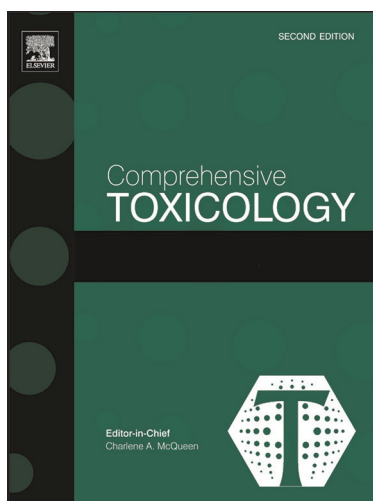


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14.07 Carcinogenic Mycotoxins

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Nomenclature

ppb (parts per billion) $\mu\text{g kg}^{-1}\text{diet}$

ppm (parts per million) $\text{mg kg}^{-1}\text{diet}$

Abbreviations

| | | | |
|------------------------|---|------------------------|---|
| AFB₁ | aflatoxin B ₁ | JECFA | Joint FAO/WHO Experts Committee on Food Additives |
| CDC | Centers for Disease Control and Prevention | NTP | National Toxicology Program |
| CYP | cytochrome P450 | OR | odds ratio |
| EN | endemic nephropathy | OT_α | ochratoxin α |
| ESF | European Scientific Committee | OTA | ochratoxin A |
| FB₁ | fumonisin B ₁ | OTB | ochratoxin B |
| GSH | glutathione | OTC | ochratoxin C |
| HBsAg | HBV surface antigen | RR | relative risk |
| HBV | hepatitis B virus | Sa | sphinganine |
| HCC | hepatocellular carcinoma | SCE | sister chromatid exchange |
| HCV | hepatitis C virus | So | sphingosine |
| IARC | International Agency for Research on Cancer | TNF_α | tumor necrosis factor α |
| IPCS | International Programme on Chemical Safety | USFDA | US Food and Drug Administration |

14.07.1 Introduction

Mycotoxins are metabolites of molds considered to be a major risk factor affecting human and animal health. It is estimated that 25% of the world's crop production is contaminated with mycotoxins. Mold contamination occurs extensively in fields and it is particularly severe in tropical countries. Due to world trade of grains, exposure to mycotoxins is ubiquitous.

The co-occurrence of mycotoxins is frequent in food and feed, because of frequent infection with several fungal strains and particular fungal strain may produce different mycotoxins (Binder *et al.* 2007). Animals and humans are mostly exposed to mycotoxins by ingestion of contaminated food, although dermal and inhalation routes of exposure have been reported. The contamination by these molds may be effectively stopped by pesticide use, but pesticides cannot eliminate mycotoxins produced before their application. There is no single method of mycotoxin elimination, and each method increases the cost of food production.

Up to now there have been more than 300 mycotoxins identified that cause signs of toxicity in mammals (Fink-Gremmels 1999). Diseases caused by mycotoxins are referred to as mycotoxicoses and outbreaks are more readily recognized in veterinary than in human medicine. Mycotoxicoses are caused mostly by ingestion of high levels of mycotoxins, and either in humans or in animals they are seen more frequently in tropical countries (Peraica *et al.* 1999b). There are several mycotoxins suspected to be human carcinogens, but with the exception of aflatoxin B₁ (AFB₁) this has only been demonstrated in experimental animals. It is important to know by which mechanism a mycotoxin is carcinogenic in order to define the safety factor that will be used in the calculation of an acceptable daily intake (Dirheimer 2000).

14.07.2 Aflatoxins

The first outbreak of acute poisoning with aflatoxins in turkeys in the United Kingdom known as Turkey X disease focused the attention of scientists to the problem of mycotoxins. Aflatoxins were very soon found to be carcinogenic to rats and linked with hepatocellular carcinoma (HCC) in humans (Lebreton *et al.* 1962).

Aflatoxins are potent liver toxins, and the severity of effects in animals varies with dose, length of exposure, species, strain, and diet or nutritional status. They are acutely toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic compounds. They are lethal when consumed in high doses; sublethal exposures can induce chronic toxicities, and low levels of chronic exposure can result in neoplasia, primarily HCC, in humans and animals. Aflatoxins inhibit DNA synthesis, DNA-dependent RNA polymerase activity, messenger RNA synthesis, and protein synthesis. The International Agency for Research on Cancer (IARC 2002) found that there is sufficient evidence in humans for the carcinogenicity of naturally occurring aflatoxins and classified them as Group 1 carcinogens.

14.07.2.1 Occurrence

Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus*, fungi that contaminate crops before harvest, during storage and food processing. Conditions that favor their growth are temperatures between 24 and 35 °C and a moisture content exceeding 7% (10% with ventilation) (Williams *et al.* 2004). The fungi are ubiquitous and can affect many of the developing country dietary staples.

Aflatoxins are a group of approximately 20 related highly substituted coumarins containing a dihydrofurofuran moiety. The five major aflatoxins are aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁), G₂ (AFG₂), and M₁ (AFM₁) (Figure 1). The most frequently found, the most toxic, and the most potent liver carcinogen is AFB₁, while AFG₁ and AFG₂ are inactive before metabolic activation *in vivo*. AFM₁ and AFM₂ are hydroxylated derivatives of AFB₁ and AFB₂ formed and excreted in the milk of lactating animals and humans. AFM₁ is also produced in plants on the field or on stored feeds together with other aflatoxins. A precursor of the aflatoxins, sterigmatocystin, is an acutely toxic and carcinogenic mycotoxin characterized by a xanthone moiety fused to a dihydrofuran and tetrahydrofuran moiety (Sweeny and Dobson 1999).

