CHAPTER 2

Mycotoxins with Special Reference to the Carcinogenic Mycotoxins: Aflatoxins, Ochratoxins and Fumonisins

This chapter is an updated form of a review article that was published by Macmillan Reference in the second edition of *General and Applied Toxicology*, edited by Ballantyne, Marrs and Syversen in 1999.

Contribution made by the candidate

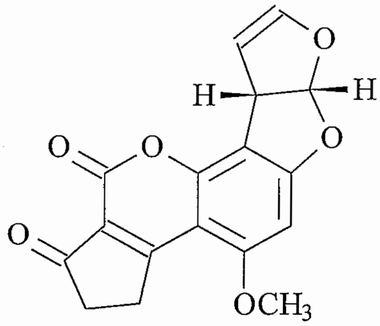
The candidate was responsible for the literature searches, research and writing of the majority of this chapter including the Ochratoxin, Aflatoxin and Fumonisin sections. Prof. P.S. Steyn co-ordinated the data compilation and wrote the sections: Mycotoxins produced by non-storage fungi and Trichothecenes.

Mycotoxins with Special Reference to the Carcinogenic Mycotoxins: Aflatoxins, Ochratoxins and Fumonisins

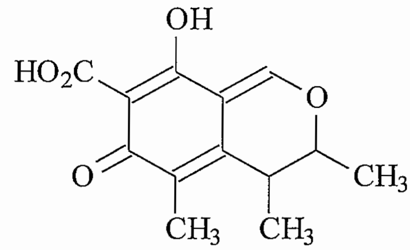
INTRODUCTION

Naturally occurring toxicants produced by microorganisms, such as bacteria and fungi (moulds), contaminate foods and feeds; these foodborne hazards pose a serious health risk to mammals, fish and poultry. This chapter is exclusively dedicated to toxins produced by fungi, *viz.* mycotoxins and to diseases caused by the ingestion of mycotoxins, called mycotoxicoses: the toxin production can take place in the preharvest and/or during the postharvest stage of the crop.

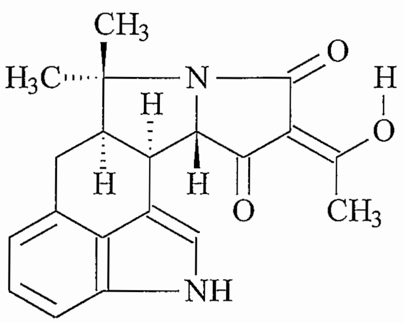
The well known mycotoxicologist, Forgacs, referred to mycotoxicoses in the early 1960s as the most neglected diseases, although many people in Russia died during World War II owing to alimentary toxic aleukia (ATA), a mycotoxicosis caused by T-2 toxin, a sesquiterpenoid mycotoxin (Ueno *et al.*, 1972; Yagen *et al.,* 1977). The resurgence of interest in mycotoxin research is directly related to the discovery of the aflatoxins during 1960, a group of structurally related hepatocarcinogens, produced on nuts and cereals by *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*, and their role in the aetiology of primary liver cancer in humans (Van Rensburg, 1986; Bressac *et al.*, 1991 and Groopman *et al.*, 1992). This event led to an unabated interest in mycotoxins as evidenced by the large number of monographs (Uraguchi and Yamazaki, 1978; Steyn, 1980; Lacey, 1985; Cole, 1986; Steyn, 1989; Natori, *et al.,* 1989; Smith and Henderson, 1991; Creppy *et al.*, 1993; Miller and Trenholm, 1994, and Jackson, *et al.*, 1996), numerous reviews (e.g. Steyn and Vleggaar, 1985; Pohland, *et al.*, 1992; Steyn, 1993; 1995; Beardall and Miller, 1994; Grove 1996; Bennet and Keller, 1997) and thousands of research papers. Mycotoxins are a chemically heterogeneous group (see **Figure 1** for the structures of representative members of the important mycotoxins) of low molecular weight compounds which are produced by the secondary metabolism of fungal genera such as *Aspergillus*, *Penicillium, Fusarium, Alternaria* and *Claviceps*. The mycotoxins are mostly produced by the so-called storage fungi; however, some unique mycotoxicoses such as ergotism, lupinosis and facial eczema are caused by some parasitic and saprophytic fungi (see later). It is, therefore, not surprising that mycotoxins induce powerful and dissimilar pathological effects as shown in **Table 1**.



