Opinion of the Scientific Panel on Contaminants in Food Chain on a request from the Commission related to fumonisins as undesirable substances in animal feed

Request No. EFSA-Q-2003-040

Adopted on 22 June

SUMMARY

Fumonisins are a distinct group of mycotoxins produced by several field fungi, including *Fusarium verticillioides* and *Fusarium proliferatum*. Fumonisins are occurring particularly in maize and maize-based products. Co-occurrence with other *Fusarium* toxins, such as zearalenone and deoxynivalenol, is regularly observed. Fumonisin B_1 is considered to be the most prevalent and most toxic derivative within the group of fumonisins. It is a prototypic inhibitor of cellular sphingosine (sphinganine) N-acetyltransferase. Inhibition of this enzyme is followed by an accumulation of sphinganine (Sa) and sometimes also sphingosine (So) and a depletion of complex sphingolipids in eukaryotic cells, which in turn results in impairment of cell cycle regulation and cellular differentiation, and in oxidative stress as well as apoptosis and necrosis. Fumonisin B_1 is carcinogenic in rodents, but it is devoid of significant genotoxic activity. *In vivo* rodent experiments suggest that fumonisins are tumour promoters. The increased Sa:So ratio in body fluids and tissues serves as a sensitive biomarker of exposure to fumonisins. Equidae and porcine species are considered to be the most sensitive animal species to fumonisins, developing species-specific clinical syndromes such as equine leukoencephalomalacia and porcine pulmonary oedema. Ruminants and poultry show a low responsiveness to fumonisins. Few data are available on the effects of fumonisins on farmed fish and on minor species, such as rabbits, goats and minks. The available data on animal exposure via feedingstuffs are limited and monitoring of feed materials is needed to improve exposure assessment. Available data on carry-over of fumonisins from animal feeds into edible tissues, including milk and eggs, indicate that transfer is limited, and thus residues in animal tissues contribute insignificantly to total human exposure.

KEY WORDS: fumonisins, animal feeds, toxicity, tissue residues

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BACKGROUND

1. General background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed[1] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[2].

The main modifications can be summarised as follows:

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination ("early warning system") and to take measures to reduce or eliminate the contamination ("pro-active approach").

In particular the introduction of the principle of non-dilution is an important and far- reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful mean to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)[3].

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003[4] provides a comprehensive overview on the possible risks for

[1] OJ L140, 30.5.2002, p. 10
[2] OJ L 115, 4.5.1999, p. 32
[3] Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)
[4] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)

animal and public health as the consequence of the presence of undesirable substances in animal feed.

It was nevertheless acknowledged by SCAN itself for several undesirable substances and by the Standing Committee on the Food Chain and Animal Health section Animal Nutrition that additional detailed risks assessments are necessary to enable a complete review of the provisions in the Annex, including the establishment of maximum levels for undesirable substances currently not listed.

2. Specific Background

Fumonisins are formed mainly by *Fusarium verticillioides* (syn. *Fusarium moniliforme)* and *Fusarium proliferatum.* At least 12 fumonisin analogues are known, the most important being the B series (fumonisins B_1, B_2 and B_3). The most significant crop, in which fumonisins occur, is maize, particularly when grown in warmer regions.

No maximum levels for fumonisins in animal feed have been established in EU legislation.

Maximum levels for fumonisins in foodstuffs and more in particular for the sum of Fumonisin B_1 and B_2, are currently under discussion at EU level.

SCAN concluded[5] that fumonisins can be responsible for serious adverse health effects in horses and pigs.

Given the high concentrations of fumonisins that may be found in maize especially in imported from warm regions, the introduction of control measures should be considered. It is therefore appropriate that a full risk assessment should be undertaken.

TERMS OF REFERENCE

The European Commission requests the EFSA to provide a detailed scientific opinion on the presence of fumonisins in animal feed.

This detailed scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of fumonisins for the different animal species of relevance (difference in sensitivity between animal species) above which
  - signs of toxicity can be observed (animal health / impact on animal health) or
  - the level of transfer/carry over of fumonisins from the feed to the products of animal origin results in unacceptable levels of fumonisins in the products of animal origin in view of providing a high level of public health protection.

[5] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 7.6. Conclusions and recommendations.

- identification of feed materials which could be considered as sources of contamination by fumonisins and the characterisation, insofar as possible, of the distribution of levels of contamination
- assessment of the contribution of the different identified feed materials as sources of contamination by fumonisins
- to the overall exposure of the different relevant animal species to fumonisins,
  - to the impact on animal health
  - to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and carry over rates[6].
- identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

ASSESSMENT

1. Introduction

Fumonisins such as B_1, B_2, B_3 and B_4 are mycotoxins produced by various fungi of the genus *Fusarium,* primarily by *Fusarium verticillioides* (formerly named *F. moniliforme*) and the related *F. proliferatum,* although other fungal species including *F. napiforme*, *F. dlamini* and *F. nygamai* are also able to produce fumonisins (US-NTP, 1999; WHO-IPCS, 2000). *F. verticillioides* and *F. proliferatum* are common fungi associated with maize causing 'Fusarium kernel rot' an important plant disease in hot climates. 'Fusarium kernel rot' may also be induced by *F. graminearum* and a strong relationship exists between insect damage, temperature stress, and fungal invasion, especially in cultivars grown outside their area of adaptation. As *F. verticillioides* and *F. proliferatum* grow over a wide range of temperatures but only at relatively high water activities ($a_w > 0.9$), fumonisins are formed in maize prior to harvest or during the early stage of storage. Except under extreme conditions, the concentrations of fumonisins do not increase during storage.

Fumonisins have been found as natural contaminants in maize and maize-based food from many parts of the world, e.g. the U.S.A., Canada, South Africa, Italy, Poland and Spain (Eriksen and Alexander, 1998; WHO-IPCS, 2000). Many countries have generated data on occurrence of fumonisins in grains (mostly maize) and grain-based foods. A SCOOP report on *Fusarium* toxins has recently become available, which includes data on fumonisins in foodstuffs from 9 European countries (EC, 2003a).

[6] Importance of the human exposure to fumonisins from foods of animal origin compared to overall human dietary fumonisin exposure can be assessed making use of the information contained in the report on a task on human exposure assessment to fumonisin which has been finalised in July 2003 at EU level within the framework of co-operation by Member States in the scientific examination of questions related to food (SCOOP – Task 3.2.10 – *Fusarium* toxins) (EC, 2003a)

Fumonisin B_1 (FB_1) is the most significant of the fumonisins in terms of toxicity and occurrence. FB_1 is chemically described as 1,2,3-propanetricarboxylic acid, 1,1'-[1-(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl]ester ($C_{34}H_{59}NO_{15}$, MW 721.838, CAS No 116355-83-0). The structure of FB_1 is shown in Figure 1. Fumonisin B_2 (FB_2) is a deoxy analogue of FB_1 in which the corresponding epimeric units on the eicosane backbone have the same configuration. The full stereochemistry of fumonisins B_3 (FB_3) and B_4 is unknown yet, but the amino terminal of FB_3 has the same absolute configuration as that of FB_1 (Bolger *et al.*, 2001; WHO-IPCS, 2000).

