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SCIENTIFIC COMMITTEE ON FOOD

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**OPINION
OF THE
SCIENTIFIC COMMITTEE ON FOOD
ON
FUSARIUM TOXINS
PART 3¹: FUMONISIN B₁ (FB₁)**

(Expressed on 17 October 2000)

¹ The present opinion deals only with FB₁ and therefore does not address all the terms of reference outlined below for fusarium toxins in general. The Committee will address the general aspects when all the relevant individual toxins have been considered.

**Opinion of the Scientific Committee on Food on
fusarium toxins part 3¹: Fumonisin B₁ (FB₁)**

(expressed on 17 october 2000)

Terms of reference

Although it is acknowledged that there are gaps in the toxicological information available, the Scientific Committee on Food is requested

- to assess the health risk associated with exposure to the different Fusarium toxins in cereals, taking into the account the current state of knowledge;
- to indicate, on the basis of current knowledge, which of these Fusarium toxins are of most concern for public health and for which there is an urgent need for further research and/or need for measures to reduce the presence of these toxins in cereals;
- to indicate, if possible, the nature of the toxicological studies to recommend in order to elucidate (more) completely the toxicology of these toxins.

In considering these issues the Committee is asked to take note, *inter alia*, of the comprehensive report "Fusarium toxins in cereals – a risk assessment" which has been prepared for the Nordic Council of Ministers.

Background

A variety of Fusarium fungi, which are common soil fungi, produce a number of different mycotoxins of the class of trichothecenes (T-2 toxin, HT-2 toxin, deoxynivalenol (DON)) and nivalenol and some other toxins (zearalenone and fumonisins). The Fusarium fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia. Fusarium toxins have been shown to cause a variety of toxic effects in both experimental animals and livestock. On some occasions toxins produced by Fusarium species have also been suspected to cause toxicity in humans.

¹ The present opinion deals only with FB₁ and therefore does not address all the terms of reference outlined below for fusarium toxins in general. The Committee will address the general aspects when all the relevant individual toxins have been considered.

Introduction

In the evaluation of *Fusarium* toxins the criteria for toxin selection have been:

- the toxins most commonly found in analytical surveys of cereals;
- the toxins for which there is a minimum of toxicological data.

The first group of toxins to be evaluated contains deoxynivalenol (SCF 1999), T-2 toxin, HT-2 toxin, nivalenol (2000b), fumonisin B₁ and zearalenone (SCF 2000a).

The present evaluation deals with fumonisin B₁. It is primarily based on the report prepared for the Nordic Council of Ministers "Fusarium toxins in cereals – a risk assessment" (Eriksen and Alexander, 1998), a report on Mycotoxins in human nutrition and health (Smith and Solomons, 1994), reviews of Environmental Health Criteria (EHC, 2000) and of USDA (Norred et al, 1998; Riley et al., 1996), and the recent NTP technical report on the Toxicology and Carcinogenesis of fumonisin B₁ in F344/N rats and B6C3F₁ mice (NTP, 1999). In addition, data from relevant recent publications were included.

The Committee noted that no previous assessment of fumonisin B₁ was available and decided to produce a concise opinion summarising the data essential for risk assessment of fumonisin B₁. Further details and the complete reference list are included in a more comprehensive annex to this opinion.

Fumonisin B₁ (FB₁)

Description

Fumonisin B₁ (FB₁) belongs to the recently (1988) discovered toxins fumonisins which are produced by *Fusarium verticilloides* (older synonym is *F. moniliforme*) and *F. proliferatum*, fungi that commonly contaminate maize. It has been also claimed that *F. napiforme*, *F. anthophilum*, *F. dlamini* and *F. nygamai* are able to produce FB₁ (EHC, 2000, NTP, 1999). FB₁ has been found as natural contaminant in maize and maize-based food from many parts of the world, e.g. the US, Canada, South Africa, Nepal, Australia, Thailand, The Philippines, Indonesia, Mexico, France, Italy, Poland, and Spain (Eriksen, and Alexander, 1998; EHC, in 2000).

Chemistry

FB₁: 1,2,3-propanetricarboxylic acid, 1,1'-[(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester, C₃₄H₅₉NO₁₅. MW: 721.838, CAS no.: 79748-81-5.

FB₁ is stable during most types of processing. Dry milling of maize results in the distribution of FB₁ into the bran, germ and flour. FB₁ is stable in polenta (maize porridge). However, the concentration of FB₁ is reduced during the manufacture of cornstarch by wet milling, since FB₁ is water-soluble. A number of factors make it difficult to extract FB₁ from processed food (Bullerman and Tsai, 1994; Scott and Lawrence, 1995; Murphy et al., 1995; Norred et al., 1998; Kuiper-Goodman et al., 1996). Nixtamalisation (calcium hydroxide processing) and ammoniation lead to hydrolysed FB₁ (AP₁ or HFB₁) and aminopentol, respectively. These treatments reduce

the fumonisin content while increasing the concentration of hydrolysed fumonisins without eliminating the toxic product (Norred et al., 1998; Kuiper-Goodman et al., 1996; Voss et al., 1996a; Flynn et al., 1997).

Exposure data on fumonisin B₁

Within the EU there is no systematic collection of exposure data available yet. For a number of countries worldwide the concentration of FB₁ is given (EHC, 2000). However, real exposure assessments have rarely been reported. In the following, concentrations are reported as mg/kg maize or maize products. In Latin America it varies from 0.07 to 38.5 mg/kg. In North America the levels varied from 0.004 to 330 mg/kg. In Africa the levels varied from 0.02 to 8.85 mg/kg. In Asia the levels varied from 0.01 to 153 mg/kg. The data available for Europe varied from 0.007 to 250 mg/kg in maize, and 0.008 to 16 mg/kg in maize products

Human exposure estimates have been derived for fumonisins for a few countries (EHC, 2000). For Canada, the exposure estimates ranged from 0.017 to 0.089 µg/kg bw/day. For the USA, a preliminary estimate of human exposure was 0.08 µg/kg bw/day. The mean daily intake in Switzerland is estimated to be 0.03 µg/kg bw/day. In the Netherlands the exposure estimates ranged from 0.006 to 7.1 µg/kg bw/day. In South Africa the estimates ranged from 14 to 440 µg/kg bw/day, showing that the exposure to FB₁ is considerably higher than in the other countries in which exposure assessments were performed (EHC, 2000).

Toxicokinetics

FB₁ is poorly absorbed, but rapidly distributed and eliminated in many animal species including laying hen, swine, cow, rat, and mouse and non-human primates (Prelusky et al., 1994; 1995; 1996a,b; Norred et al., 1993; 1996; 1998; EHC, 2000). However, a small but persistent pool of FB₁ or its metabolites appears to be retained in liver and kidney. It has been shown that FB₁ is not transferred through the placenta or into the milk in several animal species (Scoot et al., 1994; Becker et al., 1995; Voss et al., 1996 a,b,c; Laborde et al., 1997; Collins et al., 1998). However, there are no toxicokinetic data available for non-human primates and humans.

Mode of action

The mode of action of fumonisins is primarily explained by interference with the *de novo* synthesis of complex glyco-sphingolipids. This results in disturbances of cellular processes such as cell growth, cell differentiation and cell morphology, endothelial cell permeability and apoptosis. Inhibition of biosynthesis of sphingolipids is seen at different levels of the process (Merill et al., 1995; 1996; 1997, Riley et al., 1996; Norred et al., 1996, 1998; Wang et al., 1999), and is reflected in changes of the ratio sphinganine/sphingosin (Sa/So). Marginal effects on the Sa/So ratio were seen from 0.2 mg/kg bw level onwards. The inhibition of biosynthesis of glyco-sphingolipids is already seen a few hours after oral exposure to FB₁.

