dombrink-Kurtzman, M. A.; Dvorak, T. J.; Barron, M. E.; Rooney, L. W. Effect of nixtamalization (alkaline cooking) on fumonsin-contaminated corn for production of masa and tortillas. *J. Agric. Food Chem.* **2000**, *48*, 5781−5786.

(31) Voss, K. A.; Poling, S. M.; Meredith, F. I.; Bacon, C. W.; Saunders, D. S. Fate of fumonisins during the production of fried tortilla chips. *J. Agric. Food Chem.* **2001**, *49*, 3120−3126.

(32) Castelo, M. M.; Katta, S. K.; Sumner, S. S.; Hanna, M. A.; Bullerman, L. B. Extrusion cooking reduces recoverability of fumonisin $B_1$ from extruded corn grits. *J. Food Sci.* **1998**, *63*, 696−698.

(33) Castelo, M. M.; Jackson, L. S.; Hanna, M. A.; Reynolds, B. H.; Bullerman, L. B. Loss of fumonisin $B_1$ in extruded and baked corn-based foods with sugars. *J. Food Sci.* **2001**, *66*, 416−421.

(34) Katta, S. K.; Jackson, L. S.; Sumner, S. S.; Hanna, M. A.; Bullerman, L. B. Effect of temperature and screw speed on stability of fumonisin $B_1$ in extrusion-cooked corn grits. *Cereal Chem.* **1998**, *76*, 16−20.

(35) Shephard, G. S.; Leggott, N. L.; Stockenström, S.; Somdyala, N. I. M.; Marasas, W. F. O. Preparation of South African maize porridge; effect on fumonisin mycotoxin levels. *S. Afr. J. Sci.* **2002**, *98*, 393−394.

(36) Jackson, L. S.; Hlywka, J. J.; Senthil, K. R.; Bullerman, L. B. Effect of thermal processing on the stability of fumonisins. *Adv. Exp. Med. Biol.* **1996**, *392*, 345−354.

(37) Jackson, L. S.; Katta, S. K.; Fingerhut, D. D.; DeVries, J. W.; Bullerman, L. B. Effects of baking and frying on the fumonisin $B_1$ content of corn-based foods. *J. Agric. Food Chem.* **1997**, *45*, 4800−4805.

(38) Pineiro, M.; Miller, D.; Silva, G.; Musser, S. Effect of commercial processing on fumonisin concentration of maize-based foods. *Mycotoxin Res.* **1999**, *15*, 2−12.

(39) Scott, P. M.; Lawrence, G. A. Stability and problems in recovery of fumonisins added to corn-based foods. *J. AOAC Int.* **1994**, *77*, 541−545.

(40) Kim, E.-K.; Scott, P. M.; Lau, B. P.-Y.; Lewis, D. A. Extraction of fumonisins $B_1$ and $B_2$ from white rice flour and their stability in white rice flour, cornstarch, cornmeal, and glucose. *J. Agric. Food Chem.* **2002**, *50*, 3614−3620.

(41) Voss, K. A.; Plattner, R. D.; Bacon, C. W.; Norred, W. P. Comparative studies of hepatotoxicity and fumonisin $B_1$ and $B_2$ content of water and chloroform/methanol extracts of *Fusarium moniliforme* strain MRC 826 culture material. *Mycopathologia* **1990**, *112*, 81−92.

(42) Voss, K. A.; Plattner, R. D.; Riley, R. T.; Meredith, F. I.; Norred, W. P. *In vivo* effects of fumonisin $B_1$-producing and fumonisin $B_1$-nonproducing *Fusarium moniliforme* isolates are similar: Fumonisins $B_2$ and $B_3$ cause hepato- and nephrotoxicity in rats. *Mycopathologia* **1998**, *141*, 45−58.

(43) Gad, S. C.; Weil, C. S. Statistics for toxicologists. In *Principles and Methods of Toxicology*; Hayes, A. W., Ed.; Raven Press: New York, 1982; pp 435−483.

(44) Voss, K. A.; Chamberlain, W. J.; Bacon, C. W.; Norred, W. P. A preliminary investigation on renal and hepatic toxicity in rats fed purified fumonisin $B_1$. *Nat. Toxins* **1993**, *1*, 222−228.

(45) Voss, K. A.; Chamberlain, W. J.; Bacon, C. W.; Herbert, R. A.; Walters, D. B.; Norred, W. P. Subchronic feeding study of the mycotoxin fumonisin $B_1$ in $B6C3F_1$ mice and Fischer 344 rats. *Fundam. Appl. Toxicol.* **1995**, *24*, 102−110.

(46) Harrison, L. R.; Colvin, B. M.; Greene, J. T.; Newman, L. E.; Cole, J. R., Jr. Pulmonary edema and hydrothorax in swine produced by fumonisin $B_1$, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* **1990**, *2*, 217−221.

(47) Voss, K. A.; Bacon, C. W.; Meredith, F. I.; Norred, W. P. Comparative subchronic toxicity studies of nixtamalized and water-extracted *Fusarium moniliforme* culture material. *Food Chem. Toxicol.* **1996**, *34*, 623−632.

(48) Hendrich, S.; Miller, K. A.; Wilson, T. M.; Murphy, P. A. Toxicity of *Fusarium proliferatum*-fermented nixtamalized corn-based diets fed to rats: Effect of nutritional status. *J. Agric. Food Chem.* **1993**, *41*, 1649−1654.

(49) Norred, W. P.; Voss, K. A.; Bacon, C. W.; Riley, R. T. Effectiveness of ammonia treatment in detoxification of fumonisin-contaminated corn. *Food Chem. Toxicol.* **1991**, *29*, 815−819.

(50) Howard, P. C.; Couch, L. H.; Patton, R. E.; Eppley, R. M.; Doerge, D. R.; Churchwell, M. I.; Matilde Marques, M.; Okerberg, C. V. Comparison of the toxicity of several fumonisin derivatives in a 28-day feeding study with female $B6C3F_1$ mice. *Toxicol. Appl. Pharmacol.* **2002**, *185*, 153−165.

(51) Liu H.; Lu, Y.; Haynes, J. S.; Cunnick, J. E.; Murphy, P.; Hendrich, S. Reaction of fumonisin with glucose prevents promotion of hepatocarcinogenesis in female F344/N rats while maintaining normal hepatic sphinganine/sphingosine ratios. *J. Agric. Food Chem.* **2001**, *49*, 4113−4121.

(52) Lu, Z.; Dantzer, W. R.; Hopmans, E. C.; Prisk, V.; Cunnick, J. E.; Murphy, P. A.; Hendrich, S. Reaction with fructose detoxifies fumonisin $B_1$ while stimulating liver-associated natural killer cell activity in rats. *J. Agric. Food Chem.* **1997**, *45*, 803−809.

(53) Bullerman, L. B.; Ryu, D.; Jackson, L. S. Stability of fumonisins in food processing. *Adv. Exp. Med. Biol.* **2000**, *504*, 195−204.

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