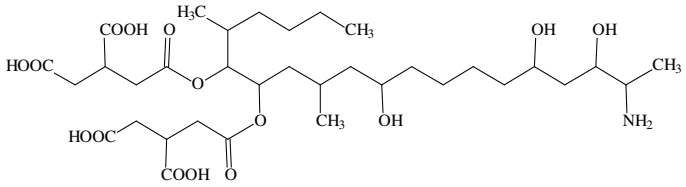

Figure 1. Chemical structure of fumonisin B_1.

Maize screenings contain higher concentrations of fumonisins than whole grain. Separation and removal of screenings reduces the levels of fumonisins prior to storage, but the screenings are used in animal feeds. Both, wet and dry milling of maize, results in distribution of fumonisins into various maize fractions with high concentrations predominantly in those fractions destined for animal feed. Fumonisins are fairly heat-stable, and the toxin content is significantly reduced only during processes in which the temperature exceeds 150˚C. There is little degradation of fumonisins during fermentation.

A range of toxic syndromes have been associated with exposure to fumonisins including Equine Leukoencephalomalacia (ELEM), Porcine Pulmonary Edema (PPE) and hepatic and renal injury in most species tested. Fumonisin exposure results also in hemodynamic alterations, considered to be involved in the pathogenesis of both, ELEM and PPE.

In resembling the structure of sphingosine, sphinganine and related complex sphingolipids fumonisins inhibit sphingosine (sphinganine) N-acetyltransferase (ceramide synthetase) thus blocking the synthesis of complex sphingolipids (Merrill *et al.*, 1996; Norred *et al.*, 1998; Wang *et al.*, 1999; Yoo *et al.,* 1992). Subsequently, an accumulation of sphinganine (and sometimes also sphingosine) and a depletion of complex sphingolipids has been observed in a variety of cultured cells (Norred *et al.,* 1998; Schroeder *et al.*, 1994; Tolleson *et al.*, 1999), and in sera, liver, kidney and urine of animals fed contaminated grains (Merrill *et al.*, 1997; Riley *et al.*, 1993; Van der Westhuizen *et al.*, 2001; Wang *et al.*, 1992). The increased ratio of sphinganine to sphingosine (Sa:So ratio) is the most sensitive indicator for exposure to fumonisin. The disruption of the sphingolipid metabolism presumably accounts also for the major biological effects exerted by fumonisins including impairment of cell growth and differentiation, cellular oxidative stress, and apoptosis and necrosis (Ciacci-Zanella and Jones, 1999; He *et al.*, 2002; Howard *et al.*, 1995; Norred *et al.*, 1998; Sharma *et al.*, 1997).

In 1993 and 2002 IARC evaluated FB_1 and classified it in Group 2B: "Possible carcinogenic to humans" (IARC, 1993, 2002), as FB_1 has been shown to produce kidney and liver tumours

in rodents (FAO/WHO, 2002, US-NTP, 1999) but there is no adequate evidence that FB_1 is genotoxic. It is assumed that FB_1 acts as tumour promotor (Gelderblom *et al.* 1996). Human exposure to FB_1 has been associated with oesophageal cancer and neural tube effects in humans (for details see EC 2003b).

Risk assessments for fumonisins have been performed by the Scientific Committee on Food (SCF) (EC, 2000, 2003b), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 2002) and a Nordic Working Group (Eriksen and Alexander, 1998). A group provisional maximum tolerable daily intake (PMTDI) for FB_1, FB_2 and FB_3 of 2µg/kg body weight was established by JECFA. A group tolerable daily intake (TDI) of 2µg/kg body weight was also established by SCF (EC 2003b). The tolerable intake established by JECFA and SCF was based on a NOEL of 0.2 mg FB_1/kg b.w./day for kidney toxicity in the rat, the most sensitive adverse effect observed.

Concerning agriculture animal species and experimental laboratory species the SCF summarised NOAEL levels from diverse studies as follows (Table 1):

Table 1. NOAEL's from diverse studies, expressed as mg FB_1/kg b.w./day

Species	**Duration**	**Target organ /effect**	**NOAEL** **(mg/kg b.w.)**	**Reference**
Rat	Short-term	Liver	< 0.75	Gelderblom *et al.,* 1994
Pig	Short-term	Lung/ PPE Lung/heart	< 4.5 0.2	Motelin *et al.,* 1994 Zamborsky-Kovács *et al,* 2002b[7]
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*., 1995
Mouse	Chronic	Liver	0.6	US-NTP, 1999
Rat	Chronic	Kidney	0.25	US-NTP, 1999

2. Methods of analysis

For the monitoring of the occurrence of fumonisins in food and feed commodities, mainly chromatographic methods of analysis are used, reviewed by Shephard (1998) and updated by

[7] This study was not considered by SCF

the same author (FAO/WHO, 2001). There is evidence that some fumonisins are matrix-bound. They may also occur bound to proteins and sugars in the matrix (Humpf and Voss, 2004; Kim *et al.,* 2003; Seefelder *et al.,* 2003). Extraction conditions need to be sufficiently rigorous to ensure good recovery of the unbound and/or hydrolysed toxins (Park *et al.,* 2004; Seefelder *et al.,* 2001). Most methods for fumonisins include solid phase extraction (SPE) or, more often, immunoaffinity (IA) cleanup, followed by pre-column derivatisation with o-phtaldialdehyde as derivatisating agent, and liquid chromatography (LC) separation with fluorescence detection. For screening purposes enzyme-linked immunosorbent assays (ELISA) can be used. Two LC-methods and an ELISA have received AOAC Official Method status (AOAC International, 2000). They were validated for maize and certain maize products. LC followed by mass spectrometric (MS) detection is gaining popularity (Musser *et al.*, 2002) but a formal interlaboratory validation has not been conducted yet. An alternative is the application of reverse-phase thin-layer chromatography (TLC). A collaborative study with maize as test material has shown good performance of this approach for semi-quantitative purposes (Shephard and Sewram, 2004). Formally validated methods for (mixed) feedingstuffs are currently not available.

Certified matrix reference materials are not available for fumonisins. Calibrants of FB_1 and FB_2 are commercially available. The Food Analysis Performance Assessment Scheme (FAPAS®) organises proficiency tests for FB_1 and FB_2 twice yearly. Recent FAPAS studies (FAPAS, 2000, 2001a,b, 2002, 2003) indicate that LC-fluorescence is the preferred analytical technique, whereas ELISA, TLC and LC-MS are used to a much lesser extent. The studies showed that "satisfactory Z-scores" for the participants ranged from 44 % to 76 % for FB_1 and from 55 % to 82 % for FB_2 in various maize test materials and one blended maize/wheat test material, with assigned values ranging from 650 µg/kg to 4879 µg/kg for FB_1 and from 306 µg/kg to 2220 µg/kg for FB_2. Further studies are necessary to improve the analytical performance of test laboratories analysing for fumonisins.

