FUMONISIN B_1

This substance was considered by a previous working group in June 1992 (IARC, 1993). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

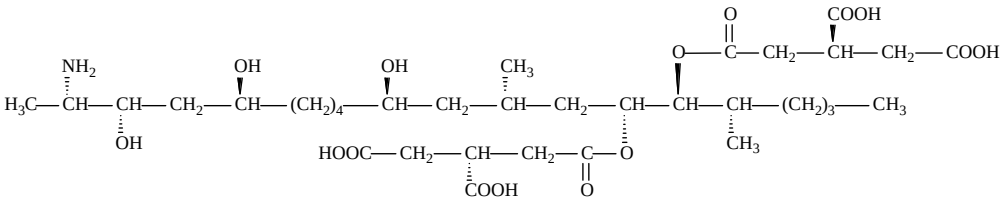
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 116355-83-0
Chem. Abstr. Serv. Name: 1,2,3-Propanetricarboxylic acid, 1,1′-[1-(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester
Synonyms: FB_1; macrofusine

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{34}H_{59}NO_{15}$ Relative molecular mass: 721

1.1.3 *Chemical and physical properties of the pure substance*

From WHO (2000) unless otherwise noted
(*a*) *Description*: White hygroscopic powder
(*b*) *Melting-point*: Not known (has not been crystallized)
(*c*) *Spectroscopy*: Mass spectral and nuclear magnetic resonance spectroscopy data have been reported (Bezuidenhout *et al.*, 1988; Laurent *et al*., 1989a; Plattner *et al*., 1990; Savard & Blackwell, 1994)

(*d*) *Solubility*: Soluble in water to at least to 20 g/L (National Toxicology Program, 2000); soluble in methanol, acetonitrile–water

(*e*) *Octanol/water partition coefficient* (log P): 1.84 (Norred *et al*., 1997)

(*f*) *Stability*: Stable in acetonitrile–water (1:1) at 25 °C; unstable in methanol at 25 °C, forming monomethyl or dimethyl esters (Gelderblom *et al*., 1992a; Visconti *et al*., 1994); stable in methanol at –18 °C (Visconti *et al*., 1994); stable at 78 °C in buffer solutions at pH between 4.8 and 9 (Howard *et al*., 1998)

1.1.4 *Analysis*

Methods for the analysis of fumonisins have been extensively reviewed (WHO, 2000). Six general analytical methods have been reported: thin-layer chromatographic (TLC), liquid chromatographic (LC), mass spectrometric (MS), post-hydrolysis gas chromatographic, immunochemical and electrophoretic methods (Sydenham & Shephard, 1996; Shephard, 1998).

An LC method for the determination of fumonisins B_1 and B_2 in maize and corn (maize) flakes was collaboratively studied. The method involves double extraction with acetonitrile–methanol–water (25:25:50), clean-up through an immunoaffinity column, and LC determination of the fumonisins after derivatization with *ortho*-phthaldialdehyde. This method has been proposed as the AOAC Official Method 2001.14, First Action (Visconti *et al*., 2001).

The majority of studies have been performed using LC analysis of a fluorescent derivative (WHO, 2000). There are no validated biomarkers for human exposure to fumonisin B_1.

Many studies, both *in vivo* and *in vitro*, have demonstrated a correlation between disruption of sphingolipid metabolism — as measured by an increase in free sphinganine — and exposure to fumonisin B_1 (WHO, 2000, 2002).

Sphingolipids are a highly diverse class of lipids found in all eukaryotic cells. Their biological functions are equally diverse: the compounds serve as structural components required for maintenance of membrane integrity, as receptors for vitamins and toxins, as sites for cell–cell recognition and cell–cell and cell–substrate adhesion, as modulators of receptor function and as lipid second messengers in signalling pathways responsible for cell growth, differentiation and death (Merrill *et al.*, 1997).

Ceramide synthase is a key enzyme in the biosynthesis of sphingolipids. Alterations in the ratio free sphinganine/free sphingosine, a consequence of ceramide synthase inhibition, are now used as a biomarker for exposure to fumonisins in domestic animals and humans. The mechanistic aspects of the effects of fumonisins on sphingolipid metabolism are discussed more fully in Section 4.5.1.

Methods have been reported for extraction of fumonisin B_1 from human urine (Shetty & Bhat, 1998), plasma and urine of rats (Shephard *et al*., 1992a, 1995a), bile of rats and vervet monkeys (Shephard *et al*., 1994a, 1995b), faeces of vervet monkeys (Shephard

et al., 1994b), liver, kidney and muscle of beef cattle (Smith & Thakur, 1996) and milk (Maragos & Richard, 1994; Scott *et al*., 1994; Prelusky *et al*., 1996a).

1.2 Formation

Fumonisin B_1 was isolated in 1988 by Gelderblom *et al*. (1988). It was chemically characterized by Bezuidenhout *et al*. (1988), and shortly thereafter as 'macrofusine' by Laurent *et al*. (1989a), from cultures of *Fusarium verticillioides* (Sacc.) Nirenberg (formerly known as *Fusarium moniliforme* Sheldon) (Marasas *et al.*, 1979) as well as *Gibberella fujikuroi* (Leslie *et al.*, 1996). The absolute stereochemical configuration of fumonisin B_1 (see section 1.1.2) was determined by ApSimon (2001).

Fumonisin B_1 is produced by isolates of *F. verticillioides, F. proliferatum, F. anthophilum, F. beomiforme, F. dlamini, F. globosum, F. napiforme, F. nygamai, F. oxysporum, F. polyphialidicum, F. subglutinans* and *F. thapsinum* (*Gibberella thapsina*) isolated from Africa, the Americas, Oceania (Australia), Asia and Europe (Gelderblom *et al*., 1988; Ross *et al*., 1990; Nelson *et al*., 1991; Thiel *et al*., 1991a; Chelkowski & Lew, 1992; Leslie *et al*., 1992; Nelson *et al*., 1992; Miller *et al*., 1993; Rapior *et al*., 1993; Desjardins *et al*., 1994; Visconti & Doko, 1994; Abbas & Ocamb, 1995; Abbas *et al*., 1995; Logrieco *et al*., 1995; Miller *et al*., 1995; Leslie *et al*., 1996; Klittich *et al*., 1997; Musser & Plattner, 1997; Sydenham *et al*., 1997). *Alternaria alternata* f. sp. *lycopersici* has also been shown to synthesize B fumonisins (Abbas & Riley, 1996). Fumonisins can be produced by culturing strains of the *Fusarium* species that produce these toxins on sterilized maize (Cawood *et al*., 1991) and yields of up to 17.9 g/kg (dry weight) have been obtained with *F. verticillioides* strain MRC 826 (Alberts *et al*., 1990). Yields of 500–700 mg/L for fumonisin B_1 plus fumonisin B_2 have been obtained in liquid fermentations and high recoveries of the toxins are possible (Miller *et al*., 1994). The predominant toxin produced is fumonisin B_1. Fumonisin B_1 frequently occurs together with fumonisin B_2, which may be present at levels of 15–35% of fumonisin B_1 (IARC, 1993; Diaz & Boermans, 1994; Visconti & Doko, 1994).

F. verticillioides and *F. proliferatum* are among the most common fungi associated with maize. These fungi can be recovered from most maize kernels including those that appear healthy (Bacon & Williamson, 1992; Pitt *et al*., 1993; Sanchis *et al*., 1995). The level of formation of fumonisins in maize in the field is positively correlated with the occurrence of these two fungal species, which are predominant during the late maturity stage (Chulze *et al*., 1996). These species can cause *Fusarium* kernel rot of maize, which is one of the most important ear diseases in hot maize-growing areas (King & Scott, 1981; Ochor *et al*., 1987; De León & Pandey, 1989) and is associated with warm, dry ears and/or insect damage (Shurtleff, 1980).

1.3 Use

Fumonisin B_1 is not used commercially.

1.4 Occurrence

Fumonisins have been found worldwide, primarily in maize. More than 10 compounds have been isolated and characterized. Of these, fumonisins B_1, B_2 and B_3 are the major fumonisins produced. The most prevalent in contaminated maize is fumonisin B_1, which is believed to be the most toxic (Thiel *et al.*, 1992; Musser & Plattner, 1997; Food and Drug Administration, 2001a,b). A selection of data on the occurrence of fumonisin B_1 in maize and food products is given in Table 1.

(*a*) *Formation in raw maize*

The concentrations of fumonisins in raw maize are influenced by environmental factors such as temperature, humidity, drought stress and rainfall during pre-harvest and harvest periods. For example, high concentrations of fumonisins are associated with hot and dry weather, followed by periods of high humidity (Shelby *et al.*, 1994a,b). Magan *et al.* (1997) have studied the effects of temperature and water activity (a_w) on the growth of *F. moniliforme* and *F. proliferatum*. Growth increases with a_w (between 0.92 to 0.98) and is maximum at 30 °C for *F. moniliforme* and at 35 °C for *F. proliferatum*.

High concentrations of fumonisins may also occur in raw maize that has been damaged by insects (Bacon & Nelson, 1994; Miller, 2000). However, maize hybrids genetically engineered to carry genes from the bacterium *Bacillus thuringiensis* (Bt maize) that produce proteins that are toxic to insects, specifically the European maize borer, have been found to be less susceptible to *Fusarium* infection and contain lower concentrations of fumonisins than the non-hybrid maize in field studies (Munkvold *et al.*, 1997, 1999).

(*b*) *Occurrence in processed maize products*

One of the major factors that determine the concentration of fumonisins in processed maize products is whether a dry- or wet-milling process is used. The whole maize kernel consists of the following major constituents: (i) starch, which is the most abundant constituent from which maize starches and maize sweeteners are produced; (ii) germ, which is located at the bottom of the centre of the kernel from which maize oil is produced; (iii) gluten, which contains the majority of the protein found in maize kernel; and (iv) hull (pericarp), which is the outer coat of the kernel from which maize bran is produced.

Dry milling of whole maize kernel generally results in the production of fractions called bran, flaking grits, grits, meal and flour. Because fumonisins are concentrated in the germ and the hull of the whole maize kernel, dry milling results in fractions with different concentrations of fumonisins. For example, dry-milled fractions (except for the bran fraction) obtained from degermed maize kernels contain lower concentrations of fumonisins than dry-milled fractions obtained from non-degermed or partially degermed maize. Industry information indicates that dry milling results in fumonisin-containing fractions in the following order of descending fumonisin concentrations: bran, flour,

Table 1. Worldwide occurrence of fumonisin B_1 in maize-based products[a]

Product	Region/Country	Detected/ total samples	Fumonisin B_1 (mg/kg)
	North America		
Maize	Canada, USA	324/729	0.08–37.9
Maize flour, grits	Canada, USA	73/87	0.05–6.32
Miscellaneous maize foods[b]	USA	66/162	0.004–1.21
Maize feed	USA	586/684	0.1–330
	Latin America		
Maize	Argentina, Brazil, Uruguay	126/138	0.17–27.05
Maize flour, alkali-treated kernels, polenta	Peru, Uruguay, Venezuela	5/17	0.07–0.66
Miscellaneous maize foods[b]	Uruguay, Texas–Mexico border	63/77	0.15–0.31
Maize feed	Brazil, Uruguay	33/34	0.2–38.5
	Europe		
Maize	Austria, Croatia, Germany, Hungary, Italy, Poland, Portugal, Romania, Spain, United Kingdom	248/714	0.007–250
Maize flour, maize grits, polenta, semolina	Austria, Bulgaria, Czech Republic, France, Germany, Italy, Netherlands, Spain, Switzerland, United Kingdom	181/258	0.008–16
Miscellaneous maize foods[b]	Czech Republic, France, Germany, Italy, Netherlands, Spain, Sweden, Switzerland, United Kingdom	167/437	0.008–6.10
Imported maize, grits and flour	Germany, Netherlands, Switzerland	143/165	0.01–3.35
Maize feed	France, Italy, Spain, Switzerland, United Kingdom	271/344	0.02–70
	Africa		
Maize	Benin, Kenya, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zambia, Zimbabwe	199/260	0.02–117.5
Maize flour, grits	Botswana, Egypt, Kenya, South Africa, Zambia, Zimbabwe	73/90	0.05–3.63
Miscellaneous maize foods[b]	Botswana, South Africa	8/17	0.03–0.35
Maize feed	South Africa	16/16	0.47–8.85

Table 1 (contd)

Product	Region/Country	Detected/ total samples	Fumonisin B_1 (mg/kg)
	Asia		
Maize	China, Indonesia, Iran[c], Nepal, Philippines, Thailand, Viet Nam	380/633	0.01–155
Maize flour, grits, gluten	China, India, Japan, Thailand, Viet Nam	44/53	0.06–2.60
Miscellaneous maize foods[b]	Japan, Taiwan	52/199	0.07–2.39
Maize feed	Korea (Republic of), Thailand	10/34	0.05–1.59
	Oceania		
Maize	Australia	67/70	0.3–40.6
Maize flour	New Zealand	0/12	—

[a] Adapted from WHO (2000) and Plattner *et al.* (1990); Shephard *et al.* (1990); Sydenham *et al.* (1990a,b); Wilson *et al.* (1990); Lew *et al.* (1991); Ross *et al.* (1991a,b); Sydenham *et al.* (1991); Thiel *et al.* (1991b); Bane *et al.* (1992); Colvin & Harrison (1992); Minervini *et al.* (1992); Osweiler *et al.* (1992); Park *et al.* (1992); Pittet *et al.* (1992); Rheeder *et al.* (1992); Stack & Eppley (1992); Sydenham *et al.* (1992); Caramelli *et al.* (1993); Chamberlain *et al.* (1993); Holcomb *et al.* (1993); Hopmans & Murphy (1993); Murphy *et al.* (1993); Price *et al.* (1993); Scudamore & Chan (1993); Sydenham *et al.* (1993a,b); Ueno *et al.* (1993); Wang *et al.* (1993); Chu & Li (1994); Doko & Visconti (1994); Doko *et al.* (1994); Kang *et al.* (1994); Lee *et al.* (1994); Pestka *et al.* (1994); Sanchis *et al.* (1994); Shelby *et al.* (1994a); Sydenham (1994); Usleber *et al.* (1994a,b); Viljoen *et al.* (1994); Yoshizawa *et al.* (1994); Zoller *et al.* (1994); Bottalico *et al.* (1995); Doko *et al.* (1995); Miller *et al.* (1995); Pascale *et al.* (1995); Trucksess *et al.* (1995); Visconti *et al.* (1995); Wang *et al.* (1995); Yamashita *et al.* (1995); Bryden *et al.* (1996); Burdaspal & Legarda (1996); Doko *et al.* (1996); Dragoni *et al.* (1996); Hirooka *et al.* (1996); Meister *et al.* (1996); Ramirez *et al.* (1996); Castella *et al.* (1997); Gao & Yoshizawa (1997); Patel *et al.* (1997); Piñeiro *et al.* (1997); Rumbeiha & Oehme (1997); Tseng & Liu (1997); Ueno *et al.* (1997); Ali *et al.* (1998); Fazekas *et al.* (1998); de Nijs *et al.* (1998a,b); Ostrý & Ruprich (1998); Scudamore *et al.* (1998); Stack (1998)

[b] Includes maize snacks, canned maize, frozen maize, extruded maize, bread, maize-extruded bread, biscuits, cereals, chips, flakes, pastes, starch, sweet maize, infant foods, gruel, purée, noodles, popcorn, porridge, tortillas, tortilla chips, masas, popped maize, soup, taco and tostada

[c] From Shephard *et al.* (2000)

meal, grits and flaking grits. Consequently, maize products such as corn bread, maize grits and maize muffins made from the grits and flour fractions may contain low concentrations of fumonisins. Ready-to-eat breakfast cereals made from flaking grits, such as corn (maize) flakes and puffed type cereals, contain very low concentrations (from non-detectable to 10 ppb) of fumonisins (Stack & Eppley, 1992).

