TOXINS DERIVED FROM *FUSARIUM MONILIFORME*: FUMONISINS B_1 AND B_2 AND FUSARIN C

Fusarium moniliforme and a number of related species are ubiquitous on maize. These fungi produce fumonisins and fusarins. Six fumonisins have so far been isolated from *F. moniliforme* and characterized. Fumonisin B_1, fumonisin B_2 and fumonisin B_3 are the major ones produced in nature, while fumonisin B_4 is produced in relatively minor quantities (Thiel *et al.*, 1993). Only fumonisins B_1 and B_2 are considered in this monograph. A review on fumonisins is available (Riley & Richard, 1992). Fusarin C is a member of a family of unstable compounds which includes fusarins A, B, C, D, E and F (Savard & Miller, 1992).

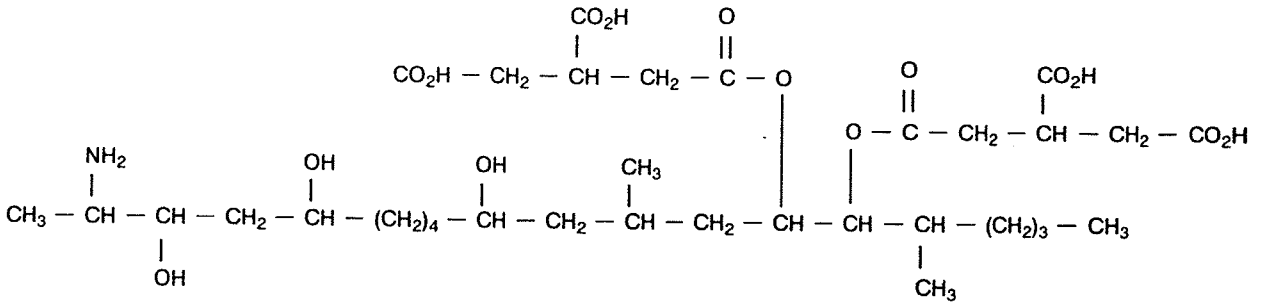
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Fumonisin B_1

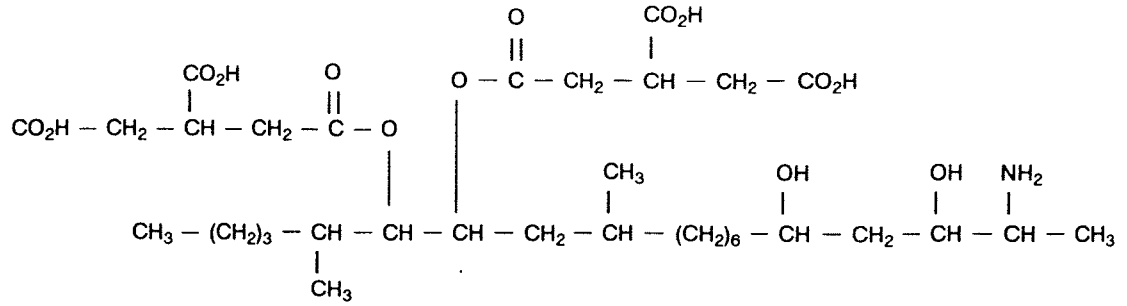
Chem. Abstr. Services Reg. No.: 116355-83-0
Chem. Abstr. Name: 1,2,3-Propanetricarboxylic acid, 1,1′-[1-(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester
Synonym: Macrofusine



$C_{34}H_{59}NO_{15}$ Mol. wt: 721

Fumonisin B_2

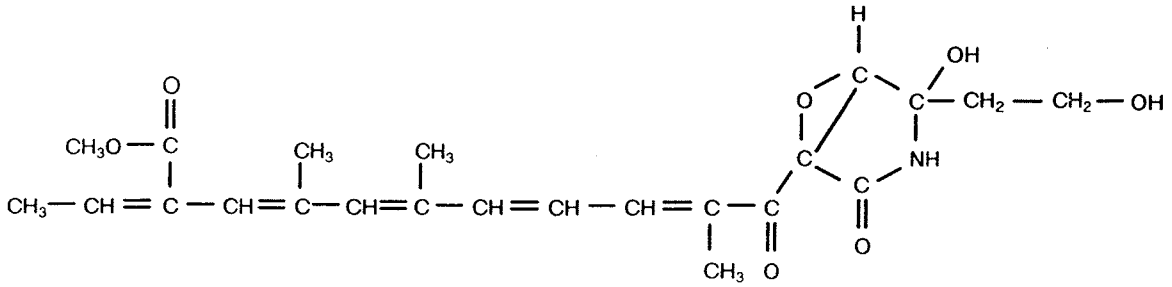
Chem. Abstr. Services Reg. No.: 116355-84-1
Chem. Abstr. Name: 1,2,3-Propanetricarboxylic acid, 1,1′-[1-(12-amino-9,11-dihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester



$C_{34}H_{59}NO_{14}$ Mol. wt: 705

Fusarin C

Chem. Abstr. Services Reg. No.: 79748–81–5
Chem. Abstr. Name: 3,5,7,9-Undecatetraenoic acid, 2-ethylidene-11-[4-hydroxy-4-(2-hydroxyethyl)-2-oxo-6-oxa-3-azabicyclo-[3.1.0]hex-1-yl]-4,6,10-trimethyl-11-oxo, methyl ester, [1R-[1α(2E,3E,5E,7E,9E),4α,5α]]-
Synonym: 6-Oxa-3-azabicyclo[3.1.0]hexane, 3,5,7,9-undecatetraenoic acid derivative