Aflatoxins contaminate agricultural products (rice, maize, cassava, nuts, peanuts, chillies, and spices), meat and meat products, milk and dairy products, and eggs. Inhalation exposure to AFB₁ is occupational, and the presence of AFB₁-contaminated dust particles in grain mills may pose an occupational risk for lung cancers (Kelly *et al.* 1997).

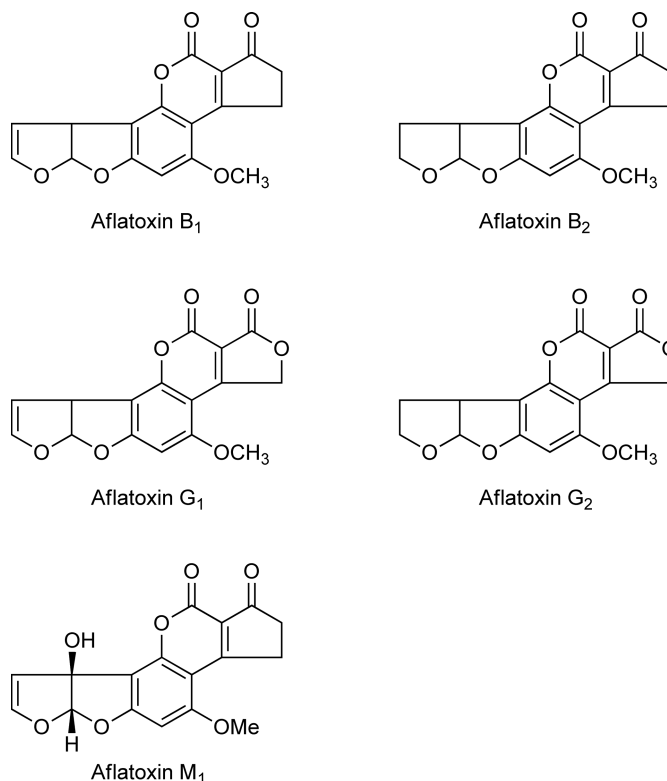


Figure 1 Chemical structure of aflatoxin B₁, aflatoxin B₂, aflatoxin G₁, aflatoxin G₂, and aflatoxin M₁.

The European Union agreed that the maximum level of AFB₁ in all cereals and products derived from cereals, groundnuts, nuts, and dried fruits intended for direct human consumption or use as ingredient in foodstuffs should be 2.0 $\mu\text{g kg}^{-1}$ (Commission Regulation EC No. 1881/2006). If cereals, groundnuts, nuts, and dried fruits are subjected to sorting or other physical treatment before human consumption or use as ingredient in foodstuffs, the maximum level of AFB₁ is 5.0 $\mu\text{g kg}^{-1}$. Similarly, the maximum level of the sum of AFB₁, AFB₂, AFG₁, and AFG₂ in all cereals and products derived from cereals, groundnuts, nuts, and dried fruits intended for direct human consumption is lower (4.0 $\mu\text{g kg}^{-1}$) than that in the same foodstuffs intended for physical treatment before human consumption (10 $\mu\text{g kg}^{-1}$).

14.07.2.2 Experimental Carcinogenesis

AFB₁ induces liver, kidney, lung, and colon carcinoma in rats, fish, rodents, dogs, and nonhuman primates. Susceptibility in experimental animals is species-, sex-, and age-dependent (Hengstler *et al.*

1999). Rats are more susceptible to AFB₁ carcinogenesis than mice, in which males are more susceptible than females, and young animals develop malignancies after shorter exposure than older ones. Experiments with chemically pure toxin showed that HCC was induced in sensitive species when AFB₁ was fed at levels as low as 2.0 ppb ($\mu\text{g kg}^{-1}$) in the diet (Wogan *et al.* 1974), and that even a single AFB₁ dose of 5.1 mg kg^{-1} might cause hepatomas several months after administration (Carnaghan 1967).

Intermittent feeding with diet containing 2 ppm AFB₁ caused HCC in six males and three out of six female treeshrews (*Tupaia glis*) between 74 and 172 weeks after the beginning of the experiment (Reddy *et al.* 1976). In another experiment 35 out of 47 Old World (rhesus, cynomolgus) and African green monkeys die after receiving AFB₁ i.p. (intraperitoneal) (0.125–0.25 mg kg^{-1}) and/or p.o. (per os) (0.1–0.8 mg kg^{-1}) for 2 months and longer (Sieber *et al.* 1979). Out of these, 13 developed one or more malignant neoplasms, and five of these neoplasms were primary liver tumors (two HCCs and three hemangioendothelial sarcomas). There were also two osteogenic

sarcomas, six gall bladder or bile duct carcinomas, three tumors of pancreas or their ducts, and one papillary carcinoma of the urinary bladder.

14.07.2.3 Metabolism and Biomarkers

Differences in susceptibility to AFB₁ across species and between people depend largely on the fraction of the dose that is directed into the various possible biotransformation pathways. AFB₁ is bioactivated in the phase I oxidative pathway catalyzed by cytochrome P450 (CYP)-dependent monooxygenase to various hydroxylated derivatives and to an unstable highly reactive epoxide metabolite *exo*-8,9-epoxide with reactivity to DNA 1000-fold greater than *endo*-epoxide (Guengerich 2003). AFB₁ *exo*-epoxide has a $t_{1/2}$ of only 1 s in aqueous buffer but reacts with DNA to yield 98% of the N⁷-Gua adduct. In The Gambia, the polymorphism of CYP3A5, human hepatic CYP involved in bioactivation of AFB₁ to AFB₁-*exo*-8,9-epoxide, was studied in humans exposed to dietary AFB₁. It was demonstrated that CYP3A5 haplotypes reflecting high CYP3A5 protein expression were associated with increase of the mutagenic AFB₁-*exo*-8,9-epoxide (Wojnowski *et al.* 2004). AFB₁-8,9-epoxides hydrolyze to genotoxic dihydrodiol which is further catalyzed to dialdehyde (Guengerich 2003). The amount of AFB₁-DNA adducts is greater in the liver than in other organs and it generally correlates with AFB₁ dosage and species susceptibility to hepatocarcinogenesis (Hengstler *et al.* 1999).