Recently it has been shown that FB₁ administered to different animal species is able to produce increased apoptosis in various tissues (Lim et al., 1996; Tolleson et al., 1996; Gelderblom et al., 1996; Voss et al., 1996a; Sharma et al., 1997; Bucci et al., 1998; Ciacca-Zanella and Jones, 1999; NTP, 1999; Lemmer et al., 1999; Haschek-Hock (1999). Increased apoptosis in particular seems to play an important role in the toxic effects including tumour induction by FB₁. In animal experiments (when determined),

apoptosis is seen at all dose levels of FB₁, causing other toxic including carcinogenic effects. In most studies apoptosis is one of the observations on which the NOAEL is based. The dose level causing apoptosis depends on the duration of exposure and can vary in rodents from 0.9 to 12 mg FB₁/kg bw (respectively in long-term and short-term experiments).

Oxidative damage has also been indicated in the aetiology of the toxic effects (Abel and Gelderblom, 1998).

General toxicity and carcinogenicity

FB₁ has a low acute oral toxicity in several animal species (Eriksen and Alexander, 1998).

In addition to the studies with rodents, and pigs and horses, there are several subacute toxicity studies performed with many animal species (poultry, rabbits, hamsters, non-human primates, lamb, mink, cattle) not directly useful for quantitative dose response assessment, i.e. not providing a NOAEL. Many of these studies were performed with contaminated feed rather than pure fumonisin (Javed et al., 1995; Bryden et al., 1987; Weibking et al., 1993; Kuiper-Goodman et al., 1996; Norred et al., 1996, EHC, 2000). The major target organs are liver and kidney in almost all animal species, but particularly in mouse and rat. For the rat whether the liver or the kidney is the most sensitive organ might even depend on the strain or gender.(NPT, 1999; Gelderblom et al., 1996a). In addition, in pigs and horses some other typical effects are seen, like porcine pulmonary edema (PPE) in pigs (Kriek et al., 1981; Colvin et al., 1993; Diaz and Boermans, 1994; Fazekas et al., 1998; Casteel et al., 1993,1994; Rotter et al. 1996; Gumprecht et al., 1998; Haschek-Hock, 1999) and equine leukoencephalomalacia (ELEM) in horses (Marasas et al., 1988a; Kriek et al 1981; Kellerman et al., 1990; Wilson et al., 1992, Ross et al., 1993,1994; Uhlinger, 1997). The NOAEL for PPE in pigs fed FB₁ is somewhat lower 5.0 mg FB₁/kg bw/day. The minimum dose that causes ELEM ranges from between 0.2 and 0.44 mg FB₁/kg bw/day(Ross et al., 1994). The NOAEL for horses is thus estimated at 0.2 mg FB₁/kg bw/day.

The lowest NOAEL for FB₁ in a subchronic study with rats was 0.2 mg FB₁/kg bw/day and the lowest NOAEL for mice was 1.8 mg FB₁/kg bw/day (Voss et al, 1995).

The NOAELs established in the long-term toxicity/carcinogenicity studies in mouse and rats were are 0.25 mg FB₁/kg bw/day (male rats) for kidney lesions and 0.7 mg FB₁/kg bw/day (female mice) for liver lesions. The kidney adenomas and carcinomas in rats were only seen at higher doses than the other toxic effects including apoptosis, similarly the hepatocellular neoplasms were only seen at the higher dose levels in female mice (NTP, 1999). The lowest dose level at which increased kidney tumour incidences were observed in rats (males) is 2.5 mg/kg bw, and was 7.0 mg/kg bw in mice (females) for increased liver tumour incidence.

Table 1: NOAELs from diverse studies, expressed as mg FB₁/kg body weight

<u>Species</u>	<u>Duration</u>	<u>Target organ/effect</u>	<u>NOAEL</u>	<u>Reference</u>
Rat	short-term	liver	<0.75	Gelderblom et al, 1994
Pig	short-term	lung/ PPE	<4.5	Motelin et al, 1994
Horse	short-term	brain/ELEM	0.2	Ross et al., 1994
Mouse	subchronic	liver	1.8	Voss et al., 1995
Rat	subchronic	kidney	0.2	Voss et al., 1995
Rat	chronic	liver	1.25	Gelderblom et al. 1995a
Mouse	chronic	liver	0.6	NTP, 1999
Rat	chronic	kidney	0.25	NTP, 1999

Genotoxicity

On the basis of negative genotoxicity data from experiments covering several endpoints (Ames test and *in vitro* and *in vivo* UDS assays (Gelderblom et al., 1989, 1992b, 1995a; Gelderblom and Snyman, 1991; Park et al., 1992; Norred et al., 1990, 1992a) and positive results in a non-validated type of bacteria test (Sun and Stahr, 1993) and a very limited *in vitro* study in which chromosomal aberration and micronucleus tests were performed (Knasmüller et al., 1997, for details see the annex to this opinion), the overall conclusion is that there is no adequate evidence that FB₁ is genotoxic.

Reproductive toxicity and developmental toxicity studies

Studies on reproduction and developmental toxicity, with the exception of one study (Floss et al., 1994a,b), showed either no developmental or reproductive effects or effects only observed at dose levels which also clearly showed maternal toxicity (Laborde et al., 1995, 1997; Gross et al., 1994; Lebepe-Mazur et al., 1993; Voss et al., 1996a; Collins et al., 1996,1997, 1998a,b; Reddy et al., 1995, 1996 and Penner et al., 1998). There is some evidence *in vitro* for developmental toxicity but, except for chicken, no teratogenic effects were reported in either *in vitro* or *in vivo* studies. (Bacon et al. 1995; Flynn et al., 1994, 1997, Javed et al., 1993). Several animal studies indicate there is little or no transfer through the placenta or to milk. As the human placenta differs in properties from that of most laboratory animals, it has to be considered whether FB₁ could cross the human placenta. However, even when considering the effect levels of FB₁ causing developmental or embryotoxic effects *in vitro*, and a worst case scenario of distribution of FB₁ assuming complete transfer through human placenta, it is considered unlikely that FB₁ will cause developmental effects in humans.

Special studies; immunotoxicity, neurotoxicity and cardiovascular toxicity

Other toxic effects of FB₁ such as neurotoxic (only observed after subcutaneous injection; Kwon et al., 1995, 1997a,b) and immunotoxic effects (primary IGM response in spleen of sheep, IgG, T lymphocytes surface antigen expression; Tryphonas et al., 1997; Martinova et al., 1998; Eriksen and Alexander, 1998) occur at a higher dose level than the hepato- and nephrotoxicity in rats and mice. In some cases these effects may be secondary to the hepato- and nephrotoxicity. On the other hand,

there is also some evidence that both effects in brains of horses (ELEM) and the lungs of pigs (PPE) are secondary to cardiovascular dysfunction caused by FB₁. Cardiovascular effects were observed in pigs (Casteel et al., 1994; Harvey et al., 1996; Haschek et al., 1995, Haschek-Hock et al., 1998; Smith et al., 1996, 1999) in vervet monkeys (Fincham et al., 1992; Nair et al., 1998; EHC, 2000), and in baboons (Kriek et al., 1981). An increase in serum cholesterol concentration has been found in many species given FB₁ including pigs (as low as 1mg/kg feed/day; Rotter et al, 1996), calves (148 mg/kg feed/day; Osweiler et al., 1992), lambs (6 mg/kg feed; Edrington et al., 1995), mice (1 mg/kg feed/day; Bondy et al 1997), rats (5 mg/kg feed; Bondy et al., 1998), mink (8 mg/kg feed; Restum et al., 1995), and broiler chicken (61 mg/kg/kg; Javed et al., 1995)

Evaluation of experimental data

On the basis of the observation of apoptosis, changed Sa/So ratios and increased kidney weights, the overall NOAEL in rodents was 0.2 mg/kg bw/day and the LOAEL 1 mg/kg bw/day. In horses the NOAEL was also approximately 0.2 mg/kg bw. The observation of ELEM was reported after short-term exposure and no chronic data are available. Many of the studies in horses are observations from poisoning cases in which the degree of contamination could only be recorded as an approximate estimate. The Committee also considered recent indications that cardiovascular effects of FB₁ could play a role in the development of other toxic effects observed.