Wet milling of whole maize generally results in the production of fumonisin-containing fractions in the following order of descending fumonisin concentrations: gluten, fibre, germ and starch. No fumonisins have been detected in the starch fraction obtained from wet milling of fumonisin-contaminated maize. The starch fraction is further processed for production of high-fructose maize syrups and other maize sweeteners (JECFA, 2001). Therefore, products of these types do not contain any detectable concentration of fumonisins. Maize oil, extracted from maize germ and refined, does not contain any detectable fumonisins (Patel *et al*., 1997). The gluten and fibre fractions from the wet-milling process do contain fumonisins; these fractions are used to produce animal feed, such as maize gluten meal and maize gluten feed (JECFA, 2001).

Another process to which whole maize may be subjected is nixtamalization, which consists of boiling the raw maize kernels in aqueous calcium hydroxide solution (lye), cooling and washing to remove the pericarp and excess calcium hydroxide. The washed kernels are then ground to produce the 'masa', from which maize chips and tortillas are made. This process has been shown to reduce concentrations of fumonisins in raw maize kernels (Dombrink-Kurtzman & Dvorak, 1999). However, the reaction product, hydrolysed fumonisin B_1 (HFB_1) is highly hepatotoxic and nephrotoxic (Voss *et al*., 1996a, 1998, 2001a).

Available data indicate the presence of low concentrations (4–82 ppb (μg/kg)) of fumonisins in sweet maize (Trucksess *et al*., 1995). Fumonisins can be present in beer but at low concentrations (4.8–85.5 ppb (μg/L)) (from maize-based brewing adjuncts) (Torres *et al*., 1998; Hlywka & Bullerman, 1999), but distilled spirits made from maize do not contain fumonisins (Bennett & Richard, 1996). The fermentation process does not destroy fumonisins and 85% of the toxin may be recovered in fermented products. Products from ethanol fermentations generally used as animal feeds may be detrimental if consumed by pigs or horses (Bennett & Richard, 1996).

Broken kernels of maize which have been screened from bulk lots of maize before any milling process contain higher concentrations of fumonisins than whole kernels, and are often used in animal feeds. Higher fumonisin concentrations are found in maize screenings. Fumonisin-contaminated maize at concentrations of 330 and 160 mg/kg has caused porcine pulmonary oedema and equine leukoencephalomalacia, respectively (Ross *et al*., 1991a,b). However, fumonisin residues in milk (Maragos & Richard, 1994; Scott *et al*., 1994; Becker *et al*., 1995; Richard *et al*., 1996), eggs (Vudathala *et al*., 1994) and meat (Prelusky *et al*., 1994, 1996a,b; Smith & Thakur, 1996) have been either undetectable or were detected at extremely low concentrations.

(*c*) *Formation in commodities other than maize*

Fumonisins have also been reported in other food products, notably sorghum in Botswana, Brazil, India, South Africa and Thailand (Bhat *et al.*, 1997; Siame *et al.*, 1998; Vasanthi & Bhat, 1998; Suprasert & Chulamorakot, 1999; da Silva *et al.*, 2000; Gamanya & Sibanda, 2001). In these countries, about 40% of the samples screened contained low concentrations of fumonisin B_1 (0.11–0.55 mg/kg). However, higher amounts (up to 7.8 mg/kg) were observed in rain-damaged sorghum, still lower than the concentrations reported for maize (up to 65 mg/kg) (Vasanthi & Bhat, 1998). Other commodities in which fumonisin B_1 has been detected include millets, rice, wheat, barley, cereal-based food products, soybean and pastes and animal feeds (Nelson *et al.*, 1992; Castella *et al.*, 1997; Abbas *et al.*, 1998; Siame *et al.*, 1998; Hlywka & Bullerman, 1999; Chulamorakot & Suprasert, 2000; Gamanya & Sibanda, 2001; JECFA, 2001).

Fumonisin B_1 has been found in black tea (80–280 mg/kg) as well as in some medicinal plants, such as leaves of the orange tree (350–700 mg/kg) and leaves and flowers of the linden tree (20–200 mg/kg) (Martins *et al.*, 2001).

Twenty-five asparagus plants affected by crown rot were analysed for *Fusarium* infestation and fumonisin contamination. *F. proliferatum* was found in all plants. Fumonisin B_1 was detected in crowns and stems at concentrations of 7.4 and 0.83 mg/kg dry weight, respectively (Logrieco *et al.*, 1998).

Co-occurrence of fumonisins and aflatoxins in maize is reviewed in the monograph on aflatoxins in this volume.

1.5 Human exposure to fumonisins

A number of estimates of human exposure to fumonisins have been made. In a preliminary estimate for the Food and Drug Administration (FDA) in the USA, exposure to fumonisins for consumers of maize in the USA was estimated as 0.08 μg/kg bw per day (Humphreys *et al.*, 1997; WHO, 2000). In Canada for the period 1991 to early 1995, Kuiper-Goodman *et al.* (1996) estimated human exposure to be 0.017–0.089 μg/kg bw per day. In Switzerland, mean daily intake of fumonisins has been estimated to be 0.03 μg/kg bw (Zoller *et al.*, 1994).

As a conservative estimate, de Nijs *et al.* (1998c) found that in the Netherlands 97% of individuals with gluten intolerance had a daily exposure of at least 1 μg fumonisin B_1 and 37% of at least 100 μg; in the general population it was estimated that 49% and 1%, respectively, were exposed to these levels of fumonisin B_1.

Human exposures in the Transkei, South Africa, were earlier estimated to be 14 and 440 μg/kg bw fumonisin B_1 per day for good quality and mouldy maize, respectively (Thiel *et al.*, 1992). More recent estimates of probable daily intake by South Africans vary from 1.2 to 355 μg/kg bw per day in a rural population in Transkei consuming home-grown mouldy maize (Marasas, 1997).

Since fumonisin B_1 is present in the spores and mycelia of *F. verticillioides* (Tejada-Simon *et al.*, 1995), occupational inhalation exposure could be a problem, but data are lacking on airborne levels of fumonisins during the harvesting, processing and handling of fumonisin-contaminated maize.

In a study conducted in China, urine was collected from volunteers before and after consumption of a fumonisin B_1-contaminated diet for one month. The ratio of free sphinganine to free sphingosine (Sa/So) was increased threefold in the urine of the men, but was unchanged in that of the women. This increase was also apparent when the data were pooled for men and women and grouped into those individuals who had estimated intakes of fumonisin B_1 greater than or less than 110 μg/kg bw per day (Qiu & Liu, 2001).

This result is similar to that reported for swine, where the sphinganine/sphingosine ratio became significantly different from controls at a fumonisin B_1 intake of 500 μg/kg bw per day (Rotter *et al.*, 1996).

1.6 Regulations and guidelines

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recommended a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg bw for fumonisins B_1, B_2 and B_3, alone or in combination (JECFA, 2001).

An official tolerance value for dry maize products (1 mg/kg fumonisin B_1 plus fumonisin B_2) has been issued in Switzerland (Canet, 1999).

The recommended maximum levels for fumonisins in human foods and in animal feeds in the USA that the FDA considers achievable with the use of good agricultural and good manufacturing practices are presented in Tables 2 and 3. Human exposure to fumonisins should not exceed levels achievable with the use of such practices (Food and Drug Administration, 2001c).

2. Studies of cancer in humans

Studies on the relationship between *Fusarium verticillioides* (formerly known as *F. moniliforme*) toxins (of which fumonisin B_1 and fumonisin B_2 are the major toxic secondary metabolites) and oesophageal cancer in areas of South Africa and China were summarized in Volume 56 of the *IARC Monographs* (IARC, 1993). The evidence in humans was judged to be '*inadequate*' at that time.

The only subsequent study that investigated the relationship between fumonisins and cancer was carried out in the People's Republic of China. Yoshizawa and Gao (1999) collected 76 corn samples from the homes of oesophageal cancer patients, selected at random in Linxian, China (a high-risk area for oesophageal cancer) as well as 55 samples from homes of peasant families with no oesophageal cancer patient in Shangqiu (a low-

Table 2. Maximum levels of fumonisins in human foods and in animal feeds in the USA[a]

Product	Total fumonisins ($B_1 + B_2 + B_3$) ppm (mg/kg)
Human foods	
Degermed dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of < 2.25%, dry weight basis)	2
Whole or partially degermed dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of ≥ 2.25%, dry weight basis)	4
Dry milled corn bran	4
Cleaned corn intended for masa production	4
Cleaned corn intended for popcorn	3
Animal feeds	
Corn and corn by-products intended for:	
Equids and rabbits	5[b]
Swine and catfish	20[c]
Breeding ruminants, breeding poultry and breeding mink[d]	30[c]
Ruminants ≥ 3 months old being raised for slaughter and mink being raised for pelt production	60[c]
Poultry being raised for slaughter	100[c]
All other species or classes of livestock and pet animals	10[c]

[a] From Food and Drug Administration (2001a)
[b] No more than 20% of diet on a dry weight basis
[c] No more than 50% of diet on a dry weight basis
[d] Includes lactating dairy cattle and hens laying eggs for human consumption

risk comparison area). Homegrown samples of corn intended for human consumption were collected in 1989, 1995 and 1997 (Yoshizawa *et al*., 1994; Yoshizawa & Gao, 1999). Fumonisins B_1, B_2 and B_3 were analysed by high-performance liquid chromatography (HPLC). Mean concentrations of fumonisin B_1 in the high- and low-risk areas, respectively, were 872 and 890 ng/g in 1989, 2730 and 2702 ng/g in 1995, and 2028 and 2082 ng/g in 1997. Maximum concentrations were 2960, 21 000 and 8290 ng/g in the high-risk area and 1730, 8470 and 5330 ng/g in the low-risk area, respectively, in the three years studied. There was no significant difference in any of the measured fumonisin levels between the two areas ($p > 0.05$). The percentages of samples with detectable

Table 3. Levels of total fumonisins ($B_1 + B_2 + B_3$) in corn, corn by-products and the total ration for various animal species recommended in the USA[a]

Animal or class	Recommended maximum level of total fumonisins in corn and corn by-products (ppm; mg/kg)	Feed factor[b]	Recommended maximum level of total fumonisins in the total ration (ppm)
Horse[c]	5	0.2	1
Rabbit	5	0.2	1
Catfish	20	0.5	10
Swine	20	0.5	10
Ruminants[d]	60	0.5	30
Mink[e]	60	0.5	30
Poultry[f]	100	0.5	50
Ruminant, poultry and mink breeding stock[g]	30	0.5	15
All others[h]	10	0.5	5

[a] From Food and Drug Administration (2001c)
[b] Fraction of corn or corn by-product mixed into the total ration
[c] Includes asses, zebras and onagers
[d] Cattle, sheep, goats and other ruminants that are ≥ 3 months old and fed for slaughter
[e] Fed for pelt production
[f] Turkeys, chickens, ducklings and other poultry fed for slaughter
[g] Includes laying hens, roosters, lactating dairy cows and bulls
[h] Includes dogs and cats

fumonisins in the high-risk area were 48% in 1989, 79% in 1995 and 73% in 1997; the corresponding figures in the low-risk area were 25% in 1981, 50% in 1995 and 47% in 1997. Based on local dietary habits, the estimated daily intake of fumonisin B_1 was 1.6–1.9 times higher in the high-risk than in the low-risk area for oesophageal cancer. The authors noted that aflatoxin B_1 was detected at very low levels in corn samples from both of the areas. [The Working Group noted that cancer families in high-risk areas were compared with non-cancer families in low-risk areas.]

3. Studies of Cancer in Experimental Animals

Toxins derived from *Fusarium moniliforme* were considered by a previous Working Group in 1992 (IARC, 1993). Since that time, new data have become available and these have been incorporated into the monograph and taken into consideration in the present evaluation.

3.1 Oral administration of fumonisin mixtures

3.1.1 *Studies using naturally contaminated maize (fumonisins including other* Fusarium *mycotoxins)*

Rat: A group of 12 male Fischer 344 rats (average body weight, 125 g) was fed a maize diet, naturally contaminated with *Fusarium verticillioides* (*F. moniliforme*) over a period of 4–6 months. The maize sample was obtained from feed being fed to horses during an outbreak of equine leukoencephalomalacia (ELEM). A group of 12 control rats was fed a commercial rodent chow (Purina 5001) (Wilson *et al*., 1985). No aflatoxins were detected in the maize diet (detection limit, < 0.9 μg/kg), which was deficient in many nutrients including choline and methionine. Maize samples contained moniliformin (2.82 mg/kg) and fusarin C (0.39 mg/kg), but no trichothecene or aflatoxin (Thiel *et al*., 1986). Retrospective mycological and chemical analyses indicated the presence of *F. verticillioides* and *Aspergillus flavus* as major fungal contaminants and a total fumonisin B (fumonisin B_1 and B_2) concentration of 33.1 mg/kg, while only trace amounts of aflatoxin B_1 and B_2 ranging between 0.05 and 0.1 μg/kg were detected (JECFA, 2001). A mean fumonisin B intake of between 1.6 and 2.0 mg/kg bw per day was estimated based on an apparent feed intake of 50–60 g/kg bw per day (Wilson *et al*., 1985; JECFA, 2001). One of the treated rats died after 77 days, three were killed on days 123, 137 and 145, and the remaining eight animals were killed on day 176: all animals showed multiple hepatic nodules, large areas of adenofibrosis and cholangiocarcinomas. The controls had no liver lesions.

3.1.2 *Studies using fungal culture material*

Rat: A group of 31 female Wistar rats [age unspecified] was fed a diet containing maize bread inoculated with *F. verticillioides*. After 554–701 days of feeding, four papillomas and two carcinomas had developed in the forestomach. No epithelial lesion of the forestomach was seen in a control group of 10 female rats fed conventional maize bread, not inoculated with the mould, for 330–700 days (Li *et al*., 1982). [The Working Group noted the inadequate reporting of the study.]

Groups of 20 male BD IX rats (weighing 80–100 g) were fed commercial rat feed containing *F. verticillioides* MRC 826 mouldy meal (freeze-dried or oven-dried) at levels of either 8% for up to 57 or 75 days or 4% for 286 days followed by 2% until 763 days (Marasas *et al*., 1984). The estimated intakes of fumonisin B were 138 (8% in diet), 69 (4%) and 32 (2%) mg/kg bw per day for the respective diets, with an average feed intake of 32 g feed/kg bw per day (JECFA, 2001). All rats given 8% diet between 57 and 75 days had severe liver damage. Among rats fed the 4%/2% freeze-dried diet regimen and surviving beyond 450 days, 12/14 had hepatocellular carcinomas and 10/14 had hepatic ductular carcinoma. In the rats fed the 4%/2% oven-dried material, 12/16 had hepatocellular carcinoma and 9/16 had hepatic ductular carcinoma. In both treatment groups

with freeze-dried or oven-dried diet, three rats had pulmonary metastases. No liver tumours were seen in rats fed control diet (Marasas *et al*., 1984).

Groups of 30 male BD IX rats, weighing approximately 110 g, were fed for 27 months freeze-dried culture material of *F. verticillioides* MRC 826 at levels from 0.25% to 0.75% in either a semi-synthetic diet (marginally deficient in certain vitamins and minerals) or a semi-synthetic diet containing 5% culture material of *F. verticillioides* MRC 1069 (to contain 18.2 mg/kg fusarin C). Thirty control rats received commercial maize (5%) in the diet. Fumonisin B intake of between 4 and 13 mg/kg bw fumonisin B_1 in the rats fed MRC 826 was estimated assuming consumption of 32 g/kg bw feed per day. In animals fed the MRC 826 diet and necropsied between 23 and 27 months, neoplastic nodules (21/21), hepatocellular carcinoma with lung metastases (2/21), adenofibrosis (19/21) and cholangiocarcinoma (8/21) were observed. In the group fed the MRC 1069 diet, there were 1/22 neoplastic nodule and 1/22 adenofibrosis. No such lesions were seen in the control group. Forestomach papillomas were observed in 13/21 MRC 826-treated, 3/22 MRC 1069-treated and 5/22 control animals, respectively, and forestomach carcinomas were observed in 4/21 MRC 826-treated animals versus none in the other groups. Basal-cell hyperplasia of the oesophageal epithelium was observed in 12/21 rats fed the MRC 826 diet (Jaskiewicz *et al*., 1987).