In rats the AFB₁-N⁷-Gua adduct removed from DNA ($t_{1/2}$ of 8–10 h) is excreted exclusively in urine (Bennett *et al.* 1981) and 80% of the total excretion occurs in the first 48 h after dosing, making the AFB₁-N⁷-Gua adduct a good biomarker of AFB₁ exposure (Groopman *et al.* 1992b). Twenty-four hours after exposure to AFB₁ the relationship between AFB₁ dose and the excretion of AFB₁-N⁷-Gua had a correlation coefficient of 0.99, and the correlation coefficient of AFB₁ liver DNA adducts and AFB₁-N⁷-Gua excreted in urine was 0.98.

Another biomarker of AFB₁ exposure is the serum AFB₁-albumin adduct that correlates well with urine AFB₁-DNA adducts (Groopman *et al.* 1992b). Since the average half-life of albumin in humans is about 20 days, an accumulated dose of AFB₁ can be present in albumin long after dietary exposure ceased. A good correlation of AFB₁ intake with AFB₁-N⁷-Gua and AFB₁-albumin level measured in serum was found in China (Gan *et al.* 1988; Groopman *et al.* 1992c),

confirmed in Africa (Groopman *et al.* 1992a; Wild *et al.* 1992), and methods were validated in experimental and human samples analyses (Wild *et al.* 1990).

The major route of the detoxification of AFB₁ metabolites is through conjugation with glutathione (GSH) to form the AFB₁-GSH conjugate excreted in urine or bile, and this reaction determines species resistance (Klein *et al.* 2000).

14.07.2.4 Molecular, Biochemical, and Pathological Alterations

Acute aflatoxicosis manifests either in humans or in animals with acute loss of appetite, vomiting, weakness, and lethargy. Pathological findings such as acute necrosis and bile duct hyperplasia are seen in domestic and experimental animals exposed to aflatoxins. In 1974, during an outbreak in India, people of rural origin with maize as their staple food presented with jaundice, brief febrile episode, vomiting, and anorexia (Krishnamachari *et al.* 1975; Tandon *et al.* 1977). In mild cases, the recovery was complete, while in others, ascites appeared rapidly within a period of 2–3 weeks, followed by edema of the lower extremities. Death was sudden, usually preceded by massive gastrointestinal bleeding, and the fatality rate was 10–29% according to different authors (Bhat and Krishnamachari 1977; Krishnamachari *et al.* 1975; Tandon *et al.* 1977). Morphological changes in the liver were characterized by severe cholangiolar proliferation, scarring of the efferent vein with varying degrees of occlusion of the hepatic veins, severe canalicular and cholangiolar cholestasis, presence of syncytial giant cells, and diffuse parenchymal injury. It was estimated that the affected people could have consumed between 2 and 6 mg of aflatoxins daily over a period of a month (Krishnamachari *et al.* 1975). In 1981, aflatoxicosis caused similar clinical signs in 20 patients in Kenya (with 12 fatalities) (Ngindu *et al.* 1982). In 2004, in the same part of Kenya out of 317 patients with acute aflatoxicosis 125 died (CDC 2004). A total of 182 (53.2% of 342) samples of maize and maize products had >20 ppb of aflatoxins (limit for food suggested by Kenyan authorities) reaching 4400 ppb in some of them (Azziz-Baumgartner *et al.* 2005).

In approximately 50% of HCC patients from regions with high aflatoxins exposure in China and Africa, there is a characteristic G → T transversion at the third base of codon 249 in the p53 tumor suppressor gene. This results in the insertion of serine at

position 249 in the mutant protein (Bressac *et al.* 1991; Hsu *et al.* 1991; Yang *et al.* 1997). In areas with low exposure to aflatoxins (Europe, the United States, and Japan), such mutations were not found (Challen *et al.* 1992; Montesano *et al.* 1997), but in the high AFB₁ exposure areas of the United States, Thailand, and China, the frequency of such mutations were in correlation with the aflatoxins exposure (Aguilar *et al.* 1994).

In plasma samples collected in Qidong, a region of China with high incidence of HCC in the period from 1997 to 2001, Jackson *et al.* (2003) studied the occurrence of a missense mutation in the p53 tumor gene at codon 249. The codon 249 mutation was detected in plasma samples obtained after diagnosis in seven of the fifteen cases (46.7%) at least 1 year prior to diagnosis and in one case 5 years prior to diagnosis indicating that this mutation may be a pre-diagnosis biomarker. It is evident that hepatitis B virus (HBV) infection plays an important role in HCC development. However, a meta-analysis on 48 published studies examining the interrelationship among aflatoxin exposure, HBV infection, and p53 mutations in HCC revealed a positive correlation between the 249 codon mutation and aflatoxin exposure ($P=0.0001$), but brief evidence for an HBV–aflatoxin interaction modulating the presence of this or any type of codon 249 p53 mutation is found (Stern *et al.* 2001).