$C_{23}H_{29}NO_7$ Mol. wt: 431.5

1.1.2 *Chemical and physical properties*

Fumonisins B_1 and B_2

(*a*) *Description*: Powder, very hygroscopic
(*b*) *Melting-point*: Not known (has not been crystallized)
(*c*) *Spectroscopy data*: Mass spectral and nuclear magnetic resonance data have been reported (Bezuidenhout *et al.*, 1988; Laurent *et al.*, 1989; Plattner *et al.*, 1990).
(*d*) *Solubility*: Soluble in methanol (Sydenham *et al.*, 1992a)
(*e*) *Stability*: Stable in acetonitrile:water (1:1) and to light

Fusarin C

From Farber and Scott (1989), unless otherwise specified
(*a*) *Description*: Yellow oil
(*b*) *Melting-point*: Not known
(*c*) *Optical rotation*: $[\alpha]_D^{23}$ +47.04° (2% in methanol)

(*d*) *Spectroscopy data*: Ultraviolet, infrared, nuclear magnetic resonance and mass spectral data have been reported (Wiebe & Bjeldanes, 1981).
(*e*) *Solubility*: Soluble in ethanol and methanol
(*f*) *Stability*: Unstable on exposure to light and heat; decomposes rapidly as pH increases (Zhu & Jeffrey, 1992)

1.1.3 *Analysis*

Fumonisins B_1 and B_2

The analysis of fumonisins in maize presents major difficulties. A water/methanol extraction of grain followed by ion-exchange chromatography is used to extract fumonisins (Shephard *et al.*, 1990; Sydenham *et al.*, 1992a). Analytical, thin-layer chromatographic methods exist, but these are not useful for quantification (Sydenham *et al.*, 1992a). There are two principal approaches for quantification: (i) hydrolysis followed by derivatization and detection by gas chromatography/mass spectroscopy of the esterified tricarballylic acid (propane-1,2,3-tricarboxylic acid) or the derivatized aminopentol backbone (Plattner *et al.*, 1990; Sydenham *et al.*, 1990a; Scott, 1992; Shephard *et al.*, 1992; Thiel *et al.*, 1993), which has the disadvantage that all fumonisins are determined together; and (ii) preparation of a fluorescent derivative followed by high-performance liquid chromatography (HPLC)–fluorescence detection (Shephard *et al.*, 1990; Plattner *et al.*, 1991). Two derivatives have been tested: The first was a fluorescamine derivative, which unfortunately results in two peaks (Sydenham *et al.*, 1990a); the other was *ortho*-phthaldialdehyde (Shephard *et al.*, 1990). Use of the method has proven satisfactory for the analysis of fumonisins in maize and mixed horse-feed samples, with a limit of detection of 50 ng/g for fumonisin B_1 and 100 ng/g for fumonisin B_2 (Shephard *et al.*, 1990; Thiel *et al.*, 1993). As the *ortho*-phthaldialdehyde derivative has been reported to be unstable, use of a 4-fluoro-7-nitrobenzofurazan derivative was investigated and has also proven useful (Scott & Lawrence, 1991).

A method has been reported for the analysis of fumonisin B_1 in plasma and urine involving solid-phase anion-exchange clean-up, precolumn derivatization with *ortho*-phthaldialdehyde and reverse-phase HPLC with fluorescence detection, at a detection limit of 50 ng/ml (Shephard *et al.*, 1992).

Fusarin C

A detailed review of the few available analytical methods is provided by Farber and Scott (1989). Thin-layer chromatographic methods have been developed, but reliable quantification requires the use of HPLC with ultraviolet detection. Serious questions have been raised, however, as to whether these methods are adequate, in view of the instability of fusarins under extraction conditions: The mutagenicity of extracts containing fusarin C is greater than can be explained on the basis of measured fusarin C concentrations. Rearrangements of the co-occurring fusarins (E and F) under analytical conditions may result in underestimates of the amount of fusarins present in *Fusarium*-infected maize (Savard & Miller, 1992).

1.2 Production and use

1.2.1 *Production*

Fumonisins B_1 and B_2

Fumonisins B_1 and B_2 were first isolated in 1988 by Bezuidenhout *et al.* (1988); shortly thereafter, fumonisin B_1 was isolated as 'macrofusine' by Laurent *et al.* (1989) from cultures of *Fusarium moniliforme* (*F. verticillioides*) (Marasas *et al.*, 1981). Fumonisins can be produced at concentrations of several grams per kilogram by culturing strains of the fungi that produce this toxin on sterilized maize (Vesonder *et al.*, 1990; Cawood *et al.* 1991): *F. moniliforme* MRC 826 produced 7100 mg/kg fumonisin B_1 and 3000 mg/kg fumonisin B_2 (Thiel *et al.*, 1991a). They can also be produced in liquid cultures (Jackson & Bennett, 1990). In Southeast Asian strains, 0.66–192 mg/l fumonisin B_1 plus fumonisin B_2 have been obtained in liquid fermentations, and high recoveries of the toxins are possible (Miller *et al.*, 1993).

Of 90 strains of *F. moniliforme* that have been grown on autoclaved maize, 61 were found to contain fumonisin B_1, at levels ranging from 48 to 6400 mg/kg (Nelson *et al.*, 1991). Fumonisins B_1 and B_2 are also produced by some related species, including *F. anthophilum*, *F. dlamini*, *F. proliferatum*, *F. napiforme* and *F. nygamai*. These strains have been isolated in Africa, Australia, Nepal, New Caledonia and the USA (Nelson *et al.*, 1991; Thiel *et al.*, 1991a; Nelson *et al.*, 1992) and in Indonesia, Italy, the Philippines, Poland and Thailand (Miller *et al.*, 1993; Nelson *et al.*, 1992; Visconti, 1992). Fumonisins B_1 and B_2 invariably occur together; however, because B_2 always represents 15–35% of B_1, low levels of B_1 may appear to contain 'no detectable' B_2.