3. Current legislation

Whereas in 1995 fumonisins were only subject of regulations in one country (FAO, 1997) this number has now increased to six, with limits for maize ranging from 1000 – 3000 µg/kg (FAO, 2004). In Europe, fumonisins are now regulated in Bulgaria (FB_1 and FB_2 in maize and maize products), in France (FB_1 in cereals and cereal products), and in Switzerland (FB_1 and FB_2 in maize). Specific EU-harmonised limits for fumonisins in food or feed have not been established yet. Limits for FB_1, FB_2 and FB_3 in animal feeds currently only exist in the USA, as guidance levels for industry. Different limits are applied, depending on the destination of the feed, and are specified for equidae, rabbits, swine, catfish, ruminants, poultry, mink and pet animals (Table 2).

Table 2. Industry guidance levels for the sum of fumonisins B_1, B_2 and B_3 in animal feedstuffs as applied in the USA (US-FDA, 2001).

Type of feed	Limit (µg/kg)	Remarks
Corn and corn byproducts intended for equids and rabbits	5000	No more than 20 % of diet on dry weight basis
Corn and corn byproducts intended for swine and catfish	20,000	No more than 50 % of diet on dry weight basis
Corn and corn byproducts intended for breeding ruminants, breeding poultry and breeding mink (includes lactating dairy cattle and hens laying eggs for human consumption	30,000	No more than 50 % of diet on dry weight basis
Ruminants over 3 months old being raised for slaughter and mink being raised for pelt production	60,000	No more than 50 % of diet on dry weight basis
Poultry being raised for slaughter	100,000	No more than 50 % of diet on dry weight basis
All other species or classes of livestock and pet animals	10,000	No more than 50 % of diet on dry weight basis

4. Occurrence of fumonisins in feed materials

Fumonisins are unique amongst the mycotoxins in being almost exclusively contaminants of maize, rather than occurring in a wider range of cereals or other commodities (Bullerman and Tsai, 1994). Only incidentally fumonisins have been found in wheat, asparagus, tea, and cowpea (Trucksess, 2003). Extensive world-wide survey data (Placinta *et al.*, 1999) on fumonisins in maize indicate that the vast majority of maize is contaminated with fumonisins. It is in fact very difficult to obtain uncontaminated maize as even when contamination is not significant, fumonisins can still be found at low background levels. In general, where contamination is found, levels of fumonisins range from 0.02 mg/kg (the limit of detection in many investigations) to sometimes tens of mg/kg. In most samples, FB_1 was the most prevalent toxin, with co-occurrence of FB_2 and FB_3. For 21 countries in Africa, North- and South America, Asia, and Europe, FB_1 was reported at levels from 0.02 to 25.9 mg/kg with FB_2 co-occurring at levels from 0.05 to 11.3 mg/kg (Placinta *et al.*, 1999). Samples of maize and distiller's dried grains from Denmark showed levels for the sum of FB_1, FB_2 and FB_3, ranging from < 0.025 up to 1.93 mg/kg (Friis-Wandall, 2004). Most survey data that have been published were for raw maize, but the intended destination has not been specified and often sampling and analysis was confined to materials intended for human consumption. However, animal feed in South Africa was reported to contain 4.0 to 11.0 mg/kg of FB_1 (Dutton and Kinsey, 1995), from Uruguay from 0.2 to 6.3 mg/kg (Pineiro *et al.*, 1997). Lower levels were reported in samples of poultry feed (0.02 - 0.26 mg/kg) from India (Shetty and Bhat, 1997). In contrast, Jindal *et al.* (1999) reported FB_1 concentrations in maize samples from India up to 87 mg per kg; whereas mixed poultry feed samples contained maximum concentrations of 28 mg FB_1 per kg feed. In 35 samples of pig feed from France prepared with home-grown maize, fumonisins were found in all samples at levels up to 2.1 mg/kg for FB_1 and 0.9 mg/kg for FB_2 (Dragoni *et al.,* 1996). In Poland, in 1997 fumonisins were detected in

78 samples of poultry mixed feed at levels from 0.010 to 0.178 mg/kg (Wisniewska-Dmytrow *et al.,* 2004).

In studies on the fate of fumonisins during dry milling of maize when the contamination was 5.09 mg/kg in the raw maize, levels of 9.5 mg/kg, 8.1 mg/kg and 6.8 mg/kg were found in the germ, bran and animal feed flour respectively, whilst levels were reduced to 0.42 mg/kg, 1.0 mg/kg and 0.45 mg/kg in the large grits, small grits and flour respectively (Brera *et al*., 2004). Thus, the fractions destined for animal feed can be expected to contain higher levels of fumonisins than the raw materials. Fumonisins in maize components of animal feed were found at average levels of 24 mg/kg, 8.1 mg/kg, 5.7 mg/kg and 1.1 mg/kg in samples of maize screenings, maize meal, maize germ and maize germ bran respectively (Scudamore *et al*., 1998). A similar picture was found when 27 of 32 samples of maize gluten used as raw ingredients for animal feed were found to contain FB_1 at levels from 0.1 to 4.5 mg/kg (Scudamore *et al.,* 1997). Maize germ, screenings and other maize derived fractions were similarly contaminated at levels up to 1.1 mg/kg, 2.7 mg/kg and 1.0 mg/kg respectively. The concentration of maize in the final feed was not reported. Information about the possible occurrence of fumonisins in silage is slender, but incidental studies suggest that maize silage may be contaminated with fumonisins at low levels (up to approximately 0.6 mg FB_1/kg) (Kim *et al.*, 2004).

The most recent compilation of data for fumonisins in cereals in Europe can be found in the SCOOP Report on *Fusarium* toxins (EC, 2003a). As the SCOOP Report on *Fusarium* toxins primarily focussed on assessing human exposure to fumonisins and other *Fusarium* toxins, inevitably the analysis centred on grain and grain products presumably destined for human food.

5. Estimating the intake of fumonisins by farm livestock

Maize and maize based feedstuffs are extensively used in feeding of farm animals. The main feedstuffs, which are made of maize, are maize grain, maize silages (whole plant, corn cob mix (CCM), maize stover) and products of the refining industry. Maize oil, maize gluten, maize germ meal and maize germ bran are the most important maize by-products in animal feeding.

In ruminant nutrition, whole plant maize silage is a basic component of the daily ration in many farms. Its proportion comprises usually between approximately 30 and 50 % of the daily ration, but it can be fed up to approximately 80 %, especially to beef cattle. Fumonisin degrading microorganisms have been isolated from silage (Camilo *et al.,* 2000), but it is not known if this degradation is of any significance for reduction of fumonisin concentrations in silage. Additionally, maize grain is a common component of the concentrate portion and might reach up to approximately 20 % of the total daily dry matter intake.

Maize grain is a basic component in diets for monogastric animals because of its high energy content. In fattening animals (broilers, pigs and turkeys), its dietary proportion is usually higher at the beginning of the fattening period than in the finishing period. Starter diets might

contain up to 70 % maize grains. In contrast, the maize grain content in finisher diets is limited up to approximately 20 % in most feeding systems.

On a dry matter basis, CCM can replace maize grain in most situations, both in ruminants and in monogastric animals.