In many cases of HCC in China and Africa, a double mutation in the HBV genome, a A → T transversion at nucleotide 1762 and a G → A transition at nucleotide 1764 (1762^T/1764^A) have been found in tumors (Kuang *et al.* 2004). The potential predictive value of this biomarker was proved by its finding in plasma of HCC patients in Qidong up to 8 years before diagnosis.

14.07.2.5 HCC Epidemiology and Prevention

Primary liver cancer is the fifth most common cancer in the world and the third most common cause of cancer mortality. In most countries 75–90% of primary liver cancer are HCCs (McGlynn and London 2005), and the great majority of liver cancer (>80%) occurs in either sub-Saharan Africa or Eastern Asia, with one country alone, China, accounting for over 50% of the cases. The Cancer Registry reporting the highest liver cancer rates in the world is Qidong, China, where male incidence is 95.7 per 100 000 and female incidence is 29.6 per 100 000. In contrast,

liver cancer incidence in the United States is about 1.5 per 100 000 (Kensler and Groopman 1997). In most populations, the incidence increases with age, with a peak in the fifth and sixth decades. In high-incidence areas, there is a marked shift toward the younger age groups, with peaks in Africa occurring in the third through fifth decades of life. The early onset of HCC is attributed to exposure to HBV, aflatoxins, and hepatitis C virus (HCV) at or soon after birth (Ogunbiyi 2001). High prevalence of HBV positivity was noticed in HCC patients in Africa (75–90%), Korea (82%), and Japanese Americans in Hawaii (50%), whereas the HCV prevalence was higher in Japan (61.5%), Spain (60%), and the United States (41.5%) (Ogunbiyi 2001).

Indeed, as long ago as the 1960s, the appearance of HCC in Africa was linked with AFB₁ exposure, and it was found that the increased AFB₁ exposure from 3 to 222 ng kg⁻¹ b.w. day⁻¹ increased liver cancer incidence from 2.0 to 35.0 cases per 100 000 (Van Rensburg *et al.* 1974).

In Swaziland where the proportion of HBV-exposed people is 86% in the whole country, differences in incidence of HCC varied over a fivefold range correlating with the estimated daily intake of aflatoxins (Peers *et al.* 1987). This study is in accordance with investigations on HCC performed in Guanxi, China (Yeh and Shen 1986). During the period between the 1960s and the 1970s persons with positive HBV surface antigen (HBsAg) and heavy exposure to aflatoxins the incidence of HCC was 649 per 100 000. The incidence of HCC in persons with positive HBsAg but lower exposure to aflatoxins was about 10 times lower (66 per 100 000).

A strong interaction between the HBsAg-positive findings and aflatoxin exposure and an increase in HCC risk was observed in prospective studies performed in China and Taiwan (Quian *et al.* 1994; Wang *et al.* 1996; Yu *et al.* 1997). In China, the relative risk (RR) for HCC in individuals with detected urinary aflatoxin was significantly increased (3.4) and it was even higher in individuals positive for HBsAg (7.3). When both risk factors were present, RR for developing HCC was about 59. In Taiwan, HBV-infected males had adjusted odds ratio (OR) of 2.8 for detectable AFB₁–albumin adducts compared with nondetectable AFB₁–albumin adducts and 5.5 for high levels compared with low levels of aflatoxin metabolites in urine (Wang *et al.* 1996). In another prospective study on men with chronic HBV hepatitis in China, in the 10-year period the risk of HCC increased 3.3-fold in patients with detectable urinary

AFM₁ (>3.6 ng l⁻¹) (Sun *et al.* 1999). Concomitant infection with HCV increased the risk of HCC 5.8-fold, adjusted for age and AFM₁ status.

In a recent study performed in Taiwan the combination of polycyclic aromatic hydrocarbon exposure and AFB₁-albumin adducts above the mean combined with chronic HBV infection resulted in an OR of 8.2 ($P < 0.0001$) compared to those with low adducts and no viral infection (Wu *et al.* 2007).

The prevention of HCC includes the vaccination against HBV and HCV infection and the reduction of aflatoxin exposure. There is a safe vaccine against HBV infection that has already been introduced in health programs in some countries. Given estimates that approximately 70% of HCC in developing countries is attributable to HBV then vaccination could prevent more than 250 000 cases per year. HCV is genetically heterogeneous, which makes vaccine production more problematic, but according to the same estimation, HCV vaccination would reduce the number of cases of HCC by approximately 93 000 per year (24% of all HCC cases). In South Africa, the implementation of vaccination against HBV at birth has already shown a positive impact in the reduction of HBsAg rate in children >5 years of age (Tsebe *et al.* 2001).

Food surveys aimed at evaluating aflatoxin contamination should involve pre- and postharvest control, low-technology postharvest measures to limit fungal growth, or genetic engineering of crops to be resistant to fungal infection or mycotoxin production.

Food processing may reduce the aflatoxin content in grain. Mixing of highly contaminated with low-contaminated grains (the so-called dilution) reduces concentration of aflatoxins. The efforts to throw out the smaller and more shriveled maize seeds (known to be more contaminated) have resulted in the consumption of such seeds by the poor or using them as animal feed.

Chemoprotection involves the use of compounds that either increase the detoxification or prevent the production of epoxides (Kensler *et al.* 2004). Some compounds, such as oltipraz and chlorophyll, decrease the biological effective dose. Animal feed may also be mixed with esterified glucomanoses and other yeast extracts that increase the detoxification of aflatoxins. Chemoprotection is highly expensive for use in developing countries as is the use of chemosorbents that selectively absorb aflatoxins in food, thus preventing their absorption.