Fusarin C

Fusarin C was first isolated from a culture of *F. moniliforme* grown on sterilized corn (Wiebe & Bjeldanes, 1981). The absolute configuration was determined in 1984 (Gelderblom *et al.*, 1984a), and it was reported as a natural product of maize in 1984 (Gelderblom *et al.*, 1984b). Fusarin C can be produced by culturing strains of the species that produce this toxin on sterilized maize (Gelderblom *et al.*, 1983). It can also be produced in liquid culture (Farber & Scott, 1989).

Fusarin C is produced by several species of *Fusarium*, including *F. moniliforme*, *F. poae*, *F. avenaceum*, *F. crookwellense*, *F. culmorum*, *F. graminearum* and *F. sambucinum* (Marasas *et al.*, 1984a; Thrane, 1989).

1.3 Occurrence

Fumonisins B_1 and B_2

Fumonisins are widely distributed in maize products, including those from Europe, although limited numbers of analyses have been published outside South Africa and the USA (Thiel *et al.*, 1992) (Table 1).

No data are available about the effects of milling and baking on levels of fumonisins nor on their transmission into milk, meat and eggs. Ammonia treatment was ineffective in

reducing fumonisin concentrations in maize (Norred *et al.*, 1991b). After fermentation of maize containing fumonisins, most of the toxin was recovered in the spent grains (Bothast *et al.*, 1992).

Table 1. Natural occurrence of fumonisins B_1 and B_2

Country	Product	Year	Fumonisin B_1		Fumonisin B_2		Reference
			Positive samples/ total no.	Content (mg/kg)	Positive samples/ total no.	Content (mg/kg)	
North America							
USA	Maize feed	1989	3/3	37–122	3/3	2–23	Wilson *et al.* (1990)
USA	Damaged maize kernels		2/2	144, 148	2/2	31, 41	
USA	Maize feed	1983–86	14/14	1.3–27.0	14/14	0.1–12.6	Thiel *et al.* (1991b)
USA	Maize feed	1989–90	177/232	1–330			Ross *et al.* (1991)
USA	Maize feed	NR	2/2	20, 150			Plattner *et al.* (1990)
USA	Maize meal	1990–91	15/16	av. 1.0	13/16	0.3	Sydenham
USA	Maize grits		10/10	av. 0.6	5/10	0.4	*et al.* (1991)
USA	Cornflakes		0/2		0/2		
Canada	Maize meal		1/2	0.05	0/2		
South America							
Brazil	Maize	1985, 1990	20/21	0.2–38.5	18/21	0.1–12.0	Sydenham *et al.* (1992b)
Peru	Maize meal	1990–91	1/2	0.66	1/2	0.13	Sydenham *et al.* (1991)
Europe							
Austria	Maize	1988–89	3/9	< 15			Lew *et al.* (1991)
Italy	Maize feed	NR	23/25	0.01–8.4	13/25	0.01–1.33	Minervini *et al.* (1993)
Africa							
Transkei	Maize	NR	3/3	< 10–83			Sydenham *et al.* (1990a)
Transkei	Maize	1985	39/48	0.2–46.9	37/48	0.15–16.3	Sydenham *et al.* (1990b)
Transkei	Maize	1985, 1989	61/74	0.05–117.5	55/74	0.05–22.9	Rheeder *et al.* (1992)
South Africa	Maize meal	1990–91	46/52	Mean, 0.14	11/52	Mean, 0.08	Sydenham
South Africa	Maize grits		10/18	Mean, 0.13	4/18	Mean, 0.09	*et al.* (1991)

NR, not reported

Fusarin C

Fusarin C was determined in two samples of maize from southern Africa at concentrations of 0.02 and 0.28 mg/kg (Gelderblom *et al.*, 1984b). One maize sample from the USA was reported to contain 0.39 mg/kg (Thiel *et al.*, 1986), but analysis of 12 maize samples from Canada revealed no fusarin C (Farber & Scott, 1989). Natural occurrence in maize has also been reported from China (Cheng *et al.*, 1985).

1.4 Regulations and guidelines

No regulation or guidelines exists for these compounds (van Egmond, 1989).

2. Studies of Cancer in Humans

Ecological studies of the relationship between exposure to *Fusarium* toxins and oesophageal cancer are summarized in the monograph on toxins derived from *F. graminearum*, *F. culmorum* and *F. crookwellense*, p. 409. Most of the studies refer to mixtures of many toxins from many species of fungi on maize.

In the study of Marasas *et al.* (1981), the proportion of kernels in both mouldy and healthy maize samples infected by *F. moniliforme*, one of the most prevalent fungi in maize in the Transkei, was significantly correlated with oesophageal cancer rates.

In the study of Marasas *et al.* (1988a), described in detail on p. 410, the mean proportions of maize kernels infected with *F. moniliforme* in both healthy and mouldy maize samples from households in the high-incidence oesophageal cancer area were significantly higher (42% and 68%, respectively) than those in the low-incidence area (8% and 35%, respectively). A similar survey was conducted one year later, with the same criteria for high and low incidence but adding 24 households from a study area with an intermediate incidence of oesophageal cancer. Although the proportion of kernels infected with *F. moniliforme* in healthy maize from the latter area was in between those from the high- and low-incidence areas, further subdivision of households in the intermediate-incidence area into 12 situated in a low-risk zone and 12 in a high-risk zone (with an estimated six-fold difference in oesophageal cancer rates) did not reveal a difference in the proportion of infected kernels in the corresponding samples (26 and 24%, respectively). Furthermore, there was no difference in the prevalence of cytological abnormalities of the oesophagus in adult occupants of the low- and high-risk zones of the intermediate-incidence area. [The Working Group noted that the sampling strategy was different in the high- and low-risk areas.]