Studies in humans

Epidemiological studies performed in South Africa and China revealed that there might be a correlation between the intake of FB₁ and increased oesophageal cancer incidence (Rheeder et al., 1992; Chu and LI, 1994; IARC, 1993; Jaskewicz et al., 1987; Scott et al., 1995; Marasas et al., (1979, 1981, 1988b; Sydeham et al., 1990a,b; Zhen et al., 1984; Yoshizawa et al., 1994; IARC, 1993). Similar studies performed in Italy did not establish any correlation between the intake of FB₁ and the oesophageal cancer incidence (Pascale et al., 1995; Logrieco et al., 1995; EHC, 2000).

In 1993 IARC evaluated FB₁ and classified it in Group 2B: “possibly carcinogenic to humans”. It was concluded that for FB₁ there was inadequate evidence in humans for carcinogenicity. The available studies are largely inconclusive and no quantitative data enabling a risk assessment on human data are available.

Conclusions

The Committee concluded that there is no adequate evidence that FB₁ is genotoxic and that information on the mode of action justifies a threshold approach. The Committee also took into account the approximate NOAEL in horses of 0.2 mg FB₁/kg bw/day. The Committee considered ELEM in horses as a severe effect which does not need long-term exposure to reach fatal expression, and would expect that this effect if induced in humans, would be observed after short-term exposure. Therefore, the Committee concluded that there was no need for an additional uncertainty factor and allocated, on the basis of the overall NOAEL from subchronic toxicity study in rats and the long-term toxicity/carcinogenicity study in rats equivalent to 0.2 and 0.25 mg/kg bw/day, respectively, a TDI of 2 µg/ kg bw, using a safety factor of 100.

The Committee also considered recent indications that cardiovascular toxic effects of FB₁ could play a role in the development of other toxic effects observed.

Recommendations

In order to assess whether there is a public health problem for the EU population from exposure to fumonisin B₁ the Committee recommends the collection of data on occurrence of this contaminant in foodstuffs and notes that such work is already scheduled in the Scientific Co-operation Programme (SCOOP). Monitoring for residues of fumonisin B₁ in animal products is currently not recommended, as there is no indication of significant carry-over of fumonisin B₁ or its metabolites in animal products such as milk, meat and eggs. (Jonker et al., 1999).

The Committee would welcome studies which provide further information on the kinetics of FB₁ in humans, including transplacental transfer in non-human primates or humans, and studies given further information whether some ultimate effects in horses, pigs and monkeys are consequential to cardiovascular toxic effects as is suggested in some recent studies. The Committee was informed that additional studies on the cardiovascular effects in mini pigs are programmed.

Note: All cited references are listed in the annex to this opinion.

ANNEX

TOXIC EFFECTS OF FUMONISINS, PARTICULARLY FUMONISIN B₁

1. Fumonisin B₁ (FB₁)

1.1 Description

Fumonisin B₁ (FB₁) belongs to the recently (1988) discovered toxins fumonisins which are produced by *Fusarium verticilloides* (older synonym is *F. moniliforme*) and *F. proliferatum*, fungi that commonly contaminate maize. But it has been claimed also that *F. napiforme*, *F. anthophilum*, *F. dlamini* and *F. nygamai* are able to produce FB₁ (EHC, 2000, NTP, 1999). FB₁ has been found as natural contaminant in maize and maize-based food from many parts of the world, e.g. the US, Canada, South Africa, Nepal, Australia, Thailand, The Philippines, Indonesia, Mexico, France, Italy, Poland, and Spain (Eriksen, and Alexander, 1998; EHC, 2000).

1.2 Chemistry

FB₁: 1,2,3-propanetricarboxylic acid, 1,1'-[(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester, C₃₄H₅₉NO₁₅. MW: 721.838, CAS no.: 79748-81-5.

FB₁ is stable during most types of processing. Dry milling of maize results in the distribution of FB₁ into the bran, germ and flour. FB₁ is stable in polenta (maize porridge). However, FB₁ is reduced by the manufacture of cornstarch by wet milling, since FB₁ is water-soluble. A number of factors make it difficult to extract FB₁ from processed food (Bullerman and Tsai, 1994; Scott and Lawrence, 1995; Murphy et al., 1995; Norred et al., 1998; Kuiper-Goodman et al., 1996). FB₁ is also reduced as it is hydrolysed by nixtamalisation (calcium hydroxide processing) with the formation of the aminopentol or hydrolysed FB₁ (AP₁ or HFB₁). Treatment with base (nixtamalisation) and ammoniation reduces fumonisin concentrations while increasing the concentration of hydrolysed fumonisins without eliminating the toxic product (Norred et al., 1998; Kuiper-Goodman et al., 1996; Voss et al., 1996a; Flynn et al., 1997)

2. Biochemical mode of action

Studies have shown that fumonisins are competitive inhibitors of de novo sphingolipid biosynthesis and metabolism. Fumonisins are structurally similar to sphingoid bases such as sphingosine, which is a component of the sphingolipid molecule, and are able to inhibit sphingosine-sphinganine-transferase and ceramide synthase. In mammals sphingolipid biosynthesis can occur in all kinds of tissue. The biosynthesis consists of a cascade of reactions that are regulated and catalysed by several enzymes. Briefly the biosynthesis starts with serine and palmitoyl-CoA forming 3-ketosphinganine, then sphinganine which is converted to sphingosine, which in turn can be converted to glycosphingolipids such as ceramide, which can be converted further into sphingomyelin or other complex glycosphingolipids. The first classes of sphingolipids were named for the tissues from which they were isolated (e.g. sphingomyelin, cerebroside) leaving the false impression that these compounds are unique to neuronal tissues. However, sphingolipids can be found in all eukaryotic cells, where they primarily occur in cell membranes and related intracellular membranes, such as Golgi and lysosomal membranes.

In addition to biosynthesis, metabolism of sphingolipids also occurs. In the intestines sphingomyelin and other complex glycosphingolipids are digested and the gut cells absorb ceramide and sphingosine.

The different intermediates of sphingolipids have various effects on cellular processes. Sphingosines play a role in the regulation of cell growth, cell differentiation, cell morphology, apoptosis and endothelial cell permeability. The glycosphingolipid ceramide plays a role in the regulation and differentiation of cells, apoptosis and protein secretion, induction of cellular senescence and other processes. The ultimate effects are dependent of concentration and cell type. Another feature of the sphingoid bases (sphinganines, sphingosines) is the inhibition of cell transformation (Merrill et al., 1995; 1996; 1997; Riley et al., 1996). Important for the involvement of sphingolipids is that inhibition of the biosynthesis is already seen a few hours after exposure to FB₁ (Riley et al., 1996).