3.2 Oral administration of purified fumonisin B_1

3.2.1 *Mouse*

Groups of 48 male and female $B6C3F_1$ mice, four weeks of age, were fed fumonisin B_1 (> 96% pure, ammonium salt) at concentrations of 0, 5, 15, 80 or 150 mg/kg of diet (males) and 0, 5, 15, 50 or 80 mg/kg of diet (females) in NIH 46 diet over a period of two years (equivalent to average daily doses of ~0.6, 1.7, 9.5 and 17 mg/kg bw for males or 0.7, 2.1, 7.0 and 12.5 mg/kg bw for females). Survival of the female mice was significantly reduced in the group treated with 80 mg/kg fumonisin B_1 (60%), while that in treated male mice was not significantly different from the controls. The low incidence of spontaneous liver tumours in control female (11%) and male (26%) mice compared with historical controls was ascribed to feed restriction, as previously suggested by Haseman *et al.* (1998). After two years, the incidences of hepatocellular adenomas in female mice were 5/47, 3/48, 1/48, 16/47 [$p = 0.0047$] and 31/45 [$p = 0.001$] and those of hepatocellular carcinomas were 0/47, 0/48, 15/48 [$p = 0.0007$], 10/47 [$p = 0.0007$] and 9/45 for the groups treated with 0, 5, 15, 50 and 80 mg/kg of diet, respectively. The incidences of hepatocellular adenomas and carcinomas in treated males were not significantly increased compared with control males (National Toxicology Program, 2000). [No analyses to determine the presence of *Helicobacter hepaticus* were reported.]

3.2.2 *Rat*

Two groups of 25 male BD IX rats, weighing between 70 and 80 g, were fed a semi-purified diet (intentionally marginally deficient in minerals and vitamins) in the absence or presence of 50 ppm (mg/kg diet) fumonisin B_1 (90% pure) for 26 months (Gelderblom *et al*., 1991). An average daily fumonisin B_1 intake of 1.6 mg/kg bw was calculated on the basis of a mean average feed intake of 32 g/kg bw per day (JECFA, 2001). Groups of five treated and five control rats were killed at 6 and 12 months. At 6 months, regenerative nodules were observed in all animals and cholangiofibrosis was observed in all but one; at 12 months, regenerative nodules and cholangiofibrosis were observed in all animals. In all 15 rats that died or were killed between 18 and 26 months, hepatocyte nodules, cholangiofibrosis and cirrhosis were observed. Hepatocellular carcinomas, two of which metastasized — one to the heart and lungs and one to the kidneys — were also observed in 10/15 animals (Gelderblom *et al*., 1991).

Four groups of 20 male BD IX rats, weighing approximately 100 g, were fed a semi-purified diet (intentionally marginally deficient in minerals and vitamins) containing 0, 1, 10 or 25 mg/kg of diet fumonisin B_1 (purity, 92–95%) over a period of two years. The mean intakes of fumonisin B_1 (mg/kg bw) were 0.005, 0.03, 0.3 and 0.8 for control, low-, mid- and high-dose groups, respectively. The survival rates at two years were 16/20, 14/20, 18/20 and 17/20 in the four groups, respectively. There was a significant ($p < 0.05$) increase in the incidence of portal fibrosis (5/17), ground glass foci (7/17) and hepatocyte nodules (9/17) in the liver of the rats fed fumonisin B_1 at 25 mg/kg of diet. One rat had a large focal area of adenofibrosis. Some of these hepatic changes were detected to a smaller extent in rats treated with fumonisin B_1 at 1 and 10 mg/kg of diet. No such lesions were observed in the livers of control rats (Gelderblom *et al*., 2001a).

Groups of 40–48 male and 40–48 [40 for the 15-mg/kg group] female Fischer 344 rats, eight weeks of age, were fed fumonisin B_1 (> 96% pure, ammonium salt) at concentrations of 0, 5, 15, 50 or 100 mg/kg of diet for females and 0, 5, 15, 50 or 150 mg/kg of diet for males in a powdered NIH 36 diet that was available *ad libitum* over a period of two years (equivalent to average daily doses of 0, 0.25, 0.8, 2.5 and 7.5 mg/kg bw for females and 0, 0.3, 0.9, 3.0 and 6.0 mg/kg bw for males). Survival rates were similar in the treated and control rats. In males at two years, the incidences of renal tubule adenomas were 0/38, 0/40, 0/48, 2/48 and 5/48, those of carcinomas were 0/48, 0/40, 0/48, 7/48 and 10/48 and those of adenomas and carcinomas combined were 0/48, 0/48, 0/48, 9/48 [$p = 0.001$] and 14/48 [$p = 0.0001$] for the controls and increasing doses, respectively. The occurrence of renal tumours in males was accompanied by an increased incidence of renal tubule epithelial cell hyperplasia at two years (2/48, 1/40, 4/48, 14/48 and 8/48 of the male rats receiving fumonisin B_1 at 0, 5, 15, 50 and 150 mg/kg of diet, respectively). In female rats, there were no significant fumonisin B_1-dependent changes in the incidence of tumours. One renal adenoma was detected in a female rat fed fumonisin B_1 at 50 mg/kg of diet, and one renal tubule carcinoma was detected in a female rat fed 100 mg/kg (National Toxicology Program, 2000).

A re-evaluation of the renal pathology of the National Toxicology Program (2000) study characterized toxic lesions as cytotoxic/regenerative (graded from 0 to 4) and atypical tubule hyperplasia (Hard *et al*., 2001). There was a progressive increase in the grade of severity of the former lesion in the rats fed 15, 50 and 150 mg fumonisin B_1 per kg of diet. Atypical hyperplasia was observed in 4/48 and 9/48 rats fed the 50- and 150-mg/kg diets. Adenomas (4/48) and carcinomas (6/48) were observed in the kidneys of the rats fed 50 mg/kg and also in the group fed 150 mg/kg (8/48 adenomas, 10/48 carcinomas). Two of 8 and 5/8 carcinomas in the 50- and 150-mg/kg treatment groups, respectively, metastasized to the lung. Only one of the 18 carcinomas displayed the conventional reasonably differentiated phenotype. Cellular pleomorphisms were noticed in 3/8 and 1/10 carcinomas in rats fed 50 and 150 mg/kg, respectively. Among the carcinomas observed in these studies, 61% were an anaplastic variant. [The Working Group noted that this re-evaluation did not affect the conclusions of the National Toxicology Program (2000) study.]

3.3 Administration with known carcinogens and other modifying factors

3.3.1 *Mixtures of fumonisins*

Rat: Groups of six male Fischer rats, 10 days of age, were given an intraperitoneal injection of *N*-nitrosodiethylamine (NDEA) (15 mg/kg bw) and fed ground maize containing culture material of *F. proliferatum* (50 mg/kg fumonisin B_1 in the diet) or nixtamalized [calcium hydroxide-treated] corn culture material (8–11 mg/kg hydrolysed fumonisin B_1 (HFB_1)), in the absence or presence of nutrient supplementation for a period of 30 days, at which time animals were killed. No aflatoxins were detected in the maize, while nutritional modulation stimulated the toxic effects in the rats treated with the nixtamalized and untreated maize cultures in rats. Hepatocellular adenomas developed in 83% and 14% of the rats on diets containing the untreated and nixtamalized maize cultures, respectively. Cholangiomas were induced in 33% of the animals in both groups (with or without nixtamalization) (Hendrich *et al*., 1993). [The Working Group noted the small number of animals.]

3.3.2 *Purified fumonisins*

Mouse: Three groups of 15 female SENCAR mice, seven weeks of age, were treated with a single application of 390 nmol 7,12-dimethylbenz[*a*]anthracene (DMBA) on their shaven backs. After one week, fumonisin B_1 was applied at doses of 0, 1.7 or 17 nmol twice a week and continued for 20 weeks. The highest dose of fumonisin B_1 induced skin tumours in all the mice with an average of 3.6 tumours per animal. In a similar experiment, groups of 10 female SENCAR mice were given a single intraperitoneal injection of 1.8 mg NDEA per mouse followed by fumonisin B_1 treatment (0.0025 % in the drinking-water) for 20 weeks. Lung tumours were found in 90% of the mice treated

with NDEA and fumonisin B_1, while none were observed in the mice treated with NDEA alone (Nishino *et al*., 2000).

Rat: In an initiation/promotion study in male BD IV rats, the promotional activity of fumonisin B_1 was tested with *N*-nitrosomethylbenzylamine as initiator. Fumonisin B_1 did not show any activity as a tumour promoter in the oesophagus over a 48-week period (Wild *et al.*, 1997).

Rainbow trout: Groups of 150 three-month-old rainbow trout fry were fed diet containing 0, 3, 23 or 104 mg/kg fumonisin B_1 (> 90% pure) for 34 weeks. No liver tumours were seen when the fish were killed at 60 weeks. Groups of 150 three-month-old rainbow trout fry pretreated with 100 mg/kg aflatoxin B_1 were fed diets containing 0, 3, 23 or 104 mg/kg fumonisin B_1 for 42 weeks. At 60 weeks, promotion of liver tumours was seen at 23 mg/kg (61% of fish had gross or confirmed tumours) and 104 mg/kg (74% of fish had gross or confirmed tumours). Groups of three-month-old rainbow trout fry pretreated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG; 35 mg/kg) were fed diets containing 0, 3, 23 or 104 mg/kg fumonisin B_1. At 60 weeks, promotion of liver tumours was seen in fish given 104 mg/kg fumonisin B_1 (55%) compared with 33% of fish treated with MNNG only (Carlson et al., 2001).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No studies of the absorption, distribution, metabolism and excretion of fumonisin B_1 in humans have been reported. Chelule *et al*. (2001) measured fumonisin B_1 in staple maize and in faeces in rural and urban populations in KwaZulu Natal, South Africa. Faecal concentrations were of the same order of magnitude as those in the maize consumed.

4.1.2 *Experimental systems*

Studies have been conducted with fumonisins B_1 and B_2 biosynthesized using deuterated or ^{14}C-labelled methionine, resulting in fumonisins labelled at C-12 and C-16 or C-21 and C-22 (the methyl groups and adjacent carbons; see section 1.1.2), respectively (Plattner & Shackelford, 1992; Alberts *et al*., 1993). 1,2-[^{14}C]Acetate has also been used, resulting in fumonisin labelled uniformly along the backbone with some label in the two tricarboxylic acid side-chains (Blackwell *et al*., 1994). In these studies, the two forms of [^{14}C]fumonisin B_1 had specific activities of 36 and 650 μCi/mmol, respectively, and radiochemical purities of > 95%.

The kinetics of absorption of fumonisin B_1 and of fumonisin B_2 in rats are similar, involving rapid distribution and elimination (Shephard *et al*., 1995c). In vervet monkeys (*Cercopithecus aethiops*), the bioavailability of fumonisin B_2 may be less than that of fumonisin B_1 and proportionally less fumonisin B_2 is excreted in bile (Shephard & Snijman, 1999).

The quantity of fumonisin B_1 detected in plasma after oral administration to pigs, laying hens, vervet monkeys, dairy cows and rats was very low. In rats (BD IX, Fischer 344, Sprague-Dawley, Wistar) given [^{14}C]fumonisin B_1 orally, accumulation of radioactivity in tissues was also very low. This demonstrates that absorption is very poor (< 4% of dose) (Shephard *et al*., 1992b,c; Norred *et al.*, 1993; Shephard *et al*., 1994c). Fumonisins are also poorly absorbed (2–< 6% of dose) in vervet monkeys, dairy cows and pigs (Prelusky *et al*., 1994; Shephard *et al*., 1994a,b; Prelusky *et al*., 1996b). In orally dosed laying hens and dairy cows, systemic absorption based on plasma levels and accumulation of radioactivity in tissues was estimated to be < 1% of the dose (Scott *et al*., 1994; Vudathala *et al*., 1994; Prelusky *et al*., 1995).

In rats and pigs given [^{14}C]fumonisin B_1 via the diet or by gavage, ^{14}C was distributed to various tissues, with the liver and kidney containing the highest concentration of radiolabel (Norred *et al*., 1993; Prelusky *et al*., 1994, 1996b). In chickens given a single oral dose of [^{14}C]fumonisin B_1, trace amounts of radioactivity were recovered in tissues, but no residues were detectable in eggs laid during the 24-h period after dosing (Vudathala *et al*., 1994). No fumonisin B_1 or aminopentol hydrolysis products were recovered in milk from cows that had received an oral dose of fumonisin B_1 (Scott *et al*., 1994). In pregnant rats dosed intravenously with [^{14}C]fumonisin B_1, approximately 14% and 4% of the dose was recovered in liver and kidney, respectively, after 1 h. In contrast, the uteri contained 0.24–0.44%, individual placentae contained 0–0.04% and total fetal recovery of radioactivity was ≤ 0.015% of the dose per dam (Voss *et al*., 1996b).

When [^{14}C]fumonisin B_1 was administered by intraperitoneal or intravenous injection to rats (BD IX, Sprague-Dawley, Wistar), initial elimination (subsequent to the distribution phase) was rapid (half-life, approximately 10–20 min) with little evidence of metabolism (Shephard *et al*., 1992b; Norred *et al*., 1993; Shephard *et al*., 1994c). In rats, the elimination kinetics based on intraperitoneal or intravenous dosing of fumonisin B_1 are consistent with a one- (Shephard *et al*., 1992b) or two-compartment model (Norred *et al*., 1993). However, one study using Wistar rats dosed orally with fumonisin B_1 indicated that the kinetics were probably best described by a three-compartment model (Martinez-Larranaga *et al*., 1999), as was the case in swine (see below).

In vervet monkeys, as in rats, the radioactivity was widely distributed and rapidly eliminated (mean half-life, 40 min) after intravenous injection of [^{14}C]fumonisin B_1 (Shephard *et al*., 1994a). The elimination kinetics after oral dosing in non-human primates have not been determined; however, peak plasma levels of fumonisin B_1 and B_2 occurred between one and several hours after a gavage dose of 7.5 mg/kg bw in vervet monkeys and the plasma concentrations ranged from 25–40 ng/mL for fumonisin B_2 to nearly 210 ng/mL for fumonisin B_1 (Shephard *et al.*, 1995b; Shephard & Snijman, 1999).

In pigs, clearance of [^{14}C]fumonisin B_1 from blood after an intravenous injection was best described by a three-compartment model (half-lives, 2.2, 10.5 and 182 min, respectively, averaged over five animals). Cannulation of the bile duct (which prevents enterohepatic circulation) resulted in much more rapid clearance, which was best described by a two-compartment model. A similar effect of bile removal was observed whether the dosing was intravenous or intragastric. The elimination half-life in pigs dosed intragastrically without bile removal was 96 min (averaged over four animals). The studies with pigs clearly show the importance of enterohepatic circulation of fumonisin B_1 in pigs. As with rats, over 90% of radioactivity was recovered in the faeces, with less than 1% recovered in urine after an oral dose of [^{14}C]fumonisin B_1 (Prelusky *et al.*, 1994).

After intraperitoneal injection in rats, fumonisin B_1 was excreted unchanged in bile (Shephard *et al.*, 1994c). In vervet monkeys after intravenous injection, there was evidence of metabolism to partially hydrolysed fumonisin B_1 and to a much lesser extent the fully hydrolysed aminopentol backbone in faeces. In urine, 96% of the radioactivity was recovered as fumonisin B_1 (Shephard *et al.*, 1994a). In further experiments, it was shown that metabolism was likely to be mediated by the bacteria in the gut, since partially hydrolysed and fully hydrolysed fumonisin B_1 were recovered in faeces but not bile of vervet monkeys (Shephard *et al.*, 1995b).

In-vitro studies using primary rat hepatocytes with microsomal preparations (Cawood *et al.*, 1994) and with a renal epithelial cell line (Enongene *et al.*, 2002a,b) indicated that there was no metabolism of fumonisin B_1 in these systems.