In the study of Sydenham *et al.* (1990b), significantly higher mean numbers of kernels infected with *F. moniliforme* and correspondingly higher levels of the mycotoxins fumonisin B_1 and fumonisin B_2 were found in mouldy maize samples in the high-risk oesophageal cancer area than in the low-risk area ($p < 0.01$). Fumonisin B_1 and B_2 levels in healthy maize samples from the low-risk area were approximately 20 times lower than those in healthy samples from the high-risk area, and only three out of 12 samples contained these toxins. [The Working Group noted that the sampling strategy was different in the high- and low-risk areas.]

Rheeder *et al.* (1992) reanalysed the samples of healthy and mouldy home-grown maize collected from high-risk and low-risk oesophageal cancer areas during 1976–86 which had been examined by Marasas *et al.* (1979, 1981, 1988a) and by Sydenham *et al.* (1990b). The material was supplemented by samples obtained during 1989 from the same study areas. Samples collected from high-risk areas in 1985 and 1986 were taken from preselected households (Marasas *et al.*, 1988a); all other samples were taken at random. During the entire period, the percentages of kernels infected by *F. moniliforme* in healthy as well as mouldy samples were significantly higher in the high-risk oesophageal cancer area than in the low-risk area. The samples collected in 1985 and 1989 were analysed for the presence of fumonisin B_1 and fumonisin B_2: Both toxins occurred in more samples and at significantly higher levels in healthy and mouldy maize obtained in 1985 and at significantly higher levels in mouldy maize obtained from high-risk areas than from low-risk areas in 1986.

Zhen *et al.* (1984) cultured and isolated fungal strains from samples of wheat, maize, dried sweet potato, rice and soya beans in five counties with a high incidence of oesophageal cancer and three with a low incidence, in Henan Province, northern China. Mortality rates for males ranged between 26.5 and 37.0/100 000 in the low-risk counties and between 76.6 and 161.3 in the high-risk counties. The frequency of contamination by *F. moniliforme* was higher in samples from high-risk counties (6.8% out of 2009 measurements) than in samples from low-risk areas (5.4% out of 830 measurements) ($p < 0.001$). The frequency of contamination by all other fungi analysed was also significantly higher in samples from high-risk counties.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

A group of 29 female DBA mice, 8–10 weeks of age, were given 0.5 mg fusarin C [purity unspecified] by gavage twice a week; when toxic effects became apparent, the dose was decreased to 0.05 mg twice a week. A control group of 20 mice was available. Animals were evaluated for development of forestomach and oesophageal tumours and were observed to a maximum of 655 days after initiation of dosing. Dysplasia in the forestomach and oesophagus was observed in 2/28 treated animals, papillomas of the forestomach and oesophagus in 3/28 and carcinomas of the forestomach and oesophagus in 3/28. There was no evidence of such lesions in the control group (Li *et al.*, 1992).

3.1.2 *Rat*

A group of 31 female Wistar rats [age unspecified] were fed a diet containing maize bread inoculated with *F. moniliforme*. After 554–701 days of feeding, four papillomas and two early carcinomas had developed in forestomachs. No epithelial lesion of the forestomach was seen in a control group of 10 female rats given conventional maize bread for 330–700 days (Li *et al.*, 1982). [The Working Group noted the inadequate reporting of the study.]

Groups of 20 male inbred BDIX rats [age unspecified] were fed commercial rat feed containing 0 (8% uninoculated maize) or 4% freeze-dried or 4% oven-dried culture material that had been inoculated with *F. moniliforme* MRC 826 for 286 days and then 2% until termination of the study at day 763. The incidence of liver tumours (hepatocellular and cholangiocellular carcinomas combined) was increased: control, 0/20; 4% freeze-dried, 13/20; and 4% oven-dried, 16/20 (Marasas *et al.*, 1984b).

Two groups of 12 male Fischer rats, weighing approximately 125 g, were fed either commercial rodent chow or maize contaminated with *F. moniliforme* that had caused an outbreak of leukoencephalomalacia in horses (see p. 454). Individually treated rats were necropsied on days 123–145 and the remaining 8 treated and 12 control rats on day 176 after the start of the experiment. All treated rats had hepatic nodules, cholangiofibrosis or cholangiocarcinomas [numbers not given], while no such lesion was found in the controls (Wilson *et al.*, 1985).

Groups of 30 male inbred BDIX rats, weighing approximately 110 g, were fed semi-purified diets containing 0 (5% maize meal), 0.5% *F. moniliforme* MRC 826 culture material (containing 364 mg/kg fusarin C and later found to produce fumonisins B_1 and B_2) or 5% *F. moniliforme* MRC 1069 culture material (containing 104 mg/kg fusarin C). In rats treated with 0.5% MRC 826 and examined at 23–27 months, neoplastic hepatic nodules occurred in all 21 surviving animals and in none of 22 controls; in addition, two hepatocellular and eight cholangiocellular carcinomas were observed, and there were increased incidences of forestomach papillomas (13/21 *versus* 5/22) and carcinomas (4/21 *versus* 0/22). In rats treated with 5% MRC 1069, the incidence of neither liver nor forestomach tumours was significantly increased (Jaskiewicz *et al.*, 1987).

Groups of 25 male inbred BDIX rats, weighing 70–80 g, were fed a modified cereal-based diet containing 0 or 50 mg/kg fumonisin B_1 (purified from culture material of *F. moniliforme* MRC 826). Five rats from each group were killed at 6, 12 and 20 months to assess the progression of liver lesions; the remaining rats were killed at 26 months. No hepatocellular carcinoma was observed in treated or control rats at 6 or 12 months; at 18–26 months, however, 10/15 treated rats and 0/15 controls had hepatocellular carcinomas (Gelderblom *et al.*, 1991).