Recently it has been shown that FB₁ administered to different animal species is able to produce increased apoptosis in various tissues (Lime et al., 1995; Tolleson et al., 1996; Gelderblom et al., 1996; Voss et al., 1996; Sharma et al., 1997; Bucci et al., 1998; Ciacci-Zanella, J.R. and Jones, 1999; NTP, 1999, Lemmer et al., 1999; Haschek, 1999). Apoptosis is seen after exposure to FB₁ both *in vitro* and *in vivo*. In animal experiments (where determined) the apoptosis is seen at all dose levels of FB₁ causing other toxic including carcinogenic effects. In most studies it is one of the effects considered in establishing the NOAEL. Apoptosis is seen early in time already in short-term toxicity studies in rat. It is seen in hepatocytes at the lowest dose level in a 28-day study tested which was 99mg FB₁/kg feed equivalent to 12 mg FB₁, and the prevalence and severity increased with increasing dose (Tolleson et al., 1996). Taken into consideration all the hepatic effects, the data indicated a threshold of effect (NOEL) after 28-day feeding with FB₁ between 99 and 163 mg FB₁/kg feed in the female rat liver. In the 28-day feeding study apoptosis and degeneration of the kidney were observed in all exposed male rats (12 mg FB₁/kg bw and greater) and in females rats exposed to 20 mg FB₁/kg and greater (NTP, 1999). Incidences of bile duct hyperplasia were only significantly increased in the high dose group 56-mg FB₁/kg bw. In a 2-year rat study apoptosis in kidney tubule epithelium cells was observed at dietary levels of 0.9 mg FB₁/kg bw and higher in the male rats, the only other change at this level in males was an increased kidney weights, the other effects were seen at a higher level in males whereas in females no effects were observed. In mice hepatocellular apoptosis was seen in females at dose levels of 9.5 mg FB₁/kg bw or higher, at these dose levels also hepatocellular neoplasm was increased also. Only hepatocellular hypertrophy is observed at a lower level (1.7 mg FB₁/kg bw.) in females, whereas in males no effects at all were reported.

Disruption of sphingolipid metabolism by FB₁ in liver and kidneys may directly cause tissue damage, whereas the damage in the brain and lung may possibly be indirect due to disruption of endothelial functions (Gumprecht et al., 1995; Riley et al., 1996; Eriksen and Alexander, 1998; Tsunoda et al., 1998).

In addition other investigators reported results indicating that oxidative damage could also play a role in the mode of action (Abel and Gelderblom, 1998).

As the effects of FB₁ on sphingolipid metabolism can be considered as a early event of several different toxic effects, depending on the animal species tested, biochemical parameters indicative for sphingolipid metabolism e.g. the sphinganine/sphingosine ratio (Sa/So) maybe used as indicators of FB₁ exposure (Merrill et al., 1996; Wang et al., 1999).

3.Toxicokinetics

3.1. Absorption, distribution and excretion

FB₁ is poorly absorbed when given orally (less than 6 %) and rapidly eliminated by biliary excretion in several animal species including laying hen, swine, cow, rat, mouse and non-human primates. There are no human data available. Enterohepatic recycling is clearly important in some animal species. Small amounts (less than 1 %) are excreted in urine. A small but persistent (and biologically active) pool of [¹⁴C]-labelled FB₁ or its metabolites appears to be retained in liver and kidneys. Prelusky et al., (1996a) have shown that feeding pigs a feed contaminated with FB₁ (2-3mg/kg feed) over a period of four weeks resulted in accumulation of FB₁ or its metabolites in liver and kidneys (EHC, 2000; Norred et al, 1998; Eriksen and Alexander, 1998). There are no studies of fumonisin absorption following inhalation and/or dermal exposure. After intraperitoneal (ip) or intravenous (iv) administration of FB₁ to rats the initial elimination of FB₁ is rapid, $t_{1/2\text{ el}}$ is approximately 10 –20 minutes. In rats the elimination kinetics after ip or iv administration of FB₁ are consistent with a one or two compartment model (Norred et al; 1993; 1996). [¹⁴-C]-FB₁ administered ip to rats resulted in 32 % of the radioactivity in the urine, and 66 % in the faeces (Prelusky et al.,1996b).

In non-human primates, as in rats, the [¹⁴C]-label is widely distributed and rapidly eliminated ($t_{1/2\text{ el}} = 40$ minutes) after iv injection of FB₁ (Shephard et al., 1994a,b). In pigs, clearance of [¹⁴ C]-labelled FB₁ from blood following an iv injection was best described by a 3-compartment model ($T_{1/2\text{ el}} = 2.5, 10.5$ and 183 minutes, respectively) and cannulation of the bile duct (bile removed) resulted in much more rapid clearance. The effect of bile removal was observed whether dosing was iv or intragastric (ig) (Prelusky et al., 1994; EHC, 2000). The $t_{1/2}$ in pigs dosed ig without bile removal was determined to be 96 minutes (Prelusky et al., 1995). After administration of [¹⁴C]-FB₁ to pigs in the diet from day 1- 24 and followed by a 9-day withdrawal period. In the tissues analysed (day 3-day 24), residues were found to accumulate only in liver and kidney. Once pigs were placed on non-contaminated feed, tissue levels declined very rapidly; down to approximately 35 % of peak levels after 3 days and only marginally above the detection limit 9 days after of withdrawal (Prelusky et al., 1996a).

Toxicokinetic studies in cows given FB₁ iv or orally revealed a rapid distribution; $t_{1/2\alpha}$ was 1.7 minutes (iv) and 15 – 18 minutes (oral). No FB₁ was detected in the plasma 120 min after dosing and no FB₁ was detected in tissues (Prelusky et al., 1995b; 1996b). Other studies have also shown that there is no distribution of FB₁ to the maternal milk (Scott et al., 1994; Becker et al., 1995).

Voss et al. (1996c) injected pregnant rats on gestation day 15 iv with [¹⁴C]-FB₁. After 1 hr, which allowed for approximately 98% of the dose to be cleared from the maternal blood, negligible amounts of radioactivity were found in the foetuses. Other studies confirmed that FB₁ did not cross the placenta in rats, mice and rabbits (Voss et al., 1996a; Laborde et al., 1997; Collins et al., 1998). Studies on other species such as the monkey with different type of placenta (similar to the human) were not available. Also an increased Sa/So ratio indicative of exposure to FB₁ was not observed (Voss et al., 1996b; Laborde et al., 1997).

Becker et al. (1995) studied the effects of feeding FB₁ in lactating sow and their suckling pigs. When sows ingested sub-lethal concentrations of FB₁ (100 mg/kg feed) for 17 days, no detectable amounts of FB₁ in the sows milk were found.

3.2. Metabolism

Only very sparse information concerning metabolism is available. No metabolites were detected in rat studies independent of the route of administration, whereas analysis of non-human primate's faeces extract revealed a metabolite of FB₁ (Norred et al, 1996; Eriksen and Alexander, 1998). This metabolism most likely occurred in the gut since partially hydrolysed and fully hydrolysed FB₁ were recovered in faeces but not in bile of Vervet monkey (Shephard et al., 1995; EHC, 2000). In pigs it was suggested that similar metabolism occurred, but this was not demonstrated (Prelusky et al., 1994).

4. Toxicity

4.1. Acute and subacute toxicity

Rat and mouse

There is little information on the acute toxicity of FB₁ and the LD₅₀ of FB₁ is unknown. No information at all is available on the toxicological effects of single dose exposure to FB₁ by inhalation or dermal routes (EHC,2000).

In contrast there are several subacute oral toxicity studies (varying from a few days to several weeks) with FB₁ performed in rodents (Gelderblom et al., 1988, 1992,1993,1994,1998; Bondy et al 1995,1996; Badria et al., 1996; Suzuki et al., 1995; Voss et al., 1993; Howard et al., 1993; Bucci et al., 1998). In all rat studies nephrotoxic and hepatotoxic effects were observed. The hepatotoxicity was characterised by scattered single-cell necrosis accompanied by mild fatty changes, hydropic degeneration and hyaline droplet degeneration. At high dose levels and or longer exposure the effect became more severe including proliferation of bile ducts, fibrosis that caused distortion of the lobular structure of the liver, which together with the development of hyperplastic nodules gave the liver a distinctly nodular appearance. The nephrotoxicity consisted of fatty changes, scant necrosis in the proximal convoluted tubules, focal proximal tubular epithelial basophilia, hyperplasia and single cell necrosis or pyknosis. The incidence and severity of ultrastructural alterations in kidneys and liver were closely correlated with increased sphinganine concentrations in tissues, serum and urine (Riley et al., 1994). Sometimes other toxic effects of FB₁ were also reported in the rat, such as severe dissemination of acute myocardial necrosis and severe pulmonary oedema (Gelderblom et al., 1993,1994 and 1998). The “no observed adverse effect level” (NOAEL) for renal toxicity (< 15 mg/kg diet) was lower than for the NOAEL for hepatotoxicity in the rat (> 150 mg/kg diet). Degenerated hepatocyte and renal proximal tubular cells were shown to contain apoptotic bodies, suggesting that FB₁ induces an accelerated programmed cell death in both kidney and liver. In mice toxic effects were recorded in the liver which were similar to the effects seen in rats, but no toxic effects in kidney were seen (Bondy et al., 1997).

