Potential of Alkaline Hydrolysis for the Removal of Fumonisins from Contaminated Corn

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Studies have shown that fumonisin B_1 (FB_1) may undergo alkaline hydrolysis to yield its aminopentol (AP_1) and tricarballylic acid moieties. Treatment of fumonisin-contaminated ground corn with 0.1 M calcium hydroxide, over a period of 24 h at room temperature, resulted in the transfer of the majority of the FB_1 (mean = 74.1%) to the easily separable aqueous fraction, where it was present predominantly as the AP_1 moiety. Following similar treatment of intact corn kernels, only 5.1% of the original FB_1 concentration was retained in those kernels devoid of their outer pericarp.

Keywords: *Fumonisin B_1; alkaline hydrolysis; decontamination; corn*

INTRODUCTION

The fumonisin mycotoxins consist of a group of seven structurally related analogues (Bezuidenhout et al., 1988; Gelderblom et al., 1988; Cawood et al., 1991; Branham and Plattner, 1993). Originally isolated from culture material of *Fusarium moniliforme* Sheldon (Gelderblom et al., 1988), a common contaminant of corn, the fumonisins have subsequently been shown to be produced by several other morphologically related *Fusarium* species (Thiel et al., 1991; Nelson et al., 1992). Of the seven known fumonisin analogues, only three, fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3), have been reported to occur naturally at significant concentrations in corn and corn-based products (Sydenham et al., 1991; Thiel et al., 1992; Ross et al., 1992; Pittet et al., 1992; Murphy et al., 1993; Ueno et al., 1993).

The major naturally occurring fumonisin analogue, FB_1, is known to be both hepatotoxic and carcinogenic to rats (Gelderblom et al., 1988, 1992) and to induce leukoencephalomalacia in horses (Marasas et al., 1988; Kellerman et al., 1990) and pulmonary edema in swine (Harrison et al., 1990). Combined fumonisin levels in animal feeds of ≥ 10 and ≥ 100 μg/g have been suggested as potentially harmful to horses and swine, respectively (Marasas et al., 1993). Toxic effects of the fumonisins have also been studied in broiler chicks (Ledoux et al., 1992), turkey poults (Weibking et al., 1993), and feeder calves (Osweiler et al., 1993). In addition, FB_1 is both cytotoxic (Shier et al., 1991; Gelderblom et al., 1993) and phytotoxic (Abbas et al., 1993; Lamprecht et al., 1994), while FB_1 and FB_2 have been shown to be inhibitors of sphingosine (sphinganine) *N*-acyltransferase in rat primary hepatocytes (Wang et al., 1991). Both FB_2 and FB_3 have subsequently been shown to exhibit cancer-initiating activity and hepatotoxic effects in rats, similar to those previously observed for FB_1 (Gelderblom et al., 1993).

In those areas of the Transkei region of southern Africa known to exhibit a high incidence of human esophageal cancer, people consume moldy home-grown corn contaminated with mean combined fumonisin levels of up to 67 μg/g (Sydenham et al., 1990; Rheeder et al., 1992). Commercially available corn-based products have been found to be contaminated with fumonisins at levels of up to 4.7 μg/g (Sydenham et al., 1993), while Murphy et al. (1993) observed mean combined fumonisin levels of between 3.4 and 4.6 μg/g in commercial corn harvested in three states of the United States between 1988 and 1991.

Existing hazard assessment data suggest that fumonisin contamination of corn is cause for concern, especially in view of the fact that corn is a major dietary staple for both animals and humans worldwide (Thiel et al., 1992). This concern was recently emphasized with the designation, by the International Agency for Research on Cancer, of "toxins derived from *F. moniliforme*" as group 2B carcinogens (i.e. possibly carcinogenic to humans) (Vainio et al., 1993).