A group of 20 female Wistar rats, weighing 80–120 g, were given 2 mg fusarin C [purity unspecified] by gavage twice a week; as body weights increased, the dose of fusarin C was increased to 3 mg twice a week. A control group of 25 rats was available. Animals were observed to a maximum of 742–814 days. Dysplasia of the forestomach and oesophagus was observed in 1/20 treated animals, papillomas of the forestomach and oesophagus in 5/20 and carcinomas of the forestomach and oesophagus in 5/20. There was no evidence of such lesions in the control group (Li *et al.*, 1992).

3.2 Administration with known carcinogens

3.2.1 *Mouse*

Groups of 10 female ICR/Ha mice, seven weeks of age, were each treated with a single application of 220 or 500 μg fusarin C [purity unspecified] in 0.1 ml acetone on the shaved back, followed one week later by twice weekly skin applications of 2 μg 12-*O*-tetradecanoyl-

phorbol 13-acetate (TPA) in 0.1 ml acetone for 16 weeks. A group of nine positive controls each received a single application of 50 μg 7,12-dimethylbenz[*a*]anthracene (DMBA) on the dorsal skin, followed one week later by twice weekly applications of 2 μg TPA for 16 weeks. A group of eight mice each received an application of 50 μg DMBA followed by acetone, and 10 mice each received an application of 0.1 ml acetone followed by TPA. Nine of nine mice treated with DMBA followed by TPA had multiple skin papillomas (34), while no skin papilloma was seen in mice painted with DMBA plus acetone or acetone plus TPA. One mouse that received 220 μg fusarin C followed by TPA had two skin papillomas, but there was no skin tumour in mice that received 500 μg fusarin C (Gelderblom *et al.*, 1986).

3.2.2 *Rat*

Groups of five to seven male BDIX and four to five female Wistar rats, seven weeks old, were fed a synthetic diet and subjected to a two-thirds partial hepatectomy. On the day after surgery, the rats were given a single intraperitoneal injection of 0, 50 or 100 mg/kg bw fusarin C [purity unspecified] in dimethyl sulfoxide and one week later were maintained on 0.05% phenobarbital in drinking-water for 14 weeks. At that time, their livers were examined for the presence of foci of altered hepatocytes, as revealed by γ-glutamyltranspeptidase-positive (GGT^+) staining. Fusarin C did not increase the number of foci of altered hepatocytes (Gelderblom *et al.*, 1986).

Groups of four to five male inbred BDIX rats, weighing approximately 150 g, were given an intraperitoneal injection of 0 or 200 mg/kg bw *N*-nitrosodiethylamine (NDEA), were held for one week and were then fed diets containing 0 or 0.1% (1 g/kg diet) fumonisin B_1 (purity, 92%) for four weeks. Rats fed the control diet following exposure to NDEA or no treatment had no detectable foci of altered hepatocytes, determined by GGT^+ staining. Rats fed only fumonisin B_1 had an average of 20 ± 8 foci/cm^2, while those fed fumonisin B_1 following NDEA treatment had an average of 55 ± 10 foci/cm^2 (Gelderblom *et al.*, 1988a).

Groups of four male Fischer rats, weighing 100–120 g, were fed a diet containing 0.1% fumonisin B_1 (purity, 90–95%) for 26 days and then subjected to partial hepatectomy. The resected liver lobes contained 2.9 ± 0.7 foci/cm^2 of altered hepatocytes (identified by GGT^+ staining) and a total area of foci of 0.42 ± 0.34% of liver section area; untreated controls had 1.0 ± 0.2 foci/cm^2 and a total area of 0.01 ± 0.00%. These differences were significant. Two weeks after partial hepatectomy, rats received a daily dose of 2-acetylaminofluorene (20 mg/kg bw by gavage) for three consecutive days, followed by an oral dose of 2 ml/kg bw carbon tetrachloride on the fourth day. Ten days later they were sacrificed and found to have 7.1 ± 0.8 foci/cm^2 comprising 15.6 ± 8.2% of section area in the liver. Controls subjected to the same treatment without fumonisin B_1 had 1.2 ± 0.3 foci/cm^2 and a total area of 0.07 ± 0.05%. These differences were significant (Gelderblom *et al.*, 1992a).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Lu *et al.* (1990) studied the distribution and elimination of ^{3}H-fusarin C given by gavage to rats. The highest levels of radiolabel were found in the intestines, stomach and liver; lower levels were found in the kidney, bladder, oesophagus and spleen. Levels of radiolabel in the lungs and brain were low. Those in the blood reached a peak at 3 h after administration, but about 50% remained in the blood even after 24 h. Total urinary excretion of radiolabel was found to be about 31% within 48 h, and about 28% was excreted in the faeces. Only 5.4% unchanged fusarin C was excreted in the urine, and metabolites accounted for 94.6% of the total urinary radiolabel.

Two rat liver microsomal enzymes, carboxylesterase and a monooxygenase, are involved in the metabolism of fusarin C. The carboxyesterase catalyses the conversion of fusarin C to the water-soluble fusarin PM_1 (see Fig. 1) (Gelderblom *et al.*, 1988b). The conversion of fusarin C to an active mutagenic metabolite(s) is catalysed by a monooxygenase (Gelderblom *et al.*, 1984c, 1988b). It has been suggested that esterases metabolize fusarin C to a less mutagenic form: the mutagenicity of fusarin C to *Salmonella typhimurium* TA100 could be doubled by pretreating the microsomes with an esterase inhibitor (1 μM diisopropyl fluorophosphate) (Lu *et al.*, 1989).

Glutathione interacts *in vitro* both chemically and enzymatically with fusarin C, resulting in the formation of fusarin A (see Fig. 1), which does not have the C13–C14 epoxide group of fusarin C, and a compound that lacks the 2-pyrrolidone moiety, suggesting an interaction at the C13–C14 epoxide (Gelderblom *et al.*, 1988c).