14.07.3 Fumonisin

14.07.3.1 Occurrence

Fumonisin are produced by a variety of *Fusarium* fungi, which are common soil fungi in temperate and warm countries. They are produced not only by *Fusarium verticillioides* (formerly *F. moniliforme*), but also by some other *Fusarium* species such as *F. proliferatum*, *F. nygamai*, and *Alternaria alternata* f. sp. *Lycopersici* (Bennett and Klich 2003). Fumonisin are produced before harvest or during the early stage of drying and their concentration does not increase during grain storage (IPCS 2001).

There are 14 different fumonisins known so far. Their structure is based on a hydroxylated hydrocarbon chain which contains methyl and either amino or acetyl groups (Figure 2). Fumonisin B₁ (FB₁), B₂ (FB₂), and B₃ (FB₃) are the major contaminants of food and feed. The most abundant and the most toxicologically significant is FB₁ although the toxicological profile of other fumonisins is very similar to FB₁ (IPCS 2001).

FB₁ is detected mostly in maize and maize-based products worldwide, but its concentration is lower in processed maize foods (cornflake breakfast cereals, corn tortillas, tortillas corn chips, and popcorn) (Peraica *et al.* 2002). Fumonisin are less frequent in other cereal grains, such as sorghum and rice, but can be detected in beer when contaminated maize-based brewing adjuncts are used (IPCS 2001). Fumonisin are detected in Africa, America, Europe, Asia, and Oceania, and in some cases, the concentration was up to about 4000 mg kg⁻¹ (for review see Soriano and Dragacci 2004). Different *Fusarium* species are found in moisture-damaged buildings, leading to the possibility of fumonisin exposure by inhalation (Tuomi *et al.* 2000).

The Scientific Committee for Food of the European Union has agreed a maximum level for the sum of FB₁ and FB₂ in various foodstuffs. Thus, the maximum level for the sum of these mycotoxins in unprocessed maize is set at 4000 µg kg⁻¹, in maize intended for human consumption at 1000 µg kg⁻¹, in maize-based breakfast cereals at 800 µg kg⁻¹, and in maize-based foods and baby foods for infants and young children at 200 µg kg⁻¹ (Commission Regulation EC No. 1126/2007). The Center for Food Safety and Nutrition has set guidance for limiting fumonisin concentrations in animal feed that range from 1 to 50 mg kg⁻¹ (USFDA 2001).

| Fumonisin | R_1 | R_2 | R_3 |
|--------------------------|-------|-------|--------------------|
| Fumonisin A ₁ | OH | OH | CH ₂ CO |
| Fumonisin A ₂ | H | OH | CH ₂ CO |
| Fumonisin B ₁ | OH | OH | H |
| Fumonisin B ₂ | H | OH | H |
| Fumonisin B ₃ | OH | H | H |

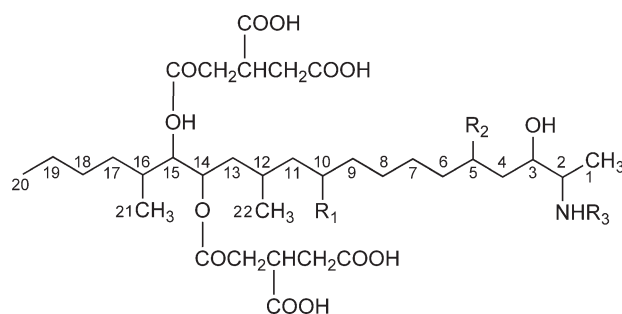


Figure 2 Chemical structure of fumonisin A₁, fumonisin A₂, fumonisin B₁, fumonisin B₂, and fumonisin B₃.

14.07.3.2 Acute Toxicity

Experiments on different animal species demonstrated that fumonisins are not acutely toxic (IPCS 2001). In a recent study on F344 rats treated orally with 1.0–46.4 mg FB₁ kg⁻¹ b.w.), no lethal effect was observed although higher doses of FB₁ (21.5 and 46.4 mg kg⁻¹ b.w.) resulted in toxic effects such as loss of feed consumption and lameness (McKean *et al.* 2006). In the same study acute toxicity was tested on mosquitofish (*Gambusia affinis*), and the LC₅₀ (lethal concentration) value was calculated to be 4.64 mg FB₁ l⁻¹.

In animals, fumonisins are poorly absorbed and rapidly eliminated through a two- or three-compartment model, and they are not metabolized (Martinez-Larranga *et al.* 1999). FB₁ does not appear to accumulate in the tissues, but low levels can be detected in the kidneys and liver of FB₁-treated rats (Norred *et al.* 1993). There is no evidence that the mechanism of action of fumonisins depends on their metabolic activation.

The toxic effects of fumonisins are species-, strain-, and sex-specific (Voss *et al.* 2007). High doses of FB₁ can cause leukoencephalomalacia in equines, rats, and rabbits, and pulmonary edema and hydrothorax in swine, while it is hepatotoxic and nephrotoxic in rodents (IPCS 2001). Fumonisin is structurally similar to sphingoid bases and their toxicity is the consequence of disruption of sphingolipid metabolism due to competitive inhibition of ceramide synthase, a key enzyme in sphingolipid metabolism (Wang *et al.* 1999)

(Figure 3). An immediate consequence of ceramide synthase inhibition is reversible accumulation of the enzyme substrates, sphinganine (Sa) and sphingosine (So), followed by accumulation of their metabolites, Sa and So 1-phosphate. The increase of Sa, So, and Sa and So 1-phosphate is dose- and time-dependent, and occurs prior to kidney lesions, which make them good biomarkers for fumonisin exposure (Peraica *et al.* 2008). The use of the ratio of Sa and So as an exposure biomarker in human populations has been limited and its usefulness for epidemiological studies is not established (Voss *et al.* 2007). Sa and So exert proapoptotic, cytotoxic, and growth inhibitory effects. On the other hand, reduced ceramide and increased So 1-phosphate have been shown to inhibit apoptosis and promote mitosis and regeneration. Sa and So and their metabolites are known to be important signaling molecules implicated in regulating cell growth, differentiation, survival, and apoptosis (Merrill *et al.* 2001).