Recent studies have considered the development of decontamination procedures for fumonisin-contaminated corn. Alberts et al. (1990) observed that heat treatment of fumonisin-contaminated culture material of *F. moniliforme* did not result in a reduction in either the fumonisin levels or the cancer-initiating potential of the heat-treated material. The heat stability of the fumonisins was subsequently confirmed by Doko and Visconti (1993) and Dupuy et al. (1993). Viljoen et al. (1993) reported the progressive reduction in mean fumonisin levels during the refinement of commercial corn in the milling process, while Sydenham et al. (1994) reported that the removal of "fines" (screenings), from bulk corn consignments, could reduce combined fumonisin levels by between 29 and 69%. Bothast et al. (1992) detected negligible degradation of FB_1 during the fermentation to ethanol of fumonisin-contaminated corn. Most of the toxin was recovered in the distillers' grains, silage, and soluble fractions. No FB_1 was, however, detected in either the distilled alcohol or centrifuge solids (Bothast et al., 1992). Norred et al. (1991) studied the effect of ammonia, at relatively low temperatures and pressures, on FB_1 levels in culture material of *F. moniliforme*, recording only partial reductions in FB_1 concentrations, while the toxicity of the treated material was retained (Norred et al., 1991). In contrast, treat-

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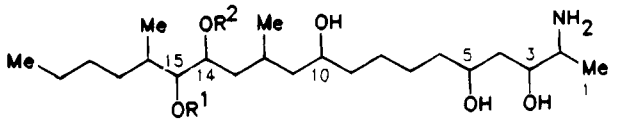

[1] R^1 AND R^2 = CO-CH_2-CH(CO_2H)-CH_2-CO_2H
[2] R^1 AND R^2 = H

Figure 1. Chemical structures of fumonisin B_1 (**1**) and its hydrolyzed aminopentol moiety (AP_1) (**2**).

ment with ammonia at higher temperatures and pressures resulted in an 80% reduction in FB_1 content (Park et al., 1992).

Sydenham et al. (1991) reported that alkali-treated corn-based commodities (tortilla preparations) contained very low levels of fumonisins and suggested that treatment of corn with calcium hydroxide [$Ca(OH)_2$] might cause a reduction in fumonisin levels. Preliminary studies, involving reactions between $Ca(OH)_2$ and aqueous solutions of FB_1, resulted in the alkaline hydrolysis of FB_1 (Figure 1) and the formation of its hyrolyzed aminopentol (AP_1) (Figure 1) and tricarballylic acid (TCA) moieties (Sydenham, 1994). As expected, the rate of hydrolysis was influenced by the concentration of the hydroxonium ion and the incubation temperature (Sydenham, 1994). In a series of comparative experiments, the Ca^{2+} ion (in contrast to either the sodium or potassium cation) enhanced the rate of hydrolysis of FB_1 (Sydenham, 1994). Almost complete hydrolysis of FB_1 was recorded following treatment with a saturated solution (0.1 M) of $Ca(OH)_2$ (incubated at 23 °C for 24 h) (Sydenham, 1994). These reaction conditions were therefore applied to fumonisin-contaminated corn samples, and this paper reports the results of these studies.

EXPERIMENTAL PROCEDURES

Analytical Standards. A pure standard of FB_1 was prepared from culture material of *F. moniliforme*, as previously described (Cawood et al., 1991). The aminopentol (AP_1) moiety was prepared by the alkaline hydrolysis of FB_1, in accordance with the method of Plattner et al. (1990).

Corn Samples. Two corn samples naturally contaminated with the fumonisins were collected from households in the Transkei. FB_1 levels were determined according to the method of Sydenham et al. (1992). One of the samples (ca. 750 g) was ground in a laboratory mill, mixed to improve homogeneity, analyzed, and found to contain a mean concentration of 8590 ng/g FB_1 (RSD = 4.1% based on four determinations). A second sample, found to contain 8200 ng/g FB_1, was retained as intact kernels.