4.2 Toxic effects

4.2.1 *Humans*

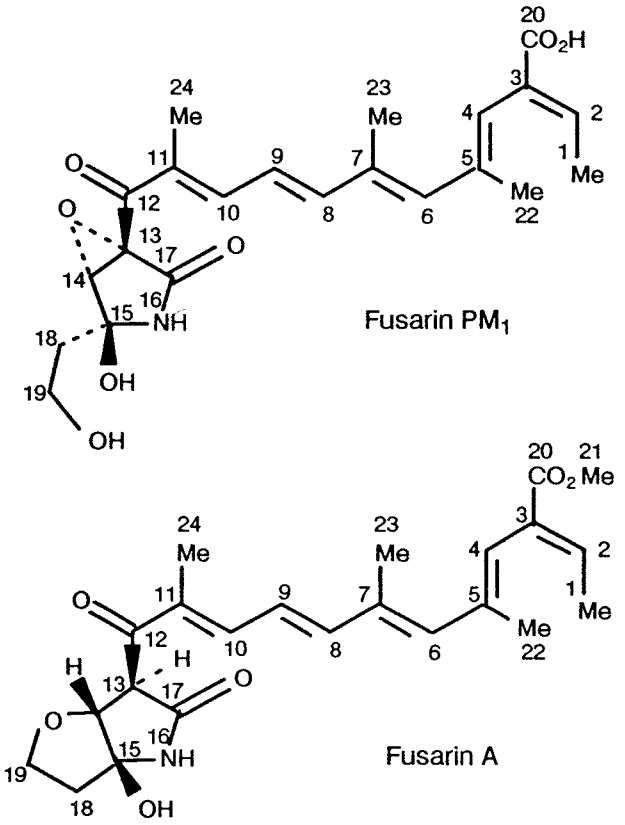
No data were available to the Working Group.

4.2.2 *Experimental systems*

Equine leukoencephalomalacia is a neurotoxic disease of horses, donkeys and mules and is characterized by multifocal liquefactive necrosis predominantly of the white matter in the cerebral hemispheres. This disease was reproduced experimentally by feeding pure cultures of *F. moniliforme* MRC 826 containing fumonisin B_1 to horses. A horse injected intravenously seven times from day 0 to day 9 with 0.125 mg/kg bw fumonisin B_1 per day showed clinical signs of neurotoxicosis on day 8. On day 10, the horse was killed while in tetanic convulsion. The principal lesions were severe oedema of the brain and early, bilaterally symmetrical leukoencephalomalacia in the brain stem (Marasas *et al.*, 1988b). The characteristic lesions of leukoencephalomalacia were also induced in two horses by oral administration of fumonisin B_1 (Kellerman *et al.*, 1990).

Three pigs were treated by daily intravenous injections of 0.17 mg/kg bw fumonisin B_1 for seven days, 0.4 mg/kg bw fumonisin B_1 for four days or 0.3 mg/kg fumonisin B_2 for five days. The highest dose of fumonisin B_1 was lethal on day 5; necropsy showed marked pulmonary oedema, hydrothorax and pancreatic lesions (focal to massive necrosis, acinar-cell

Fig. 1. Metabolites of fusarin C



From Gelderblom *et al.* (1988b)

dissociation and rounded individual acinar cells). No liver damage was detected. No such alteration was found after seven injections of the lower dose of fumonisin B_1 or with fumonisin B_2 (Harrison *et al.*, 1990).

Fumonisin B_1 was administered intravenously to two female SPF cross-bred swine, weighing 6–13 kg (total dose, 4.6 and 7.9 mg/kg or 67 and 72 mg per pig); and corn screenings naturally contaminated with 166 mg/kg fumonisin B_1 and 48 mg/kg fumonisin B_2 were given orally to three swine (total doses of fumonisins B_1 and B_2 ranged from 176 to 645 mg per pig). All treated pigs had pulmonary and hepatic changes. At the lower doses, slowly progressive hepatic disease was the most prominent feature, while at the higher doses, acute pulmonary oedema was superimposed on hepatic injury. Pancreatic acinar cell degeneration and many other types of ultrastructural change were noted. The target organs in the pig were concluded to be the lung, liver and pancreas (Haschek *et al.*, 1992).

Rats fed diets containing 1 g/kg fumonisin B_1 for four weeks had a significant reduction in weight gain and developed an insidious, progressive toxic hepatitis similar to that induced by culture material of *F. moniliforme* MRC 826. Fatty changes and scant necrosis were present in the proximal convoluted tubules of the kidney. Prominent lymphoid necrosis in Peyer's patches and scattered focal, superficial and mid-zonal epithelial necrosis occurred in the mucosa of the stomach. Severe, disseminated acute myocardial necrosis and severe

pulmonary oedema were observed in two of the four rats (Gelderblom *et al.*, 1988a). Rats and mice were fed diets containing seed maize inoculated with *F. moniliforme* (containing 99 mg/kg fumonisin B_1) or ammoniated seed maize inoculated in the same way (containing 75 mg/kg fumonisin B_1) for four weeks. Hepatotoxicity, renal toxicity and adrenal cortical vacuolation were noted in rats and hepatotoxicity in mice, irrespective of ammoniation (Voss *et al.*, 1992). Fumonisin B_2, isolated and characterized along with fumonisin B_1 from *F. moniliforme* MRC 826, given at dietary levels of 0.05–0.1% induced early liver lesions in rats, similar to those induced by fumonisin B_1 (Gelderblom *et al.*, 1992b).

In a long-term experiment, rats were fed a diet containing 50 mg/kg fumonisin B_1 for 26 months. Treatment significantly increased the levels of the serum enzymes alanine aminotransferase, GGT and alkaline phosphatase, as well as those of creatinine and bilirubin (conjugated and non-conjugated). The results were considered to corroborate the histopathological observation that the liver is the main target organ of fumonisin B_1 (Gelderblom *et al.*, 1991).