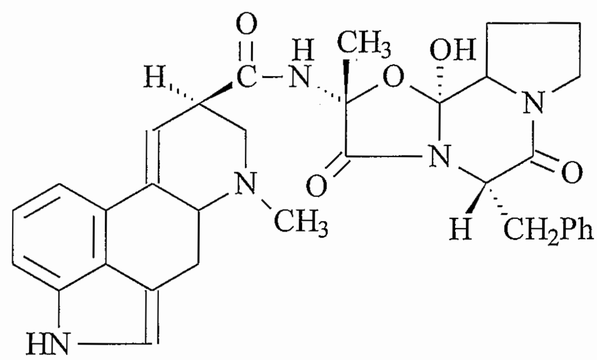
Aflatoxin B_1



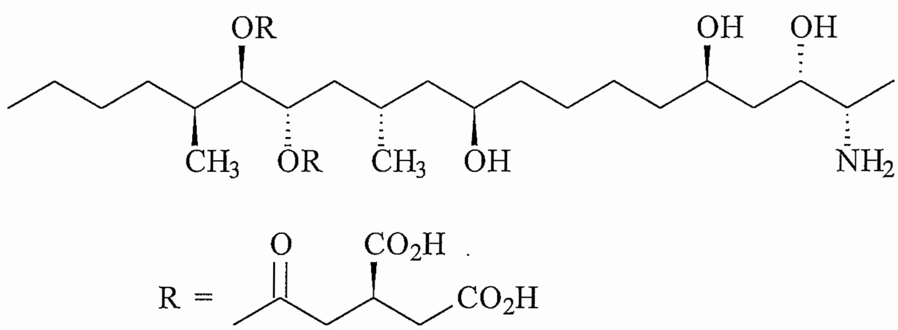
Citrinin



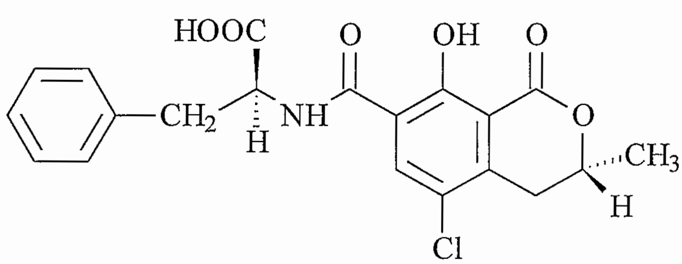
Cyclopiazonic acid



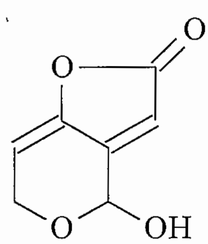
Ergotamine



Fumonisin B_1

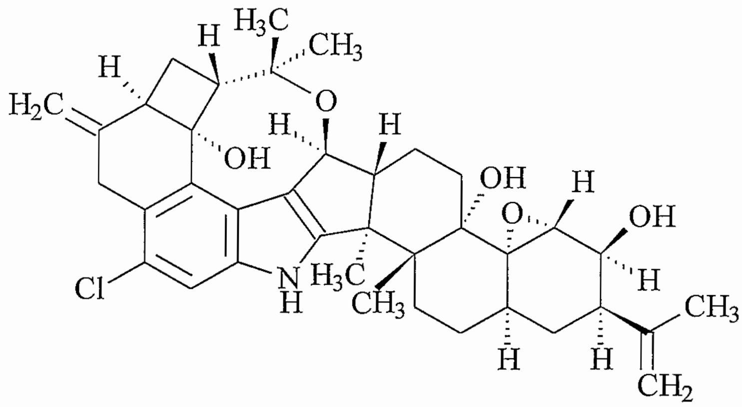


Ochratoxin A

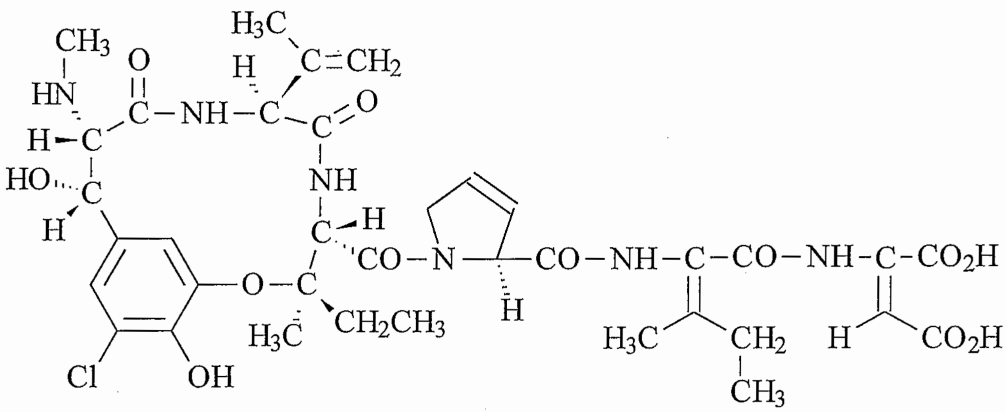


Patulin

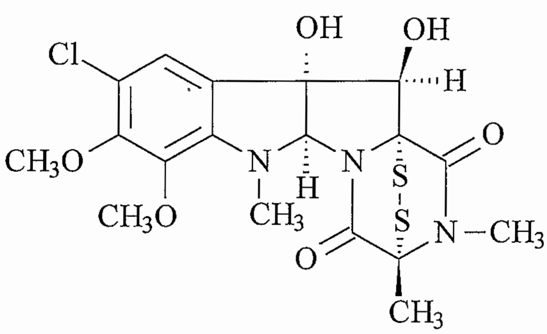
Figure 1: Structures of representative mycotoxins



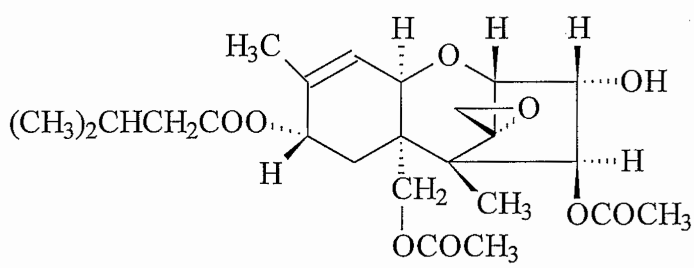
Penitrem A



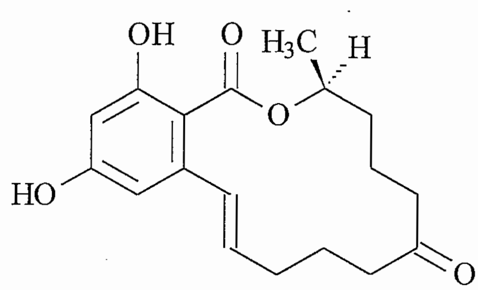
Phomopsin A



Sporidesmin



T2-toxin



Zearalenone

Figure 1: Continues

Table 1: Diverse biological activity displayed by some representative mycotoxins.

Mycotoxin	Biological Activity	Producing Genus	
Aflatoxin B_1	Carcinogenicity, teratogenicity	*Aspergillus*	Büchi *et al.* (1966); Van Rensburg (1986), Groopman *et al.* (1992)
Citrinin	Nephrotoxicity	*Penicillium, Aspergillus*	Betina (1984)