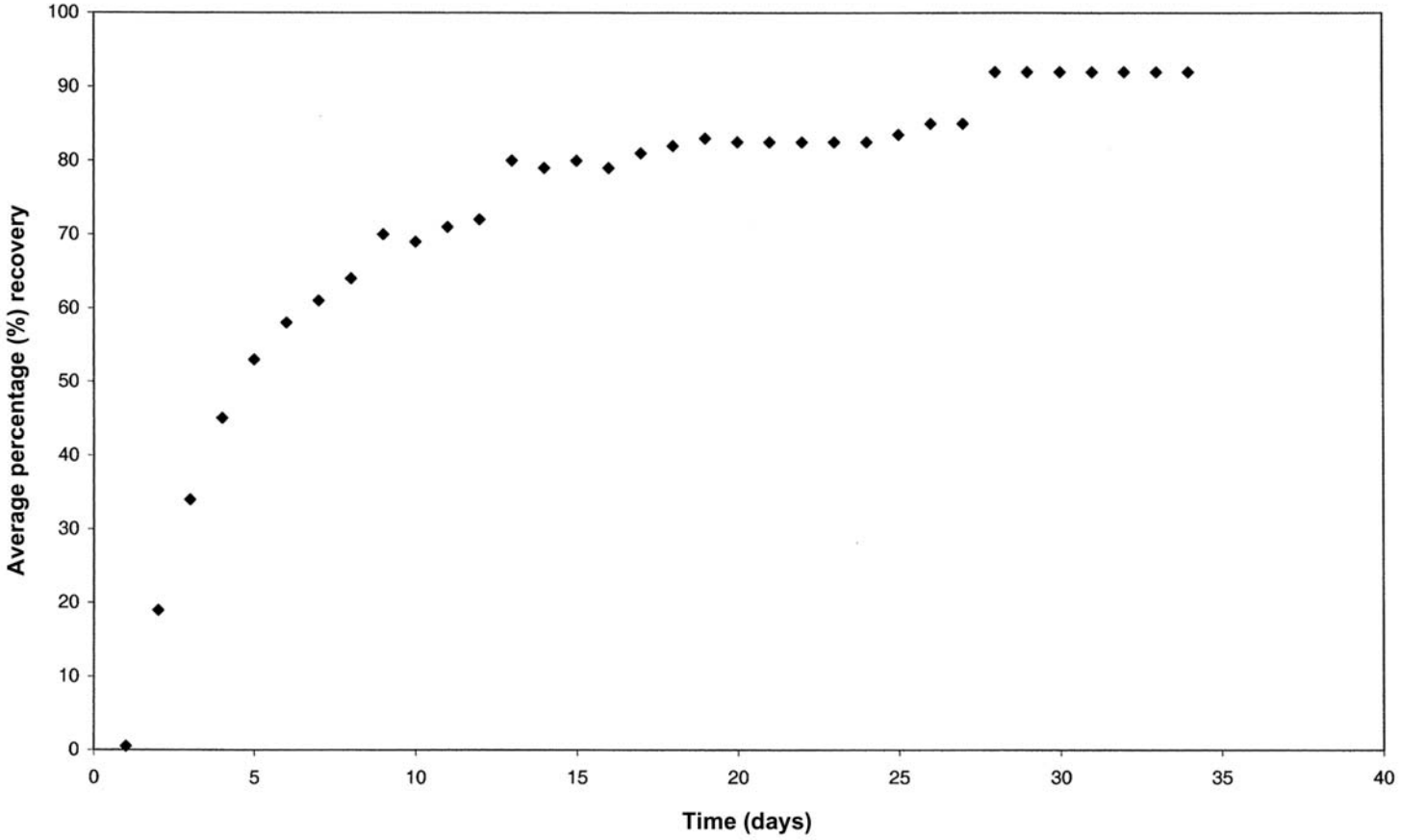
In rats given three oral doses of [^{14}C]fumonisin B_1 at 24-h intervals, the specific radioactivity in liver and kidney increased with each successive dose and remained unchanged for at least 72 h after the last dose (Norred *et al.*, 1993). In pigs, it was estimated that exposure to dietary fumonisin B_1 at 2–3 mg/kg feed would require a withdrawal period of at least two weeks for the [^{14}C]radiolabel to be eliminated from liver and kidney (Figure 1; Prelusky *et al.*, 1996b). Fumonisins B_1, B_2 and B_3 and aminopolyol hydrolysis products were detected in the hair of vervet monkeys exposed to fumonisin B_1 in the feed and of Fischer rats after oral exposure to culture material of *F. verticillioides* containing fumonisins (Sewram *et al.*, 2001).

Fumonisins do not appear to be metabolized in animal systems *in vitro* or *in vivo*, apart from some evidence for removal of the tricarboxylic acid side-chains. This is thought to be effected by the microbial flora of the gut.

4.1.3 *Comparison of humans and animals*

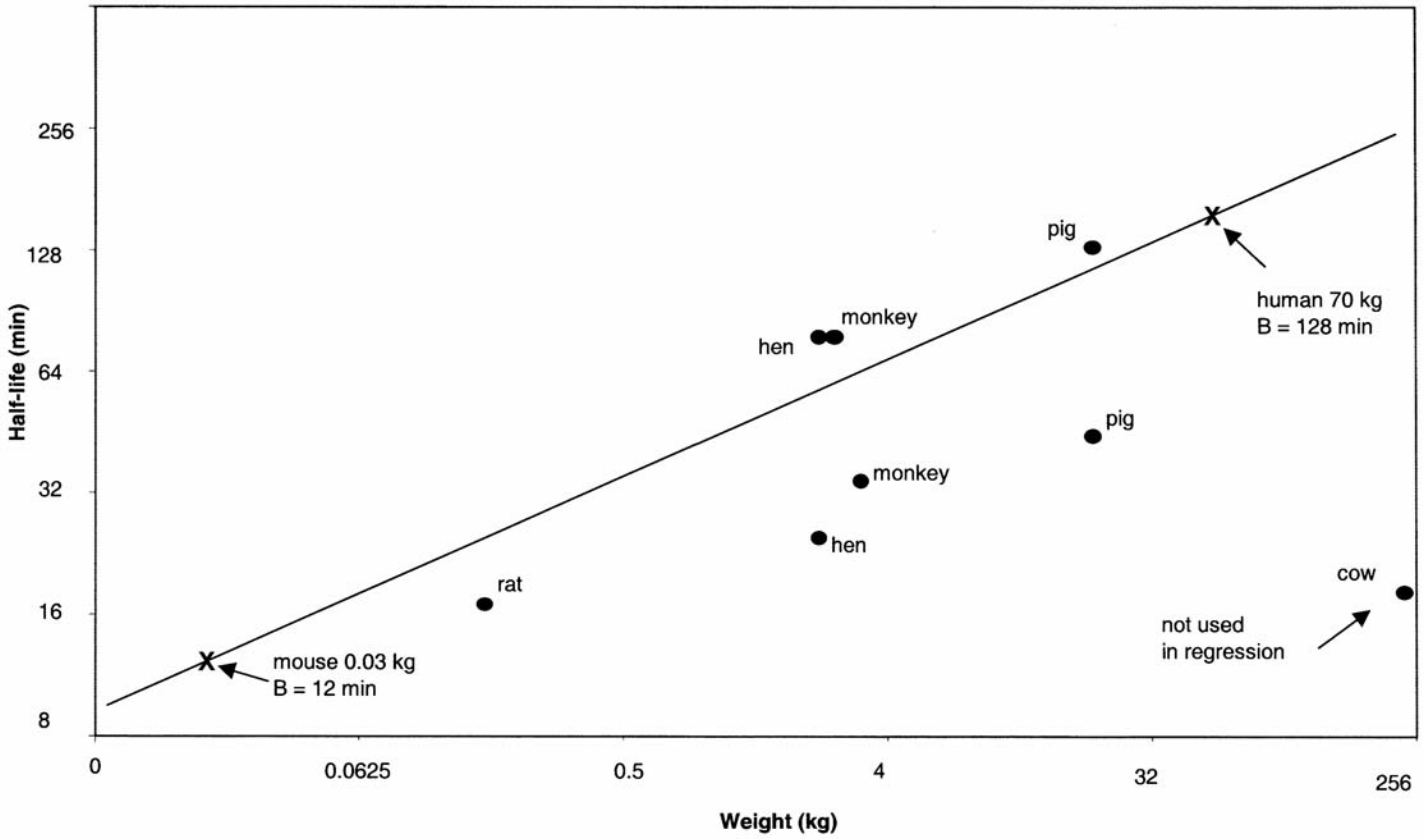
Several experiments have indicated that the rate of elimination of fumonisin B_1 is a function of body weight. In mice, elimination is very rapid, whereas fumonisin B_1 is predicted to be retained much longer in humans (Figure 2; Delongchamp & Young, 2001).

Figure 1. Percentage cumulative recovery of fumonisin B_1 in urine and faeces of swine fed [^{14}C]fumonisin B_1 (uniformly labelled) at 3 mg/kg diet from days 1 to 12, then 2 mg/kg diet from days 13 to 24 and clean feed from days 25 to 33



Adapted from Prelusky *et al*. (1996b)

Figure 2. Allometric relationship between body weight and fumonisin B_1 half-life for elimination



Adapted from Delongchamp & Young (2001)
The data for mouse and human are extrapolated from the linear regression model.

4.2 Toxic effects

4.2.1 *Humans*

As noted above, Chelule *et al*. (2001) reported that fumonisin levels in faeces were of the same order of magnitude as those in the maize consumed. In a study conducted in China, urine was collected from volunteers before and after consumption of a fumonisin B_1-contaminated diet for one month. The ratio of free sphinganine to free sphingosine (Sa/So) was increased threefold in the urine of the men, but was unchanged in that of the women. This increase was also apparent when the data were pooled for men and women and grouped into those individuals who had estimated intakes of fumonisin B_1 greater than or less than 110 μg/kg bw per day (Qiu & Liu, 2001). For a detailed discussion of the effects of fumonisin B_1 on sphingolipid metabolism, see section 4.5.1.

One report from India described gastric and other symptoms possibly associated with high exposures to fumonisins from consumption of rain-damaged mouldy sorghum or maize (Bhat *et al*., 1997).

4.2.2 *Experimental systems*

Studies on culture material were reviewed in IARC (1993), WHO (2000) and in the background papers for the 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001). Because, for most species, there are now adequate studies using fumonisin B_1 with reported purities of 96–98%, these will be emphasized here (Bondy *et al*., 1996; Rotter *et al*., 1996; National Toxicology Program, 2000).

The single-dose LD_{50} of fumonisin B_1 is unknown. Single gavage doses of 50, 100 and 200 mg/kg bw fumonisin B_1 significantly inhibited hepatocyte proliferation when given to male Fischer rats six hours after partial hepatectomy (Gelderblom *et al*., 1994). In male Sprague-Dawley rats, intravenous injection of fumonisin B_1 at 1.25 mg/kg bw resulted in histological changes in the outer medulla of the kidney, with an increased number of mitotic figures and apoptosis followed by severe nephrosis (Lim *et al*., 1996).

Equine leukoencephalomalacia (ELEM) syndrome is caused by ingestion of fumonisin B_1 in contaminated feed and is characterized by the presence of liquefactive necrotic lesions in the white matter of the cerebrum (Marasas *et al*., 1988). The first symptoms are lethargy, head pressing and inability to eat or drink, followed by convulsions and death after several days. In addition to the brain lesions, histopathological abnormalities in liver and kidney have been reported in horses orally dosed with pure fumonisins (Kellerman *et al*., 1990). Marasas *et al*. (1988) reported that high doses of fumonisin B_1 induced fatal hepatotoxicity with mild brain lesions, while low doses caused mild hepatotoxicity and severe brain lesions. Fatal liver disease in the absence of any brain lesions was induced in a mare by intravenous injection of large doses of fumonisin B_1, while gastric intubation of the mycotoxin had no effect (Laurent *et al*., 1989b). However, signs of neurotoxicosis and liver lesions in the absence of elevated clinical chemistry parameters, and ELEM concurrent with significant liver disease have been

observed in horses and ponies after ingestion of feeds naturally contaminated with fumonisins at low concentrations (Wilson *et al*., 1992; Ross *et al*., 1993). The development of brain lesions in the absence of major liver lesions does not preclude a contribution of biochemical dysfunction in non-brain tissue to the development of brain lesions. Length of exposure, level of contamination, individual animal differences, previous exposure or pre-existing liver impairment may all contribute to the appearance of the clinical disease (Ross *et al*., 1993).

The lowest dietary dose observed to induce ELEM was 22 mg/kg fumonisin B_1 in a diet formulated with naturally contaminated maize screenings: one pony died of ELEM after consumption of contaminated diet for 235 days, of which the final 55 days' diet contained 22 ppm fumonisin B_1 (Wilson *et al*., 1992). Analysis of feeds from confirmed cases of ELEM indicated that consumption of feed with a fumonisin B_1 concentration greater than 10 mg/kg diet is associated with increased risk of development of ELEM, whereas a concentration less than 6 mg/kg is not (Ross *et al*., 1994). The minimum toxic dose of pure fumonisins is unknown.

In swine, fumonisin B_1 causes damage to the liver, lungs and cardiovascular and immune systems. Liver lesions have been induced with fumonisin-contaminated maize screenings at 1.1 mg/kg per day (fumonisins B_1 and B_2; 17 mg/kg fumonisin B_1 and 6 mg/kg fumonisin B_2 in the diet). Intravenous exposures resulted in changes similar to those recorded in rodents including necrosis and cell proliferation (Motelin *et al*., 1994; Haschek *et al*., 2001). When pure fumonisin B_1 was fed to Yorkshire swine at dietary levels of 0.1, 1 or 10 mg/kg (0.005, 0.052 or 0.496 mg/kg bw), apart from reduced organ weights (pancreas, adrenals), no histopathological signs of organ damage were observed. There were changes in sphingolipid ratios in lung, liver and kidney at the highest dose, as well as increased serum cholesterol (Rotter *et al*., 1996). Fumonisin B_1 given to young adult swine at several doses up to 1 mg/kg in the diet resulted in changes in serum cholesterol and in altered carcass fat distribution at 0.05 mg/kg bw (Rotter *et al*., 1997).

Lung oedema occurs in pigs following very high fumonisin B_1 exposure (≥ 100 ppm in diet, or ≥ 16 mg/kg bw per day). Clinical signs of lung oedema typically occur 2–7 days after exposure, and usually include dyspnoea, weakness, cyanosis and death (Osweiler *et al*., 1992; Haschek *et al*., 2001). At necropsy, the animals exhibit varying degrees of interstitial and interlobular oedema, with pulmonary oedema and hydrothorax, with varying amounts of clear yellow fluid accumulating in the pleural cavity (Colvin & Harrison, 1992; Colvin *et al*., 1993). Fumonisin B_1 is believed to be a negative osmotropic agent causing decreased cardiac contractility. It has been hypothesized that the cardiovascular alterations are a consequence of sphingosine-induced inhibition of L-type calcium channels. Pulmonary oedema results from left-sided heart failure (Smith *et al*., 1996, 2000; Haschek *et al*., 2001). Porcine pulmonary oedema was produced within 3–4 days after pigs started consuming a diet of culture material that provided 20 mg/kg bw fumonisin B_1 per day (Smith *et al.*, 1999). There are no published studies on pulmonary oedema induced by oral exposure to pure fumonisin B_1.