14.07.3.3 Carcinogenicity

FB₁ is a rodent carcinogen classified as possibly carcinogenic to humans (Group 2B) (IARC 2002). In male BD IX rats, 50 mg FB₁ kg⁻¹ in the diet for 26 months caused an increased incidence of HCC (in 60% of rats) and cholangiocarcinomas (Gelderblom *et al.* 1991). In an National Toxicology Program (NTP) study, chronic dietary FB₁ exposure resulted in a significant increase of kidney tumors in male F344/N rats and liver tumors in female B6C3F₁ mice (NTP 1999).

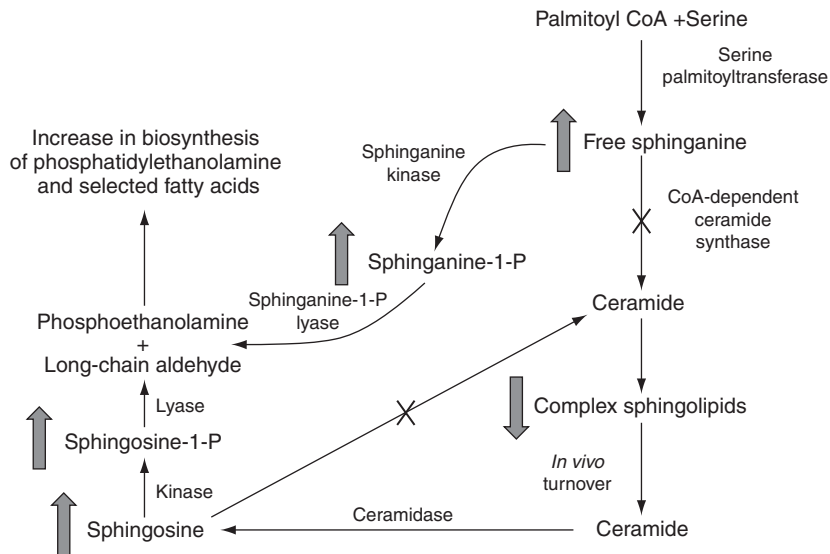


Figure 3 Summary schematic representation of sphingolipid metabolism showing the inhibition of ceramide synthase (X) by fumonisins. The consequences of ceramide synthase inhibition include increased tissue and serum concentrations (up arrows) of sphinganine (to a lesser extent), sphingosine, and their 1-phosphate metabolites, and decreased (down arrows) complex sphingolipids downstream of ceramide. Reproduced from Voss, K. A.; Smith, G. W.; Haschek, W. M. *Anim. Feed Sci. Technol.* **2007**, *137*, 299–325, with permission from Elsevier.

Prolonged treatment with FB₁ in normal diet (250 mg kg⁻¹ diet for 5 weeks, followed by 100 mg kg⁻¹ diet up to 25 weeks) caused HCC and cholangiocellular carcinoma in male F344 rats (Lemmer *et al.* 2004). FB₁ appears to be a unique carcinogen that causes regenerative hyperplasia in hepatocytes and promotion of tumors despite striking proapoptotic effects. The molecular mechanism of FB₁ hepatocarcinogenicity suggests a role for activation of specific apoptotic and oncogenic pathways. The cytokine tumor necrosis factor α (TNF α) has been implicated as a modifier of FB₁ toxicity. Results of studies on mice lacking a functional TNF receptor 1 (p55) or TNF receptor 2 (p75), and on mice that overexpress the human TNF α transgene plus studies on TNF α null mice given FB₁ show that TNF α signaling pathways are not obligatory for FB₁ toxicity (Voss *et al.* 2006).

The previous studies on experimental animals and cell cultures suggest that oxidative stress is a key mechanism of fumonisins genotoxicity (Abel and Gelderblom 1998), but recent studies on rats have indicated that oxidative stress is rather the consequence and not the cause of FB₁ genotoxicity (Domijan *et al.* 2007). The mechanism of FB₁ carcinogenicity is still under study and direct binding of FB₁ to DNA is not confirmed. Experimental data suggest that overall FB₁ has a strong promoting potential (Gelderblom *et al.* 2002).

14.07.3.4 Public Health Significance

Human exposure to fumonisins occurs worldwide, associated with the consumption of contaminated maize and products of maize. The estimated mean daily intake of FB₁ in a European-type diet is 0.2 $\mu\text{g kg}^{-1}$ b.w. and 2.4 $\mu\text{g kg}^{-1}$ b.w. in the African diet, and the maximum intake may be as high as 2500 $\mu\text{g day}^{-1}$ (IPCS 2001). The FAO/WHO Expert Committee on Food Additives determined the maximum tolerated daily intake for FB₁, FB₂, and FB₃ alone or in combination as 2.0 $\mu\text{g kg}^{-1}$ b.w.

The single acute human FB₁ mycotoxicosis that occurred in India caused only gastrointestinal symptoms (abdominal pain and diarrhea) (Bhat *et al.* 1997).