Pigs and horses

Although most animal species show at certain dose levels nephrotoxic and hepatotoxic effects, the most relevant effect of FB₁ in pigs is the porcine edema syndrome (PPE), which is characterised by dyspnoea, weakness, cyanosis and death (Kriek et al., 1981; Colin et al., 1993; Diaz and Boermans, 1994; Fazekas et al., 1997, 1998; Casteel et al., 1993, 1994; Rotter et al., 1996; Gumprecht et al., 1998). Several outbreaks of PPE were observed with concurrent hepatotoxicity mostly occurring in the USA (Harrison

et al., 1990; Osweiler et al., 1992; Ross et al., 1992; Motelin et al., 1994; Ross, 1994). There are several hypotheses proposed on the aetiology of this effect. Most likely the cardiovascular toxic effects are involved in PPE (Haschek et al., 1995, 1998; Smith et al., 1996, 1999). Daily intakes of FB₁ of 4.5 – 6.3 mg/kg bw induced PPE (Motelin et al., 1994). The NOAEL for PPE in pigs is thus lower than 4.5 mg FB₁/kg bw.

In horses and ponies (equids) the most relevant toxic effect of FB₁ is equine leukoencephalomalacia (ELEM) syndrome. ELEM is characterised by the presence of liquefactive necrotic lesions in the white matter of the cerebrum (Ross et al., 1993; Marasas et al., 1988a; Kriek et al., 1981; Kellerman et al., 1990; Wilson, 1992). The first symptoms of ELEM are lethargy, head pressing and inappetence, followed by convulsions, ataxia and death after several days (Wilson et al., 1992; Uhlinger, 1997). Also liver toxicity was recorded (Wilson et al., 1992). In studies with horses, the toxic effects were preceded by elevation in the serum sphinganine to sphingosine (Sa/So) ratio (Wang et al., 1992; Riley et al., 1994a). A recent study in horses suggests that ELEM was secondary to cardiovascular toxic effects of FB₁ (Constable et al., 2000b). The minimum dose that causes ELEM ranges between 10 and 22 mg FB₁/kg diet which is equivalent to 0.2 and 0.44 mg FB₁/kg bw (Ross et al., 1994; Eriksen and Alexander, 1998). The NOAEL for horses is thus estimated at 0.2 mg FB₁/kg bw.

A study in Mexico showed that the level of FB₁ contamination in food causing ELEM in donkeys ranged from 0.67 to 13.3 mg/kg food, which is equivalent to 0.01 to 0.26 mg/kg bw (Rosiles et al., 1998). However, the feed might have contained other mycotoxins.

Other animal species

Several reports have been published implicating *F. moniliforme* contamination of feed in diseases of poultry (Bryden et al., 1987; Jeschke et al., 1987; Brown et al., 1992; Dombink-Kurtzman et al., 1993; Kubena et al., 1995a,b; Javed et al., 1993; Javed et al., 1995; Lebourg et al., 1992; Weibking et al., 1993 a,b, 1995). In addition to hepatotoxicity and nephrotoxicity immunosuppressive effects were also reported (Marijanos et al., 1991). Espada et al. (1994) reported toxicity in broiler chicks fed a diet containing 10 mg pure FB₁/kg diet. Prathap Kumar et al. (1997) reported a disease outbreak in laying hens arising from the intake of FB₁-contaminated feed. In two farms respectively 6700 and 3000 hens were affected. Reduced egg production (20 %), black sticky diarrhoea, severe reduction of food intake and body weights followed by lameness and mortality (10 %) were observed.

Other species that have been studied using fumonisins, contaminated maize screenings or maize culture material include non-human primates, rabbits, hamsters, catfish, lamb, mink and cattle (Kuiper-Goodman et al., 1994; Norred et al., 1996; EHC, 2000). In all cases where toxicity was evident it involved liver and or kidneys. In a rabbit study, two of five pregnant rabbits that died 9 and 13 days after being gavaged with purified FB₁ at 1.75 mg/kg bw/day, showed focal small haemorrhages in cerebral white matter, with malacia and haemorrhage also present in the hippocampus of one. The lesions were bilateral. Both animals also had marked degeneration of renal tubule epithelium and of hepatocytes. Apoptosis was the dominant change in kidney and liver (Bucci et al., 1996a,b).

4.2. Subchronic toxicity

Rat and mouse

F344 rats and B6C3F1 mice were fed diets containing 0, 1, 3, 9, 27 or 81 mg FB₁/kg diet for 13 weeks. In rats, toxicity was confined to the kidneys. Lesions of the proximal tubule located in the outer medulla were found in males fed 9 mg FB₁/kg diet (equal to 0.6 mg /kg bw.) or higher and in females fed 81 mg FB₁/kg diet (Voss et al., 1995). Qualitatively these lesions were of the same type as found in the 4-week study (Voss et al., 1993). No differences in the incidence or severity of nephropathy between rats examined (5/sex/group or 13 (10/sex/group) after 4 weeks were found. Decreased relative kidney weights were found in males fed 27 mg/kg diet or higher for 4 weeks and in both sexes fed 9 mg/kg bw. for 13 weeks. Serum creatinine was increased after 13, but not after 4 weeks in males fed 27 mg/kg diet or higher and females fed 81mg/kg diet. The NOAEL in this study is 0.2 mg FB₁/kg bw./day.

In mice, hepatopathy and serum chemical evidence of liver dysfunction were found after 13 weeks in females fed 81 mg/kg diet. Liver lesions in female mice were primarily centrilobular, although some midzonal involvement and apparent ‘bridging’ between adjacent central areas was evident. Single cell hepatocyte necrosis, cytomegaly, increased numbers of mitotic figures, some mixed infiltration of neutrophils and macrophages were present and, in more advanced lesions, the loss of hepatocytes caused an apparent collapse around the central vein. Hepatotoxicity was not found in male mice and FB₁- related kidney lesions did not occur in either sex. A few macrophages containing minimal to mild amounts of cytoplasmic pigment, presumably ceroid, were also found in the adrenal cortex of high-dose (81 mg/kg diet) females only (Voss et al., 1995). The NOAEL in this mouse study was 1.8 mg FB₁/kg bw/day. Female mice exhibit hepatic effects at lower doses than males. In Fischer rats, however, the kidney is the main target organ and, in contrast to liver effects in the mouse, the male rats were affected at lower doses than females.

Other species

In a study with Vervet monkeys 0.5 % *F. verticillioides* culture material containing fumonisins was added to the diet. The animals were fed about 0.2 and 0.4 mg of total FB/kg bw/day for more than four years. FB₁ contributed about 70 % of the total dose equal to 0.15 and 0.3 mg/kg bw/day. Mild portal fibrosis in the liver and changes in blood parameters (indicators for vascular diseases) were induced at both dose levels (Fincham et al., 1992; Nair et al., 1992; EHC 2000).