Because poultry are very resistant to fumonisins, toxicity studies all involve culture material obtained from fermentation of autoclaved corn inoculated with cultures of a fumonisin-producing fungus (WHO, 2000). Approximately 200 broiler chicks were fed *F. verticillioides* M-1325 culture material from hatching to 21 days. The concentrations of fumonisin B_1 were 75, 150, 225, 300, 375, 450 and 525 mg/kg feed. The total dietary fumonisin levels (B1 + B2 + B3) were reported as 89, 190, 283, 389, 481, 592 and 681 mg/kg feed. Broilers fed diets containing 89 and 190 ppm of the mixture showed no significant difference from controls in feed intake, weight gain, feed conversion, organ weights, haematology or clinical chemistry. Compared with controls, all chicks fed diets containing fumonisins had significantly increased ($p < 0.05$) serum sphinganine:sphingosine ratios. Histopathological examination of an extensive array of tissues showed no lesions or incidental findings in any treatment group. Isolated foci of hepatic necrosis with mild heterophil and macrophage infiltration, moderate diffuse hepatocellular hyperplasia, mild biliary hyperplasia and moderate to severe periportal granulocytic cell proliferation were noted only in broilers fed at least 283 mg/kg total fumonisins (Weibking *et al.*, 1993).

In an attempt to identify food-borne carcinogens contributing to the high incidence of oesophageal cancer in the Transkei region of the Eastern Cape Province, South Africa, various food mixtures, collected from households of local inhabitants, were fed to BD IX rats for 829–980 days. Diets consisted of maize, beans and a salt mixture with or without the addition of edible wild vegetables called *imifino* (Purchase *et al.*, 1975). Based on reported fumonisin levels in maize from the Transkei during six different seasons and an apparent mean dietary intake of the BD IX rats of 32 g/kg bw feed per day, a total fumonisin intake of 0.65–1.4 mg/kg per day was estimated (Rheeder *et al.*, 1992). Advanced toxic lesions in the liver included extensive bile duct proliferation, hepatocellular degeneration and development of hyperplastic liver nodules (Purchase *et al.*, 1975).

In male BD IX rats fed 1 g/kg fumonisin B_1 in the diet for 33 days, major changes seen in the liver included bile duct proliferation, fibrosis and hepatocyte nodules. Changes in kidney were mild and included fatty changes and scant necrosis in the proximal convoluted tubule. Gavage dosing with 238 mg/kg bw fumonisin B_1 per day caused the death of three of four rats and produced lesions in liver with minor changes in kidney. Severe disseminated acute myocardial necrosis and severe pulmonary oedema were observed in two rats. At lower dosages but with longer exposures (9–12 days), pathological changes were observed in liver. Early signs of bile duct proliferation and fibrosis, radiating from the portal areas, were noticed and the nuclei of a few hepatocytes were enlarged (Gelderblom *et al.*, 1988).

Additional feeding studies were performed using concentrations of 25–750 mg fumonisin B_1 per kg diet. Histopathological lesions in the liver (hepatocyte nodules) after 21 days were observed in rats that had received the high doses (750, 500 and 250 mg/kg diet), with severity decreasing with decreasing dose. A few necrotic cells were detected in the liver of rats treated with 50 mg fumonisin B_1 per kg diet, whereas no lesions were observed at the 25-mg/kg dietary level (Gelderblom *et al.*, 1994, 1996a).

Hepatocyte injury was investigated in male Fischer 344 rats fed a diet containing fumonisin B_1 at 250 mg/kg for five weeks. Fumonisin B_1 induced hepatocyte necrosis and apoptosis mainly in zone 3 of the liver lobule. Hepatocyte injury and death were reflected by desmin-positive hepatic stellate cell proliferation and marked fibrosis, with changes in architecture and formation of regenerative nodules. Oval cell proliferation was noted from week 2 and occurred in parallel with continuing hepatocyte mitotic activity. Nodules developed and, at later time points, oval cells were noted inside some of the nodules (Lemmer *et al*., 1999a).

Male and female Fischer 344 rats were fed doses of approximately 12, 20, 28 or 56 mg/kg bw fumonisin B_1 per day for 28 days (National Toxicology Program, 2000). Body weight in both males and females was decreased at doses ≥ 20 mg/kg bw per day. The kidney was more sensitive to fumonisin B_1-induced changes in males than in females, but the liver was more affected in females than in males. The earliest cellular response in both liver and kidney was increased apoptosis accompanied by increased cell proliferation. Structural degeneration as a result of apoptosis was noted in both liver and kidney. In females, the lowest effective dose for bile duct hyperplasia and decreased liver weight was 56 mg/kg bw per day and that for liver degeneration and increased hepatocellular mitosis was 28 mg/kg bw per day. The lowest effective dose for increased hepatocellular apoptosis was 20 mg/kg bw per day. Decreased kidney weight, increased structural degeneration and increased renal tubule epithelial cell apoptosis were seen even at 12 mg/kg bw per day in males but only at ≥ 20 mg/kg per day in females (Howard *et al*., 2001).

Male and female Sprague-Dawley rats were fed 15, 50 or 150 mg fumonisin B_1 per kg of diet over a period of four weeks. The estimated daily intake of fumonisin B_1 was 1.4, 4.4 and 13.6 mg/kg bw for males and 1.4, 4.1 and 13.0 mg/kg bw for females. In liver, mild histopathological changes were observed by light microscopy only in rats fed the high dose. Nephrotoxic changes were found in the proximal convoluted tubules in males fed diets containing ≥ 15 mg fumonisin B_1 per kg and in females at diets containing ≥ 50 mg/kg. Serum levels of enzymes, cholesterol and triglycerides were increased at dietary fumonisin B_1 concentrations of 150 mg/kg (Voss *et al*., 1993, 1995a).

Male and female Sprague-Dawley rats were given oral doses of 1, 5, 15, 35 or 75 mg/kg bw fumonisin B_1 daily for 11 days. Histopathological changes in the kidneys were similar to those seen in other studies, males being more sensitive, with a lowest effective dose of 1 mg/kg bw per day versus 5 mg/kg bw for females. Hepatotoxicity was associated with reduced liver weight, as well as increased vacuolization of adrenal cortex cells, which occurred in female and male rats treated at doses ≥ 15 mg/kg bw per day. Elevated cholesterol concentrations in serum were observed in female rats at doses ≥ 5 mg/kg bw per day, but only at the highest dose (75 mg/kg bw per day) in males. Serum glucose was significantly reduced and alanine transaminase, aspartate transaminase and creatinine were significantly elevated at the highest dose in males, and in the two highest-dose groups in females. Single-cell necrosis and mitosis were seen at doses of 15 to 75 mg/kg bw per day in both males and females. Mild lymphocytosis in the

thymic cortex of the fumonisin B_1-treated rats was evident at ≥ 5 mg/kg bw per day in males and at 75 mg/kg bw per day in females (Bondy *et al.*, 1996, 1998).

Male RIVM rats were treated with fumonisin B_1 at 0.19, 0.75 or 3 mg/kg bw per day by gavage for 28 days. The treatment had no effect on body weight, but kidney weight was significantly reduced in the highest-dose group. Increased apoptosis in the medulla of the kidney and renal tubule cell death were seen in the mid- and high-dose groups but not at the 0.19-mg/kg bw dose. There was no histological indication of liver toxicity (de Nijs, 1998).

Male BALB/c mice were given five subcutaneous injections of fumonisin B_1 in sterile water over a period of five days at doses of 0.25, 0.75, 2.25 or 6.75 mg/kg bw per day. Apoptosis was detected in the liver at doses above 0.75 mg/kg bw and in the kidneys at all doses. The relative kidney weights (% of bw) were decreased at all dose levels except 0.75 mg/kg bw per day, while no effect was observed with respect to relative liver weights (Sharma *et al*., 1997; Tsunoda *et al*., 1998).

In adult male and female B6C3F_1 mice treated with daily doses of 1, 5, 15, 35 or 75 mg/kg bw fumonisin B_1 by gavage during 14 days, hepatotoxicity was observed in both sexes, but kidney toxicity was seen only in females. Females were more sensitive than males to the effects in liver and kidney. Single-cell necrosis was detected in the liver at doses ≥ 35 mg and ≥ 15 mg/kg bw per day in males and females, respectively. Hepatocyte mitosis was elevated in males at 75 mg/kg bw per day and in females at ≥ 5 mg/kg bw per day. Mild single-cell necrosis in the kidney was detected in the cortical and medullary tubules only in female mice at 15–75 mg/kg bw per day. Males (≥ 35 mg/kg bw per day) and females (≥ 15 mg/kg bw per day) exhibited moderate diffuse vacuolization of adrenal cortical cell cytoplasm. Mild thymic cortical lymphocytolysis was noticed in a few female mice that received ≥ 35 mg/kg bw per day (Bondy *et al*., 1997).

Male and female B6C3F_1 mice were fed 99, 163, 234 or 484 mg fumonisin B_1 per kg of diet over a period of 28 days (National Toxicology Program, 2000). The average daily intake of fumonisin B_1 was slightly higher in females than in males. Males developed liver lesions at 484 mg/kg in the diet, while such changes were seen in females at all dose levels. The lowest effective doses with respect to liver pathology were 93 mg/kg bw per day in males and 24 mg/kg bw per day in females.

Fumonisin B_1 at 1, 3, 9, 27 or 81 mg/kg of diet was fed to male and female B6C3F_1 mice over a period of 90 days. The mean daily intake of fumonisin B_1 was 0.3, 0.8, 2.4, 7.4 or 23 mg/kg bw for males and 0.3, 1, 3, 9.7 or 29 mg/kg bw for females. Serum levels of cholesterol, alanine transaminase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase and total bilirubin were significantly increased in the high-dose female mice, while no effect was reported in male mice. The clinical findings paralleled histological observations in the liver of the female mice, which were mainly restricted to the centrilobular zone. No lesions were reported in the kidneys of the mice (Voss *et al*., 1995b).

4.2.3 *Related studies*

Alkaline hydrolysis of fumonisins B_1 and B_2 removes the carboxylic acid side-chains producing hydrolysed fumonisin B_1 (HFB_1) and hydrolysed fumonisin B_2 (HFB_2). HFB_1 and HFB_2 are major breakdown products in nixtamalized corn. Feeding nixtamalized *F. verticillioides* corn culture material containing 58 mg/kg HFB_1 to rats during four weeks caused lesions in the liver and kidney that were indistinguishable from those caused by feeding culture material that was not nixtamalized and contained predominantly fumonisin B_1 (71 mg/kg). Liver lesions included apoptosis, sloughing of epithelial cells into the limina and an increased nucleus-to-cytoplasm ratio. However, the extent and severity of the liver lesions, the decrease in weight gain and the elevation of free sphingoid bases were less in animals that received the nixtamalized culture material than in rats that received non-treated material, even though the molar concentration of HFB_1 (58 μg/g [143 nmol/g]) was greater than that of fumonisin B_1 (71 μg/g [98.5 nmol/g]) in the culture material diets that had not been nixtamalized (Voss *et al*., 1996c).

Male Fischer 344 rats (8–10 animals per group) were treated by gavage with 1.4, 4.2, 14.3, 21.0 or 35.0 mg/kg bw fumonisin B_1 (92–95% pure) per day for 14 days. After 14 days, degenerative changes in the liver were seen in the two high-dose groups and included apoptosis, mild proliferation of oval cells and increased mitotic figures. One week after the start of the fumonisin B_1 treatment, separate groups were treated either intravenously with 100 μmol/kg bw lead nitrate, by partial hepatectomy or with a single gavage dose of 2 mL/kg bw carbon tetrachloride to stimulate cell proliferation. Three weeks after the fumonisin B_1 treatment, rats were subjected to 2-acetylaminofluorene (2-AAF)/partial hepatectomy or 2-AAF/carbon tetrachloride promotion treatmentsduring four days and the incidence of placental glutathione *S*-transferase (GSTP)-positive lesions was monitored two weeks later. In groups receiving partial hepatectomy or carbon tetrachloride during the initiation phase followed by 2-AAF/carbon tetrachloride or 2-AAF/partial hepatectomy, respectively, enhanced induction of GSTP-positive lesions was observed in the high-dose groups. This effect was not seen in the group treated with the mitogen lead nitrate followed by 2-AAF/partial hepatectomy (Gelderblom *et al*., 2001b).

A total of 38 male Fischer 344 rats were divided into four groups and fed 250 mg fumonisin B_1 per kg diet (92–95% pure; fumonisin intake, 16.4 mg/kg bw per day) for five weeks in the absence or presence of 1–2% dietary iron in a modified American Institute of Nutrition (AIN) 76 diet. One group received dietary iron but no fumonisin B_1 and one group received the control diet. The dietary iron treatment included one week at 2%, one week on control diet followed by two weeks at 1% to avoid excessive toxic effects. Two animals in each treatment group and one control rat were killed at three and four weeks, and the remaining rats (six per group) were sacrificed after five weeks. Hepatocyte necrosis, mitosis and apoptosis and GSTP-positive hepatic lesions were noted in the fumonisin B_1-treated group (5.34 ± 1.42 lesions/cm^2), while the fumonisin

B_1/iron-treated group showed fewer GSTP-positive lesions (1.50 ± 0.52 lesions/cm^2). The concentration of alanine transaminase in serum was increased, reflecting hepatotoxicity, in both the fumonisin B_1- and fumonisin B_1/iron-treated groups. Body weight gain was decreased in the fumonisin B_1-, iron- and fumonisin B_1/iron-treated groups, while relative liver weights were decreased only in the fumonisin B_1-treated rats. Lipid peroxidation in the liver was increased in fumonisin B_1/iron- and iron-treated rats (Lemmer *et al*., 1999b).

Male Fischer 344 rats (5–8 per group) were fed modified AIN-76 diets containing 250 mg fumonisin B_1 per kg diet for three weeks. Other groups received 17 µg/kg bw aflatoxin B_1 per day by gavage for 14 days (total dose, 240 µg/kg bw) or a single intraperitoneal injection of 200 mg/kg bw *N*-nitrosodiethylamine (NDEA). The three groups were compared using the resistant hepatocyte model (Semple-Roberts *et al*., 1987) which consisted of treatment with 20 mg/kg bw 2-AAF by gavage on each of three consecutive days followed by partial hepatectomy on day 4. The induction of GSTP-positive lesions was monitored three weeks after the latter treatment. GSTP-positive lesions were increased by treatment with both fumonisin B_1 and aflatoxin B_1 in combination with the 2-AAF/partial hepatectomy promoting stimulus, but to a much lesser extent than by treatment with NDEA (ratio 1:3:10 for fumonisin B_1, aflatoxin B_1 and NDEA). In a second set of experiments, the separate and combined effects of aflatoxin B_1 and fumonisin B_1 on the induction of GSTP-positive lesions were determined in the absence of the 2-AAF/partial hepatectomy promoting treatment. When rats were treated sequentially with aflatoxin B_1 followed three weeks later by fumonisin B_1, a synergistic interaction was found based on increased numbers and size of the GSTP-positive lesions in the liver (total of 72, 5 and 1.6 lesions consisting of more than 5 cells/cm^2 for aflatoxin B_1 + fumonisin B_1, aflatoxin B_1 and fumonisin B_1, respectively (Gelderblom *et al*., 2002).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

A specific role for fumonisins in the development of neural tube defects was suggested after the appearance of a cluster of such defects in Texas associated with consumption of corn from the heavily fumonisin-contaminated 1989 corn crop (Hendricks, 1999). More recent studies have shown that fumonisin B_1 inhibits folate metabolism in cultured cells (Stevens & Tang, 1997). The relationship between folate deficiency and neural tube defects is well established, but there are no specific studies to confirm the association with exposure to fumonisins.

4.3.2 *Experimental systems*

(*a*) *Developmental and reproductive toxicity studies*

Pregnant CD CRL rats were given oral doses of 0, 1.875, 3.75, 7.5 or 15 mg/kg bw fumonisin B_1 per day on gestation days 3–16. Feed consumption and body weight gain were significantly decreased at the 15-mg/kg bw dose. Fetal body weights at day 17 were similar in control and treated groups, but in day-20 fetuses, female weight and crown–rump length were significantly decreased at the highest dose. In day-17 animals, dose-related increases in sphinganine/sphingosine ratios were seen in maternal livers, kidneys and serum. Sphinganine/sphingosine ratios in maternal brains were not affected, nor were those of fetal kidneys, livers or brains (Collins *et al*., 1998a). In a similar study using dose levels of 0, 6.25, 12.5, 25 or 50 mg/kg bw fumonisin B_1 per day, maternal toxicity and fetal toxicity were seen at the 50-mg/kg bw dose. The effects on the fetuses included increased numbers of late deaths, decreased body weight and crown–rump length and increased incidence of hydrocephalus and skeletal anomalies. Dose-related increases in sphinganine/sphingosine ratios were seen in maternal livers, kidneys, serum and brain, but not in fetal livers, kidneys or brain (Collins *et al*., 1998b). The data from these two studies suggest either that fumonisin B_1 does not cross the placenta, the observed fetal toxicity being a secondary consequence of maternal toxicity, or that a potential direct effect of fumonisin B_1 on fetal development is not related to changes in sphinganine/sphingosine ratios in the fetuses.

