Discovery and Occurrence of the Fumonisins: A Historical Perspective

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This article describes the events leading to the discovery of the fumonisins in South Africa in 1988 and highlights the first 10 years (1988–1998) of fumonisin research. The predominant fungus isolated from moldy corn implicated in a field outbreak of equine leukoencephalomalacia (ELEM) in South Africa in 1970 was *Fusarium verticillioides* (*F. moniliforme*). This fungus was also prevalent in moldy home-grown corn consumed by people in high-incidence areas of esophageal cancer (EC) in the Transkei region of South Africa. Culture material on corn of *F. verticillioides* strain MRC 826, which was isolated from moldy corn in Transkei, was shown to cause ELEM in horses, porcine pulmonary edema (PPE) syndrome in pigs, and liver cancer in rats. A short-term cancer initiation/promotion assay in rat liver was used to purify the carcinogen(s) in the culture material. These efforts finally met with success when fumonisins B_1 and B_2, novel mycotoxins with cancer-promoting activity in rat liver, were isolated from culture material of *F. verticillioides* MRC 826 at the Programme on Mycotoxins and Experimental Carcinogenesis of the Medical Research Council in Tygerberg, South Africa. Following the elucidation of the chemical structure of the fumonisins, these carcinogenic mycotoxins were shown to occur naturally in moldy corn in Transkei. Shortly thereafter, high levels of fumonisins in the 1989 U.S. corn crop resulted in large-scale field outbreaks of ELEM and PPE in horses and pigs, respectively, in the United States. Subsequently the fumonisins were found to occur naturally in corn worldwide, including corn consumed as the staple diet by people at high risk for EC in Transkei and China. These findings, together with the fact that the fumonisins cause field outbreaks of mycotoxicoses in animals, are carcinogenic in rats, and disrupt sphingolipid metabolism, have resulted in much worldwide interest in these compounds during the first 10 years after the discovery of the fumonisins in 1988. *Key words*: corn, esophageal cancer, fumonisins, *Fusarium moniliforme*, *Fusarium verticillioides*, leukoencephalomalacia, porcine pulmonary edema syndrome, South Africa. — *Environ Health Perspect* 109(suppl 2):239–243 (2001).
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The fungus *Fusarium verticillioides* (Sacc.) Nirenberg (synonym: *F. moniliforme* Sheldon; teleomorph *Gibberella moniliformis* Wineland) is one of the most prevalent seed-borne fungi associated with corn (maize, *Zea mays* L.) intended for human and animal consumption throughout the world (*1*). The fumonisins, a family of foodborne carcinogenic mycotoxins, were first isolated in 1988 from cultures of *F. verticillioides* strain MRC 826 at the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC) of the Medical Research Council (MRC) in Tygerberg, South Africa, by Gelderblom et al. (*2*). Also in 1988, the structures of the fumonisins were also elucidated in a collaborative effort between the PROMEC and the Council for Scientific and Industrial Research (CSIR) in Pretoria (*3*). Fumonisin B_1 (FB_1), in a collaborative effort between PROMEC and the Onderstepoort Veterinary Research Institute in South Africa (*4*), was shown to cause equine leukoencephalomalacia (ELEM).

The isolation and chemical characterization of the fumonisins in South Africa in 1988 was the culmination of 18 years (1970–1988) of intensive dedicated research by a multidisciplinary team with members at each of the above-mentioned three institutions. In this historical perspective, we describe the events during this period that led to the isolation of the fumonisins in 1988, and highlight the first 10 years (1988–1998) of fumonisin research.

Events Leading to the Characterization of the Fumonisins in South Africa: 1970–1988

F. verticillioides was the predominant fungus isolated from moldy corn associated with a field outbreak of ELEM in South Africa during 1970 characterized by liquefactive necrotic lesions in the white matter of the cerebral hemispheres of horses (*1,5*). The causative role of *F. verticillioides* in ELEM (*6*) was subsequently confirmed with several South African isolates of the fungus, and the pathognomonic pathologic changes were described in detail (*5,7*). The occurrences of bile duct proliferation, increased numbers of mitotic figures, multinucleated hepatocytes, and large, bizarre hyperchromatic nuclei in the livers of these horses (*5,7*) were the first indications that *F. verticillioides* might be a carcinogenic fungus.