Maize oil can generally be used in preparing of supplementary feedingstuff for nutritional or technological reasons (dust binding). The plant oil proportion is usually not higher than 3 to 5 % of the supplementary feedingstuff.

The proportion of maize gluten, maize germ meal and maize germ bran in diets for ruminants and monogastric animals accounts usually for less than 20 % of the daily dry matter intake.

Potential intakes that would arise at different levels of contamination of complete diets for pigs may be calculated on the basis that young, rapidly growing animals daily consume an amount of feed equivalent to approx. 10 % of their body weight, declining to approx. 5 % in adult animals. If it is assumed that the complete diet contains the hypothetical levels of up to 1 mg/kg feed, intake by young animals fed exclusively on such diets would be less than 0.1 mg/kg b.w./day.

6. Adverse effects on livestock

Intoxications following fumonisin exposure have been described for various animal species, and the differences in sensitivity and clinical symptoms are described below. Culture material of *Fusarium* has been used as the source of FB_1 in most of the feeding studies cited below. The culture material may contain also FB_2 and FB_3, but this has not been investigated in all cases, and hence the described pathological alterations might be attributable to the entire group of *Fusarium verticillioides* toxins.

6.1. Adverse effects in pigs

Fumonisin toxicosis in pigs is characterised by pulmonary, cardiovascular and hepatic symptoms. Moreover, hyperplastic oesophagitis, gastric ulceration, hypertrophy of the heart and hypertrophy of the pulmonary arteries have been described (Casteel *et al.,* 1994; Gumprecht *et al*., 1998, 2001; Smith *et al*., 1996, 1999). Lethal pulmonary oedema and hydrothorax has been observed in pigs exposed to feed containing > 12 mg FB_1/kg feed (corresponding to 0.6 mg/kg b.w./day) (Haschek *et al*., 2001). Gross pathology reveals a severe pulmonary oedema (heavy, wet lungs) with widened interlobular septa, but without further signs of inflammation. When exposure was consistent over a period of 8 weeks, levels as low as 1 mg FB_1/kg feed have produced proliferation of the connective tissue, primarily around the lymphatic vessels and in the subpleural and interlobular connective tissue, extending into the peribronchial and peribronchiolar area. However, these alterations were not accompanied by clinical signs (Zomborszky-Kovács *et al*., 2002a,b). These changes were seen in one of 4 animals at the lowest level of 1 mg/kg feed, 2 of 5 animals at 5 mg/kg feed and 3 of 4 animals at 10 mg/kg feed. The weight of the lungs of the pigs from the 5 and 10 mg/kg feed groups showed also a significant dose-dependent increase.

It has been suggested that the pulmonary injury is preceded by cardiovascular abnormalities and haemodynamic changes with pulmonary hypertension and left heart insufficiency (Smith *et al*., 1999).

When pigs are exposed to fumonisins they develop also hepatic injury with hepatic necrosis and cholestasis. Affected animals become anorexic; show signs of encephalopathy, loose body weight and show hepatic nodular hyperplasia. These changes are accompanied by alterations in serum biochemical parameters, including an increase in circulating bile acids, elevated bilirubin concentration, and increased values for serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (γGT) and lactate dehydrogenase (LDH) activity (Zomborsky-Kovács *et al.,* 2002a,b). These effects were concentration- and time-dependent. Motelin *et al.* 1994 found elevation of serum bilirubin, cholesterol, γGT, ALP, alanine amino transferase (ALT), AST and arginase in pigs fed diets containing 101 or 175 mg FB_1/kg feed although levels were only significantly higher than control for bilirubin (at 5 days), and γGT and ALT (at 14 days) in the group fed the highest FB_1 level. The findings were extrapolated to a so called "no observed effect level" NOAEL of < 12 mg FB_1/kg feed for ALP. In the feeding studies of Zomborszky-Kovács *et al.* (2002a,b) serum AST activities were only significantly higher at 4 weeks in the highest dosed groups administered 20 and 40 mg FB_1/kg feed. Elevated levels outside the normal physiological ranges were observed for ALP, AST and ALT in some of the animals from the groups fed 5 and 10 mg FB_1/kg feed that had pathological changes in the lungs. In contrast, an increase in serum cholesterol concentration was observed after exposure to ≥ 1 mg pure FB_1/kg feed to male pigs for 2 weeks (Rotter *et al.,* 1996). It was, however, not significantly higher in the 1 and 10 mg FB_1/kg feed group after 8 weeks.

A dose- and time dependent increase in the concentration of free sphinganine in serum and tissues is also observed early resulting in an increase in the Sa:So (sphinganine:sphingosine) ratio. Particularly in lung, liver and kidney this ratio is increased and significant changes have been observed after exposure to 5 mg FB_1 per kg feed (corresponding to an exposure of 0.2 mg/kg b.w./day) for a period of two weeks. Riley *et al.* (1993) found elevated free sphinganine in liver, lung and kidney tissues from pigs if the animals were exposed to concentration ≥ 23 mg total FB_1 + FB_2 per kg feed, and an increased Sa:So ratio in serum from pigs occurred when concentrations were ≥ 5 mg/kg feed. Rotter *et al.* (1996) found the Sa:So ratio to be increased in lung, kidney and liver from pigs fed 10 mg (10 ppm) but not 1 mg (1 ppm) pure FB_1/kg feed. Increased serum Sa:So ratios were found by Zomborszky-Kovács *et al.* (2002a) in pigs fed a rations with ≥ 5 mg/kg feed, but not in the group fed 1 mg FB_1/kg feed.

Under field conditions, the increase in the Sa:So ratio can be used to establish a diagnosis, as this phenomenon is unique for a fumonisin toxicosis (Riley *et al*., 1993). At the same time alterations in the Sa:So ratio in organs are a sensitive biomarker of the onset of adverse effects. Hence monitoring of the Sa:So ratio (for example in serum or urine samples) has a dual function in monitoring exposure and assessing the onset of adverse effects.

In vitro studies with different cell cultures have confirmed the unique (diagnostic) value of the Sa:So ratio, which may also account for cellular apoptosis and necrosis of alveolar macrophages and endothelial cells in lung tissues of pigs (Zomborsky-Kovács *et al.*, 2002b). These latter findings correspond to previous data in mice (Martinova *et al.*, 1995), but are particularly relevant to pigs as these animals - in contrast to other species - have high numbers of macrophages residing in the lung, serving as first line of defence towards infectious agents, comparable to the function of Kupffer cells in the liver. Immune-modulatory effects have been described in pigs (Osborne *et al.,* 2002) but also in chickens (Li *et al.,* 1999; Marijanovic *et al.,* 1991; Qureshi *et al.*, 1995), and in rodents (Tryphonas, 1997).

Considering the Sa:So ratio as the most sensitive parameter in the assessment of adverse effect exerted by fumonisins the lowest observed adverse effect level was found to occure when pigs were exposed to feed containing 5 mg of fumonisins per kg feed (which is corresponding to approximately 0.2 mg/kg b.w./day). Lung lesions that are typical for fumonisins toxicity in pigs were observed at a dose of 0.4 mg/kg b.w./day (Zomborszky-Kovács *et al.,* 2002a, Riley *et al.,* 1993).