Treatment of FB_1-Contaminated Ground Corn. Three 100 g subsamples of the ground corn were treated (with continual stirring) for 24 h with 400 mL volumes of 0.1 M $Ca(OH)_2$. Following this treatment, the aqueous and solid phases were separated by filtration, and each fraction was retained. The solid residue was subsequently washed with distilled water, prior to being dried at 100 °C for 4 h. Each fraction was analyzed for FB_1 and its AP_1 hydrolysis product.

Treatment of FB_1-Contaminated Corn Kernels. A 100 g subsample of the corn kernels was treated with 0.1 M $Ca(OH)_2$ (using the conditions previously cited). Following treatment and filtration, the kernels were manually separated into those fractions exhibiting (a) partial and (b) total removal of their outer pericarp. The separated fractions were dried, ground in a laboratory mill, and analyzed for FB_1 and AP_1.

Determination of FB_1 and AP_1 in Aqueous Phases. Aliquots (10 mL) of the aqueous phase following $Ca(OH)_2$ treatment of corn fractions were adjusted to pH 2.5 by the addition of 0.1 M HCl. The solutions were then applied to BondElut C_{18} solid phase extraction (SPE) cartridges (containing 500 mg of sorbent; Varian, Harbor City, CA) which had been preconditioned with 5 mL of methanol and 5 mL of water. The cartridges were washed with water (3 mL) followed by methanol/water (1:3; 5 mL), and the FB_1 and AP_1 were eluted with methanol (15 mL). The eluates were evaporated to dryness, redissolved in methanol (200 μL), and retained for analyses.

Table 1. $Ca(OH)_2$ Treatment of FB_1-Contaminated Corn (8590 ng/g)

sample no.	FB_1[a] (ng/g)	% of original	AP_1[a,b] (ng/g)	FB_1 equiv[a] (ng/g)	% of original	total % of original
			Aqueous $Ca(OH)_2$ Fraction			
1	1050	12.2	3280	5820	67.7	79.9
2	ND[c]	ND	3420	6080	70.8	70.8
3	ND	ND	3460	6150	71.6	71.6
mean	350	4.1	3360	6015	70.0	74.1
			Solid Corn Residue			
1	ND	ND	510	910	10.6	10.6
2	755	8.8	790	1400	16.3	25.1
3	ND	ND	830	1480	17.2	17.2
mean	250	2.9	710	1265	14.7	17.6

[a] Results rounded to the nearest 10 ng/g. [b] FB_1 concentration calculated from the corresponding AP_1 level. [c] ND, not detected (<100 ng/g).

Determination of FB_1 and AP_1 in Solid Residues. Following treatment with $Ca(OH)_2$, subsamples (25 g) of the dried corn were extracted with a mixture of methanol and 0.01 M ethylenediaminetetraacetic acid (EDTA) (1:1; 50 mL), by means of blending for 3 min in a homogenizer. The suspension was filtered and the pH of a 10 mL aliquot of the filtrate adjusted to pH 2.5 with 0.1 M HCl. Precipitates were removed by centrifugation, prior to purification of the supernatants on preconditioned BondElut C_{18} SPE cartridges. The cartridges were washed and the toxins eluted as described for the analyses of FB_1 and AP_1 in the aqueous phases.

Chromatographic Analyses of FB_1 and AP_1. Aliquots (50 μL) of the reconstituted residues were derivatized with *o*-phthaldialdehyde (OPA) as previously described (Sydenham et al., 1992) and separated on a Phenomenex column (250 × 4.6 mm i.d.) packed with 5 μm of Ultracarb ODS 30 material. The mobile phase of methanol/0.1 M sodium dihydrogen phosphate (80:20), adjusted to pH 3.35 with orthophosphoric acid, was pumped at a flow rate of 1 mL/min. The eluate was monitored by fluorescence detection at an excitation wavelength of 335 nm and an emission wavelength of 440 nm.

RESULTS AND DISCUSSION

$Ca(OH)_2$ Treatment of Ground Corn. Following treatment of ground corn with $Ca(OH)_2$ (in triplicate), the separated fractions were analyzed for FB_1 and AP_1, and the results are presented in Table 1.