4.3. Chronic toxicity and carcinogenicity

Rats were fed diets containing 4 % (Marasas et al., 1984b^e) or 0.5 % (Jaskiewicz et al., 1987) culture materials of *F. verticillioides* known to produce high amounts of fumonisins, primarily FB₁. After 23-27 months an increased incidence of hepatocellular carcinomas and cholangiocarcinomas was observed (Eriksen and Alexander, 1998)

A semi-purified maize-based diet containing 50 mg FB₁ /kg diet (purity > 90 %; equivalent to 2.5 mg/kg bw) was fed to 25 inbred male BD IX rats over a period of 26 months. A control group received the same diet with a background of approximately 0.5 mg FB₁/kg diet (equivalent to 0.025 mg/kg bw) and containing no aflatoxin B₁. Five rats from each group were killed at 6, 12, 20 and 26 months. All FB₁ dosed rats

that died or were killed from 18 months onwards suffered from micro- and macronodular cirrhosis and had large expansile nodules of cholangiofibrosis at the hilus of the liver. The pathological changes terminating in cirrhosis and cholangiofibrosis were already present in the liver of rats killed after 6 months, and included fibrosis, bile duct hyperplasia and lobular distortion. The severity of the hepatic lesions increased with time and was consistent with those of a chronic toxic hepatitis progressing to cirrhosis. Ten out of 15 FB₁-dosed rats (66 %) that were killed or died between 18 and 26 months developed primary hepatocellular carcinoma. Metastases to the heart, lungs or kidneys were present in 4 of the rats with hepatocellular carcinoma. Apart from the hepatocellular carcinoma, FB₁ also induced cholangiofibrosis consistently from 6 months onward, and toward the end of the study it induced cholangiocarcinoma (Gelderblom et al., 1991). Although this study was too limited (only 25 animals per group, only one dose group and only one gender) to be considered as an adequate carcinogenicity study, the study clearly indicates that FB₁ was carcinogenic and provided further support for the progressive development from chronic liver toxicity to liver cancer.

A second long-term study with 0, 1, 10 and 25 mg FB₁/kg diet over a period of 24 months (equivalent to 0, 0.05, 0.5 and 1.25 mg/kg bw) was performed in BD IX rats (Gelderblom et al., 1995a). No tumours were observed in these rats, including those fed the highest level of 25 mg FB₁/kg diet. However, full details have not been published yet. In view of the finding that a dietary level of 50 mg/kg diet induced hepatocellular carcinoma (Gelderblom et al., 1991), whereas 25 mg FB₁/kg diet did not (Gelderblom et al 1995a), the NOAEL would be equivalent to 1.25 mg FB₁/kg bw.

In a 2-year toxicity/carcinogenicity study with rats (F344/N); groups of 48/sex (40 for 5 mg/kg) diets were fed containing 0, 5, 15, 50 or 150 mg FB₁/kg diet (males) or 0, 5, 15, 50 and 100 mg FB₁/kg diet (females) (equivalent to 0, 0.25, 0.8, 2.5, or 7.5 mg FB₁/kg bw. and 0, 0.3, 0.9, 3.0 or 6.0 mg FB₁/kg bw. respectively) for 105 weeks. Additional groups of 4/sex were exposed to the same concentration as the core study animals and were evaluated at 6, 10, 14 or 26 weeks. Survival, mean body weights, and feed consumption of exposed male and female rats were generally similar to the controls throughout the study. Sa/So ratios were increased in the urine of 15, 50 and 150 mg/kg diet male groups and of 50 and 100 mg FB₁/kg diet female groups exposed for up to 26 weeks. The Sa/So ratios were also increased in kidney tissue from 50 mg FB₁/kg diet onwards in both male and female rats at 2 years. Renal tubule epithelial cell proliferation was increased in 50 and 150 mg FB₁/kg diet males exposed for 26 weeks. Renal tubule epithelial cell proliferation was only marginally increased in high dose level females (100 mg FB₁/kg diet). Kidney weights of the males of the 50 and 150 mg FB₁/kg diet dose groups were less than those of the respective controls during the whole study. Kidney weights of females of 100 mg FB₁/kg diet groups were less than those of the controls at 26 weeks and at 2 years. Kidney weights of females of the 15, 50 and 100 mg FB₁/kg diet were higher than in controls.

At 2 years, there was a significant increase in the incidence of renal tubule adenoma in male rats of the 150 mg/kg diet group and of renal tubule carcinoma in male rats of the 50 and 150 mg FB₁/kg diet groups. The incidence of apoptosis of the renal tubule epithelium was generally significantly increased in males exposed to 15 mg/kg diet or higher for up to 26 weeks. The incidence of focal renal tubule epithelial hyperplasia was significantly increased at 2 years in the males of 50 and 150 mg FB₁/kg diet group. Under the conditions of this 2-year feeding study, there was clear evidence of

carcinogenic activity of FB₁ in male F344/N rats based on the increased incidence of renal tubule carcinomas and adenomas. There was no evidence of carcinogenic activity of FB₁ in the female rats. In addition to the carcinogenic effects, some toxic effects (apoptosis in renal tubule epithelial cells, Sa/So ratio in urine and kidney weights) were seen at a lower dose level (15 mg/kg diet). A NOAEL was established at 5 mg FB₁/kg diet (equivalent to 0.25 mg/kg bw in male and 0.3 mg FB₁/kg bw. in female rats (NTP, 1999).

In a 2-year toxicity/carcinogenicity study with B6C3F₁ mice groups of 48/sex were fed diets containing 0, 5, 15, 80 or 150 mg/kg diet (males) or 0, 5, 15, 50 or 80 mg FB₁/kg diet (females) (equivalent to 0.6, 1.7, 9.5 or 17 mg/kg bw. and 0.7, 2.1, 7.0, or 12.5 mg FB₁/kg bw) for 105 weeks. Additional groups of 4/sex exposed to the same concentrations as the core study animals, were evaluated at 3, 7, 9, or 24 weeks.

Survival of males in the 15 mg/kg diet and in the 5mg FB₁/kg diet group was significantly greater and survival of males and females in 80 mg/kg diet group was significantly less than that of the control groups. Mean body weights and feed consumption of exposed mice were generally similar to the controls. After two years of exposure liver weights were increased in the females of the 50 and 80 mg FB₁/kg diet groups, and incidences of hepatocellular neoplasms in the female of the 50 and 80 mg/kg diet group were significantly greater than those in the controls and occurred with positive trends. The incidences of hepatocellular hypertrophy were significantly increased in 15, 80, and 150 mg/kg diet males and in 50 and 80 mg FB₁/kg diet females. Also the incidences of hepatocellular apoptosis were significantly increased in the females of the 50 and 80 mg Fb₁/kg diet group. Under the conditions of this 2-year feeding study there was clear evidence of carcinogenic activity of FB₁ in female B6C3F₁ mice and no evidence of carcinogenic activity of FB₁ in male B6C3F₁. In addition to the carcinogenic effect in the females some toxic effects (reduced survival rate and hepatocellular hypertrophy) were seen in both males and females at a lower dose level (15 mg/kg diet), i.e. tumours were only seen above the maximum tolerated dose (MTD). A NOAEL was established at 5 mg FB₁/kg diet (equivalent to 0.6 mg/kg bw in males and 0.7 mg FB₁/kg bw. in female mice)(NTP, 1999).

4.4. Genotoxicity

FB₁ (98 % pure) as well as FB₂ and FB₃ (98 and 90 % pure, respectively) were non-mutagenic in the *Salmonella* assay against the tester strains TA 97a, TA 98, TA 100 and TA 102, either in presence or absence of the S-9 microsomal preparation at concentrations of 0, 0.2, 0.5, 1, 5 and 10 mg/plate (Gelderblom and Snyman, 1991). The non-mutagenicity of FB₁ (approximately 90 % pure) to *Salmonella* tester strain TA 100 at concentrations up to 100 mg/plate was confirmed by Park et al., (1992).