Human exposure to maize-based food contaminated with FB₁ was at first connected to the high incidence of esophageal cancer in the Transkei region of South Africa and also in northeast Italy but epidemiological studies failed to connect FB₁ exposure to esophageal cancer (IPCS 2001). The same occurred in Linxian, a Chinese region with high incidence of esophageal cancer (Abnet *et al.* 2001).

The simultaneous exposure to aflatoxins and fumonisins was suggested to be the contributing factor to the development of HCC in the Chinese regions Haimen and Guanxi where the

incidence of this tumor is very high (Li *et al.* 2001; Ueno *et al.* 1997). Experimental evidence for synergistic effects of these two mycotoxins in HCC development supports these findings (Gelderblom *et al.* 2002).

Consumption of FB₁-contaminated maize is assumed to be a risk factor for neural tube defect in South Texas (the United States), Mexico, Guatemala, China, and South Africa (Marasas *et al.* 2004). Epidemiological studies and studies on experimental animals confirmed this theory (Gelineau-van Waes *et al.* 2005; Missmer *et al.* 2006).

14.07.4 Ochratoxins

14.07.4.1 Occurrence

Ochratoxins are a group of mycotoxins produced by *Penicillium verrucosum* and different species of *Aspergillus* molds (*A. alliaceus*, *A. auricomus*, *A. carbonarius*, *A. glaucus*, *A. melleus*, *A. niger*) that contaminate crops in the field leading to field and storage ochratoxins contamination (Bennett and Klich 2003).

Ochratoxins are derivatives of an isocoumarin moiety linked to phenylalanine by an amide bond. The most important ochratoxins are ochratoxin A (OTA), ochratoxin B (OTB), ochratoxin C (OTC), and ochratoxin α (OT α) (Figure 4). The most frequently found and the most toxic is OTA.

Ochratoxins occur worldwide, but most data are from European countries (IPCS 2001). Ochratoxins contaminate cereals (barley, maize, oats, rice, rye, wheat) and other plant products (coffee beans, nuts, dried peanuts, spices, dried fruits, raisins, wine, grape juice, and beer). Through a carryover effect, they might be found as residues in food of animal origin (pork and poultry meat, milk, cheese) which may

contribute to human exposure. Inhalation in the workplace is a possible route of exposure (Brera *et al.* 2002). Ochratoxins are relatively heat stable; baking and roasting reduces the content by 20%, while boiling has no effect (O'Brien and Dietrich 2005).

Maximum levels for ochratoxins in foodstuffs have been set by the Commission Regulation (EC) No. 1881/2006, and the tolerance levels for infant food, wine, roasted coffee, and unprocessed cereals are 0.5, 2.0, 5.0, and 5.0 $\mu\text{g kg}^{-1}$, respectively.

14.07.4.2 Acute Toxicity

OTA is toxic in all tested animal species, except adult ruminants. The target organ of OTA toxicity is the kidneys, but it also affects the heart and the liver, causing aberrations in coagulation factors, lesions in the gastrointestinal tract and the lymphoid tissue, and myelotoxicity (for review see Pfohl-Leskowicz and Manderville 2007).

The toxicity of OTA varies widely depending on the animal species, sex, and route of administration. Oral LD₅₀ (lethal dose) values range from approximately 20 and 46–58 mg kg^{-1} b.w. in rats and mice, respectively, to 0.2–1.0 mg kg^{-1} b.w. in pigs, cats, rabbits, and dogs (O'Brien and Dietrich 2005). In general, the symptoms included multifocal hemorrhaging in all major organs and fibrin thrombi in the spleen, brain, liver, kidneys, and heart. Nephrosis, necrosis in the liver and lymphoid tissues, and enteritis with coincident villous atrophy were also apparent in all species examined (IPCS 2001).

OTA is rapidly absorbed both from the stomach and from the small intestine in rats and mice. The concentration of OTA and its metabolites in blood depends on dose, route, and duration of administration, binding to serum proteins and enterohepatic

| Ochratoxins | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ |
|---------------------|--------------------------|----------------|----------------|----------------|----------------|
| Ochratoxin A | Phenylalanyl | Cl | H | H | H |
| Ochratoxin B | Phenylalanyl | H | H | H | H |
| Ochratoxin C | Phenylalanyl-ethyl-ester | Cl | H | H | H |
| Ochratoxin α | OH | Cl | H | H | H |

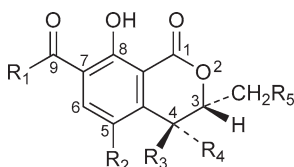


Figure 4 Chemical structure of ochratoxin A, ochratoxin B, ochratoxin C, and ochratoxin α .

recirculation. Organic anion transporters in kidney proximal tubules enhance reabsorption of OTA, thus probably causing nephrotoxicity (Zepnik *et al.* 2003). The biotransformation of OTA has not been entirely elucidated and toxicokinetics and toxicodynamics of OTA have been recently reviewed (Ringot *et al.* 2006). Toxicokinetic studies on human volunteers showed that the *in vivo* data are described well by a two-compartment open model consisting of a central compartment. The calculated human plasma half-life is 35.55 days (Studer-Rohr *et al.* 2000). There are several mechanisms proposed to be involved in OTA toxicity: inhibition of protein synthesis, production of reactive oxygen species, disruption of calcium homeostasis, inhibition of mitochondrial respiration, and DNA damage (Ringot *et al.* 2006).

