Occurrence of Fumonisins B_1 and B_2 in Corn-Based Products from the Swiss Market†

Alain Pittet,* Véronique Parisod, and Marija Schellenberg

Nestlé Research Centre, Nestec Ltd., Vers-chez-les-Blanc, P.O. Box 44, CH-1000 Lausanne 26, Switzerland

Corn-based food and feed products purchased in Switzerland were analyzed for fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) using high-performance liquid chromatography. Forty-four samples (36.7%) of 120 were found positive for FB_1 (55–790 ng/g), with only 15 containing detectable levels of FB_2 (50–160 ng/g). The highest frequency of positive samples and also the highest FB_1 concentrations were found in corn grits (61.8%, 790 ng/g), followed by corn-based poultry feed (27.3%, 480 ng/g). Thirteen corn grits samples (23.6%) were positive for both FB_1 and FB_2, with mean concentrations of 460 and 100 ng/g, respectively. Of the 22 poultry feed samples examined, 6 were positive for FB_1 with an average content of 235 ng/g, while only 2 contained measurable levels of FB_2.

INTRODUCTION

The fungus *Fusarium moniliforme* Sheldon occurs worldwide on a variety of plant hosts and is one of the most prevalent fungi associated with corn intended for human and animal consumption (Marasas et al., 1984b). It has long been known to be the causative agent of equine leukoencephalomalacia (ELEM), a fatal neurotoxic syndrome of horses and related animals (Wilson and Maronpot, 1971; Marasas et al., 1976; Kriek et al., 1981a). *F. moniliforme* is also suspected of being involved in other animal diseases, particularly porcine pulmonary edema syndrome (Kriek et al., 1981a), and has been shown to be highly toxic to a variety of experimental animals (Kriek et al., 1981a,b; Jaskiewicz et al., 1987a). Moreover, the high rate of human esophageal cancer in the Transkei, southern Africa (Marasas et al., 1981, 1988a), and in China (Yang, 1980) appears to be correlated with the incidence of *F. moniliforme* in corn. Experimentally, culture material of *F. moniliforme* strain MRC 826, originally isolated from corn in a high esophageal cancer risk area in the Transkei, has been demonstrated to be hepatocarcinogenic in rats (Jaskiewicz et al., 1987b; Marasas et al., 1984a).

The intense interest in the toxicity of *F. moniliforme* recently culminated in the isolation of the fumonisins, a new group of structurally related mycotoxins (Gelderblom et al., 1988). To date, six different fumonisins have been identified and characterized (Gelderblom et al., 1988; Bezuidenhout et al., 1988; Cawood et al., 1991). Among them, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), and fumonisin B_3 (FB_3) are the major toxins produced in fungal cultures or present in naturally contaminated corn samples, while the other three, fumonisin B_4 (FB_4), fumonisin A_1 (FA_1), and fumonisin A_2 (FA_2), are produced only in minor amounts (Cawood et al., 1991). Recent studies have shown that FB_1 and FB_2 may also be produced by isolates of *Fusarium proliferatum* (Matsushima) Nirenberg (Ross et al., 1990) and *Fusarium nygamai* Burgess & Trimboli (Thiel et al., 1991a).

FB_1 is the only fumonisin for which toxicity data have been reported. The pure compound exhibits hepatotoxic effects in rats similar to that of the culture material of *F. moniliforme* MRC 826 (Gelderblom et al., 1988). In addition, FB_1 has been shown to have cancer-promoting activity in rats (Gelderblom et al., 1988), to induce leukoencephalomalacia in a horse by both intravenous injection (Marasas et al., 1988b) and per os administration (Kellerman et al., 1990), and to reproduce porcine pulmonary edema by intravenous injection (Harrison et al., 1990). The characterization of the other fumonisins is still too recent for any toxicological assessment to have been undertaken as yet. However, the data already available on the toxicity and carcinogenicity of FB_1 indicate that these mycotoxins should be regarded as potential risks to human health.

To assess these risks, it is necessary to obtain data on the distribution and contamination levels of fumonisins in human foodstuffs. Various analytical methods have been developed for this purpose, allowing a simultaneous determination of FB_1 and FB_2 in cereals and mixed feeds (Sydenham et al., 1990a; Plattner et al., 1990; Shephard et al., 1990). Although the majority of papers published so far were only concerned with analysis of animal feed samples implicated in outbreaks of ELEM (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, and a significant correlation between fumonisin concentrations and the high incidence of human esophageal cancer was established (Sydenham et al., 1990b). Moreover, both FB_1 and FB_2 have recently been found in commercially available corn products intended for human consumption (Sydenham et al., 1991). Consequently, the aim of the present study was to investigate the levels of FB_1 and FB_2 in a variety of corn-based food and feed products purchased in Switzerland.

EXPERIMENTAL PROCEDURES

Analytical Standards. FB_1 and FB_2 were purchased from CSIR, Division of Food Science and Technology, Pretoria, South Africa.

Corn-Based Samples. Food products were collected in September 1991 as single random purchases from retail outlets in Switzerland or obtained from local factories. Samples of corn-based poultry feed were supplied by the Federal Research Station for Animal Production, Posieux, Switzerland.