Groups of pregnant Fischer 344 rats were dosed by gavage daily on gestation days 8 to 12 with 30 or 60 mg/kg bw purified fumonisin B_1 or with a fat-soluble extract of *F. proliferatum*/corn culture that would provide a dose of approximately 60 mg fumonisin B_1 per kg body weight. Lower fetal litter weight and delayed ossification were observed in the rats given 60 mg/kg bw fumonisin B_1, but not in rats given 30 mg/kg bw fumonisin B_1 or the fat-soluble extract (Lebepe-Mazur *et al*., 1995).

The neurobehavioural and developmental effects of fumonisin B_1 were studied in Sprague-Dawley rats treated by gavage on gestation days 13–20 with 0, 0.8 or 1.6 mg/kg bw fumonisin B_1 obtained from culture material or 0, 1.6 or 9.6 mg/kg bw purified fumonisin B_1. There was no effect on reproductive outcomes or offspring body weight through adulthood in either experiment. Some effects on acoustic startle response and play behaviour were found in male but not in female offspring prenatally treated with any dose of purified fumonisin B_1. Fumonisin B_1 treatment had no effect on complex maze performance or open field and running wheel activity (Ferguson *et al.*, 1997).

Pregnant Charles River CD-1 mice were treated orally with a semipurified extract of *F. verticillioides* culture providing 0, 12.5, 25, 50 or 100 mg/kg bw fumonisin B_1 daily on gestation days 7–15. Maternal mortality was observed at doses of 50 and 100 mg/kg bw. Signs of liver damage and decreased maternal body weight gain were observed at $\geq$ 25 mg/kg bw. The percentage of implants resorbed was increased at all doses in a dose-dependent manner. The number of live fetuses per litter and the mean fetal body weight

were decreased and the incidence of ossification deficits, short and wavy ribs and hydrocephalus was increased at the 50- and 100-mg/kg bw doses (Gross *et al*., 1994).

Pregnant Charles River CD-1 mice were administered 0 to 100 mg/kg bw pure fumonisin B_1 by gavage on gestation days 7–15. Doses ≥ 25 mg/kg bw induced maternal liver lesions and a dose-dependent increase in the incidence and severity of hydrocephalus in the fetuses. Reduced fetal body weight was found at ≥ 50 mg/kg bw, while increased frequency of resorptions and decreased litter size were present only at 100 mg/kg bw. Doses ≥ 25 mg/kg bw increased the sphinganine/sphingosine ratios in maternal but not fetal livers. The effects of fumonisin B_1 on the fetuses and the alteration of the sphinganine/sphingosine ratio in maternal but not fetal liver suggest that the effects of fumonisin B_1 on the fetuses are not mediated by changes in sphinganine/sphingosine ratios in the fetuses. The association with effects on the maternal liver may indicate that developmental effects are mediated by maternal hepatotoxicity (Reddy *et al*., 1996).

Six groups of Syrian hamsters were dosed with 0–18 mg/kg bw purified fumonisin B_1 by gavage daily on days 8–12 of gestation and killed on day 15. The treatment caused fetal death, decreased fetal body weight and skeletal variations consistent with delayed development in a dose-dependent manner, without causing maternal toxicity (Penner *et al*., 1998).

In timed-bred Syrian hamsters dosed daily with 0–12 mg/kg bw fumonisin B_1 by gavage on gestation days 8–10 or 12, reduced maternal weight gain was observed at doses ≥ 8 mg/kg bw. Maternal aspartate transaminase and total bilirubin, used as indices of maternal hepatotoxicity, showed no significant difference between groups. At doses higher than 2 mg/kg bw fumonisin B_1, there was an increased incidence of prenatal loss (death and resorptions). At 12 mg/kg bw, all litters were affected and 100% of the fetuses were dead and resorbing (Floss *et al*., 1994a).

A significant increase in litters with fetal deaths occurred in Syrian hamsters given 18 mg/kg bw purified fumonisin B_1 or culture-extracted fumonisins (18 mg fumonisin B_1 plus 4.5 mg fumonisin B_2) by gavage on gestation days 8 and 9. There were no clinical signs of maternal intoxication (Floss *et al*., 1994b).

New Zealand White rabbits were dosed by gavage on gestation days 3–19 with purified fumonisin B_1 at 0.1, 0.5 or 1.0 mg/kg bw. Maternal lethality occurred at the 0.5- and 1.0-mg/kg bw doses (10–20%), but there was no difference in maternal weight gain during pregnancy. Fetal weight and liver and kidney weights were decreased at 0.5 and 1.0 mg/kg bw. Increased sphinganine/sphingosine ratios were found in maternal serum, liver and kidney, but there was no significant effect of fumonisin B_1 on the sphinganine/sphingosine ratio in fetal brain, liver or kidney (LaBorde *et al*., 1997).

Diet formulated with culture material of *F. verticillioides* strain MRC 826 to provide 0, 1, 10 or 55 mg fumonisin B_1 per kg diet was fed to male and female rats beginning 9 and 2 weeks before mating, respectively, and continuing throughout the mating, gestational and lactational phases of the study. Nephropathy was found in males at dietary doses of ≥ 10 mg/kg and in females fed 55 mg/kg diet. No significant reproductive effects were found in males or dams and fetuses examined on gestation day 15, or dams

and litters on postnatal day 21. Litter weight gain in the 10- and 55-mg/kg groups was slightly decreased; however, gross litter weight and physical development of offspring were not affected. Increased sphinganine/sphingosine ratios were found in the livers of dams from the high-dose group on gestation day 15. However, sphinganine/sphingosine ratios in abdominal slices containing liver and kidney of fetuses from the control and high-dose groups did not differ. In an additional experiment, two dams were given an intravenous injection of 101 μg [^{14}C]fumonisin B_1 on gestation day 15. After 1 h, about 98% of the dose had disappeared from the maternal blood, but only negligible amounts of radioactivity were found in the fetuses (Voss et al., 1996b).

(*b*) *Mechanistically oriented developmental toxicity studies*

Doses of 0.8 or 8 mg/kg bw of fumonisin B_1 were given subcutaneously to male Sprague Dawley rats on postnatal day 12. Brain tissue and blood were collected at ten time points up to 24 h after fumonisin B_1 administration. The sphinganine levels in brain and plasma showed dose-dependent increases; the brain sphinganine level during the 24 h was much higher than plasma sphinganine, with an area under the concentration–time curve (AUC) ratio of 40:1. In addition, fumonisin B_1 was found in the brain tissue after the higher dose. These data indicate that alterations of the brain sphinganine levels are the result of a direct action of fumonisin B_1 on the brain rather than transport of peripheral sphinganine to the brain (Kwon *et al.*, 1997a).

Subcutaneous dosing of Sprague-Dawley rats with 0.4 or 0.8 mg/kg bw fumonisin B_1 from postnatal day 3 to day 12 resulted in reduced body weight gain and decreased survival. Both sphinganine concentration and sphinganine/sphingosine ratios in the brain were increased at the higher dose. To investigate the effects of limited nutrition on sphinganine levels and myelinogenesis, rats were given 0.8 mg/kg bw fumonisin B_1 or subjected to limited nutrition (temporary removal from dam in the postnatal period) and compared with a saline control group. Sphinganine levels were increased in rats treated with 0.8 mg/kg fumonisin B_1, but not in those given limited nutrition. Myelin deposition was decreased in both the nutritionally limited and the fumonisin B_1-exposed rats. These data indicate that sphingolipid metabolism in developing rats is vulnerable to fumonisin B_1, while hypomyelination associated with fumonisin B_1 may be mediated by limited nutrition (Kwon *et al.*, 1997b).

Concentration- and time-dependent increases in sphinganine/sphingosine ratios were found in developing chick embryos after injection of 72 or 360 μg of fumonisin B_1 per egg. A close correlation was observed between disruption of sphingolipid metabolism and tissue lesions detectable by light microscopy (Zacharias *et al.*, 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 4 for references)

Fumonisin B_1 was not mutagenic in *Salmonella typhimurium*/microsome assays with strains TA100, TA98 or TA97 or in the SOS repair test with *Escherichia coli*, whereas a positive result was reported from a Mutatox® assay (luminescence induction) in the absence of metabolic activation. The compound did not induce unscheduled DNA synthesis in liver cells of rats *in vitro* or *in vivo* and no evidence for DNA-adduct formation with oligonucleotides *in vitro* was found; however, positive results were obtained in chromosomal aberration assays and in the micronucleus test with rat hepatocytes. Furthermore, evidence for induction of DNA damage by fumonisin B_1 was found with C6 rat brain glioma cells and human fibroblasts *in vitro*, and in spleen and liver cells isolated from fumonisin B_1-exposed rats. The in-vivo effect could be reversed with α-tocopherol and selenium (Atroshi *et al*., 1999). Positive results were obtained in micronucleus assays *in vitro* with human-derived hepatoma (HepG2) cells but not with rat hepatocytes. In bone marrow of mice, an increase in formation of micronuclei was found after intraperitoneal injection of fumonisin B_1, whereas in a transformation study with a mouse embryo cell line, no response was observed.

4.5 Mechanistic considerations

There are no published data demonstrating that fumonisins form DNA adducts (WHO, 2002). Early studies indicated that fumonisin B_1 gave negative results in bacterial mutation assays and in the unscheduled DNA synthesis assay using primary rat hepatocytes (IARC, 1993). More recent studies with rat hepatocytes *in vitro* and *in vivo* using the Comet assay (DNA migration) have shown that fumonisin B_1 induces DNA damage in rodent- and human-derived cells (Atroshi *et al*., 1999; Erlich *et al*., 2002; Galvano *et al*., 2002) and also chromosomal aberrations or micronucleus formation in human hepatoma cells (Erlich *et al*., 2002) and primary rat hepatocytes (Knasmüller *et al*., 1997). In some studies, addition of antioxidants reduced the amount of DNA damage, leading to the conclusion that oxidative stress is the cause of the DNA damage (Atroshi *et al*., 1999; Mobio *et al*., 2000b).

Numerous studies since the previous evaluation of fumonisins (IARC, 1993) have demonstrated that fumonisins alter signalling pathways that control cell behaviour. Thorough reviews of the biochemical and cellular mechanisms implicated in fumonisin B_1 toxicity and carcinogenicity are available (WHO, 2000; Allaben *et al*., 2001; WHO, 2002) and form the basis for much of what follows.

Two biochemical modes of action proposed to explain fumonisin-induced diseases in animals, including cancer, invoke disruption of lipid metabolism as the initial phase. Both hypothesized mechanisms are supported by data on carcinogenicity in animal models (Gelderblom *et al*., 20001a,b,c; Merrill *et al*., 2001; Riley *et al*., 2001; WHO, 2002) and are similar in many respects.

Table 4. Genetic and related effects of fumonisin B_1

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SOS repair, *Escherichia coli* PQ37	–	–	500 µg/plate	Knasmüller *et al*. (1997)
Escherichia coli rec strains, differential toxicity	–	–	500 µg/plate	Knasmüller *et al*. (1997)
Salmonella typhimurium TA100, TA102, TA97a, TA98, reverse mutation	–	–	5000 µg/plate[c]	Gelderblom & Snyman (1991)
Salmonella typhimurium TA100, reverse mutation	–	–	100 µg/plate	Park *et al*. (1992)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	500 µg/plate	Knasmüller *et al*. (1997)
Salmonella typhimurium TA100, TA102, TA98, reverse mutation	–	–	114 µg/plate	Aranda *et al*. (2000)
Salmonella typhimurium TA100, TA102, TA1535, TA1537, TA98, reverse mutation	NT	–[d]	200 µg/plate	Ehrlich *et al.* (2002)
Luminescence induction, *Vibrio fischeri*, mutation *in vitro*	+	NT	5	Sun and Stahr (1993)
DNA strand breaks (DNA-unwinding method), rat liver cells *in vitro*	+	NT	29	Sahu *et al*. (1998)
DNA strand breaks (Comet assay), C6 rat brain glioma cells *in vitro*	+	NT	2.2	Mobio *et al*. (2000a)
DNA adduct formation, oligonucleotides *in vitro*[e]	–	NT	360	Pocsfalvi *et al*. (2000)
Unscheduled DNA synthesis, rat primary hepatocytes, *in vitro*	–	NT	58	Gelderblom *et al*. (1992b)
Unscheduled DNA synthesis, rat primary hepatocytes, *in vitro*	–	NT	180	Norred *et al*. (1992)
DNA hypermethylation, C6 rat brain glioma cells, *in vitro*	+	NT	6.5	Mobio *et al*. (2000b)
Micronucleus formation, rat hepatocytes *in vitro*	–	NT	100	Knasmüller *et al*. (1997)
Chromosomal aberrations, rat hepatocytes *in vitro*	+	NT	1	Knasmüller *et al*. (1997)
Cell transformation, BALB/3T3 A31-1-1 mouse embryo cells	–	NT	1000	Sheu *et al*. (1996)
DNA damage (Comet assay), human hepatoma (HepG2) cells *in vitro*	+	NT	25	Ehrlich *et al.* (2002)
DNA damage (Comet assay), human fibroblasts *in vitro*	+	NT	7.2	Galvano *et al*. (2002)
Micronucleus formation, human hepatoma (HepG2) cells *in vitro*	+	NT	25	Ehrlich *et al.* (2002)

Table 4 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA fragmentation, male Sprague-Dawley rat liver and spleen *in vivo*	+		1.55 × 1 iv	Atroshi *et al*. (1999)
Unscheduled DNA synthesis, male Fischer 344 rat hepatocytes *in vivo*	–		100 × 1 po	Gelderblom *et al*. (1992b)
Micronucleus formation, male CF1 mouse bone-marrow cells *in vivo*	+		25 × 1 ip	Aranda et al. (2000)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; iv, intravenous; po, oral; ip, intraperitoneal
[c] A dose of 10 mg/plate was inactive in the pre-incubation assay and toxic in the plate-incorporation assay.
[d] Metabolic activation with S9 from human hepatoma (HepG2) cells
[e] Analysed by HPLC-mass spectrometry

The first proposed lipid-based mechanism involves inhibition of ceramide synthase (Wang *et al*., 1991), a key enzyme in the biosynthesis of sphingolipids. In line with findings in human cell lines (Biswal *et al*., 2000; Charles *et al*., 2001), human primary cell cultures (Tolleson *et al*., 1999), non-human primates (Van der Westhuizen *et al*., 2001) and all other animals tested (reviewed in WHO, 2000, 2002), human exposure to fumonisins is also associated with evidence of disruption of sphingolipid metabolism (Qiu & Liu, 2001). Alterations in the free sphinganine/free sphingosine ratio, a consequence of ceramide synthase inhibition, are now used as a biomarker for exposure to fumonisins in domestic animals (Riley *et al*., 1994a,b) and humans (Van der Westhuizen *et al*., 1999; Qiu & Liu, 2001; Ribar *et al*., 2001). Turner *et al*. (1999) reviewed potential problems of using sphingoid base ratios as a functional biomarker for exposure to fumonisin B_1 in humans.

The second biochemical mechanism proposes changes in polyunsaturated fatty acids and phospholipid pools (Gelderblom *et al*., 1996b). This mechanism is supported by data from studies with rat liver (reviewed in WHO, 2002) and human cell lines (Pinelli *et al*., 1999; Seegers *et al*., 2000).