14.07.4.3 Carcinogenicity

IARC has classified OTA into Group 2B (carcinogenic in experimental animals with limited evidence for its carcinogenicity in humans) (IARC 1993). The carcinogenicity of OTA was demonstrated in F344/N rats of both sexes treated orally for 2 years with OTA (21, 70, and 210 $\mu\text{g kg}^{-1}$) (NTP 1989). Higher doses induced increases in uncommon tubular cell adenomas and tubular cell carcinomas of the kidneys of males and females, and in female rats increased incidence and multiplicity of fibroadenomas of mammary gland were seen. In the same study, OTA caused non-neoplastic renal changes including tubular cell hyperplasia, tubular cell proliferation, cytoplasmic alteration, karyomegaly, and degeneration of the renal tubular epithelium. The carcinogenicity of OTA was confirmed on Lewis and Dark Agouti rats (Castegnaro *et al.* 1998). The results on male F344 rats fed with OTA-contaminated diet (100 $\mu\text{g day}^{-1}$) suggested that OTA is less carcinogenic when administered in feed than through oral gavage (Mantle *et al.* 2005). In a recent study carried out under the same conditions as the NTP study, it was observed that higher OTA doses induced in rats time- and dose-dependent cell proliferation in the medulla and the outer stripe of the outer medulla of the kidneys (Rached *et al.* 2007). The authors concluded that the coincidence of cell proliferation and induction of tumors in the same kidney regions indicates that epigenetic mechanisms are involved in OTA carcinogenicity.

Currently, the mechanism of OTA carcinogenicity is not known. Mutagenicity testing, sister chromatid exchange (SCE), unscheduled DNA

synthesis, and micronucleus tests all gave controversial results mostly because of very different experimental conditions used (O'Brien and Dietrich 2005). OTA binding to DNA and formation of OTA-DNA adducts were also proposed as mechanisms of action (Obrecht-Pflumio and Direhimer 2000). Studies on mice, rats, pigs, and chickens using ^{32}P -postlabeling have shown the presence of OTA-DNA adducts after metabolic activation of OTA in a dose- and time-dependent manner (Pfohl-Leszkowicz and Castegnaro 2005). OTA adducts were also found in human kidney tumors obtained from France, Belgium, Croatia, Bulgaria, and Serbia (Pfohl-Leszkowicz *et al.* 2007). However, some studies on biotransformation *in vivo* and *in vitro* indicate that OTA is poorly metabolized and does not form reactive intermediates capable of interacting with DNA (Mally and Dekant 2005). Using the comet assay, time- and dose-dependent DNA strand breakage was found in the kidneys of OTA-treated rats (Želježić *et al.* 2006). The partial involvement of oxidative stress in DNA damage *in vivo* was proved by the comet assay with formamidopyrimidine glycosylase, which is known to convert oxidative DNA lesions into DNA strand breaks (Domijan *et al.* 2006).

14.07.4.4 Public Health Significance

Acute human ochratoxicosis is rather rare (Di Paolo *et al.* 1994), but humans are exposed daily to low levels of OTA. It was calculated that daily human exposure to OTA in Europe might range from 0.7 to 4.7 $\text{ng kg}^{-1}\text{b.w.}$ and a tolerable weekly intake proposed by the Joint FAO/WHO Experts Committee on Food Additives (JECFA) and accepted by the European Scientific Committee (ESF) is 100 $\text{ng OTA kg}^{-1}\text{b.w.}$ (and a tolerable daily intake is 14 $\text{ng OTA kg}^{-1}\text{b.w.}$) (IPCS 2001).

It was proven that OTA causes porcine nephropathy in Scandinavian countries. This raised the still unproven theory of the involvement of OTA in the development of the human disease endemic nephropathy (EN) (Krogh 1974). EN is a disease that occurs in particular areas of Bosnia and Herzegovina, Bulgaria, Croatia, Romania, and Serbia. The main characteristics of EN are focused and limited to geographical distribution, occurrence in farming households, and high mortality from uremia. The area of EN coincides with that of high incidence of otherwise rare urothelial tumors (Miletić-Medved *et al.* 2005; Puchlev *et al.* 1960). In studies on OTA exposure in areas with EN performed in Bulgaria and Croatia, the level of OTA or

the percentage of OTA-positive samples measured in various commodities of plant and animal origin, as well as in human blood, were higher than in control areas (for review see Pfohl-Leszkowicz and Manderville 2007). The low OTA concentrations are also a frequent finding in blood, urine, and human milk of apparently healthy persons in all countries where it was expected (Peraica *et al.* 1999a; Pfohl-Leszkowicz and Manderville 2007), showing geographical and seasonal variations (Peraica *et al.* 2001). Although OTA was found in low concentrations in blood of healthy people in many countries, EN might be caused by OTA together with other nephrotoxic mycotoxins such as FB₁ and citrinin which are also found frequently in areas with EN.

14.07.5 Conclusions

Human and animal exposure to mycotoxins cannot be avoided. The maximum tolerable levels of mycotoxins in foods should not be so low as to prohibit legitimate international trade of food, but these levels must protect consumers from the toxic and carcinogenic effects of mycotoxins. Despite extensive efforts, the mechanism of carcinogenicity of most mycotoxins remains to be elucidated. Some mycotoxins (such as aflatoxins) are proven to be carcinogens in humans whereas others (fumonisins and ochratoxins) are suspected to be human carcinogens based on data from experimental animals. One particular issue to address is that humans are very frequently exposed to different mycotoxins with additive or even synergistic adverse effects in experimental animals or on cell cultures. The possible interaction of mycotoxins with genotoxic and/or tumor-promoting features is an area requiring significant research effort. Overall, epidemiological studies on exposure to specific mycotoxins are also required.

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