Determination of FB_1 and FB_2. Each sample was analyzed for FB_1 and FB_2 according to the high-performance liquid chromatography (HPLC) method of Shephard et al. (1990), which may be summarized as follows. Finely ground test portions were blended with methanol/water and filtered. An aliquot of the

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Table I. Incidence and Levels of Fumonisins Determined in Corn-Based Food and Feed Products from Switzerland

product	toxin	incidence (positives[a]/total)	range, ng/g	mean-positives, ng/g
corn grits[b]	FB_1	34/55	0–790	260
	FB_2	13/55	0–160	100
cornflakes	FB_1	1/12	0–55	55
	FB_2	0/12	nd[c]	
cornmeal	FB_1	2/7	0–110	85
	FB_2	0/7	nd	
sweet corn	FB_1	1/7	0–70	70
	FB_2	0/7	nd	
miscellaneous[d]	FB_1	0/17	nd	
	FB_2	0/17	nd	
poultry feed	FB_1	6/22	0–480	235
	FB_2	2/22	0–115	90

[a] Contamination above 50 ng/g. [b] Swiss equivalent known locally as "polenta" or corn semolina. [c] nd, not detected. [d] Food samples including cornstarch (6), popcorn (4), tortillas (4), corn cookies (2) and corn noodles (1).

filtrate was applied to a Bond-Elut strong anion-exchange (SAX) cartridge previously equilibrated with methanol/water. Subsequently, the cartridge was washed with methanol/water followed by methanol, and the toxins were eluted with an acetic acid/methanol solution. The eluate was evaporated to dryness, the residue redissolved in methanol, and an aliquot derivatized with o-phthaldialdehyde (OPA) prior to separation on a reversed-phase HPLC system coupled with fluorescence detection.

Confirmation of FB_1 Identity. Aliquots (40 μL, corresponding to 1 g of corn) of the purified sample extracts and FB_1 reference standard were applied to RP-18 WF_{254} S HPTLC plates (Merck). The plates were developed in methanol/water (70:30), dried, sprayed with a solution of 0.5% vanillin in 97% sulfuric acid/ethanol (4:1), heated for 10 min at 120 °C, and visually inspected. FB_1 appeared as a blue-purple spot with an R_f value of 0.60 (limit of detection was 250 ng/g).

RESULTS AND DISCUSSION

The range and means of fumonisin concentrations together with the number of samples found to be positive for each fumonisin are given in Table I.

Forty-four samples (36.7%) of 120 were found to be contaminated with FB_1, in concentrations ranging from 55 to 790 ng/g (mean 235 ng/g). Among these 44 positive samples, only 15 (34.1%) contained also detectable levels of FB_2, between 50 and 160 ng/g (mean 100 ng/g). As expected, FB_1 was always the major fumonisin in positive samples, and its percentage of the total fumonisin concentration varied between relatively narrow limits (78–86%). This confirms previous data reported in feed samples associated with outbreaks of ELEM (Thiel et al., 1991b).

The highest frequency of positive samples and also the highest FB_1 concentrations were found in corn grits (61.8%, 790 ng/g), followed by corn-based poultry feed (27.3%, 480 ng/g). Thirteen corn grits samples (23.6%) were positive for both FB_1 and FB_2, with mean concentrations of 460 and 100 ng/g, respectively. Of the 22 poultry feed samples examined, 6 were positive for FB_1 with an average content of 235 ng/g, while only 2 contained measurable levels of FB_2. However, as corn was not a major ingredient in these feedstuffs, the levels reported in Table I do not actually reflect those at which the fumonisins might be present in corn intended for animal consumption. Lower fumonisin levels and a smaller contamination incidence were recorded in samples of cornflakes, cornmeal, and canned sweet corn, while no fumonisins could be detected in any of the 17 miscellaneous commodities examined.

Table II. Distribution of the Combined Fumonisin Levels in 98 Corn-Based Food Products Purchased in Switzerland

total fumonisin concn, ng/g	total no. of samples	% of samples
<100	74	75.5
101–300	10	10.2
301–500	6	6.1
501–700	3	3.1
701–900	4	4.1
>900	1	1.0

Table II shows the distribution pattern for the combined fumonisin levels determined in the 98 corn-based food products.

Although more than 75% of these products had total fumonisin concentrations below 100 ng/g, 8 samples (8.2%) contained the toxins in excess of 500 ng/g, the highest value being 955 ng/g. These results are consistent with the overall levels previously recorded in 81 South African commercially available corn products by Sydenham et al. (1991), who reported that about 60% of the samples contained less than 100 ng/g total fumonisins, while none contained more than 600 ng/g. However, this contrasted sharply with additional data obtained on a series of 35 U.S. samples, of which only 48.6% had total fumonisin concentrations below 500 ng/g, while 26.8% contained in excess of 1000 ng/g (Sydenham et al., 1991).

In conclusion, the present survey clearly indicates that consumers of corn-based commercially available foodstuffs in Switzerland are exposed to the fumonisins. Consequently, there is an obvious need to assess the toxicological significance of the reported levels and to obtain more data on the occurrence of these mycotoxins. For this purpose, further studies are currently underway to investigate the possible contamination of other cereal products, with a special consideration given to commodities imported from tropical regions.

ABBREVIATIONS USED

ELEM, equine leukoencephalomalacia; FB_1, fumonisin B_1; FB_2, fumonisin B_2; FB_3, fumonisin B_3; FB_4, fumonisin B_4; FA_1, fumonisin A_1; FA_2, fumonisin A_2; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin-layer chromatography; SAX, strong anion exchange.

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