Recoveries of FB_1 and AP_1 from the aqueous fractions were 98.4% (RSD = 6.0%) and 96.3% (RSD = 7.2%), respectively, based on triplicate analyses at spiking levels of 470 ng/mL FB_1 and 450 ng/mL AP_1. In the solid fractions, recoveries of 93.6% (RSD = 4.7%) and 94.7% (RSD = 2.1%) were obtained for FB_1 and AP_1, respectively, based on five determinations at spiking levels of 470 ng/g FB_1 and 490 ng/g AP_1.

Only one of the three aqueous fractions contained FB_1, corresponding to 12.2% of the original concentration. Most of the FB_1 initially present in corn was recovered in the aqueous fraction as the fully hydrolyzed AP_1 moiety (i.e. between 67.7 and 71.6%, mean 70.0%; Table 1). Between 70.8 and 79.9% (mean 74.1%; Table 1) of the FB_1 in the corn had been transferred to the easily separable aqueous $Ca(OH)_2$ fraction.

Between 10.6 and 25.1% (mean 17.6%) of the FB_1 in the ground corn was retained as AP_1 in the solid corn

Table 2. Fumonisin B_1 Levels in Corn Kernels before and after Treatment with $Ca(OH)_2$

fraction	FB_1 (ng/g)	AP_1 and $(FB_1)^a$ (ng/g)	total (ng/g)
untreated	8200	ND[b] (ND)	8200
pericarp partially removed	1950	340 (600)	2550
pericarp fully removed	100	180 (320)	420

[a] Corresponding FB_1 levels (in parentheses) calculated from AP_1 concentrations. [b] ND, not detected (<100 ng/g).

residue. Following treatment with $Ca(OH)_2$, between 88.8 and 95.9% (mean 91.7%) of the initial FB_1 present in the nontreated ground corn (Table 1) could be accounted for in the three trial treatments.

$Ca(OH)_2$ Treatment of Corn Kernels. Treatment of corn kernels with $Ca(OH)_2$ is used during the preparation of "masa" (tortilla flour) to soften the shells of kernels prior to further processing (Hendrich et al., 1993). Following a similar treatment of intact corn, the kernels were separated into fractions showing (a) partial and (b) total removal of their outer cellulose pericarp. These two fractions were subsequently analyzed for FB_1 and AP_1, and the results are presented in Table 2.

Those kernels exhibiting partial removal of their pericarp retained 2550 ng/g (31.1%) of the original FB_1 concentration, predominantly as the intact toxin (23.8%) and a smaller amount (7.3%) as AP_1. Those kernels in which the pericarps were fully removed retained only 420 ng/g (5.1%) of the original FB_1, with the majority being present as AP_1 (3.9%) (Table 2).

These data provide additional evidence that the fumonisins are associated with the outer layer(s) of naturally contaminated corn kernels and indicate that removal of pericarps may be associated with a reduction of fumonisin levels in contaminated corn. The data also substantiate the finding of Sydenham et al. (1994) that significantly higher fumonisin levels occur in corn screenings than in the intact kernels, since the screenings might be expected to contain a significant proportion of their mass from the outer layers of kernels.