α-Cyclopiazonic acid	Neurotoxicity	*Penicillium, Aspergillus*	Holzapfel (1968)
Ergotoxins (ergotamine)	Vasoconstriction, neurotoxicity	*Claviceps*	Stoll (1952); Scott *et al.* (1992)
Fumonisin B_1	Carcinogenicity, neurotoxicity	*Fusarium*	Bezuidenhout *et al.* (1988); Jackson *et al.* (1996)
Ochratoxin A	Carcinogenicity, nephrotoxicity	*Aspergillus, Penicillium*	Van der Merwe *et al.* (1965); Pohland *et al.*,(1992); Creppy *et al.* (1993).
Patulin	Mutagenicity, antibacterial	*Penicillium*	Engel and Teuber (1984)
Penitrem A	Neurotoxicity	*Penicillium*	De Jesus *et al.* (1983); Steyn and Vleggaar (1985)
Phomopsin A	Hepatotoxicity	*Phomopsis*	Culvenor *et al.* (1989)
Sporidesmin A	Hepatotoxicity, photosensitivity	*Pithomyces*	Mortimer *et al.* (1978)
Trichothecenes (T-2 toxin)	Dermotoxicity, hematopoietic	*Fusarium*	Wannemacher *et al.* (1991); Plattner *et al.* (1989); Grove (1996)
Zearalenone	Estrogenism, reproductive irregularities	*Fusarium*	Urry *et al.* (1966)

