Fumonisin Contamination of Commercial Corn-Based Human Foodstuffs

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Corn-based human foodstuffs from retail outlets in five countries were analyzed for fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2). The highest mean concentrations occurred in two Egyptian samples (2380 ng/g FB_1 and 595 ng/g FB_2). Only one of four Peruvian samples contained 660 ng/g FB_1 and 68 ng/g FB_2, while only one of two Canadian samples contained a detectable level of FB_1. The 16 cornmeal (CM) and 10 corn grits (CG) products from the United States contained mean concentrations of 1048 ng/g FB_1 and 298 ng/g FB_2 and 601 ng/g FB_1 and 375 ng/g FB_2, respectively, while the mean concentrations in 52 CM and 18 CG samples from South Africa were 138 ng/g FB_1 and 83 ng/g FB_2 and 125 ng/g FB_1 and 85 ng/g FB_2, respectively. Only 1 of 10 cornflakes/lime-treated samples contained a low level of FB_1. Of several samples obtained from a high esophageal cancer (EC) risk area in the United States 7/7 contained FB_1 (105–1915 ng/g) and 6/7 FB_2 (70–460 ng/g).

INTRODUCTION

The fungus *Fusarium moniliforme* Sheldon occurs worldwide on corn (*Zea mays* L.) intended for human and animal consumption (Marasas et al., 1984b). The incidence of *F. moniliforme* in home-grown corn has been correlated with the incidence of human esophageal cancer (EC) in the Transkei, southern Africa (Marasas, 1982; Marasas et al., 1981, 1988a) and China (Li et al., 1980; Yang, 1980, Zhen, 1984). Cultures on corn of *F. moniliforme* strain MRC 826, which was originally isolated from corn in a high EC risk area in Transkei, have been shown to be hepatocarcinogenic in rats (Jaskiewicz et al., 1987; Marasas et al., 1984a). The isolation of a group of structurally related compounds, the fumonisins, from culture material of *F. moniliforme* strain MRC 826 was reported by Gelderblom et al. (1988). Six fumonisin mycotoxins have subsequently been isolated and characterized (Gelderblom et al., 1988; Bezuidenhout et al., 1988; Cawood et al., 1991). Three of these, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), and fumonisin B_3 (FB_3), have been reported to be the major fumonisins produced in corn cultures of *F. moniliforme* strain MRC 826, while the others, fumonisin B_4 (FB_4), fumonisin A_1 (FA_1), and fumonisin A_2 (FA_2), are produced in relatively minor quantities (Cawood et al., 1991). Production of the fumonisins is not restricted to *F. moniliforme*, as recent studies have revealed the coproduction of both FB_1 and FB_2 by two other *Fusarium* species, *F. proliferatum* (Matsushima) Nirenberg and *F. nygamai* Burgess & Trimboli (Ross et al., 1990; Thiel et al., 1991a).

Toxicological investigations on the fumonisins, to date, have focused on FB_1, the major fumonisin produced in nature. The neurotoxic disease equine leukoencephalomalacia (LEM) has been reproduced in horses by both intravenous (iv) and per os administration of FB_1 (Marasas et al., 1988b; Kellerman et al., 1990), while Harrison et al. (1990) reported that iv administration of FB_1 reproduced pulmonary edema syndrome in a pig. A study of the toxicological effects of FB_1 fed to rats at a dietary concentration of 50 μg/g over a period of 26 months (Gelderblom et al., 1991a) concluded that the liver was the main target organ in the FB_1-treated rats and that FB_1 was responsible for the hepatotoxic and carcinogenic effects previously observed in rats fed a diet containing

Table I. Number and Type of Commercial Corn-Based Foodstuffs Purchased in Canada, Egypt, Peru, South Africa, and the United States

	no. of samples				
product type	Canada	Egypt	Peru	South Africa	U.S.
cornmeal	2	2	4	52	17
corn grits[a]				18	10
cornflakes				3	2
miscellaneous[b]				8	6

[a] South African equivalent known locally as corn "samp" or corn "rice". [b] Includes corn flour, corn mix, and breakfast cereals that include some corn.

culture material of *F. moniliforme* MRC 826 (Marasas et al., 1984; Jaskiewicz et al., 1987). The full toxicological implications of the other fumonisins are as yet unknown; however, future studies will certainly have to consider the contributions of FB_2 and FB_3, as these metabolites appear to exhibit toxicological and cancer-initiating properties similar to those of FB_1 (Gelderblom et al., 1991b) and appear in measurable concentrations in corn.