Subsequently, we became involved in a study of the possible role of fungal toxins in the etiology of human esophageal cancer (EC) in the Transkei region of South Africa. The incidence rate of EC in males and females in the southern part of Transkei is among the highest in the world, whereas the rate in the northern part of Transkei is low (*8,9*). The staple diet in both areas is home-grown corn, and *F. verticillioides* was shown to be the most prevalent fungus in corn consumed by people in areas with a high incidence of EC (*10*).

In continuing investigations on the toxicology of *F. verticillioides* isolates from corn associated with field outbreaks of ELEM in horses, isolates from corn in areas in Transkei with a high risk of EC were included. One of these Transkeian isolates, designated *F. verticillioides* MRC 826, was soon found to cause ELEM experimentally in horses and porcine pulmonary edema (PPE) in pigs (*11*) and to be highly hepatotoxic and cardiotoxic in rats (*11,12*). In 1984, culture material of *F. verticillioides* MRC 826 was shown to be hepatocarcinogenic in rats and to cause primary hepatocellular carcinoma and cholangiocarcinoma (*13*).

Although chemical investigations on the mycotoxin(s) produced by *F. verticillioides* MRC 826 began in South Africa in July 1970, the chemical nature of the metabolite(s) responsible for ELEM still had not been identified in 1984 when the fungus was shown to be carcinogenic. The isolation and chemical characterization of the mycotoxin(s) and carcinogen(s) produced by *F. verticillioides* then became a matter of paramount importance.

The urgency of the matter was accentuated further when researchers in the United States reported that corn implicated in field outbreaks of ELEM and naturally infected by *F. verticillioides* was also hepatocarcinogenic in rats (*14*). The pathologic changes in these rats were identical to those that had been described in 1984 in rats fed culture material of *F. verticillioides* MRC 826 by Marasas et al. (*13*). This provided evidence that the unidentified carcinogen(s) produced by *F. verticillioides*

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was present not only in culture material of *F. verticillioides* MRC 826 but also occurred naturally in corn in the United States.

In 1984 the following mycotoxins were known to be produced by *F. moniliforme*, according to the literature: deoxynivalenol; diacetoxyscirpenol, fusaric acid; fusarins A, B, C, and D; fusariocins; gibberellins; moniliformin; T-2 toxin; and zearalenone (*1*). We knew, however, that *F. verticillioides* MRC 826 did not produce moniliformin, trichothecenes, or zearalenone (*1,12,15*). We also knew that a chloroform/isopropanol extract of culture material of *F. verticillioides* MRC 826 was highly mutagenic to *Salmonella typhimurium* in the Ames test (*16,17*). Consequently, intensive efforts were made to isolate and characterize the mutagen(s) because most mutagens are also carcinogens. A group of structurally related compounds, the fusarins, were isolated. One of these, fusarin C, was very promising, as it was highly mutagenic in the Ames test and occurred naturally in Transkeian corn (*17,18*) as well as in corn from the United States that had been shown to be hepatocarcinogenic in rats (*19*). Consequently, short-term carcinogenicity assays with fusarin C and long-term trials in rats with culture material of *F. verticillioides* MRC 1069 that contained high levels of fusarin C were performed (*20,21*). However, no evidence could be found of the carcinogenicity of fusarin C. It was concluded that fusarin C was not the carcinogenic metabolite present in culture material of *F. verticillioides* MRC 826. In view of the findings that fusarin C was not carcinogenic and because it is heat and light sensitive, it was concluded that fusarin C is not a threat to human health.

Thus, the search continued for the elusive *F. verticillioides* carcinogen. During our investigations on the carcinogenicity of fusarin C, a short-term initiation/promotion assay in rat liver was developed. This involved partial hepatectomy of rats, followed by administration of an initiator such as diethylnitrosamine (DEN), followed by administration of a promotor such as phenobarbital. After 14 weeks, the gamma glutamyltranspeptidase (GGT) activity was determined histochemically in the liver (*2,20,22*). During the application of this short-term carcinogenicity assay, it was found that culture material of *F. verticillioides* MRC 826 initiated the formation of early lesions in the liver and exhibited cancer-promoting activity. The short-term cancer initiation/promotion assay, with DEN as initiator and the ability of fractions of extracts of the culture material to selectively stimulate the development of GGT-altered foci in rat liver, was used as a bioassay in the isolation of the active principle(s) (*2*).