FB₁ and FB₂ were non-genotoxic in an *in vitro* rat hepatocyte DNA repair assay at concentrations of 0.04 – 80 µM/plate (and FB₂ at 0.04 – 40 µM/plate) as well as *in vivo* at a concentration of 100 mg/kg of FB₁ or FB₂ (Gelderblom et al., 1989; 1992b). The finding that FB₁ does not induce unscheduled DNA synthesis was confirmed in an *in vitro* assay in primary hepatocytes at concentrations from 0.5 – 200 µM (Norred et al., 1990,1992a).

In contrast, Sun and Stahr (1993) using a commercial bioluminescent bacterial (*Vibrio fischeri*) genotoxicity test, claimed that FB₁ showed genotoxic activity without the metabolic activation of S-9 fraction at the concentration range of 5-20 µg/ml.

However the non-genotoxicity of FB₁ has more recently been confirmed in a series of *in vitro* systems including the *Salmonella* mutagenicity assay, the *umu*-microtest with *Salmonella*, DNA repair assay with *Escherichia coli* and assays for induction of micronuclei and mitotic activity in rat hepatocytes (Gelderblom et al., 1995a). Another recent study confirmed FB₁ as non-mutagenic, but some clastogenic effects were observed (Knasmüller et al., 1997). FB₁ was negative in a gene mutation assays with *Salmonella typhimurium* strains TA 98 and TA 100 (0.7, 2.1, 6.2, 19, 55, 167, 500 µg/plate) and in SOS chromotest with *E. coli* strain PQ37 (5, 16, 50, 166, 500 µg/assay) in the presence and absence of metabolic activation. In the micronucleus test with primary rat hepatocytes at a low concentration (1 µg/ml) moderate increases in micronucleus frequencies were seen, but no clear dose-response effects were noted. In the chromosomal aberration assay with primary rat hepatocytes a concentration of 1 µg FB₁/ml caused a 6-fold increase in aberrations above the background. The effect was clear dose-dependent. However, this study had some limitations: only 20 metaphases were analysed and the results were expressed as expressed as aberrations /plate and not as usual as the number of cells with aberrations. Due to these limitations these experiments of Knasmüller (1997) are discarded.

4.5. Cytotoxicity

In vitro studies have shown that FB₁ is moderately cytotoxic to rat hepatocytes, rat hepatoma cells, and dog and pig kidney cells. In addition FB₁ is cytotoxic to chicken macrophages (Eriksen and Alexander, 1998).

The effects of FB₁ on lipid peroxidation and protein and DNA synthesis were studied in monkey kidney cells (Vero cells). FB₁ was found to be a potent inducer of malondialdehyde (secondary product formed during lipidperoxidation). (Abado-Becognee et al., 1998).

4.6. Reproductive toxicity and developmental toxicity studies

In vitro

Fumonisin, *Fusarium verticilloides* (*moniliforme*) and *F. proliferatum* culture materials containing known amounts of fumonisins have been shown to be embryotoxic *in vitro* (Bacon et al., 1995; Flynn et al., 1994, 1997; Javed et al., 1993). Javed et al. (1993) found that injection of purified FB₁ into fertile chicken eggs resulted in time and dose dependent embryopathic and embryocidal effects. Embryonic changes include hydrocephalus, enlarged beaks, and elongated necks. Pathological changes were noted in most organs. Bacon et al. (1995) found effects of FB₁ in fertile eggs similar to those reported by Javed et al. (1993).

At the low FB₁ concentration (0.7 µg/ml) stimulation of embryo development *in vitro* has also been reported. This was also the case in presomite rat embryos exposed to 0.5 – 1.0 µg/ml of hydrolysed FB₁ (Flynn et al., 1994). At higher concentrations of hydrolysed FB₁ (Flynn et al., 1994) and all concentrations of FB₁ 0.2 µg/ml inhibited growth and development of presomite rat embryos was seen. Johnson et al. (1993) reported that FB₁ was a weak developmental toxicant to organogenesis stage rat embryo (day 10.5); the LOAEL was 0.5 mM, which is equivalent to 0.4 µg/ml). FB₁ (0.2 µg/ml or higher) inhibited reaggregation and growth of chicken embryo neural retina cells, an *in vitro* assay for screening potential developmental toxins (Bradlaw et al., 1994). It should be noted that at the early stage of day 9.5 the embryo was reportedly more sensitive to the action of FB₁ (at concentrations as low as 0.2 µg/ml

culture medium) than at a later stage (1 day later, day 10.5) when higher concentrations were needed to yield an abnormal response.

In vivo

In most reproduction and developmental toxicity studies performed in CD₁ mouse, Syrian Hamster, Sprague-Dawley- or Fischer 344 rats, New Zealand rabbit and mink, reproductive and/or developmental effects, were only observed at dose levels that caused maternal toxicity. In none of these studies did the authors report morphological alterations in the progeny indicative for teratogenic effects (LaBorde et al., 1995, 1997; Gross et al., 1994; Lebepe-Mazur et al., 1993; Voss et al., 1996a; Collins et al., 1998; Gross et al., 1994; Reddy et al., 1995; and Penner et al., 1998. Floss et al. (1994a,b) have reported that FB₁ caused developmental distortions in Syrian Hamster at a dose level of 12 – 18 mg FB₁/kg bw/day while no maternal toxicity was observed.

4.7. Immunotoxicity

A study has shown reduced thymus weight, thymus necrosis and elevated immunoglobulin M (IgM) in rats after i.p. administration of 7.5 mg FB₁/kg bw. for 4 days (Eriksen and Alexander, 1998). Tryphonas et al. (1997) studied the effects of FB₁ in the immune system of Sprague-Dawley rats. Groups of rats (15/sex/group) were gavaged daily for 14 days with doses of 0, 5, 15, and 25 mg FB₁/kg bw, and the primary IgM response to sheep expressed as plaque-forming cell number/10⁶ spleen mononuclear leukocytes, PFC/10⁶ splenocytes and PFC/spleen was determined. There was a significant dose related linear trend toward decreased PFC/10⁶ splenocytes and PFC/spleen in the male rats. Body weights were significantly reduced in the male rats administered 15 and 25 mg FB₁/kg bw.. There was a significant dose-related increase in *Listeria monocytogenes* number in spleen at 24 hrs post infection, also indicating decreased immune function. There was a weakly significant dose-related increase in immunoglobulin class G1 (IgG1). There was no effect seen on organ weights, haematology, mitogen-induced leukocyte transformation, calcium mobilisation, killer cell activity, and phagocytosis.

Martinova et al. (1998) showed that both sphingomyelin cycle products and FB₁ affect the T lymphocyte surface antigen expression, disrupt balance between different subpopulation of lymphocytes, inhibit DNA synthesis in normal lymphocytes, and suppress an immune response to T-dependent antigens *in vivo*.

4.8. Effects on the nervous system

The ELEM syndrome observed in equids has already been described in the subacute toxicity section.

A report by Kwon et al. (1995) indicated that subcutaneous injection of FB₁ in neonatal rats caused elevation in the Sa/So ratio in brain tissue and reduced myelin deposition, which could lead to a delay in development of the nervous system. Kwon et al. (1997a) showed that the AUC ratio of brain FB₁ to plasma FB₁ was 0.02, whereas the ratio brain Sa to plasma Sa was about 40, when neonate Sprague-Dawley rats (postnatal day 2) were dosed with 0.8 and 8.0 mg FB₁/kg bw sc . Also the Sa/So ratio was increased. These observations indicate that sphingolipid metabolism in the central nervous system of developing rats is vulnerable to FB₁ exposure. This was further supported by the finding that myelin deposition in the corpus callosum and 2',3'-cyclic

nucleotide 3'-phosphohydrolase (CNP) was significantly decreased when neonate Sprague-Dawley rats were dosed 0, 0.4 or 0.8 mg FB₁/kg bw. sc (Kwon et al., 1997b).