Mycotoxin decontamination procedures must, however, meet selected criteria if they are to be considered effective and viable. Of particular importance is the fact that a candidate decontamination procedure, while reducing the contamination level of one (or more) mycotoxin, should not result in the formation of products that could potentially pose a health risk (Charmley and Prelusky, 1994). In the present study, treatment of fumonisin-contaminated corn with $Ca(OH)_2$ resulted in the transfer of the majority of the FB_1 from the solid to the liquid phase, where it was present as the AP_1 moiety. This fraction probably also contained the TCA moiety derived from the hydrolysis of FB_1. Comparative cytotoxity studies in primary rat hepatocytes have indicated that AP_1 exhibits a significantly higher cytotoxic effect than the parent FB_1, while TCA exhibited no effect (Gelderblom et al., 1993). In contrast to FB_1, neither AP_1 nor TCA was found to be hepatotoxic or to act as a cancer initiator in a rat model (Gelderblom et al., 1993). It was suggested that the apparent inability of AP_1 to act as a cancer initiator might be due to a lack of absorption from the gut, and it was proposed that the presence of the TCA moiety may play an active role in the absorption of the intact FB_1 moiety (Gelderblom et al., 1993). Given the fact that fumonisin A_1 (FA_1; the *N*-acetyl derivative of FB_1) also failed to exhibit cancer-initiating properties, the authors concluded that the presence of the intact fumonisin molecule and a free amino group determined the cancer-initiating activity of the fumonisins. Hence, decontamination procedures that aim to reduce fumonisin levels in contaminated corn will have to address these issues.

To date, no data on the cancer-promoting potential of pure AP_1 have been published. Recent studies of Hendrich et al. (1993) reported that treatment of corn culture material of *F. proliferatum* with $Ca(OH)_2$ reduced FB_1 levels from 50 μg/g to yield between 7 and 10 μg/g AP_1. Subsequent feeding trials with this treated material (using diethylnitrosamine-initiated rats), led Hendrich et al. (1993) to suggest that treatment of the culture material with $Ca(OH)_2$ improved its nutritional value, resulting in an increased feed intake and body weight gain by the rats. They considered, however, that the "carcinogenic potential" of the $Ca(OH)_2$-treated material was similar to that observed for the nontreated material and concluded that treatment of corn with $Ca(OH)_2$ was not a potentially useful fumonisin detoxification strategy (Hendrich et al., 1993).

On the basis of the results of the present study, it will be necessary to compare the relative cancer-promoting potential of $Ca(OH)_2$-treated corn with that of the separated aqueous $Ca(OH)_2$ fraction, since the latter would contain significantly higher amounts of AP_1.

LITERATURE CITED

Abbas, H. K.; Gelderblom, W. C. A.; Cawood, M. E.; Shier, W. T. Biological activities of fumonisins, mycotoxins from *Fusarium moniliforme*, in jimsonweed (*Datura stramonium* L.) and mammalian cell cultures. *Toxicon* **1993**, *31*, 345–353.

Alberts, J. F.; Gelderblom, W. C. A.; Thiel, P. G.; Marasas, W. F. O.; Van Schalkwyk, D. J.; Behrend, Y. Effects of temperature and incubation period on production of fumonisin B_1 by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1990**, *56*, 1729–1733.

Bezuidenhout, G. C.; Gelderblom, W. C. A.; Gorst-Allman, C. P.; Horak, R. M.; Marasas, W. F. O.; Spiteller, G.; Vleggaar, R. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *J. Chem. Soc., Chem. Commun.* **1988**, 743–745.

Bothast, R. J.; Bennett, G. A.; Vancauwenberge, J. E.; Richard, J. L. Fate of fumonisin B_1 in naturally contaminated corn during ethanol production. *Appl. Environ. Microbiol.* **1992**, *58*, 233–236.

Branham, B. E.; Plattner, R. D. Isolation and characterization of a new fumonisin from liquid cultures of *Fusarium moniliforme*. *J. Nat. Prod.* **1993**, *56*, 1630–1633.

Cawood, M. E.; Gelderblom, W. C. A.; Vleggaar, R.; Behrend, Y.; Thiel, P. G.; Marasas, W. F. O. Isolation of the fumonisin mycotoxins: a quantitative approach. *J. Agric. Food Chem.* **1991**, *39*, 1958–1962.

Charmley, L. L.; Prelusky, D. B. Decontamination of *Fusarium* mycotoxins. In *Mycotoxins in Grain—Compounds other than Aflatoxin*; Miller, J. D., Trenholm, H. L., Eds.; Eagan Press: St. Paul, MN, 1994; pp 421–435.