All these efforts finally succeeded in 1988, when the chemical nature of the carcinogen was unraveled. FB_1 and fumonisin B_2 (FB_2), novel mycotoxins with cancer-promoting activity in rat liver, were isolated from cultures of *F. verticillioides* MRC 826 at PROMEC (*2*). The structures of FB_1 and FB_2 were elucidated in collaboration with the CSIR (*3*).

The elucidation of the chemical structure of the fumonisins, together with the demonstration in 1988 of the biologic activity of FB_1, was the end of an era and the beginning of a new one. From 1970–1988, the toxicity of culture material of *F. verticillioides* MRC 826 to horses and pigs and the carcinogenicity to rats were established. From 1988–1991, the stage was set for a new era of research on the biologic activity of chemically characterized fumonisins rather than crude fungal cultures and/or extracts.

Table 1. Incidence of *Fusarium verticillioides* in home-grown corn from Transkei.[a]

Season	Mean % kernels infected: Low incidence of EC	High incidence of EC	$p <$
Healthy corn			
1976	5.0	41.5	0.0001
1979	5.0	23.1	0.01
1985	8.3	42.0	0.001
1986	9.0	43.0	0.01
1989	8.9	41.2	0.01
Moldy corn			
1977	17.0	25.7	0.005
1979	9.8	33.4	NS
1985	34.5	67.7	0.01
1989	21.4	61.7	0.01

NS, not significant.
[a]Data from Rheeder et al. (*53*).

Fumonisins: The First 10 Years: 1988–1998

Biologic Activity and Natural Occurrence: 1988–1991

Pure FB_1 was first shown in 1988 to cause ELEM in horses by intravenous injection (*4*) and by oral dosing in 1990 (*23*). Pure FB_1 was shown in 1990 to cause PPE in pigs by intravenous injection (*24*) and to cause liver cancer in male BD IX rats at a dietary level of 50 µg/g (*25*).

Table 2. Levels of fumonisins in home-grown corn from Transkei.[a]

Season	Mean fumonisin levels (µg/g)[b]: Low incidence of EC	High incidence of EC	$p <$
Healthy corn			
1985	0.3	2.1	0.001
1989	0.6	2.0	NS
Moldy corn			
1985	9.0	31.5	0.01
1989	5.1	67.4	0.005

[a]Data from Rheeder et al. (*53*). [b]Total fumonisins in positive samples.

The natural occurrence of FB_1 in home-grown corn from Transkei was first reported in January 1990 by Sydenham et al. (*26*). The first quantitative and sensitive high-performance liquid chromatography method for the simultaneous determination of FB_1 and FB_2 in naturally contaminated corn and mixed feed was published in June 1990 by Shephard et al. (*27*). Using this analytical method, Sydenham et al. (*28*) showed that home-grown corn from areas in Transkei with a high incidence of EC contained significantly higher levels of both FB_1 and FB_2 than corresponding samples from low-incidence areas. In 1981 a correlation was shown between the incidence of the fungus *F. verticillioides* in home-grown corn and the incidence of EC in Transkei (*10*), whereas in 1990 a correlation was shown between the levels of FB_1 and FB_2 in home-grown corn and the incidence of EC in Transkei (*28*).

It is remarkable that the fumonisins were launched into international importance shortly after their discovery in South Africa in 1988 by events that occurred in the United States during 1989 and 1990. These events occurred shortly after publication of the structure of the fumonisins in 1988 (*2*) but just before the publication of a sensitive chemical analytical method for FB_1 and FB_2 in June 1990 (*27*).

The Great American Outbreaks: 1989–1990

During the fall of 1989 and the winter of 1990, widespread, large-scale outbreaks of ELEM and PPE occurred in the United States. Large numbers of horses and pigs died from consuming commercial mixed feeds containing fumonisin-contaminated corn from the 1989 U.S. corn crop (*24,29–33*).