6.2. Adverse effects in poultry

Poultry seem to be less sensitive to fumonisin exposure than pigs and horses (see below) but only a few studies testing high dosages of fumonisins are available (Bryden *et al.*, 1987; Brown and Rottinghaus, 1994). In a feeding experiment, one-day old **male broiler chickens** were exposed for a period of 14 days to either 125 or 274 mg purified FB_1/kg feed. Reduced weight gain was observed, which was more pronounced in another trial where culture material containing also FB_2 was used (Javed *et al.*, 1993). The results of the same study indicate that younger chickens are more sensitive than older animals and spontaneous death was only observed during the first three days of life. A no-effect level for chickens could not be established from these experiments.

In an early study (Ledoux *et al.,* 1992) day-old broiler chickens were fed diets containing 0, 100, 200, 300, or 400 mg FB_1 from culture material per kg feed for 21 days. Body weight and average daily weight gain decreased with increasing FB_1 concentration and the weight of liver, proventriculus and gizzard increased. Histological lesions in livers were also seen in the birds fed FB_1. Serum calcium, cholesterol, and AST levels increased at the higher FB_1 levels.

In a later study (Ledoux *et al.*, 1996) *F. moniliforme* culture material containing total amounts of fumonisins (FB_1, FB_2 and FB_3) ranging between 33 and 627 mg/kg feed was given to one-day old chickens for a period of 21 days. Birds showed a dose-dependent reduced weight gain and reduced feed conversion. Hepatocellular hyperplasia progressed from mild (99 and 132 mg/kg dose groups) to moderate to severe, (330 mg/kg group) to severe at the two highest concentrations (429 and 627 mg/kg, respectively). From these data, US-FDA (2001) deduced a safe level of 66 mg/kg feed of $FB_1 + FB_2 + FB_3$ for chickens and turkey poults, although stating clearly the limitations of this study, which was conducted only over a period of 21 days.

Weibking *et al.* (1993) fed day-old broiler chickens FB_1 from culture material at 7 levels from 75 to 525 mg/kg feed (total FB 89 - 681 mg/kg) feed for 21 days. All chicks fed diets with fumonisins had increased serum sphinganine and serum Sa:So ratio. Feed intakes and body weight gains were lower at the two highest levels (450 and 525 mg FB_1/kg). Increased liver and kidney weight together with increased mean cell haemoglobin concentrations were also observed at those levels. Histological liver lesions were seen in chickens fed ≥ 225 mg FB_1/kg feed.

Broiler chickens (5 replicates of 6 birds/treatment) have also been fed purified FB_1 at 0, 20, 40, and 80 mg/kg feed for 21 days from day 1 (Henry *et al.,* 2000). No effect was seen on body weight and growth. Liver sphinganine concentration and the liver Sa:So ratio were significantly higher at all concentrations tested and increased with the FB_1 concentration, but the serum Sa:So ratio was only elevated in the group fed 80 mg FB_1 in the feed. Total liver lipids were lower in chickens fed the two highest FB_1 levels. Chickens fed the highest FB_1 level had also significantly higher serum GOT:ASP ratio, but cholesterol, ALP and LDH were not affected.

With the aim to evaluate the immune response in chickens, FB_1 through culture material was given to one-day old chickens at feed concentrations of 50, 100 or 200 mg/kg. Three weeks later the animals were challenged intravenously with either *E. coli* or inactivated Newcastle Disease Virus vaccine and either systemic bacteraemia or primary and secondary antibody titres were measured. The results suggested that FB_1 is immunosuppressive in chickens when fed 200 mg FB_1/kg diet (Li *et al*., 1999).

In a chronic feeding study with broiler chickens and **turkeys** with *Fusarium moniliforme* culture material, representing concentrations of 25 or 50 mg FB_1/kg feed, turkeys did show a significantly lower feed intake when exposed to 50 mg FB_1/kg diet, whereas broiler chickens were not affected (Broomhead *et al*, 2002). These findings point towards species differences in sensitivity.

Turkeys have also been fed diets containing 75, 150, 225, or 300 mg FB_1 from culture material per kg feed for 21 days (Weibking *et al.,* 1995). The lowest concentration corresponding to a daily dose of approximately 9 mg/kg b.w. on average. Dose-dependent decreases in feed intake and body-weight gains were found together with increases in liver weight. Feed made from another culture material but with the same FB_1 concentrations gave different effects on the above-mentioned parameters. Serum Sa:So ratios were increased in all fumonisin fed groups with no difference due to the culture material used. Hepatocellular hyperplasia was observed in all FB_1 treated groups.

Peking ducklings fed rations containing 100, 200 or 400 mg FB_1/kg feed (corresponding to a calculated value of 121, 241, or 482 total FB, respectively) from culture material for 21 days (Bermudez *et al.,* 1995) had a dose-dependent decrease in feed intake and weight gain. Increased absolute organ weights were found for liver, heart, kidney, pancreas and proventriculus with increasing FB_1 levels. Liver Sa:So ratio was increased for all FB_1 fed groups. Two of eight ducklings in the highest group died prior to the study termination.

In **Mallard ducks** given a daily dose of 0, 5, 15 or 45 mg/kg b.w. of FB_1 from culture material extracts for 12 days, an increase in the Sa:So ratio in the liver was observed in all groups and serum biochemistry indicated liver toxicity (Bailly *et al.,* 2001; Tran *et al.,* 2003, 2005). As the onset of these alterations varied in time, the authors concluded that fumonisins exert more prominent effects when ingested at a lower dose for a long time, rather than when ingested at a higher dose for a short period (Tran *et al*., 2003). Drug metabolising enzyme activities in liver and kidney were increased in ducks from the fumonisin fed groups (Raynal *et al.,* 2001).

In conclusion, available data suggest that in broiler chickens the lowest observed adverse effect level is approximately 2 mg/kg b.w./day (calculated from Henry *et al.,* 2000). Data for ducklings and turkeys suggest that these species are not more sensitive than chickens, as LOAELs were 5 mg/kg b.w/day for Mallard ducks, 17 mg/kg b.w./day for Peking ducklings, and 9 mg/kg b.w./day for turkeys. It should be noted that these levels represent the lowest doses tested.

6.3. Adverse effects in ruminants

In contrast to many other mycotoxins FB_1 is incompletely degraded by the forestomach flora in ruminants (Gurung *et al*., 1999; Smith and Thakur, 1996; Prelusky *et al*., 1996a). Rice and Ross (1994) described initially increased concentrations of aminopolyols and aminopentols, as metabolites of FB_1 in the faeces of sheep and cattle. Later studies by Smith and Thakur (1996) and Caloni *et al.* (2002) found that FB_1 is eliminated mainly unmetabolised. Signs of intoxications have been reported in cattle, sheep and goats exposed to fumonisin contaminated feed materials:

Ingestion of feed containing 75 mg FB_1/kg feed daily for 14 days by Jersey **cows** increased serum cholesterol concentration and decreased feed intake and milk production (Richard *et al.,* 1996). Feed containing on an average 94 mg FB_1/kg feed was given daily for 253 days to Holstein steers. The treatment increased serum AST and γGT activities and induced mild histological evidence of hepatocellular injury and bilary epithelial hyperplasia (Baker and Rottinghaus, 1999).