The mycotoxicoses are not only clinically diverse, but also often extremely difficult to diagnose owing to the numerous pharmacological effects of the causative mycotoxins. Some of the diseases associated with mycotoxins are, for example, aflatoxin [human primary liver cancer and Turkey-X disease (Van Rensburg, 1986; Bressac *et al.*, 1991)]; citreoviridin (Yellow rice disease in humans); ergotoxins [ergotism, St. Anthony's Fire in humans (van Rensburg and Altenkirk, 1974]; fumonisins [encephalomalacia in horses, pulmonary oedema in swine (Bezuidenhout *et al.*, 1988)]; ochratoxins [nephropathy in pigs (Danish porcine nephropathy, Krogh, 1974) and poultry (Pohland *et al.*, 1992)]; phomopsin A [lupinosis in sheep (Culvenor *et al.*, 1989)]; sporidesmin A [facial eczema in sheep (Mortimer *et al.*, 1978)]; T-2 toxin and other trichothecene toxins [alimentary toxic aleukia (ATA)] and zearalenone (hyperestrogenism, vulvovaginitis and abortion in swine).

Mycotoxins are ubiquitous owing to the global distribution of toxinogenic fungi, thereby putting crops and consumers (man and animals) at risk and cause serious problems in the agricultural economies and international trade of nuts and cereals. The level of mycotoxin contamination of such commodities varies from year to year, depending on climatic conditions, commodity and location in a country. It has been estimated that one quarter of the world's food crops is at risk owing to mycotoxin contamination. Ammoniation can be effectively utilised to reduce aflatoxin levels in corn and cotton seeds by more than 99% (Lee *et al.*, 1992; Park, 1992).

Kuiper-Goodman, (1995, 1996), Kuiper-Goodman and Scott, (1989) and Kuiper-Goodman *et al.*, (1996) have made sterling contributions to the risk assessment of mycotoxins. The human health concerns depend on the amount of the mycotoxins consumed, the toxicity of the compound (extrapolation of the test species to humans), the body weight and physical condition of the individual, the presence of other mycotoxins as well as other dietary factors. In the case of animal mycotoxicoses, outbreaks vary according to the agricultural practice and climatic conditions in a region.

Hsieh, (1990) defined the criteria of a human mycotoxicosis as follows:

- Occurrence of the mycotoxin(s) in food supplies;
- Human exposure to the mycotoxins;
- Correlation between exposure and incidence;
- Reproducibility of characteristic symptoms in experimental animals;
- Similar mode of action in humans and animal models.

The extrapolation of toxicological data from animals to humans using safety factors or other methods to arrive at an estimate of safe intake is the most challenging aspect of such assessments (Kuiper-Goodman, 1995). The extrapolation of animal toxicity data to humans is complicated by species differences in metabolic disposition, such as differences in absorption, and binding to plasma and tissue constituents. Species differences in biotransformation as well as plasma and tissue half-life are also important.

The study of a specific mycotoxicosis requires the isolation and identification of the toxinogenic fungus involved, the chemical identification of the mycotoxin(s) and accurate and reliable laboratory methods for monitoring and regulating the mycotoxin and its metabolic products in different matrices. The toxins of most of the food-and feed-borne fungi have been characterized; the research benefited from the advent of effective and mild chromatographic techniques, high-resolution mass spectroscopy, (including MS-MS, GLC-MS and HPLC-MS), high-field nuclear magnetic resonance spectroscopy and single crystal X-ray crystallography (Cole, 1986). The routine screening of food/feed samples benefited greatly from the ELISA analysis (Morgan, 1989); reliable analytical kits are now commercially available.