Great Activity and International Agency for Research on Cancer Evaluation: 1990–1993

The disastrous consequences of the contamination of the 1989 U.S. corn crop with high levels of fumonisins triggered a great deal of interest in and research on the fumonisins in the United States and elsewhere (*34–40*). This resulted in a sharp increase in the number of publications dealing with the fumonisins (*41*), including several comprehensive

Table 3. Incidence of *F. graminearum* and levels of DON, NIV, and ZEA in moldy corn from Transkei in 1985.[a]

	Low incidence of EC	High incidence of EC	$p <$
F. graminearum (%)	34.9	8.0	0.01
DON (µg/g)	2.9	0.3	NS
NIV (µg/g)	4.6	1.8	0.05
ZEA (µg/g)	1.2	0.4	0.01

[a]Data from Sydenham et al. (*28*) and Rheeder et al. (*53*).

Table 4. Ecologic characteristics of areas with low and high incidence of EC in Transkei.[a]

Characteristic	Low incidence of EC, Bizana District	High incidence of EC, Kentani District
Altitude	600–900 meters	200–400 meters
Average rainfall	800–950 millimeters	900–1000 millimeters
Geology	Ecca, Dwyka Volcanically derived dolerite	Beaufort Sedimentary sandstones
General soil features	Red, yellow/brown apedal with orthic epipedon (Hutton, Clovelly, Griffin)	Weakly developed soils Rock (Glenrosa, Mispah, Swartland)
Vegetation	Coastal forest and Thornveld	Thornveld - Bushveld
Soil fertility factors	Soil organic matter and levels of Al and Fe significantly higher	Soil pH and levels of Mn, Ni, Mg, Ca, and K significantly higher

[a]Data from Rheeder et al. (*54*).

Table 5. EC incidence rates in Transkei.[a]

	ASIR			
	Low incidence of EC, Bizana District		High incidence of EC, Kentani District	
Period	Males	Females	Males	Females
1955–1959	2.6	1.8	54.2	30.3
1965–1969	10.5	4.4	39.7	16.1
1981–1984	19.5	15.0	45.0	23.3
1985–1990	37.0	11.7	55.6	22.1

ASIR, age standardized incidence rate/100,000/annum.
[a]Data from Makaula et al. (*9*).

Table 6. Some blood biochemical parameters of populations at risk for EC in Transkei.[a]

Blood biochemical parameters	Normal values	Transkei
Selenium (ng/mL)	112–210	58–69
Vitamin A (μg/dL)	20–70	25–40
Vitamin B_{12} (pg/mL)	220–750	227–366
Vitamin E (μg/dL)	> 6.0	3.7–5.3
Folic acid (ng/mL)	210–980	242–307

[a]Data from Jaskiewicz et al. (*55,56*).

Table 7. Incidence of esophageal cytologic abnormalities, *F. verticillioides,* and fumonisins in home-grown corn in areas with low and high incidences of EC in Transkei.[a]

	Low incidence of EC, Bizana District		High incidence of EC, Kentani District	
Advanced cytologic changes (%)[b]	4.2		36.7	
	Healthy corn	Moldy corn	Healthy corn	Moldy corn
F. verticillioides (%)[c]	8.3	34.5	42.0	67.7
Fumonisins (μg/g)[c]	0.3	9.0	2.1	31.5

[a]Data from Marasas et al. (*52*) and Rheeder et al. (*53*). [b]In occupants of 12 households in each area in 1985. [c]In samples of healthy and moldy corn from each household.

reviews (*41–48*). The First Conference on Fumonisins was held in Ames, Iowa, USA, from 6 to 7 September 1990. This was followed by other international conferences on fumonisins (*49,50*).

The International Agency for Research on Cancer in Lyon, France, evaluated the toxins produced by *F. moniliforme* as Group 2B carcinogens (i.e., *possibly carcinogenic to humans*) in 1993 (*51*).