The molecular mechanism of action of fumonisins is not known; however, these compounds bear a remarkable structural similarity to sphingosine, the long-chain (sphingoid) base backbone of sphingomyelin, cerebrosides, sulfatides, gangliosides and other sphingolipids. Sphingolipids are thought to be involved in the regulation of cell growth, differentiation and neoplastic transformation through participation in cell–cell communication and cell–substratum interactions and possibly through interactions with cell receptors and signalling systems. Incubation of rat hepatocytes with fumonisins B_1 and B_2 inhibited incorporation of ^{14}C-serine into the sphingosine moiety of cellular sphingolipids, at an IC_{50} of 0.1 μM. In contrast, fumonisin B_1 increased the amount of the biosynthetic intermediate, sphinganine, which suggests that fumonisins inhibit the conversion of ^{14}C-sphinganine to *N*-acyl-^{14}C-sphinganines, a step that is thought to precede introduction of the 4,5-*trans* double bond of sphingosine. In agreement with this mechanism, fumonisin B_1 inhibited the activity of sphingosine *N*-acyltransferase (ceramide synthase) in rat liver microsomes, with 50% inhibition at approximately 0.1 μM, and reduced the conversion of ^{3}H-sphingosine to ^{3}H-ceramide by intact hepatocytes. Fumonisin B_1 (1 μM) almost completely inhibited ^{14}C-sphingosine formation by hepatocytes (Wang *et al.*, 1991).

Fumonisins B_1 and B_2 were toxic *in vitro* to rat hepatoma and dog kidney MDCK cell lines, at IC_{50} values ranging from 2 to 10 μg/ml (Mirocha *et al.*, 1992).

Using a pig kidney cell line, LLC-PK_1, Yoo *et al.* (1992) demonstrated that both fumonisin B_1 and B_2 inhibit cell proliferation at concentrations between 10 and 35 μM, whereas concentrations > 35 μM caused cell death. Sphingolipid biosynthesis using ^{3}H-serine as the precursor was reduced at an EC_{50} of 10–15 μM; this effect was accompanied by a decrease in the ratio of ^{3}H-sphingosine: ^{3}H-sphinganine, which preceded the effect on cell proliferation. The two processes had a similar dependence on fumonisin concentration. The level of free sphinganine was elevated by 128 fold after exposure to 35 μM fumonisin B_1 for 24 h. These results support the hypothesis that inhibition of sphingolipid biosynthesis *de novo* is an early event in the toxic action of fumonisins on the pig kidney cell line LLC-PK_1, which is less sensitive than hepatocytes.

Shier *et al.* (1991) examined the effects of fumonisins B_1 and B_2 in a series of cultured mammalian cells lines. Approximate IC_{50} values for the most sensitive hepatoma line,

H4TG, were 4 and 2 μg/ml for fumonisins B_1 and B_2, respectively. An increase in the amount of free sphinganine and a reduction in complex sphingolipids were seen in serum samples from ponies given feed contaminated with 15–44 μg/g fumonisin B_1 (Wang *et al.*, 1992).

Treatment of macrophages *in vitro* with fusarin C (6 μg/ml) inhibited their activation by macrophage activating factor and muramyl dipeptide. Fusarin C also inhibited the cytotoxic activity of already activated macrophages. These effects were dose-dependent; they disappeared partially after 24 h in the absence of fusarin C, and completely after 72 h, and could be overcome by high concentrations of macrophage activating factor and antiserum, suggesting that fusarin C is not generally toxic to cells (Dong & Zhang, 1987). Fusarin C at a concentration of 2.5 μg/ml inhibited the growth of cultured lymphoma cells (Chen & Zhang, 1987). It inhibited valine incorporation into proteins of rat hepatocytes at 10^{-4}M and cell death at 10^{-3}M (Norred *et al.*, 1991a).

4.3 Reproductive and developmental toxicity

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Tables 2–4 and Appendices 1 and 2)

Fumonisins B_1 and B_2 were not mutagenic to *S. typhimurium* and did not induce unscheduled DNA synthesis in rat hepatocytes, either *in vitro* or *in vivo*.

Fusarin C induced DNA strand breakage in *S. typhimurium* TA100, but attempts to detect DNA adducts of fusarin C in the same organism by ^{32}P-postlabelling were unsuccessful. HPLC-purified fusarin C did not produce DNA adducts in calf thymus that could be measured by ^{32}P-postlabelling. HPLC-purified fusarin C induced asynchronous replication of polyoma virus in rat fibroblasts and was reported to bind *in vitro* to the DNA of rat oesophageal explants.

Fusarin C was mutagenic to *S. typhimurium* in the presence of an exogenous metabolic activation system. Crude extracts of *F. moniliforme* cultures were also reported to have direct mutagenic activity in *S. typhimurium* TA100; this activity decreased with increasing purity (Lu *et al.*, 1988). Fusarin C induced gene mutation, sister chromatid exchange, chromosomal aberrations and micronucleus formation in cultured Chinese hamster V79 cells.