Doko, M. B.; Visconti, A. Fumonisin contamination of corn and corn-based foods in Italy. In *Proceedings of the UK Workshop on the Occurrence and Significance of Mycotoxins*; Scudamore, K. A., Ed.; MAFF: London, 1993; pp 49–55.

Dupuy, J.; Le Bars, P.; Boudra, H.; Le Bars, J. Thermostability of fumonisin B_1, a mycotoxin from *Fusarium moniliforme*, in corn. *Appl. Environ. Microbiol.* **1993**, *59*, 2864–2867.

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.

Gelderblom, W. C. A.; Marasas, W. F. O.; Vleggaar, R.; Thiel, P. G.; Cawood, M. E. Fumonisins: isolation, chemical

characterization and biological effects. *Mycopathologia* **1992**, *117*, 11–16.

Gelderblom, W. C. A.; Cawood, M. E.; Snyman, S. D.; Vleggaar, R.; Marasas, W. F. O. Structure activity relationships of fumonisins in short-term carcinogenesis and cytotoxicity assays. *Food Chem. Toxicol.* **1993**, *31*, 407–414.

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.

Hendrich, S.; Miller, K. A.; Wilson, T. M.; Murphy, P. A. Toxicity of *Fusarium proliferatum*-fermented nixtamilized corn-based diets fed to rats: effect of nutritional status. *J. Agric. Food Chem.* **1993**, *41*, 357–360.

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

Lamprecht, S. C.; Marasas, W. F. O.; Alberts, J. F.; Cawood, M. E.; Gelderblom, W. C. A.; Shephard, G. S.; Thiel, P. G.; Calitz, F. J. Phytotoxicity of fumonisins and TA-toxin to corn and tomato. *Phytopathology* **1994**, *84*, 383–391.

Ledoux, D. R.; Brown, T. P.; Weibking, T. S.; Rottinghaus, G. E. Fumonisin toxicity in broiler chicks. *J. Vet. Diagn. Invest.* **1992**, *4*, 330–333.

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.

Marasas, W. F. O.; Shephard, G. S.; Sydenham, E. W.; Thiel, P. G. World-wide contamination of maize with fumonisins: foodborne carcinogens produced by *Fusarium moniliforme*. In *Cereal Science and Technology—Impact on a Changing South Africa*; Taylor, J. R. N., Randall, P. G., Viljoen, J. H., Eds.; CSIR: Pretoria, 1993; pp 791–805.

Murphy, P. A.; Rice, L. G.; Ross, P. F. Fumonisins B_1, B_2, and B_3 content of Iowa, Wisconsin, and Illinois corn and corn screenings. *J. Agric. Food Chem.* **1993**, *41*, 263–266.

Nelson, P. E.; Plattner, R. D.; Shackelford, D. D.; Desjardins, A. E. Fumonisin B_1 production by *Fusarium* species other than *F. moniliforme* in section Liseola and by some related species. *Appl. Environ. Microbiol.* **1992**, *58*, 984–989.

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.

Osweiler, G. D.; Kehrli, M. E.; Stabel, J. R.; Thurston, J. R.; Ross, P. F.; Wilson, T. M. Effects of fumonisin-contaminated corn screenings on growth and health of feeder calves. *J. Anim. Sci.* **1993**, *71*, 459–466.

Park, D. L.; Rua, S. M., Jr.; Mirocha, C. J.; Abd-Alla, E-S. A. M.; Weng, C. Y. Mutagenic potentials of fumonisin contaminated corn following ammonia decontamination procedure. *Mycopathologia* **1992**, *117*, 105–108.

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.

Plattner, R. D.; Norred, W. P.; Bacon, C. W.; Voss, K. A.; Shackelford, D. D.; Weisleder, D. A method of detection of fumonisins in corn samples associated with field cases of leukoencephalomalacia. *Mycologia* **1990**, *82*, 698–702.

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.