The available data on the toxicity and carcinogenicity of the fumonisins and the fact that FB_1 is heat stable (Alberts et al., 1990) suggest that these compounds should be regarded as potential risks to human health. To assess the overall threat that the fumonisins may pose to human health, it is necessary to obtain data on their natural occurrence in foodstuffs intended for human consumption. Methods for the analytical determination of two fumonisins (FB_1 and FB_2) in foods and mixed feeds have been developed (Sydenham et al., 1990a; Plattner et al., 1990; Shephard et al., 1990), and two high-performance liquid chromatographic (HPLC) methods are currently the subject of collaborative studies (Plattner, 1991). However, the majority of published data on the natural occurrence of the fumonisins has concerned animal feed samples associated with outbreaks of equine LEM (Voss et al., 1989; Plattner et al., 1990; Wilson et al., 1990; Shephard et al., 1990; Thiel et al., 1991b). Levels of FB_1 and FB_2 have been reported in home-grown corn samples collected from the Transkei, southern Africa, during 1985 (Sydenham et al., 1990b) and 1989 (Rheeder et al., 1991), and a significant correlation between fumonisin concentrations and the high incidence of human EC was established. The Transkei data are, however, based on the analyses of

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Table II. Range and Mean Fumonisin Levels Determined in Commercial Corn-Based Foodstuffs from Canada, Egypt, Peru, South Africa, and the United States

	fumonisin levels, ng/g									
	Canada		Egypt		Peru		South Africa		U.S.	
product	FB_1	FB_2	FB_1	FB_2	FB_1	FB_2	FB_1	FB_2	FB_1	FB_2
cornmeal										
range	0–50	0	1780–2980	470–780	0–660	0–135	0–475	0–131	0–2790	0–920
positives/total	1/2	0/2	2/2	2/2	1/2	1/2	46/52	11/52	15/16	13/16
mean–positives	50	0	2380	595	660	135	138	83	1048	298
corn grits										
range							0–190	0–120	105–2545	0–1065
positives/total							10/18	4/18	10/10	5/10
mean–positives							125	85	601	375
cornflakes										
range							0	0	0	0
positives/total							0/3	0/3	0/2	0/2
mean–positives							0	0	0	0
alkali treated[a]										
range					0	0			0–55	0
positives/total					0/2	0/2			1/3	0/3
mean–positives					0	0			55	0
miscellaneous										
range							0–91	0	85–700	0–240
positives/total							2/8	0/8	4/4	3/4
mean–positives							84	0	409	148

[a] Peruvian samples were corn kernels; the U.S. samples were tortilla preparations.

Table III. Distribution of the Combined Fumonisin Levels in 81 South African Commercial Corn-Based Foodstuffs

total fumonisin concn, ng/g	total no. of samples	% of samples
<100	49	60.5
101–200	18	22.2
201–300	8	9.9
301–400	1	1.2
401–500	3	3.7
501–600	2	2.5

Table IV. Distribution of the Combined Fumonisin Levels in 35 U.S. Commercial Corn-Based Samples

total fumonisin concn, ng/g	total no. of samples	% of samples
<500	17	48.6
501–1000	8	22.8
1001–1500	3	8.6
1501–2000	2	5.7
2001–2500	3	8.6
>2501	2	5.7

samples of corn grown, harvested, and consumed by the inhabitants of a rural area and hence do not necessarily reflect the levels at which the fumonisins might be present in commercially available corn-based foodstuffs.

To assess the extent to which consumers are exposed to the fumonisins, 124 corn-based foodstuffs were purchased from retail outlets in five countries. Each sample was analyzed for the presence of FB_1 and FB_2; 61 of the samples were also screened mycologically for the presence of *F. moniliforme*. This paper reports the results of the chemical and mycological analyses of these commercially available corn-based products intended for human consumption.

EXPERIMENTAL PROCEDURES

Corn-Based Samples. Between May 1990 and April 1991, 35 corn-based human foodstuffs were purchased from retail outlets in the United States in the states of Florida, Iowa, Maryland, Massachusetts, Missouri, North and South Carolina, Tennessee, and Texas and Washington, DC (Table I). Between June 1990 and April 1991, 81 similar samples were purchased from commercial outlets in Cape Town and Bloemfontein, South Africa, 4 samples in Lima, Peru, 2 samples in Toronto, Canada, and 2 samples in Cairo, Egypt (Table I). All samples were stored at 4 °C prior to analysis.