Table 2. Genetic and related effects of fumonisin B_1

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500.0000	Gelderblom & Snyman (1991)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	2500.0000	Gelderblom & Snyman (1991)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000.0000	Gelderblom & Snyman (1991)
SAS, *Salmonella typhimurium* TA97a, reverse mutation	–	–	2500.0000	Gelderblom & Snyman (1991)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	60.0000	Gelderblom *et al.* (1992a)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		100 × 1 po	Gelderblom *et al.* (1992a)

–, negative; 0, not tested
[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw

Table 3. Genetic and related effects of fumonisin B_2

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000.0000	Gelderblom & Snyman (1991)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	2500.0000	Gelderblom & Snyman (1991)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000.0000	Gelderblom & Snyman (1991)
SAS, *Salmonella typhimurium* TA97a, reverse mutation	–	–	5000.0000	Gelderblom & Snyman (1991)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	30.0000	Gelderblom *et al.* (1992a)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		100 × 1 po	Gelderblom *et al.* (1992a)

–, negative; 0, not tested
[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw

Table 4. Genetic and related effects of fusarin C

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, DNA strand breaks, *Salmonella typhimurium* TA100	0	+	10.0000 (TLC)	Lu *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	+	0.1000 (TLC)	Wiebe & Bjeldanes (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.1000	Gelderblom *et al.* (1984c)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	+	2.5000	Cheng *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+[b]	+	2.5000 (TLC)	Lu *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	+	0.2500	Gelderblom *et al.* (1988c)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0500 (HPLC)	Lu *et al.* (1989)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	+	0.5000	Gelderblom & Snyman (1991)
SA2, *Salmonella typhimurium* TA102, reverse mutation	-	-	5.0000	Gelderblom & Snyman (1991)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	0.0000 (TLC)	Wiebe & Bjeldanes (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	0	-	25.0000 (TLC)	Lu *et al.* (1988)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	(+)	0.0000 (TLC)	Wiebe & Bjeldanes (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	(+)	0.0000 (TLC)	Wiebe & Bjeldanes (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	+	200.0000	Cheng *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	+	2.5000	Gelderblom & Snyman (1991)
SAS, *Salmonella typhimurium* TA97a, reverse mutation	-	+	2.5000	Gelderblom & Snyman (1991)
DIA, DNA replication, polyoma-transformed rat embryo fibroblast H3	+	0	0.0200 (HPLC)	Lu *et al.* (1988)
G9H, Gene mutation, Chinese hamster V79 lung cells *hprt* locus *in vitro*	-	+	50.0000	Cheng *et al.* (1985)
SIC, Sister chromatid exchange, Chinese hamster V79 lung cells *in vitro*	-	+	25.0000	Cheng *et al.* (1985)
CIC, Chromosomal aberrations, Chinese hamster V79 lung cells *in vitro*	-	+	50.0000	Cheng *et al.* (1985)

Table 4 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MIA, Micronucleus test, Chinese hamster V79 lung cells *in vitro*	–	+	50.0000	Cheng *et al.* (1985)
BID, DNA adducts, isolated calf thymus DNA *in vitro*	–	0	50.0000 (HPLC)	Lu *et al.* (1988)
BID, Binding to DNA of rat oesophageal explants *in vitro*	+	0	0.0000	Lu *et al.* (1990)
BID, DNA adducts, *Salmonella typhimurium* TA100	–	–	10.0000 (TLC)	Lu *et al.* (1988)

+, positive; (+), weakly positive; –, negative; 0, not tested
[a]In-vitro tests, μg/ml; purified by TLC (thin-layer chromatography) or HPLC (high-performance liquid chromatography)
[b]With preincubation only; one dose (9.9 μg/ml) tested

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Fumonisin B_1, fumonisin B_2 and fusarin C are produced by *Fusarium* species that occur primarily on maize. These toxins may occur particularly when maize is grown under warm, dry conditions. Exposure occurs through dietary consumption of contaminated maize. Populations that eat milled or ground maize as a dietary staple can therefore be exposed to significant amounts of fumonisins and to lesser amounts of fusarin C.

5.2 Human carcinogenicity data

The only studies available were correlation studies, most of which indicated some relationship between oesophageal cancer rates and the occurrence of *F. moniliforme* or its toxins in maize.

5.3 Animal carcinogenicity data

Cultures of *F. moniliforme* were tested by oral administration in two experiments in male rats of one strain. A culture of *F. moniliforme* known to produce significant amounts of fumonisins B_1 and B_2 induced neoplastic nodules, hepatocellular carcinomas and cholangiocellular carcinomas; in addition, forestomach papillomas and carcinomas were observed. A culture of *F. moniliforme*, known to contain mainly fusarin C, did not induce such tumours.

Two studies in which male rats were fed maize naturally contaminated with *F. moniliforme* were inadequate for evaluation.

Fumonisin B_1 was tested for carcinogenicity by oral administration in the diet in one experiment in male rats, producing hepatocellular carcinomas. It induced the formation of foci of altered (γ-glutamyltranspeptidase-positive) hepatocytes.

No data were available to the Working Group on the carcinogenicity of fumonisin B_2.

Fusarin C was tested in one study in female mice and female rats by oral gavage. It induced papillomas and carcinomas of the oesophagus and forestomach in mice and rats.

5.4 Other relevant data

Fumonisin B_1 causes outbreaks of leukoencephalomalacia in horses and pulmonary oedema in pigs. It is toxic to the central nervous system, liver, pancreas, kidney and lung in a number of animal species. Fumonisin B_2 is hepatotoxic in rats.

Fumonisins B_1 and B_2 do not induce unscheduled DNA synthesis in rat hepatocytes *in vivo* or *in vitro* or mutation in bacteria.

In single studies, fusarin C induces chromosomal anomalies, gene mutation and DNA damage in cultured rodent cells. It induces mutations in bacteria.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of toxins derived from *F. moniliforme*.

There is *sufficient evidence* in experimental animals for the carcinogenicity of cultures of *F. moniliforme* that contain significant amounts of fumonisins.

There is *limited evidence* in experimental animals for the carcinogenicity of fumonisin B_1.

There is *inadequate evidence* in experimental animals for the carcinogenicity of fumonisin B_2.

There is *limited evidence* in experimental animals for the carcinogenicity of fusarin C.

Overall evaluation

Toxins derived from *Fusarium moniliforme are possibly carcinogenic to humans (Group 2B).*

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[1]For definition of the italicized terms, see Preamble, pp. 26–29.

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