The cellular consequences of both biochemical modes of action provide support for a non-genotoxic mechanism of carcinogenicity. It is proposed that alterations in cell growth, death and differentiation due to disruption of lipid-mediated signalling and regulatory pathways lead to an imbalance between the rates of apoptosis and proliferation and that this imbalance is a critical determinant in the process of hepato- and nephrotoxicity and tumorigenesis in animal models (reviewed in WHO, 2002).

4.5.1 *Interference with sphingolipid metabolism*

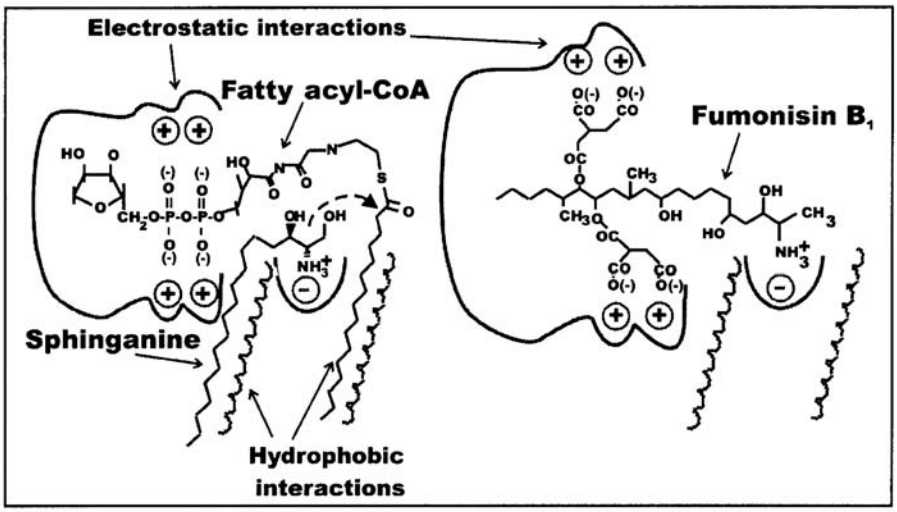
(*a*) *Sphingolipid chemistry and function*

Sphingolipids are a highly diverse class of lipids found in all eukaryotic cells. The biological functions are equally diverse: the compounds serve as structural components required for maintenance of membrane integrity, as receptors for vitamins and toxins, as sites for cell–cell recognition and cell–cell and cell–substrate adhesion, as modulators of receptor function and as lipid second messengers in signalling pathways responsible for cell growth, differentiation and death (Merrill *et al*., 1997).

(*b*) *Inhibition of ceramide synthase*

In every cell line and animal, plant or fungus in which it has been tested, fumonisin B_1 inhibits the coenzyme A (CoA)-dependent acylation of sphinganine and sphingosine via interaction with the enzyme sphinganine/sphingosine *N*-acyltransferase (ceramide synthase). This enzyme recognizes both the amino group (sphingoid-binding domain) and the tricarboxylic acid side-chains (fatty acyl-CoA domain) of fumonisin B_1 (Merrill *et al*., 2001) (Figure 3).

Figure 3. Proposed model illustrating how the tricarboxylic acid groups and free amino group of fumonisin B_1 mimic the fatty acyl–coenzyme A (CoA) and free sphinganine substrates, respectively, in the active site of ceramide synthase. For fumonisin B_1, the interaction is primarily electrostatic; for the normal substrates, there are also hydrophobic interactions involving partitioning into the lipid bilayer



Modified from Merrill *et al*. (1996, 2001)

(*c*) *Sphingoid base accumulation*

When ceramide synthase is completely inhibited, either *in vitro* or *in vivo*, the intracellular sphinganine and sometimes sphingosine concentration increases rapidly. *In vivo* there is a close relationship between the amount of sphinganine accumulated and the expression of fumonisin toxicity in liver and kidney (Riley *et al.*, 1994a,b; Tsunoda *et al.*, 1998; Riley *et al*., 2001; Voss *et al.*, 2001). Accumulated free sphingoid bases can persist in tissues (especially kidney) much longer than fumonisin B_1 (most recently shown by Enongene *et al*., 2000; Garren *et al*., 2001; Enongene *et al.*, 2002a,b). In urine from rats fed fumonisin B_1, nearly all the free sphinganine is recovered in dead cells. A sub-threshold dose in rats or mice can prolong the elevation of free sphinganine in urine or kidney caused by a higher dose (Wang *et al*., 1999; Enongene *et al*., 2002a,b). Fumonisin B_1-induced elevation of free sphingoid base levels and toxicity are both reversible, although elimination of free sphinganine from the liver is more rapid than from the kidney (Enongene *et al*., 2000; Garren *et al*., 2001; Enongene *et al*., 2002a,b).

In ponies given fumonisin B_1-contaminated feed, changes in the sphinganine/sphingosine ratio in serum were seen before hepatic enzymes were notably elevated (Wang *et al*., 1992; Riley *et al*., 1997).

(*d*) *Sphingoid base metabolite, fatty acid and glycerophospholipid imbalances*

Inhibition of ceramide synthase by fumonisin B_1 can result in the redirection of substrates and metabolites to other pathways. For example, when sphinganine accumulates, it is metabolized to sphinganine 1-phosphate. The breakdown of sphinganine 1-phosphate results in the production of a fatty acid aldehyde and ethanolamine phosphate. Both products are redirected to other biosynthetic pathways, in particular increased biosynthesis of phosphatidylethanolamine (Badiani *et al.*, 1996). Disrupted sphingolipid metabolism leads to imbalances in phosphoglycerolipid, fatty acid metabolism and cholesterol metabolism via free sphingoid base- and sphingoid base 1-phosphate-induced alterations in phosphatidic acid phosphatase and monoacylglycerol acyltransferase. Thus, fumonisin B_1 inhibition of ceramide synthase can cause a wide spectrum of changes in lipid metabolism and associated lipid-dependent signalling pathways (reviewed in Merrill *et al*., 2001).

(*e*) *Disruption of sphingolipid metabolism and in-vivo toxicity*

Disruption of sphingolipid metabolism, as shown by statistically significant increases in free sphinganine concentration, usually occurs at or below doses of fumonisin that cause liver or kidney lesions in short-term studies with rats, rabbits, mice, pigs, horses and many other species of animals and plants (reviewed in WHO, 2002). In some studies, significant increases in free sphingoid bases occur at doses that are higher than for other markers of hepatic effects (Liu *et al*., 2001). Nevertheless, many studies show a close correlation between elevation of free sphinganine levels and increased apoptosis in liver and kidney (Riley *et al*., 2001). For example, fumonisin B_1 induced an increase in the sphinganine/sphingosine ratio in kidney tissue and urine, which correlated with increased incidence of non-neoplastic and neoplastic kidney lesions in a long-term feeding study with Fischer 344/N Nctr rats (National Toxicology Program, 2000; Howard *et al*., 2001). However, in livers of female B6C3F_1/Nctr mice, elevation of free sphinganine and the sphinganine/sphingosine ratio were significantly increased only after 3 and 9 weeks at 50 and 80 mg fumonisin B_1 per kg of diet, doses that also induced liver adenoma and carcinoma (National Toxicology Program, 2000).

Fumonisin B_1-induced hepatotoxicity in both female and male Sprague-Dawley rats *in vivo* was associated with free sphinganine concentrations in liver tissue of approximately 20 nmol/g fresh tissue. At the non-hepatotoxic dietary concentration of 50 ppm [mg/kg] fumonisin B_1, the free sphinganine levels were 12 and 4 nmol/g tissue in females and males, respectively. In contrast, nephrotoxic concentrations of fumonisin B_1 in the diet (50 ppm for females, 15 ppm for males) were associated with free sphinganine levels of 146 and 129 nmol/g tissue, respectively. For the female rats, this was a 10-fold increase of free sphinganine over the level measured at the non-nephrotoxic concentration of 15 ppm fumonisin B_1 in the diet (Voss *et al.*, 1996a). This is similar to the renal free sphinganine concentrations (100–134 nmol/g fresh tissue) associated with

significantly increased nephropathy and the hepatic free sphinganine concentrations (5–15 nmol/g fresh tissue) associated with significantly increased hepatopathy in male BALB/c mice (Sharma *et al*., 1997; Tsunoda *et al*., 1998). Fumonisin B_1 is not a complete carcinogen in the rainbow trout model; however, a close correlation was reported between the elevated level of free sphinganine in liver and fumonisin B_1-mediated promotion of aflatoxin B_1-induced hepatocarcinogenicity (Carlson *et al*., 2001).

(*f*) *Free sphingoid bases as functional biomarkers in humans*

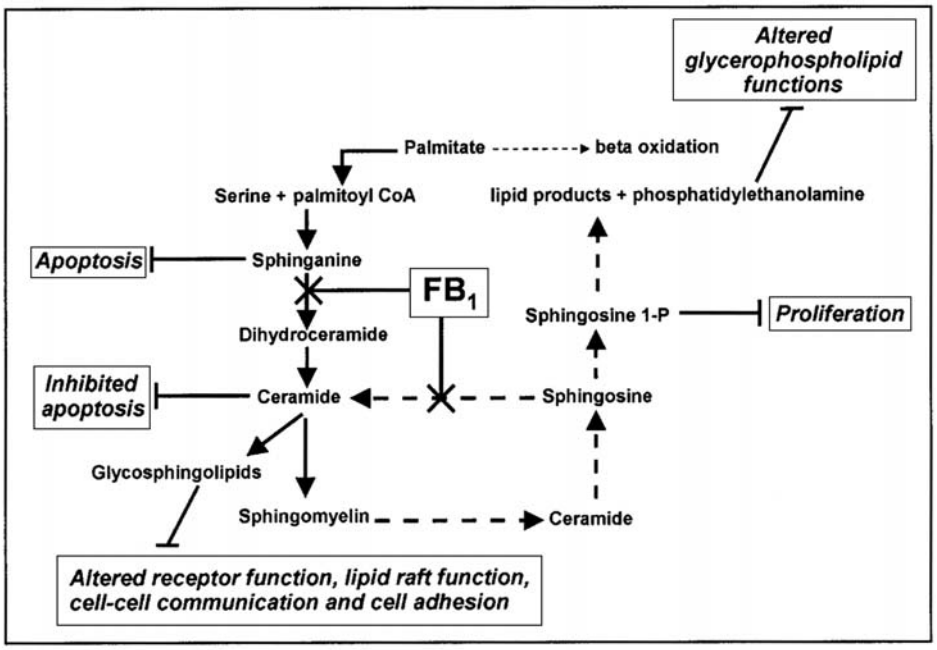
Several studies have examined the use of the elevation of free sphinganine in human urine or blood as an indicator of exposure to fumonisin B_1, with mixed success. For example, van der Westhuizen *et al*. (1999) found no relationship between urine or serum sphingoid base levels and dietary fumonisin B_1 intake. However, in this study, the levels of fumonisin B_1 in the diet were low. A recent study conducted in China found that the free sphinganine to free sphingosine ratio was significantly greater in urine collected in households with an estimated fumonisin B_1 intake above 110 μg/kg bw/day (Qiu & Liu, 2001). A study conducted in the endemic nephropathy area of Croatia found statistically significant differences (compared with control groups) in free sphingoid bases in serum and urine of individuals living in the region but not affected by endemic nephropathy. However, in this study dietary exposure to fumonisin B_1 was not established, although exposure to a mycotoxin or environmental factor that impaired sphingolipid metabolism was suggested (Ribar *et al*., 2001).

(*g*) *Sphingolipid metabolites and apoptosis*

Numerous studies using cultured cells have demonstrated sphingolipid-dependent mechanisms for inducing apoptosis. For example, accumulation of excess ceramide, glucosylceramide (Korkotian *et al.*, 1999) or sphingoid bases, or depletion of ceramide or more complex sphingolipids have all been shown to induce apoptotic or oncotic cell death (WHO, 2000; Merrill *et al.*, 2001; Riley *et al.*, 2001; WHO, 2002). Conversely, the balance between sphingosine 1-phosphate and ceramide is critical for signalling proliferation and cell survival (Spiegel, 1999). It can be expected that there will also be a diversity of alterations in cellular regulation resulting from imbalances in sphingolipid metabolite and product pools resulting from inhibition by fumonisin B_1 of ceramide synthase (Figure 4). This is best demonstrated by the numerous recent studies with fumonisin B_1 identifying cell processes that are ceramide-mediated; for example, the ability of fumonisin B_1 to protect oxidant-damaged cells from apoptosis and to alter the proliferative response (WHO, 2000; Riley *et al.*, 2001; WHO, 2002). In addition to the many studies cited previously, experiments with fumonisin B_1 have revealed new roles for de-novo ceramide production in inhibition of apoptosis and other ceramide-mediated processes (for example, Biswal *et al.*, 2000; Blázquez *et al.*, 2000; Chi *et al.*, 2000; Herget *et al.*, 2000; Kawatani *et al.*, 2000; Kirkham *et al.*, 2000; Lee *et al.*, 2000; Charles *et al.*, 2001; Dyntar *et al.*, 2001; Iacobini *et al.*, 2001; Kroesen et al., 2001; Maedler *et al.*, 2001; Wu *et al.*, 2001; Zhong *et al.*, 2001). In many of the examples cited above, short-term

treatment with fumonisin B_1 protected against ceramide-mediated cell death. In contrast, prolonged exposure to fumonisin B_1 in vivo and in vitro is toxic to cells and induces apoptosis (WHO, 2000, 2002). Perhaps the best evidence for a cause-and-effect relationship between disruption of sphingolipid metabolism and the toxic effects of fumonisin B_1 has come from studies conducted *in vitro* using inhibitors of serine palmitoyltransferase to prevent sphinganine accumulation and reverse the increased apoptosis and altered cell growth induced by fumonisin B_1 treatment (WHO, 2000; Kim *et al.*, 2001; Riley *et al.*, 2001; Yu *et al.*, 2001; He *et al.*, 2002; WHO, 2002).

Figure 4. Pathways of sphingolipid biosynthesis and turnover in a mammalian cell. In boxes are the known biological activities affected by fumonisin B_1 (FB_1) inhibition of ceramide synthase and associated with changes in the biosynthesis of various sphingolipid intermediates and products



For additional details, see Merrill *et al*. (2001); Riley *et al*. (2001)
Sphingosine 1-P, sphingosine 1-phosphate

(*h*) *Depletion of complex sphingolipids*

Depletion of more complex sphingolipids also plays a role in the abnormal behaviour, altered morphology and altered proliferation of fumonisin-treated cells (WHO, 2000; Merrill *et al*., 2001; Riley *et al*., 2001; WHO, 2002), and this has been proposed as a mechanism in fumonisin B_1-induced nephrotoxicity in male rats (Hard *et al*., 2001) through the disruption of cell–cell interactions. Numerous studies have demonstrated the

ability of fumonisin to alter the function of specific glycosphingolipids and lipid rafts (membrane associations of sphingolipids, ceramide-anchored proteins and other lipids). Examples of these functions are inhibition of folate transport, bacterial toxin binding and transport (e.g., *Shigella* and cholera toxin), cell–cell and cell–substratum contact and cell–cell communication (WHO, 2000; Riley *et al*., 2001; Merrill *et al.*, 2001; WHO, 2002).

(*i*) *Increased dihydroceramide* in vivo

In fumonisin-treated animals (pigs, horses, mice), there is an increased amount of complex sphingolipids containing sphinganine as the long-chain sphingoid-base backbone (see for example, Riley *et al*., 1993). The ceramide generated from these complex sphingolipids is dihydroceramide, which is inactive in ceramide signalling and does not induce death of oxidant-damaged hepatocytes (Arora *et al*., 1997). Dihydroceramide is also enriched in mouse hepatoma-22 cells, in which sphinganine comprised 37% of the ceramides as compared with 5% in normal rat liver (Rylova *et al*., 1999).