Esophageal Cancer in Transkei Revisited: 1981–1998

At this juncture we return to Transkei in South Africa, where we first demonstrated a link between *F. verticillioides* and EC in 1981 (*10*) and between fumonisins and EC in 1990 (*28*). These correlations between the incidence of *F. verticillioides* and fumonisin levels in home-grown corn and EC rate were confirmed in 1988 (*52*) and 1992 (*53*). The data obtained in Transkei over six seasons between 1976 and 1989 are summarized in Tables 1 and 2.

It is clear from Table 1 that *F. verticillioides* is significantly more prevalent in healthy corn as well as moldy corn from areas with a high incidence of EC than from those areas with a low incidence of EC in Transkei. Similarly, fumonisin levels in healthy as well as moldy corn from areas with a high incidence of EC are significantly higher than in areas with low incidence (Table 2). The data clearly identify the area with a high incidence of EC, comprising Kentani and Butterworth districts in southern Transkei, as an ecologic zone that favors the infection of corn ears by *F. verticillioides* and the concomitant production of fumonisins in the infected kernels. Conversely, the areas with low incidence, comprising Bizana and Lusikisiki districts in northern Transkei, are not favorable for the development of *F. verticillioides* ear rot and fumonisin production in corn. In fact, the area with a low incidence of EC was found to be much more conducive to another *Fusarium* sp. that causes ear rot of corn, i.e., *F. graminearum* Schwabe, and the production of three mycotoxins by this fungus, i.e., deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEA), in corn than the area with a high incidence of EC (Table 3). The two areas are compared in Table 4 with respect to climatic, geographic, geologic, and soil fertility factors that may be important in determining the mycotoxicologic differences between the areas.

Incidence rates of EC in high-incidence (Kentani district) and low-incidence (Bizana district) areas in Transkei from 1955 to 1990 are compared in Table 5. It is clear that from 1955 to 1959 the two areas were distinctly different with respect to EC incidence rates in both males and females, i.e., very low (2.6 and 1.8) in Bizana and very high (54.2 and 30.3) in Kentani. The rates in Kentani have consistently stayed very high and in 1985–1990 were very similar (55.6 and 22.1) to those recorded from 1955 to 1959. In Bizana, however, incidence rates in both males and females increased markedly, and from 1985 to 1990 the rates were not much different (37.0 and 11.7) from those in Kentani. Because of the numerous problems and pitfalls associated with cancer registry in remote rural areas of Africa, it is not clear whether the increased EC rates in Bizana are real or are reflections of changes in cancer registry patterns due to demographic, socioeconomic, political, and/or other factors.

In a comparative study of the incidence of esophageal cytologic abnormalities determined by means of brush biopsies in residents of the two areas in 1985, it was found that mild and advanced cytologic changes occurred much more frequently in the high EC area (Kentani, 50.0 and 36.7%, respectively) than in the area with a low incidence of EC (Bizana, 12.5 and 4.2%, respectively) (*52*). It can be assumed that residents of both areas in Transkei have nutritional deficiencies for several mineral elements and vitamins, as indicated by the blood biochemical parameters in Table 6. Esophageal cytologic changes were determined in the occupants of 12 households in each area, and samples of healthy and moldy corn were collected simultaneously from each household and analyzed for *F. verticillioides* and fumonisins (Table 7). The incidence of advanced esophageal cytologic changes was higher in the occupants of the 12 households in the area with a high incidence of EC than in the area with a low incidence of EC, as were the levels of *F. verticillioides* and of fumonisins in the corn from the households in the area with a high incidence of EC (Table 7). Although the occupants of the households in both areas probably suffered from underlying nutritional deficiencies (Table 6), the residents of Kentani district in the area with a high incidence of EC had higher levels of fumonisins in the home-grown corn stored in their houses and also had higher incidences of esophageal cytologic abnormalities than residents of Bizana district in the area with a low incidence of EC (Table 7).

Although FB_1 and FB_2 were first reported to occur naturally in corn from Transkei (*26*), fumonisins have also been reported subsequently to occur in home-grown and/or commercial corn in several other areas throughout the world with a high incidence

Table 8. Fumonisin levels reported in corn from different high-risk areas for EC.