Mycology. Subsamples (1 g) of ground corn-based products were mixed with 9 mL of sterile distilled water. Dilutions with sterile distilled water were prepared and aliquots (1 mL) added to Petri dishes containing malt extract agar (15 mL). The Petri dishes were incubated at 25 °C for 5 days, following which time the *Fusarium* colonies were counted and identified according to the system of Nelson et al. (1983).

Determination of FB_1 and FB_2. Where necessary, samples were prepared prior to analysis, by grinding in a laboratory mill. Each sample was subsequently analyzed for the presence of both FB_1 and FB_2 according to the HPLC method of Shephard et al. (1990). Briefly, subsamples were extracted with methanol/water, and an aliquot was applied to a strong anion-exchange column. The column was washed with methanol/water followed by methanol, and the fumonisins were eluted with an acetic acid/methanol solution. The eluate was evaporated to dryness and redissolved in methanol, and an aliquot derivatized with *o*-phthaldialdehyde (OPA) prior to separation on a reversed-phase HPLC system coupled with fluorescence detection. The sensitivity of the method was of the order of 50 ng/g for both FB_1 and FB_2.

RESULTS AND DISCUSSION

The levels of contamination with *F. moniliforme* found in the 61 screened samples (0–690 000 propagules/g) significantly correlated with the levels of both FB_1 ($r = 0.458$; $p < 0.001$) and FB_2 ($r = 0.406$; $p < 0.001$) detected in the samples. These results are in accordance with those previously reported in home-grown corn (Sydenham et al., 1990b).

The range and means of fumonisin concentrations together with the number of samples found to be positive for each fumonisin, separated on the basis of the origin and sample type, are given in Table II.

The levels of FB_1 detected in each sample were higher than the corresponding FB_2 levels. Similar observations have been recorded for fungal cultures of *F. moniliforme* (Gelderblom et al., 1988) as well as in samples associated with field outbreaks of equine LEM (Thiel et al., 1991b).

Table V. Range and Means of Fumonisin Levels Recorded in Samples from Esophageal Cancer Risk Areas in the United States and Transkei[a]

		fumonisin levels, ng/g			
		fumonisin B_1		fumonisin B_2	
origin	year	range	mean	range	mean
Charleston	1989	105–1915	635 (7/7)	70–460	182 (6/7)
Transkei[b,c]	1985	500–7900	1600 (12/12)	150–2250	500 (10/12)
Transkei[b,d]	1985	3450–46900	29300 (12/12)	900–16300	7550 (12/12)
Transkei[e,c]	1989	210–5380	1530 (5/6)	150–1320	420 (5/6)
Transkei[e,d]	1989	3020–117520	53740 (6/6)	750–22960	13680 (6/6)

[a] Number of positive samples is given in parentheses. [b] Data published by Sydenham et al. (1990b). [c] "Healthy" corn samples—intended for human consumption. [d] "Moldy" corn samples—intended for beer brewing or animal feed. [e] Data published by Rheeder et al. (1991).

The two Egyptian cornmeal samples were positive for both FB_1 and FB_2 with mean concentrations of 2380 and 595 ng/g, respectively, while only one Peruvian cornmeal sample contained detectable levels of FB_1 and FB_2 (at 660 ng/g FB_1 and 135 ng/g FB_2; Table II). The two Canadian samples contained the lowest fumonisin levels (for cornmeal), with only one sample containing a detectable level of FB_1 (50 ng/g; Table II); neither contained detectable levels of FB_2. Of the 52 South African samples, 46 (88.5%) and 11 (21.2%) were found to be positive for FB_1 and FB_2, respectively, with levels ranging between 0 and 475 ng/g for FB_1 and between 0 and 131 ng/g for FB_2 and mean concentrations (in the positive samples) of 138 ng/g FB_1 and 83 ng/g FB_2. These results contrasted sharply with those determined in the U.S. samples (Table II), where levels ranged between 0 and 2790 ng/g for FB_1 and between 0 and 920 ng/g for FB_2, with 93.8% and 81.3% of the samples being contaminated with FB_1 and FB_2, respectively, and mean concentrations (in the positive samples) of 1048 ng/g FB_1 and 298 ng/g FB_2. Higher fumonisin levels and a larger percentage of contamination with FB_1 and FB_2 were recorded in the U.S. corn grits and miscellaneous samples than in the equivalent South African samples (Table II). Both the range of fumonisin levels and the mean concentrations were lower in these samples than in the corresponding cornmeal samples (Table II).