Some mycotoxins, (listed in **Table 1** and **Figure 1**) are associated with specific human (Beardall and Miller, 1995) and animal mycotoxicoses (Smith and Henderson, 1991). In the following section special attention will be directed to three of the unique groups of toxins, viz. ergotoxins, sporidesmins and phomopsins.

MYCOTOXINS PRODUCED BY NON-STORAGE FUNGI

Ergotoxins

The ergotoxins (e.g. ergotamine, ergocristine, ergocryptine, and ergocornine) are among the most pharmacologically active peptides and are the main alkaloids of *Claviceps purpurea*, the etiological agent in gangrenous and convulsive ergotism. Ergotism is probably the oldest known human mycotoxicosis, known as St Anthony's Fire or the Holy Fire in the Middle Ages in Europe, and is caused by consumption of rye flour contaminated with *C. purpurea* (Stoll, 1952; Van Rensburg and Altenkirk, 1974 and Lacey, 1991). As a human disease, ergotism has almost been eliminated (Scott *et al.*, 1992), but as an animal disease it can still occur widely, in the latter case also owing to contamination with *Claviceps paspali*. An isolated case of human ergotism occurred in Wollo, Ethiopia, when 150 people died due to the consumption of wild oats (*Avena abyssinica*) contaminated by a *Claviceps* species (King, 1979). The ergot alkaloids of *C. purpurea* are firmly associated with the plant species because the causative fungus is a specific pathogen of the rye plant.

Sporidesmins

The sporidesmins, e.g. sporidesmin A (**Table 1** and **Figure 1**), are a group of epipolythiodioxopiperazines which cause photosensitization diseases among sheep in New Zealand (facial eczema) and in South Africa [yellow thick head disease ('geeldikkop' - Afrikaans)]. In New Zealand, the saprophytic fungus *Pithomyces chartarum* infects the senescent and dead material of rye grass pastures which are consumed by sheep grazing on the new spring growth. Facial eczema frequently caused major losses, e.g. $40-100 million in 1981 (Smith, 1985). In the semi-arid Karroo region of South Africa, a similar photosensitization disease among sheep was associated with consumption of a common weed, *Tribulis terrestris*, infected with the perfect or teleomorph species *Leptosphaerulina* of which the *Pithomyces* is an anamorph (Roux, 1986). A severe outbreak of 'geeldikkop' incapacitated 250 000 sheep during a serious outbreak in 1949. Facial eczema is a secondary photosensitive expression of the toxic effects of sporidesmin on the liver (Mortimer *et al.*, 1978). In addition to its direct toxic effect on the liver parenchyma, excretion of the sporidesmin in the bile results in inflammation of the bile duct epithelium, followed by progressive necrosis of the duct wall and periductal concentric lamellar fibrosis and granulation causing the ducts to eventually become occluded (Mantle, 1991). Excretion of phylloerythrin, the product of hepatic degradation of chlorophyll is thus impaired by the obliterative cholangitis, and the abnormally high level of phylloerythrin in the peripheral blood thus causes photosensitivity leading to oedema and inflammation of the exposed skin of sheep (Mantle, 1991).

Phomopsins

The phomopsins, a group of complex hexapeptides, containing β-dehydroamino acids, e.g. phomopsin A (**Table 1** and **Figure 1**), cause lupinosis in sheep in Australia and South Africa (Culvenor *et al.* 1989). The phomopsins are produced by *Phomopsis leptostromiformis* (Kühn). Bubak *ex* Lind, a fungus which in nature appears to be a specific pathogen and saprophyte of *Lupinus* spp. However, it can be cultivated on cereals (maize) and liquid media and still retain its toxigenicity. The liver is the major target for toxicity associated with the phomopsins; the affected liver accumulates lipids, turns yellow and becomes enlarged (Jago *et al.*, 1982). In case of long term exposure (low levels of the phomopsins), atrophy of the liver, fibrosis and bile duct proliferation develop. Phomopsin A acts as a mitotic drug both *in vivo* and *in vitro*, and the observed symptoms of lupinosis can be related to the specific interaction of phomopsins with tubulin and microtubules (Tönsing *et al*., 1984).