Ross, P. F.; Rice, L. G.; Osweiler, G. D.; Nelson, P. E.; Richard, J. L.; Wilson, T. M. A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. *Mycopathologia* **1992**, *117*, 109–114.

Shier, W. T.; Abbas, H. K.; Mirocha, C. J. Toxicity of the mycotoxins fumonisins B_1 and B_2 and *Alternaria alternata* f. sp. *lycopersici* toxin (AAL) in cultured mammalian cells. *Mycopathologia* **1991**, *116*, 97–104.

Sydenham, E. W. Fumonisins: chromatographic methodology and their role in human and animal health. Ph.D. thesis, University of Cape Town, South Africa, 1994.

Sydenham, E. W.; Thiel, P. G.; Marasas, W. F. O.; Shephard, G. S.; Van Schalkwyk, D. J.; Koch, K. R. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, southern Africa. *J. Agric. Food Chem.* **1990**, *38*, 1900–1903.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G.; Marasas, W. F. O.; Stockenström, S. Fumonisin contamination of commercial corn-based human foodstuffs. *J. Agric. Food Chem.* **1991**, *39*, 2014–2018.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G. Liquid chromatographic determination of fumonisins B_1, B_2, and B_3 in foods and feeds. *J. AOAC Int.* **1992**, *75*, 313–318.

Sydenham, E. W.; Shephard, G. S.; Gelderblom, W. C. A.; Thiel, P. G.; Marasas, W. F. O. Fumonisins: their implications for human and animal health. In *Proceedings of the UK Workshop on the Occurrence and Significance of Mycotoxins*; Scudamore, K. A., Ed.; MAFF: London, 1993; pp 42–48.

Sydenham, E. W.; Van der Westhuizen, L.; Stockenström, S.; Shephard, G. S.; Thiel, P. G. Fumonisin-contaminated maize: physical treatment for the partial decontamination of bulk shipments. *Food Addit. Contam.* **1994**, *11*, 25–32.

Thiel, P. G.; Marasas, W. F. O.; Sydenham, E. W.; Shephard, G. S.; Gelderblom, W. C. A.; Nieuwenhuis, J. J. Survey of fumonisin production by *Fusarium* species. *Appl. Environ. Microbiol.* **1991**, *57*, 1089–1093.

Thiel, P. G.; Marasas, W. F. O.; Sydenham, E. W.; Shephard, G. S.; Gelderblom, W. C. A. The implications of naturally occurring levels of fumonisins in corn for human and animal health. *Mycopathologia* **1992**, *117*, 3–9.

Ueno, Y.; Aoyama, S.; Sugiura, Y.; Wang, D.-S.; Lee, U.-S.; Hirooka, E. Y.; Hara, S.; Karki, T.; Chen, G.; Yu, S.-Z. A limited survey of fumonisins in corn and corn-based products in Asian countries. *Mycotoxin Res.* **1993**, *9*, 27–34.

Vainio, H.; Heseltine, E.; Wilbourn, J. Report on an IARC working group meeting on some naturally occurring substances. *Int. J. Cancer* **1993**, *53*, 535–537.

Viljoen, J. H.; Marasas, W. F. O.; Thiel, P. G. Fungal infection and mycotoxin contamination of commercial maize. In *Cereal Science and Technology—Impact on a Changing South Africa*; Taylor, J. R. N., Randall, P. G., Viljoen, J. H., Eds.; CSIR: Pretoria, 1993; pp 837–853.

Wang, E.; Norred, W. P.; Bacon, C. W.; Riley, R. T.; Merrill, A. H. Inhibition of sphingolipid biosynthesis by fumonisins. *J. Biol. Chem.* **1991**, *266*, 14486–14490.

Weibking, T. S.; Ledoux, D. R.; Brown, T. P.; Rottinghaus, G. E. Fumonisin toxicity in turkey poults. *J. Vet. Diagn. Invest.* **1993**, *5*, 75–83.

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