None of the five cornflake samples (three from South Africa and two from the United States) contained detectable levels of either FB_1 or FB_2. Previous findings (Alberts et al., 1990) have reported that, under specific conditions, FB_1 is heat stable. Therefore, taking into consideration the current results obtained for the cornmeal, corn grits, and miscellaneous samples, the absence of both FB_1 and FB_2 in cornflakes warrants further investigation.

Five of the samples (two from Peru and three from the United States) were, according to the manufacturers, treated with aqueous calcium hydroxide (lime water) as part of the manufacturing process. Of these four lime-treated samples, only one contained a low level of FB_1 (55 ng/g) bordering on the detection limit of the method, while no FB_2 was detected in any of the samples (Table II). The effect, if any, that calcium hydroxide may have on the fumonisins in corn should be given serious consideration, since the development of fumonisin decontamination procedures would be desirable.

Table III displays the distribution pattern for the combined fumonisin levels (FB_1 plus FB_2) determined in the 81 South African samples, while Table IV shows the distribution pattern for the 35 U.S. samples.

More than 60% of the South African samples contained less than 100 ng/g combined fumonisins, while none of the samples contained more than 600 ng/g (Table III). These results contrasted with those obtained for the U.S. samples (Table IV), of which only 48.6% had combined fumonisin concentrations below 500 ng/g, while 28.6% contained in excess of 1000 ng/g (Table IV). However, these figures are based on observations from only 35 U.S. and 81 South African samples, and it is not our intention to suggest that the figures presented in Tables III and IV reflect the general situation in the separate countries. The results, however, clearly indicate for the first time that consumers of corn-based commercially available foodstuffs in the United States and South Africa are exposed to elevated levels of the fumonisins.

Seven of the 35 U.S. samples were purchased in Charleston, SC. The highest incidence and mortality rates of EC in the United States occur in the black population (predominantly males) in Charleston, the adjacent Sea Islands, and the coastal mainland in the low country of South Carolina (Fraumeni and Blot, 1977; O'Brien et al., 1982; Brown et al., 1988). The study of Brown et al. (1988) identified tobacco and alcohol as the primary determinants for EC, with a significant association between EC risk and the consumption of "moonshine", a local, illicit alcoholic drink distilled from fermented corn meal. The consumption of corn as a dietary staple and/or as a home-brewed alcoholic beverage has been implicated as a risk factor for EC in Africa (Cook, 1971; Marasas, 1982; Marasas et al., 1981, 1988a; Van Rensburg et al., 1985; Segal et al., 1988; Sydenham et al., 1990a,b), China (Li et al., 1980; Yang, 1980; Zhen, 1984; Li et al., 1989; Wahrendorf et al., 1989), and Italy (Rossi et al., 1982; Franceschi et al., 1990). The fumonisin levels determined in the commercial corn-based samples from Charleston are compared, in Table V, with those obtained in samples of home-grown corn collected from high EC risk areas of the Transkei, southern Africa, during 1985 and 1989. All seven of the corn-based foodstuffs from Charleston contained FB_1 at levels between 105 and 1915 ng/g and six contained FB_2 at levels between 70 and 460 ng/g. These levels were not significantly higher than the overall levels recorded in the U.S. samples (Table II) and were considerably lower than those determined in home-grown corn samples from the Transkei (Table V). It should be noted that the number and type of samples from the two EC risk areas differed considerably. Despite the apparent disparity between the fumonisin results determined in the samples from the two EC risk areas, the fact that both FB_1 and FB_2 have now been shown to occur in commercial corn-based foodstuffs in a high EC risk area in the United States emphasizes the need to investigate further the possible role of the fumonisins in the etiology of EC.

Several conclusions may be drawn from the results. First, consumers of corn-based products in a number of countries are exposed to the fumonisin mycotoxins, and the toxicological significance of the reported levels needs to be assessed. Second, more data on the general (worldwide) occurrence of the fumonisins in corn-based products

need to be generated. Third, the levels of the fumonisins in samples from other high EC risk areas should be determined. A recent paper on the occurrence of *Fusarium* mycotoxins in corn and wheat from high and low EC areas of China (Luo et al., 1990) included data concerning various trichothecenes and zearalenone but unfortunately no data on the fumonisins.

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