TRICOTHECENES

This chapter is primarily dedicated to the carcinogenic mycotoxins: aflatoxins, ochratoxins and fumonisins. However, the trichothecenes are sufficiently important to warrant a brief description. The chemistry of the trichotecenes has been adequately covered by Grove (1996), who reported that a total of 182 trichothecenes, based on the trichothecane skeleton have been isolated from natural sources. They comprise 113 non-macrocyclic and 69 macrocyclic compounds.

The trichothecenes are produced by various species of *Fusarium, Trichoderma, Myrothecium, Verticimonisporium* and *Stachybotris* and comprise a group of closely related chemical compounds designated sesquiterpenoids. All the naturally occurring toxins contain an olefinic bond at C-9,10 and an epoxy group at C-12,13 (Grove, 1996)(See **Figure 1**). The trichothecene mycotoxins do not require metabolic activation prior to exerting their toxic effects.

The trichothecenes occur frequently in nature, and have been implicated in ATA in Russia, scabby grain toxification (Tatsuno, 1997) as well as in a number of animal diseases such as skin toxicity, bone marrow damage, haemorrhagic and ill-thrift syndromes (Wannemacher *et al*., 1991). ATA was associated with the death of more than 10% of the population in Orenburg district, close to Siberia, during the period 1942-47. The symptoms of ATA, which include vomiting, diarrhoea, skin inflammation, leukopenia, multiple heamorrhage and exhaustion of the bone marrow, are similar to those induced by T-2 toxin. Ueno *et al*. (1972) and Yagen *et al*. (1977) concluded that T-2 toxin was the likely aetiological agent in ATA. The trichothecenes, e.g. T-2 toxin and nivalenol (NIV) induce karyorrhexis in actively dividing cells, a marked reduction in bone marrow cells and have the ability to inhibit protein and DNA synthesis and induce apoptosis in HL-60 cells (Yoshino *et al.,* 1996, 1997a, b; Sugamata *et al*., 1997). In the *in vitro* study on T-2 toxin induced apoptosis in human peripheral blood lymphocytes, Yoshino *et al*. (1997a) observed that the toxin affected the human peripheral blood lymphocytes, and elicited apoptic cell death, causing, in part, a marked decrease in circulating white blood cells as observed in animals which received T-2 toxin. In the ultrastructural study of apoptic cellular damage induced by acute NIV toxicoses in mice, Sugamata *et al.* (1997) observed NIV to be a potent inducer of apoptic cell death in the thymus, spleen and liver.

The trichothecenes such as T-2 toxin, diacetoxyscirpenol (DAS), vomitoxin, and 4-deoxynivalenol (DON) are frequent contaminants of agricultural commodities (Tanaka *et al.*, 1988; ApSimon *et al.*, 1990; Gilbert, 1995; Scott, 1997). The trichothecenes, e.g. T-2 toxin are optimally produced at relatively low temperatures, viz. 8-14°C; however, Rabie *et al.* (1986) reported the production of large quantities of T-2 toxin at 25°C by *Fusarium acuminatum.* The analytical methodology for the trichothecenes in animal feedstuffs is well established (Steyn *et al.*, 1991), e.g. by applying mass spectrometry and tandem mass spectrometry (Plattner *et al.*, 1989). Yeasts (*Kluyveromyces marxianus*) and bacteria (*Bacillus brevis*) are useful indicator organisms for the bioassay of several of the common mycotoxins. Madhyastha *et al.*, (1994) evaluated the relative toxicity of 16 trichothecenes and some of their interactions by using *K. marxianus*, and found toxicity to decrease in the following order: T2-toxin > DAS > DON> NIV. Similar methodology and results were obtained by Engler *et al.,* (1999) who used a colorimetric technique.

AFLATOXINS

The deaths in 1960 in England of over 100 000 turkeys and ducklings, which consumed Brazilian peanut meal, led to the discovery of the aflatoxins, a group of hepatocarcinogenic bishydrofurano mycotoxins produced by certain strains of *A. flavus* and *A. parasiticus.* Aflatoxin B_1 (AFB_1) has the highest level of occurrence and is the most carcinogenic of the known aflatoxins, while aflatoxin M_1 (AFM_1) is excreted in the milk of cows and has toxic properties similar to those of AFB_1 (Holzapfel *et al.*, 1966).