4.9. Effects on the cardiovascular system

Evidence of fumonisin-induced cardiovascular toxicity was initially observed in chronic studies in which *F. verticillioides* culture material fed to baboons that resulted in acute congestive heart failure (Kriek et al., 1981) and to pigs that resulted in right ventricular hypertrophy of the heart and medial hypertrophy of the pulmonary arteries (Casteel et al., 1994; Harvey et al., 1996).

Smith et al. (1996) examined the cardiovascular effects of short-term FB exposure in anaesthetised and conscious male crossbred pigs (30 – 36 kg). Culture material containing fumonisins at ≤ 20 mg/kg (FB₁ and FB₂) was added to the feed of treated pigs (5) for 7 days, while controls (5) were fed a diet free of fumonisins. In pigs fed fumonisins a significant increase in mean pulmonary arterial pressure, accompanied by decreased heart rate, cardiac output, and mixed venous oxygen tension were observed. The ECG was normal, and no PPE was seen. These results suggest that pulmonary hypertension caused by hypoxic vasoconstriction may be an early event in the development of PPE observed in FB toxicity. Non-human primates (Vervet monkeys) fed *Fusarium moniliforme* -contaminated corn (about 0.15 and 0.30 mg FB₁/kg bw./day) for a prolonged period showed an atherogenic response with dose-related hypercholesterolaemia and evidence of liver damage to which the atherogenic response is thought to be secondary (Fincham, 1992; Nair, 1998). These changes are compatible with the inhibition of L-type calcium channels by increased sphingosine and/or sphinganine concentration. Fumonisin-induced pulmonary oedema in swine appears to result from acute left-sided heart failure by altered sphingolipid biosynthesis. Recent studies reviewed in a previous section indicate that both ELEM and PPE might be secondary to cardiovascular toxic effects caused by FB₁.

Increases in serum cholesterol concentration have been found many species given FB₁ including pigs (as low as 1mg/kg feed; Rotter et al., 1996), calves (148 mg/kg feed; Osweiler et al., 1992), lambs (6 mg/kg feed; Edrington et al., 1995), mice (1 mg/kg feed; Bondy et al., 1997), rats (5 mg/kg feed; Bondy et al., 1998), mink (8 mgf/kg feed; Restum et al., 1995), broiler chicks (61 mg/kg feed (Javed et al., 1995) and also in horses (Constable et al., 2000a and 2000b). FB₁ does not change cholesterol secretion (Merrill et al., 1995) but has been hypothesised to change lipid metabolism (Rotter et al., 1997).

4.10. Special studies on the etiology of toxic effects including carcinogenesis

Single doses of FB₁ (50 and 100 mg/kg bw) administered by gavage to partially hepatectomised male Fischer rats failed to initiate cancer in a short-term cancer initiation/promotion assay in rat liver (Gelderblom et al., 1992b). A single gavage dose of 50, 100 and 200 mg FB₁/kg bw significantly inhibited hepatocyte proliferation as measured by decreased incorporation of radiolabelled thymidine in male Fischer rats (Gelderblom et al., 1994a).

In a series of dose-response studies in male Fischer rat, Gelderblom et al. (1994b) reported that the lowest dietary level to effect cancer over 21 days was 250 mg FB₁/kg diet. Based on calculations of the feed intake the effective dose level (EDL) for cancer is exposure time dependent; 14.2 and 30.8 mg FB₁/kg bw over 21 days and for 333

mg/kg bw./day over 14 days. This outcome supports the hypothesis that the hepatocarcinogenicity is a consequence of the hepatotoxicity (Gelderblom et al., 1994). The cancer-promoting potential of FB₁ was investigated by feeding different dietary levels (10, 50, 100, 250 and 500 mg/kg) to diethylnitrosamine (DEN)-initiated rats for 21 days. Dietary levels containing 50 mg FB₁/kg and higher markedly increased the number and size of the placental form of glutathione-S-transferase –positive foci in the liver of the rats. The cancer-promoting activity of FB₁ was associated with an inhibitory effect on partial hepatectomy-induced regenerative hepatocyte proliferation (decreased incorporation ³H-labeled thymidine). *In vitro* studies on mitogenic activity of epidermal growth factor in primary hepatocytes supported the *in vivo* data, in that FB₁, similar to other cancer promoters (phenobarbital, 2-acetylaminofluorene), alters growth stimulatory responses. Sa/So ratios were not altered in the liver of rats fed the lowest FB₁-containing diet (50 mg/kg diet) that effected cancer promotion. It was demonstrated that FB₁ exhibited cancer-promoting activity in the absence of adverse hepatotoxic effects and at dietary levels that failed to effect cancer initiation (Gelderblom et al., 1996p)

Sheu et al. (1996) concluded on the basis of results in studies of FB₁ (10, 100, 500 and 1000 µg/ml) in BALB/3T3 A31-1-1 mouse embryo cells, that FB₁ seems to lack *in vitro* transforming activity.

As FB₁ and N-nitrosamines are suggested risk factors in the development of oesophageal cancer (see effects in humans), treating male rats with the known oesophageal carcinogen N-methylbenzyl nitrosamine (NMBA) therefore tested the hypothesis that the two would interact. Groups of rats (12) received NMBA ip either alone or in combination with 5 mg FB₁ kg bw administered by gavage or only FB₁ or only the test vehicle for up to 5 weeks. The score of oesophageal papillomas and dysplasia was similar in the NMBA and the NMBA + FB₁ groups. In the FB₁ groups sphingolipid biosynthesis was affected in the kidneys and slightly affected in the liver, but not in the oesophagus and the lung as determined by the Sa/So ration in the tissues and urine. These data show that there is no interaction between NMBA and FB₁ in the rat oesophagus when the two compounds are administered concomitantly (Wild et al., 1997).

5. Effects in human

There are no reports on the acute effects of fumonisins on humans, although instances of very high fumonisin concentrations have been reported from home grown maize in South Africa and China (118 –155 mg/kg food; Rheeder et al., 1992; Chu and Li, 1994). As is the case for swine, rats and mice, this suggests low acute toxicity in humans (EHC, 2000; Norred et al., 1998).

Very high oesophageal cancer incidences observed in human populations in the Transkei of South Africa were correlated with high intake of maize as staple food and the high concentration of fumonisins, particularly FB₁ in this food (IARC, 1993; Jaskewicz et al 1987; Scott et al., 1995; Marasas et al. (1979; 1981; 1988b; Sydenham et al., 1990a, 1990b; Rheeder et al. 1992; EHC, 2000).

Similarly, a correlation between intake of fumonisins and the incidence of oesophageal cancer in the human population of Henan Province in China (Zhen et al., 1984; Chu and Lee, 1994; Yoshizawa et al., 1994) was observed. North West Italy (Pordenone province) has the highest mortality rates for oral pharyngeal, and oesophageal cancer in Europe (EHC, 2000; Pascale et al., 1995; Logrieco et al., 1995), but a clear correlation

with FB₁ intake could not be demonstrated. A controlled study with corn pancake from 16 households in China did not show any increase in gastro-intestinal cancers (Groves et al., 1999). Turner et al. (1999) evaluated the epidemiological data and concluded that there is no direct established causal association between fumonisin exposure and cancer in humans. IARC evaluated FB₁, FB₂, fusarin C as toxins derived from *Fusarium verticillioides (moniliforme)* and classified it in group 2B; possible carcinogenic to humans. It was concluded for these toxins that there is inadequate evidence in humans for carcinogenicity, whereas there is limited (FB₁, fusarin C) evidence or inadequate evidence (FB₂) for carcinogenicity in experimental animals (IARC, 1993).

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