Eighteen feeder calves were fed diets containing low and high (26 respectively 105 mg/kg FB_1 or 33 respectively 148 mg/kg total FB) fumonisin levels based on corn screening during 31 days (Osweiler *et al.,* 1993). There was no effect on feed intake or weight gain. Significant increases in serum AST, γGT, LDH, bilirubin and cholesterol occurred from day 10 through day 31 in the high dose group. Histological changes were also found in the livers from the high dose group. Lymphocyte blastogenesis was significantly impaired at the end of feeding in the high dose group. Milk-fed calves were intravenous administered FB_1 at 1 mg/kg b.w. daily during 7 days (Mathur *et al*., 2001). They developed liver and kidney lesions with increased serum AST, ALP, γGT, and sorbitol dehydrogenase. Both, free sphinganine and the sphingosine levels in most tissues increased, except for brain where sphingosine was not increased. The highest Sa:So ratio was found in liver and kidney while brain had the lowest.

When **lambs** (average b.w. 32 kg) were dosed intra-ruminally with culture material at doses of 11.1, 22.2 or 45.5 mg/kg b.w. total fumonisins (FB_1, FB_2 and FB_3) for 4 consecutive days, spontaneous death occurred in the highest dose group (Edrington *et al*., 1995). In the other animals, an increase in alkaline phosphatase, γGT, AST and LDH activities could be observed. Moreover, serum cholesterol, triglyceride, urea nitrogen and creatinine levels were increased. Histological examination at the end of the trial revealed renal tubular necrosis and a mild hepatopathy, confirming previous findings of Kriek *et al*. (1991).

Weanling Angora **goats** tolerated feed containing 95 mg FB_1/kg feed, given for a period of 112 days, without clinical symptoms. The Sa:So ratio in the livers was significantly increased (Gurung *et al*., 1998).

In conclusion, at a mean concentrations of 94 mg FB_1/kg feed (corresponding to 2.4 – 3.5 mg/kg b.w./day) biochemical changes occurred, indicating hepatocellular injury in steers. In angora goats, an increased Sa/So ratio occurred in the liver as first sign of adverse effect exerted by fumonisins, following a concentration of 95 mg FB_1/kg feed. Moreover one study seems to indicate that in feeder calves a no effect level of approximately 26 mg FB_1/kg feed (corresponding to 0.65 mg/kg b.w./day) could be expected.

6.4. Adverse effects in horses

The first syndrome attributed to fumonisins in the 1980ies was ELEM, equine leukoencephalomalacia, characterized by fatal necrotic lesions in the cerebrum. This syndrome appears to be unique for equidae, although brain lesions have been incidentally reported in other animal species as well. Like in pigs, fumonisins induce cardiovascular dysfunction in horses with decreased heart rates, lower cardiac output, and right ventricular contractibility which may be involved in the pathogenesis of the lesions in the central nervous system (Smith *et al*., 2002). It has been hypothesized that ELEM is a result of cerebral oedema due to an inability to reduce the blood flow to the brain when the horse lowers its head to eat and drink (Constable *et al*., 2000)

Horses fed with their concentrates culture material from different *Fusarium moniliforme* strains for 27 days at concentrations of 65, 130, and 200 mg FB_1/kg showed ELEM lesions at necroscopy (Goel *et al.,* 1996). Serum Sa:So ratios were significantly elevated at day 19. Moreover, sphinganine and sphingosine tissue levels were elevated in different parts of the brain and the intestines, when compared to healthy horses. The Sa:So ratios, however, were not significantly higher in these tissue.

Experimental studies revealed that the minimum intravenous dose of pure FB_1 that induces neurological abnormalities was 0.01 - 0.05 mg/kg b.w./day (Constable *et al.,* 2000). Assuming an oral bioavailability of 5 % in equidae, the equivalent oral dose would be 0.2 - 1.0 mg/kg b.w./day, to be considered as lowest adverse effect dose.

Exposure of horses to fumonisins also induces liver and kidney damage, associated with a significant increase in the serum concentration of free sphinganine. The increased Sa:So ratio and an increase in liver specific enzymes precede the clinical signs of ELEM.

Evaluation of many cases of ELEM in the USA indicate that the consumption of feed containing more than 10 mg FB_1/kg feed (0.2 mg/kg b.w./day) is associated with an increased risk for horses to develop ELEM, whereas at concentrations < 6 mg FB_1/kg of diet (equivalent to 0.12 mg/kg b.w./day) no increased risk for target animal species is assumed (Constable *et al.*, 2000; Ross *et al.,* 1991).

Recent experimental studies in which FB_1 was injected intravenously for a period of 28 days to horses, suggest a NOAEL of 0.01 mg/kg b.w. based on the identifiable signs of neuronal abnormalities (Foreman *et al*., 2004). The NOEL for biochemical abnormalities in blood serum exceeded 0.2 mg/kg b.w./day (Constable *et al*., 2000; FAO/WHO, 2001).

Additional experiments showed that ponies (n = 5), fed concentrates that contained 8 mg/kg FB_1 (and 2.56 mg/kg FB_2) originating from contaminated maize screening, in quantities corresponding to 0.8 % of their body weight (114 - 186 kg) for 122 days followed by 58 days at 1.6 % of body weight (Wilson *et al.,* 1992) developed periodically neurological disturbances during the entire experimental period. At the end of the experiments animals were sacrificed. With gross pathology, no visible brain lesions were observed in any of the ponies. Histopathological examinations revealed the abundant presence of brain lesions similar to those found in horses in all ponies. In conclusion, equidae, and horses in particular, seem to be very sensitive to Fumonisins.

6.5. Adverse effects in other animal species

6.5.1. Rabbits

Bucci *et al*. (1996) described leukoencephalomalacia and haemorrhages in the brain of rabbits similar to those observed in horses after exposure to fumonisins. Following intravenous injection of FB1 nephrotoxic effects were also observed in rabbits (Gumbrecht *et al*., 1995). In a teratogenicity study, dosages ranging from 0.1 to 1.75 mg/kg b.w. were given orally by gavage during gestation days 3 - 19. Maternal toxicity was observed at a dose of 0.25 mg/kg b.w./day, and foetal toxicity occurred at the lowest dose tested (0.1 mg/kg b.w.), but no major malformation could be observed (Bucci *et al.,* 1996; LaBorde *et al.,* 1997). These findings are agreement with experiments in Syrian hamsters (Penner *et al.,* 1998) and in rats, in which no malformations could be observed although following prenatal exposure to FB_1 the sphinganine levels and myelination were altered in various brain areas of developing rats (Know, 1995, 1997).