Chemistry and Metabolism

Aflatoxins B_1, B_2, G_1 and G_2 are the main metabolites of *A. flavus* and *A. parasiticus.* Büchi *et al.* (1966) elucidated the structures of the aflatoxins, and confirmed these by total synthesis. Aflatoxins may be classified into two broad groups according to their chemical structure: the difurocoumarocyclopentenone series (AFB_1, AFB_2, AFB_{2a}, AFM_1, AFM_2, AFM_{2a} and aflatoxicol) and the difurocoumarolactone series (AFG_1, AFG_2, AFG_{2a}, $AFGM_1$, $AFGM_2$, $AFGM_{2a}$ and AFB_3) (Heathcote, 1984)(see **Figure 2**). Aflatoxin B_1 ($C_{17}H_{12}O_6$) is a palewhite to yellow, odourless, crystalline solid with a blue fluorescence under UV light (see **Table 2** for physical and spectroscopic data).

The aflatoxins display decreasing potency in the order $B_1 > G_1 > B_2 > G_2$, as illustrated by their LD_{50} values for day-old ducklings (**Table 3**). Structurally, the dihydrofuran moiety, containing the double bond and the substituents linked to the coumarin moiety are of importance in producing biological effects. Demethylation of AFB_1 leads to a toxic derivative, AFP_1 and hydroxylation of the bridge carbon of the furan rings to AFM_1, with similar toxic effects as AFB_1. AFM_1 is, however, considerably less carcinogenic than AFB_1.

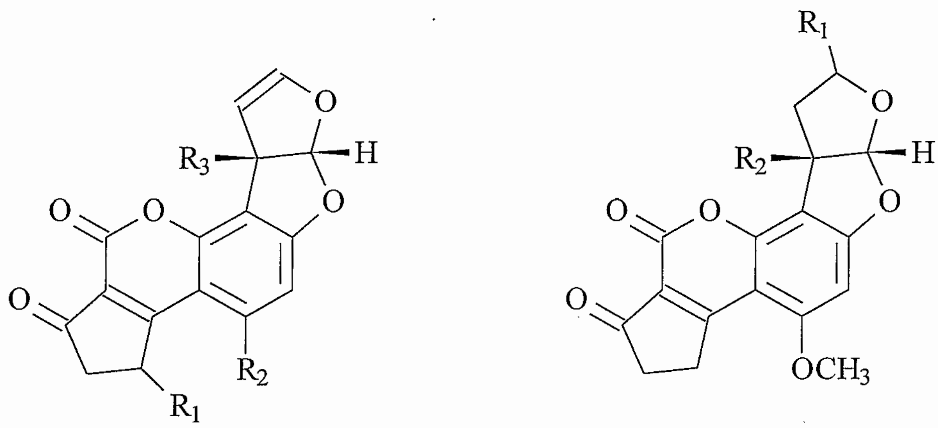
Table 2: Physical and spectroscopic data of aflatoxin B_1

Melting point	269-271 °C
Optical rotation [α]D	-559 ° (concentration 625 μmol/l in chloroform)
Infrared	Strong bands at 1770, 1600, 1570, 1390 and 1310 nm.
Molecular weight	312.3

Source: Pohland *et al.* (1982).

Aflatoxin	LD_{50} (μg per day-old duckling)
B_1	18
G_1	39
B_2	84
G_2	173
M_1	17
M_2	62

Table 3: Toxicities of the principal aflatoxins (Carnaghan *et al.*, 1963; Heathcote, 1984).