Area	Maximum total fumonisins (µg/g)	Reference
China—Linxian County	155	(*57*)
Transkei—Kentani District	117	(*53*)
Zimbabwe	4	(*58*)
Italy—Pordenone Province	4	(*59*)
United States—Charleston, South Carolina	2	(*60*)

Table 9. PDI of fumonisins in home-grown and commercial corn of rural and urban South Africans.[a]

Population group	Fumonisin levels in corn (µg/g)	Corn intake (g/70 kg bw/day)	PDI (µg/kg bw/day)
South Africa, rural (moldy corn)	54.0	460	354.9
South Africa, rural (healthy corn)	7.1	460	46.6
South Africa, urban	0.3	276	1.2

[a]Data from Marasas (*61*).

Table 10. Sa/So ratios in humans at risk for EC in Transkei.[a]

	Plasma[b]	Urine[b]
Range	0.01–2.97	0.01–5.75
Mean	0.34 ± 0.36	0.41 ± 0.72
Total fumonisins in corn samples (µg/g)[c]		
Healthy corn: 0–7.2	$\bar{x}$ = 0.6	
Moldy corn: 0.03–37.8	$\bar{x}$ = 4.8	

[a]Data from van der Westhuizen et al. (*67*). [b]Determined in 150 residents of Kentani district, Transkei, in 1997. [c]Determined in samples of healthy and moldy home-grown corn collected in Kentani in 1997.

of EC (Table 8). It is clear that fumonisins occur in corn consumed by humans at risk for EC in some areas in Africa, Asia, Europe, and the United States. The question remains whether some individuals (who take in higher levels of fumonisins in corn consumed as the staple diet than others) are at higher risk to develop cytologic abnormalities in the esophagus that may terminate in EC. The first step required to answer this question is to assess human fumonisin intake. For this assessment, two approaches are used: calculation of the probable daily intake (PDI) and measurement of biomarkers for fumonisin exposure in humans (*61*).

Human fumonisin intake can be calculated from analyses of naturally occurring levels of fumonisins in corn and data on corn intake and expressed as the PDI (Table 9). Both the level of fumonisins in the corn and the amount of corn consumed influence the PDI (i.e., the higher the level and the larger the amount consumed, the higher the PDI). Large variations are apparent in the PDI, ranging from 1.2 µg/kg body weight (bw)/day, in urban South Africans consuming commercial corn, to 354.9 µg/kg bw/day, in rural South Africans consuming moldy home-grown corn. The rural population in Transkei who are at the highest risk for EC in South Africa have the highest daily corn intake and also consume home-grown corn containing the highest levels of fumonisins. Thus, the PDI can be a useful estimate of the fumonisins intake of population groups such as the rural population in Transkei, and although it can be applied to individuals by means of food-from-the-plate analyses, this is not easy to do.

Biomarkers are more accurate as measures of individual intakes, but unfortunately, biomarkers for human fumonisin intakes are not yet available. However, fumonisins disrupt sphingolipid biosynthesis (*40*), and the resulting elevation in the sphinganine/sphingosine (Sa/So) ratio in serum, plasma, or urine has been used as a biomarker in animals, including nonhuman primates (*62,63*). Moreover, analytical techniques have been developed to determine Sa/So levels in humans, and ratios of 0.09–0.78 have been reported in serum (*64*) and ratios of 0.04–0.60 in urine (*65,66*).

In a recent article by van der Westhuizen et al. (*67*), Sa/So ratios were reported in the plasma and urine of residents of Kentani district in the area with a high incidence of EC in Transkei (Table 10). Although the mean values were similar to those reported in human serum and urine above, the upper ranges of the ratios in both plasma (2.97) and urine (5.75) were much higher than those previously reported. It remains to be determined whether:

- The Sa/So ratio is sensitive enough as a biomarker of human intake of fumonisins at the levels of contamination examined to date (Table 10).
- Variation in the ratio between individuals and within an individual over time can be accommodated.
- Genetic polymorphisms of ceramide synthase exist and contribute to individual susceptibility to fumonisins.
- Individuals with high Sa/So ratios in areas in Transkei with a high incidence of EC have high intakes of fumonisins and are at high risk for EC.

Y2K—Much more work remains to be done on the fumonisins in the Third Millennium!

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