(*j*) *Hypothesized cellular mechanism*

In cultured cells, the balance between the intracellular concentration of sphingolipid effectors that protect cells from apoptosis (decreased ceramide, increased sphingosine 1-phosphate) and the effectors that induce apoptosis (increased ceramide, increased free sphingoid bases, increased fatty acids) determines the observed cellular response (reviewed in Merrill *et al*., 2001; Riley *et al*., 2001). Cells sensitive to the proliferative effect of decreased ceramide and increased sphingosine 1-phosphate will be selected to survive and proliferate. Conversely, when the increase in free sphingoid bases exceeds the ability of a cell to convert sphinganine/sphingosine to dihydroceramide/ceramide or their sphingoid base 1-phosphate, free sphingoid bases will accumulate to toxic levels. Cells that are sensitive to sphingoid base-induced growth arrest will cease growing and insensitive cells will survive. Thus, the kinetics of fumonisin B_1 elimination (rapid), the affinity of fumonisin B_1 for ceramide synthase (competitive and reversible) and the kinetics of fumonisin-induced sphinganine elevation will influence the time course, amplitude and frequency of variations in the concentration of intracellular ceramide, sphingoid base-1 phosphates and free sphinganine in tissues of animals consuming fumonisins (Enongene *et al*., 2002a,b). This is important, because the balance between the rates of apoptosis and cell proliferation is a critical determinant in the process of hepato- and nephrotoxicity and tumorigenesis in animal models (Dragan *et al*., 2001; Howard *et al*., 2001; Voss *et al*., 2001). At the cellular level, it is hypothesized that apoptotic necrosis should be considered to be similar to oncotic necrosis (as defined in Levin *et al*., 1999), in that both will lead to a regenerative process involving sustained cell proliferation (Dragan *et al*., 2001; Hard *et al*., 2001). Numerous endogenous processes can cause DNA damage that, if unrepaired, can give rise to a mutation in the DNA. Increased cell proliferation may thus involve replication of mutated DNA, resulting in an increased risk for cancer (Dragan *et al*., 2001).

4.5.2 *Interference with fatty acid and glycerophospholipid metabolism*

(*a*) *Importance of fatty acids*

Essential fatty acids are major constituents of all cell membrane glycerophospholipids, sphingolipids and triglycerides. In addition to their important role as structural components of all cell membranes, essential fatty acids are precursors of many bioactive lipids known to regulate cell growth, differentiation and cell death.

(*b*) *Interference with fatty acid metabolism*

In rat liver and primary hepatocytes exposed to fumonisin B_1, changes in the phospholipid profile and fatty acid composition of phospholipids indicate that fumonisin B_1 interferes with fatty acid metabolism (Gelderblom *et al*., 1996b). The following summary is taken from the review by Gelderblom *et al*. (2001a) and the WHO monograph (WHO, 2002).

(*c*) *Altered lipid metabolism in rat hepatocytes* in vitro

Gelderblom *et al*. (1996b) showed that, in fumonisin B_1-treated rat hepatocytes, the pattern of changes in specific polyunsaturated fatty acids suggested disruption of the Δ6 desaturase and cyclo-oxygenase metabolic pathways (Figure 5). These changes were considered to be important in the fumonisin B_1-induced toxicity observed in primary hepatocytes (Gelderblom *et al*., 2001a; WHO, 2002).

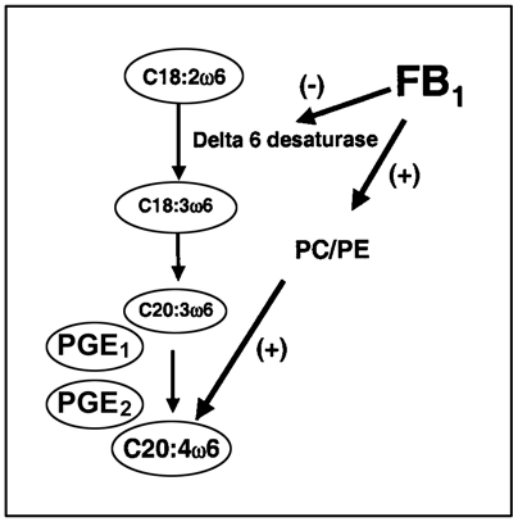
(*d*) *Altered lipid metabolism in rat liver* in vivo

In-vivo studies have confirmed that fumonisin B_1 disrupts fatty acid and phospholipid biosynthesis, but the pattern of changes is different from that observed *in vitro* (Gelderblom *et al*., 1997). Major changes are associated with both the phosphatidylethanolamine and the phosphatidylcholine phospholipid fractions, while cholesterol levels are increased in both the serum and liver (Gelderblom *et al*., 2001a; WHO, 2002). A characteristic fatty acid pattern (Figure 6) is seen in the liver of rats exposed to dietary fumonisin B_1 levels associated with the development of preneoplastic lesions and in liver of rats fed fumonisin B_1 after treatment with cancer initiators.

(*e*) *Altered signalling for cell survival*

At the fumonisin B_1 doses that have been shown to alter fatty acid and glycerophospholipid profiles in rat liver, there are numerous changes in expression of proteins known to be involved in the regulation of cell growth, apoptosis and cell differentiation (WHO, 2002). For example, expression of hepatocyte growth factor (HGF), transforming growth factor α (TGF α), TGF β_1 and the c-*myc* oncogene were all increased during short-term feeding of fumonisin B_1. Overexpression of TGF β_1 could play a role in the increased apoptosis, while the increased expression of the proto-oncogene c-*myc* could contribute to the enhanced cell proliferation that is required for the tumour progression

Figure 5. A model for the proposed interference by fumonisin B_1 (FB_1) with delta 6 ($\Delta 6$) desaturase activity and consequent effects on the fatty acid composition of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and changes in the cyclo-oxygenase metabolic pathway



For additional details, see Gelderblom *et al*. (2001a).
PGE_1 and PGE_2, prostaglandin E_1 and E_2

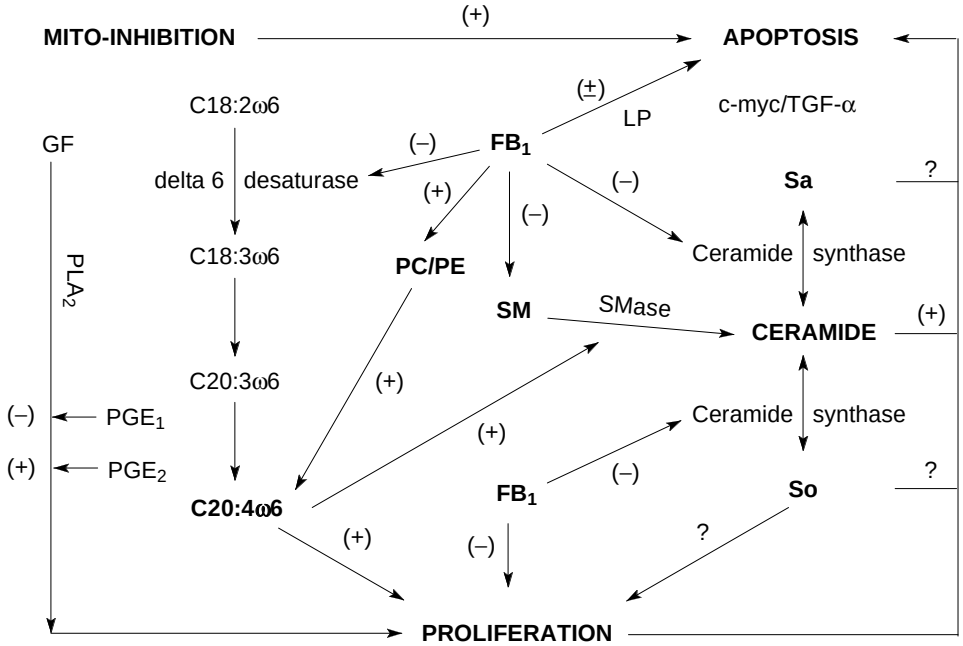
observed in the liver of rats and mice exposed to hepatotoxic levels of fumonisin B_1 (Figure 6). Increased expression of c-*myc* and TGF β_1 may also play a role in the promotion of liver tumours by fumonisin B_1 (Lemmer *et al.*, 1999a).

(*f*) *Altered cell cycle progression*

Fumonisin B_1 disruption of sphingolipid metabolism and altered membrane phospholipids in the liver of BD IX rats have been suggested to cause the changes seen in several proteins (e.g., cyclin D1, retinoblastoma protein) that regulate cell cycle progression. Accumulation of cyclin D1 was due to post-translational stabilization of the protein (Ramljak *et al.*, 2000).

Fumonisin B_1-induced alterations in cellular glycerophospholipid content and the sphingomyelin cycle have been proposed to interact so as to modify a variety of cellular processes, resulting in the increased apoptosis and altered hepatocyte proliferation that are seen in liver of rats fed toxic doses of fumonisin B_1 (Figure 6). The balance between lipid mediators generated via the cyclo-oxygenase-2 and ceramide cycle could regulate processes related to cell proliferation and apoptosis. As summarized in Figure 6, fumonisin B_1-induced changes in ceramide, prostaglandins and other lipid mediators could alter the growth and survival of normal hepatocytes. Overexpression of TGF β_1 and

Figure 6. Proposed biochemical effects and cellular responses associated with fumonisin B_1 (FB_1)-induced alterations in delta 6 (Δ6) desaturase, sphingomyelinase (SMase), and ceramide synthase activity and biosynthesis of phosphotidylcholine (PC) and phosphatidylethanolamine (PE)



Abbreviations: PLA_2, phospholipase A_2; LP, products of lipid peroxidation; GF, growth factors; PGE_1 and PGE_2, prostaglandin E_1 and E_2; Sa, free sphinganine; So, free sphingosine; +, stimulatory; –, inhibitory or decreased; ?, response may be either increased or decreased (see Section 4.5.1); SM, sphingomyelin
Modified from Gelderblom *et al.* (2001a)

c-*myc* and oxidative damage could further enhance apoptosis and alter cell growth in affected hepatocytes (Gelderblom *et al*., 2001a; WHO, 2002).

(*g*) *Hypothesized cellular mechanism*

Fumonisin B_1 has been shown to alter cell proliferation both *in vitro* and *in vivo*. The fumonisin B_1-induced effects on membrane lipids and the resultant effects on signalling pathways that involve lipid mediators could create an environment in which the growth of normal cells is impaired. Differential inhibition of cell proliferation is a possible mechanism by which hepatocytes resistant to fumonisin B_1-induced inhibition of cell growth are selectively stimulated, while growth of normal hepatocytes is inhibited. This selective inhibition of normal cell growth could increase the chances of survival of DNA-damaged hepatocytes, resulting in an increased likelihood of cancer development (Gelderblom *et al*., 2001a; WHO, 2002).

Three lines of evidence support the hypothesis that fumonisin B_1-induced alterations in lipid metabolism contribute to the establishment of a growth differential in rat liver that could influence the process of neoplastic development. First, fumonisin B_1 induces an increase in phosphatidylethanolamine and arachidonic acid (C20 : 4 ω6) (Gelderblom *et al*., 2001a), lipid mediators that are known to regulate many processes related to cell growth, such as proliferation and apoptosis (Khan *et al*., 1995; Gelderblom *et al*., 1999; Pinelli *et al*., 1999; Seegers *et al*., 2000; Abel *et al*., 2001). Second, the decrease in the concentration of long-chain polyunsaturated fatty acids in hepatocytes exposed to fumonisin B_1 will produce a more rigid membrane structure, resulting in increased resistance to lipid peroxidation. Third, lipid metabolites, and in particular glycerophospholipids, are important components of many cellular signalling systems that control the balance between cell growth and cell death. Thus, changes in these lipid pools will alter response to growth factors and other mediators of cell survival.

Fumonisin B_1-induced disruption of lipid metabolism and the consequent induction of oxidative damage and lipid peroxidation (Abel & Gelderblom, 1998; WHO, 2002) could be important events leading to DNA damage, whereas changes in the balance of the different cell regulatory molecules such as those seen in livers of rats fed fumonisin B_1 are likely to be involved in the induction of a growth differential that selectively stimulates the survival of damaged hepatocytes and the development of cancer in rats.

4.5.3 *Other biochemical mechanisms*

Several in-vitro studies with fumonisins have found changes in cellular regulation and cell functions that have been attributed to processes other than lipid metabolism (WHO, 2000, 2002). Many of these effects could be relevant to the organ toxicity of fumonisins. Examples are the direct or indirect effects on protein kinase C (Huang *et al*., 1995; Yeung et al., 1996), activity of extracellular regulated kinases (Pinelli *et al*., 1999), altered DNA methylation and increased lipid peroxidation (Mobio *et al*., 2000a,b) and alterations in the tumour necrosis factor α (TNFα) signalling pathways (He *et al*., 2001; Jones *et al*., 2001; Sharma *et al*., 2001).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Fumonisin B_1 is the most prevalent member of a family of toxins produced by several species of *Fusarium* moulds which occur mainly in maize. Fumonisin B_1 contamination of maize has been reported worldwide at mg/kg levels. Human exposure occurs at levels of micrograms to milligrams per day and is greatest in regions where maize products are the dietary staple.

5.2 Human carcinogenicity data

No new studies on the human carcinogenicity of fumonisins were available to the Working Group.

5.3 Animal carcinogenicity data

Fumonisin B_1 has been tested for carcinogenicity by oral administration in one study in mice, one study in male rats and one study in male and female rats. In female mice, it caused an increase in hepatocellular adenomas and carcinomas. In one study in male rats, it caused an increase in cholangiocarcinomas and hepatocellular carcinomas. In the other rat study, it induced renal tubule carcinomas in male rats, over half of which were classified as a rare highly malignant variant.

Fumonisin B_1 has also been shown to promote tumours in mouse skin and trout livers when 7,12-dimethylbenz[*a*]anthracene and aflatoxin B_1, respectively, were used as tumour initiators.

5.4 Other relevant data

Fumonisins are poorly absorbed, rapidly excreted and not metabolized in animal systems. The half-life for elimination in animal species is directly related to the average body weight of the species, suggesting that the half-life in humans will be longer than those determined experimentally in rats and other animals.

Fumonisin B_1 is hepatotoxic and nephrotoxic in all animal species tested. The earliest histological change to appear in either the liver or kidney of fumonisin-treated animals is increased apoptosis followed by regenerative cell proliferation. While the acute toxicity of fumonisin is low, it is the known cause of two diseases which occur in domestic animals with rapid onset: equine leukoencephalomalacia and porcine pulmonary oedema syndrome. Both of these diseases involve disturbed sphingolipid metabolism and cardiovascular dysfunction.

Fumonisin B_1 causes developmental toxicity in several animal species. In rats, mice and rabbits, developmental effects occurred at dose levels associated with disruption of sphingolipid metabolism and maternal toxicity in liver and kidney.

Postnatal dosing causes decreased survival of rat pups and results indicate that sphingolipid metabolism is vulnerable after birth.

Fumonisin B_1 is inactive in bacterial mutation assays and in the unscheduled DNA synthesis assay with rat hepatocytes, but induces DNA damage, such as micronuclei, *in vitro* and *in vivo*. In some studies, addition of antioxidants reduced the DNA-damaging effects of fumonisin B_1, suggesting that the effects may be due to oxidative stress.

Disruption of various aspects of lipid metabolism, membrane structure and signal transduction pathways mediated by lipid second messengers appears to be an important

aspect of all the various proposed mechanisms of action of fumonisin B_1, including its mechanism of carcinogenicity.

Fumonisin B_1-induced disruption of sphingolipid, phospholipid and fatty acid metabolism is observed both *in vitro* and *in vivo* in all animal models and in a single human study. Disruption of sphingolipid metabolism by fumonisin B_1 in animal and human systems *in vitro* causes cell death and regenerative cell proliferation mediated through sphingolipid signalling pathways. The kinetics of the increases and decreases in the various bioactive sphingolipid pools in liver, kidney, lung and heart are correlated with the observed toxicity.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of fumonisins.

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

Overall evaluation

Fumonisin B_1 is *possibly carcinogenic to humans (Group 2B)*.

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