6.5.2. Mink

Adult female mink have been fed a diet that contained Fusarium culture material that provided a concentration of 89 mg FB_1/kg or 108 mg total FB/kg during 87 days (Restum *et al.*, 1995). The fumonisin exposed mink showed a mild lethargy, but no other clinical signs or differences in feed consumption (first 2 week), body weights, or survivability were found. Several serum parameters e.g. cholesterol, bilirubin, ALP, ALT, AST were greater in the mink fed FB than in control. The effect of fumonisins on mink reproduction has also been studied (Powell *et al.,* 1996). Adult female mink were fed a low- or high-dose diet with

Fusarium culture material at concentrations of 86 mg FB_1/kg (115 mg total FB/kg) and 200 mg FB_1/kg (254 mg total FB/kg) respectively from two weeks prior to breeding through gestation and lactation. Only 58 % of the mated females whelped in the high dose group compared to 100 % in the low dose. The kitten's body weight at birth showed a dose-dependent decrease and the number of stillborn kittens was directly proportional to the dose. No treatment-related gross or histological lesions were observed in the kittens.

The Sa:So ratio in urine from minks fed diets containing 86 or 200 mg FB_1/kg (115 or 254 mg total FB/kg) was increased 2 to 11-fold within 7 days as compared to control (Morgan *et al.,* 1997). Lower doses of fumonisins were not tested.

6.5.3. Fish

Fumonisins are recognized to be toxic for fish, however, only few data have been published. Groups of catfish (*Ictalurus punctatus*) have been fed diets formulated by inclusion of Fusarium culture material from maize to contain FB_1 at 20, 80, 320, and 720 mg/kg during 10 weeks (Lumlertdacha *et al.*, 1995). The maize culture material also contained FB_2 (22 % of FB_1). Survival was 100 % for fish fed 20 mg/kg FB_1, but weight gain was significantly decreased by 15 % compared to control, and liver lesions were noted histologically. Catfish has also in another study been fed FB_1 from Fusarium cultured maize (Li and Robinson, 1995). There is no information on FB_2 or FB_3 levels. Eight groups of 20 fishes were fed 0, 0.7, 2.5, 5.0, 10.0, 20.0, 40.0 or 240.0 mg FB_1/kg feed, respectively, for 12 weeks. Decreased weight gain, feed consumption and feed conversion ratio as well as histological changes were found at concentrations ≥ 40 mg FB_1/kg feed. From this study an apparent no effect level of 20 mg/kg feed could be deduced.

In young carps, exposure to contaminated feed (equivalent to a daily intake of 0.5 to 50 mg FB_1/kg b.w.) resulted in a loss of body weight and alterations of haematological and biochemical parameters, indicating liver and kidney damage (Pepeljnak *et al*., 2003). Additional studies in one-year old carps demonstrated pathological alterations in liver, endocrine and exocrine pancreas, kidney, heart and brain already following exposure to 10 mg FB_1/kg diet (Petrinec *et al.,* 2004).

Nile tilapia (Oreochromis niloticus) has also been fed FB_1 from culture material in their diet for 8 weeks to study the effect on growth, histological and biochemical changes (Tuan *et al.,* 2003). Groups of 20 fishes were fed FB_1 at levels of 10, 40, 70 and 150 mg/kg feed. Average weight gain was decreased at levels ≥ 40 mg FB_1/kg. Haematocrit was reduced only in fish fed diets containing 150 mg FB_1/kg. The Sa:So ratio in liver increased dose-dependently with insignificant increase in the highest dose group. Mortality was low in all groups and no histopathological lesions were observed.

The experimental data with catfish and Nile tilapia suggest a No Effect Level of 20 mg/kg feed. Recent studies with carps indicated that signs of toxicity can be observed following to exposure of 10 mg FB_1/kg feed (Petrinec *et al*., 2004).

6.5.4. Rodents

Numerous studies have been conducted in rodents to describe the acute and chronic toxicity of fumonisins and to define the pathological alterations associated with exposure to purified toxins or fungal culture material. These data have been recently summarized (FAO/WHO, 2002). In rats, initially the liver was identified as major target organ of fumonisins toxicity, as the application of FB_1 resulted in single cell necrosis, liver fibrosis, bile duct hyperplasia and hepatocellular carcinomas (Gelderblom *et al*, 1988, Gelderblom *et al*., 1991). The apparent no-effect-level varies, however, considerable between individual studies, depending on the strain, gender and diet used. Furthermore, various studies indicated that pathological changes in the kidneys precede liver alterations and occur at lower concentrations of fumonisins (see Table 1).

7. Toxicokinetics, metabolism and tissue distribution

Fumonisins are poorly absorbed and the oral bioavailability remains generally below 5 % for FB_1, and seems to be even lower for FB_2. The absorbed fractions are rapidly distributed and eliminated. Studies in rats with radio-labelled material showed rapid distribution and renal elimination rates ($t_{1/2el}$ = 40 minutes after intravenous injection of FB_1). Tissue levels were found to be highest in kidneys, followed by the liver (Norred *et al*., 1993). FB_1 is glucuronidated and excreted with bile fluid (approximately 1.4 % of the dose), which may result in entero-hepatic re-circulation and a delayed terminal elimination phase (Dantzer *et al*., 1999).

In a more recent study with Wistar rats, dosed orally with different concentrations of FB_1, a rapid absorption (T_{max} after 1 hour) and an apparent elimination plasma half-life of 3.15 hours, was calculated. The elimination half-life was measured also in the liver (4.1 hours), and in the kidneys (7.1 hours). Taken together, these data provide no evidence for tissue accumulation of FB_1 (Martinez-Larranga *et al*., 1999).

In a kinetic study with pigs in which radio-labelled FB_1 was used, the apparent plasma half-lives were $t_{1/2}\alpha$ – 2.2 min., $t_{1/2}\beta$ – 10.5 min, and $t_{1/2}\gamma$ – 192 min (assumed to result from enterohepatic re-circulation) after intravenous application (Prelusky *et al*., 1994). Recovery of FB_1 in biliary fluids represented 70.8 % of the intravenous dose, thus being significantly higher than the biliary excretion in rats. Oral bioavailability from parallel studies with intragastric application indicated an oral bioavailability of FB_1 of 3 - 6 % of the administered dose (Prelusky *et al*., 1996b).

FB_1 may be hydroxylated, but this limited hydroxylation seems to take place pre-systemically, as studies with bovine liver microsomes did not show any significant transformation of FB_1 (Spotti *et al*., 2001). A poor rate of hydroxylation was also observed in the rumen fluid (Caloni *et al*., 2000). Although the biotransformation of FB_1 is very limited, it has been shown to inhibit certain P450 enzymes, both *in vivo* and *in vitro* (Spotti *et al*., 2000).

8. Carry-over and residues

Several studies have been published on the possibilities of carry-over of fumonisins from feed into animal products, such as milk, meat and eggs.

In a study with ^{14}C-FB$_1$, **dairy cows** were dosed orally (1 or 5 mg FB$_1$/kg b.w.) and intravenously (0.05 or 0.2 mg FB$_1$/kg b.w.) (Prelusky, 1994). FB$_1$ residues in milk were detected at negligible levels (5 - 6 ng/ml). In another study (Richard *et al.*, 1996) fungal culture material with FB$_1$, FB$_2$ and FB$_3$ was mixed into the total diet of two lactating cows. The two cows consumed an average of 3 mg FB$_1$/kg body weight per day during 14 days. In this period milk samples were collected and investigated. Fumonisin was not found in milk (LOD 5 ng/ml). In another study, three lactating cows were given an intravenous dose of 30 mg FB$_1$ (Hammer *et al.*, 1996). Analysis of milk sample showed some positive samples, and a maximum rate of 0.11 % was calculated. Spotti *et al.* (2001) studied the FB$_1$ carry-over into milk in the isolated perfused bovine udder *ex vivo*. FB$_1$ (2 mg) was injected into the perfusion fluid of 3 udders, and milk and perfused serum levels were analysed over a period of 150 minutes. Carry-over from blood-to-milk was estimated to be in the range 0.001 - 0.004 %. Previous studies had already indicated a very limited carry-over of FB$_1$ into the milk of lactating sows (Becker *et al.,* 1995).

Thirty weeks old **laying hens** received an intravenous (2 mg/kg b.w.) or an oral dose (2 mg/kg b.w.) of ^{14}C-FB$_1$ (Prelusky, 1994). Measurements showed that almost all of the radioactivity was recovered in the excreta of the hens and that its level in the different organs and tissues was negligible (< 10 - 15 ng FB$_1$/g tissue), except for some localization in the liver (530 ng/g) and kidneys (210 ng/g). Moreover, eggs were collected and separated into yolk, albumin and eggshells. The residue levels in these separated parts of eggs were negligible (< 10 - 15 ng fumonisin/g).

In a study with **pigs**, dosed 2 - 3 mg ^{14}C-FB$_1$ for a period of 24 days, concentrations of 160 and 65 ng/g were found in liver and kidney respectively. Muscle and fat tissue were free of residues of FB$_1$ (Prelusky *et al.*, 1996b). A more recent study on residue formation of FB$_1$ in porcine tissues showed that after oral administration of a very high (experimental) dose (100 mg FB$_1$ per animal per day) for 5 - 11 days, residues of FB$_1$ can be detected in the kidneys (833 ng/g), liver (231 ng/g), lung (170 ng/g), spleen (854 ng/g), muscle (26 ng/g) and fat (2 ng/g). In a final trial on which pigs were exposed to 22 mg of FB$_1$ per kg feed for five months, no residues were found in the kidneys (LOD 5 µg/kg tissue) (DeLiguoro *et al.,* 2004).

The animal studies carried out to determine the potential carry-over of fumonisins from animal feed into animal products all indicated that minor tissue levels of FB$_1$ can be detected in various tissues, but the low carry-over rate suggests that these low residue levels do not to contribute substantially to human exposure (see also below).

9. Human dietary exposure

Estimates of dietary intakes of fumonisin based on national estimates have been presented by FAO/WHO, indicating an exposure from 0.02 µg/kg b.w./day to 0.2 µg/kg b.w./day, thus remaining below the PMTDI of 2 µg/kg b.w./day set by JECFA (FAO/WHO, 2001) and the TDI set by SCF (EC, 2003b). In the JECFA evaluation only the consumption of contaminated maize or maize-containing food products was considered, as the contributions of other commodities to the intake of fumonisins are too low and too variable to affect long-term exposure significantly.

Data from the EU SCOOP Task (EC, 2003a) showed that the average daily intakes are well below the tolerable intake (0.8 % - 13.2 % of the TDI for the whole population and 0.1 - 14.1 % of the TDI for the adults). Higher intakes were noted for young children (22.3 % of the TDI). Cereals represent the major source of intake of fumonisins. Among cereals, maize and wheat dominate as main contributors to the total intake.

A total diet study has been performed in France on the basis of samples purchased in 2000 and 2001 (Leblanc *et al.,* 2005). The estimated total average intake of fumonisins of the French population is 14 ng/kg b.w./day for adults and 46 ng/kg b.w./day for children of an age of 3 to 14 years. The 95th percentile exposure is respectively 64 ng/kg b.w./day for adults (3 % of the TDI) and 175 ng/kg b.w./day for children (9 % of the TDI). In the category food of animal origin only edible offals have been analysed and levels ranging from 0.09 to 0.12 µg/kg have been found contributing for less than 3 % to the total fumonisin exposure.

CONCLUSIONS

- Fumonisins are a distinct group of mycotoxins produced by various *Fusarium* species, especially from *Fusarium verticilloioides,* invading predominantly maize plants. Fumonisins have been detected worldwide in maize and maize-based products. However, data on the occurrence of fumonisins in feed materials are scarce, and fumonisin concentrations vary from a few µg/kg to tens of mg/kg.

- Fumonisin B_1, considered to be the most prevalent and most toxic derivative, is a prototypic inhibitor of cellular sphingosine (sphinganine) N-acetyltransferase. Inhibition of this enzyme results in an accumulation of sphinganine (Sa) and sometimes also sphingosine (So) and a depletion of complex sphingolipids in eukaryotic cells. This results in increased oxidative stress, impairment of the regulation of the cell cycle and cellular differentiation, and cellular apoptosis or necrosis. The altered Sa:So ratio which is a unique indicator of fumonisin exposure serves also sensitive biomarker for the onset of adverse effects.

- Intoxications associated with the occurrence of fumonisins in animal feeds comprise distinct syndromes such as ELEM (equine leukoencephalomalacia), PPE (porcine pulmonary edema), as well as dose dependent hepatocellular injury with enzyme leakage and increased serum cholesterol levels. Moreover, progressive renal tubular

necrosis (being also the first signs of toxicity in rodent studies) has been observed in all animal studies where it was tested.

- Most reports on occurrence and toxicity refer to concentrations of fumonisin B_1 in the diet. However, it can not be excluded that other fumonisins and even other toxins produced by *Fusarium verticillioides* were present in culture materials used for diet composition, and may have contributed to the observed effects.

- Experimental data from rabbits (as well as from studies in rodents and minks) provide no evidence for teratogenic effects. Fumonisin B_1 is carcinogenic in rodents, however it is devoid of significant genotoxic activity. Animal experiments suggest that fumonisins act as tumour promoters.

- In target animal species, the lowest observed adverse effect levels (LOAEL) for fumonisin B_1 were seen in horses and pigs, showing fumonisin-related alterations at a dose of approximately 0.2 mg/kg b.w./day. In calves, the apparent NOAEL was 0.6 mg/kg b.w./day, whereas in adult ruminants the LOAEL was 2.4 mg/kg b.w./day. In poultry chicks a LOAEL of 2 mg/kg b.w. could be derived from the available experimental data. Data from ducks, ducklings and turkeys do not provide evidence that these species are more sensitive than chickens. Data from catfish and Nile tilapia suggest a NOAEL corresponding to 20 mg/kg feed, whereas in carps a LOAEL of 10 mg/kg feed was described.

- Available data on carry-over of fumonisins from animal feeds into edible tissues, including milk and eggs, indicate that transfer is limited and thus residues in animal tissues contribute insignificantly to total human exposure.

RECOMMENDATIONS

- Analytical methods for the determination of the concentrations of fumonisin B_1 including its bound forms need to be developed and validated for maize-based feedingstuffs.

- More information is needed on the effects of fumonisins on farmed fish and on minor species, such as rabbits, goats, and mink to improve risk assessment for these species.

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