R_1=H, R_2= OCH_3, R_3= H: Aflatoxin B_1

R_1=H, R_2= OCH_3, R_3= OH: Aflatoxin M_1

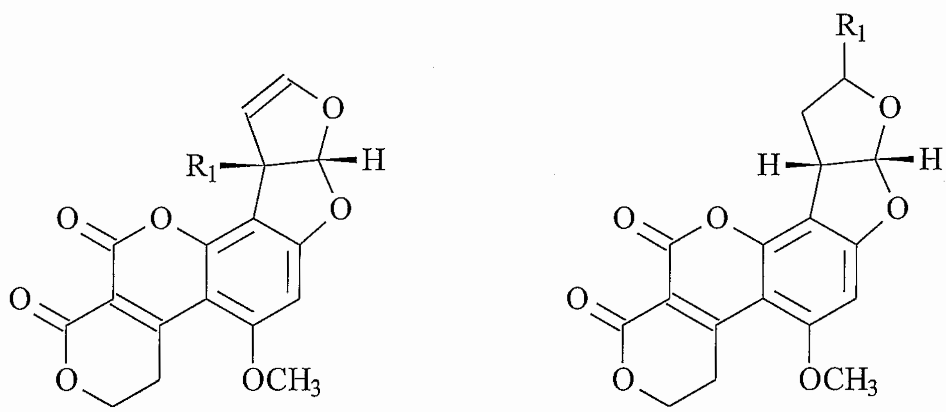
R_1=H, R_2= OH, R_3= H: Aflatoxin P_1

R_1=OH, R_2= OCH_3, R_3= H: Aflatoxin Q_1

R_1=H, R_2= H: Aflatoxin B_2

R_1=OH, R_2= H: Aflatoxin B_{2a}

R_1=H, R_2= OH: Aflatoxin M_2



R_1=H: Aflatoxin G_1

R_1=OH: Aflatoxin GM_1

R_1=H: Aflatoxin G_2

R_1=OH: Aflatoxin G_{2a}

Figure 2: Structures of the important aflatoxins.

Biosynthesis

In an effort to control aflatoxin contamination of food and feed, scientists focused on the biosynthetic pathway of aflatoxin to understand its regulation and evolution, and to eliminate the toxin from the food chain. Aflatoxin thus has one of the best studied polyketide pathways known (Trail *et al.*, 1995). The pathway involves approximately 20 enzymes: AFB_1 and AFB_2 are produced by two parallel pathways (Bennett *et al.*, 1994 and references cited). There are two possible initial steps: the first one involves condensation of acetate and nine malonate units, whereas the alternative step involves the early synthesis of a 6-carbon hexanoate (Townsend, 1986) [which is then extended by a polyketide synthetase to generate a C_{20} polyketide (Trail *et al.*, 1995 and references cited; Steyn, 1980]. (see **Figure 3** for the proposed pathway). The final step in aflatoxin biosynthesis involves the oxidative cleavage and rearrangement of O-methyl-sterigmatocystin with loss of a C_1-unit (Chatterjee and Townsend, 1994).

The detection of aflatoxigenic fungi in grains, using PCR methodology, is based on three genes from the aflatoxin biosynthetic pathway (**Figure 3**) (Shapira *et al.*, 1996). The three genes code for key enzymes involved in discrete biosynthetic steps: polyketide → norsolorinic acid (*apa-z*), versicolorin A → sterigmatocystin (*ver-1*) → O-methylsterigmatocystin (*omt-1*). The DNA sequences of the enzymes were established, and three primer pairs, each complementing the coding portion of one of the genes were generated. The PCR technology enabled Shapira *et al.* (1996) to differentiate between the aflatoxigenic strains of *A. flavus* and *A. parasiticus* and the non-aflatoxigenic *Penicillium* and *Aspergillus* species.

Production

The aflatoxins are produced by *A. flavus, A. parasiticus*, *A. nomius* and *A. tamarii* (Goto *et al.*, 1996). Watson *et al.* (1999) found the presence, but not expression, of homologues of three structural genes and a regulatory gene necessary for aflatoxin biosynthesis in *Aspergillus parasiti*cus and *A. flavus* in *A. oryzae* and *A. sojae.* The latter two fungi are important fungi used in the food and ingredient manufacture.

In addition to genetic requirements for production, the yield of aflatoxin depends on the growth conditions, such as moisture, temperature (optimal conditions for *A. flavus* are 16-24% moisture at 20-38 °C), substrate, aeration (culturing moulds on a rotary shaker greatly increases yields), and other factors which affect the qualitative state of development of the mould.

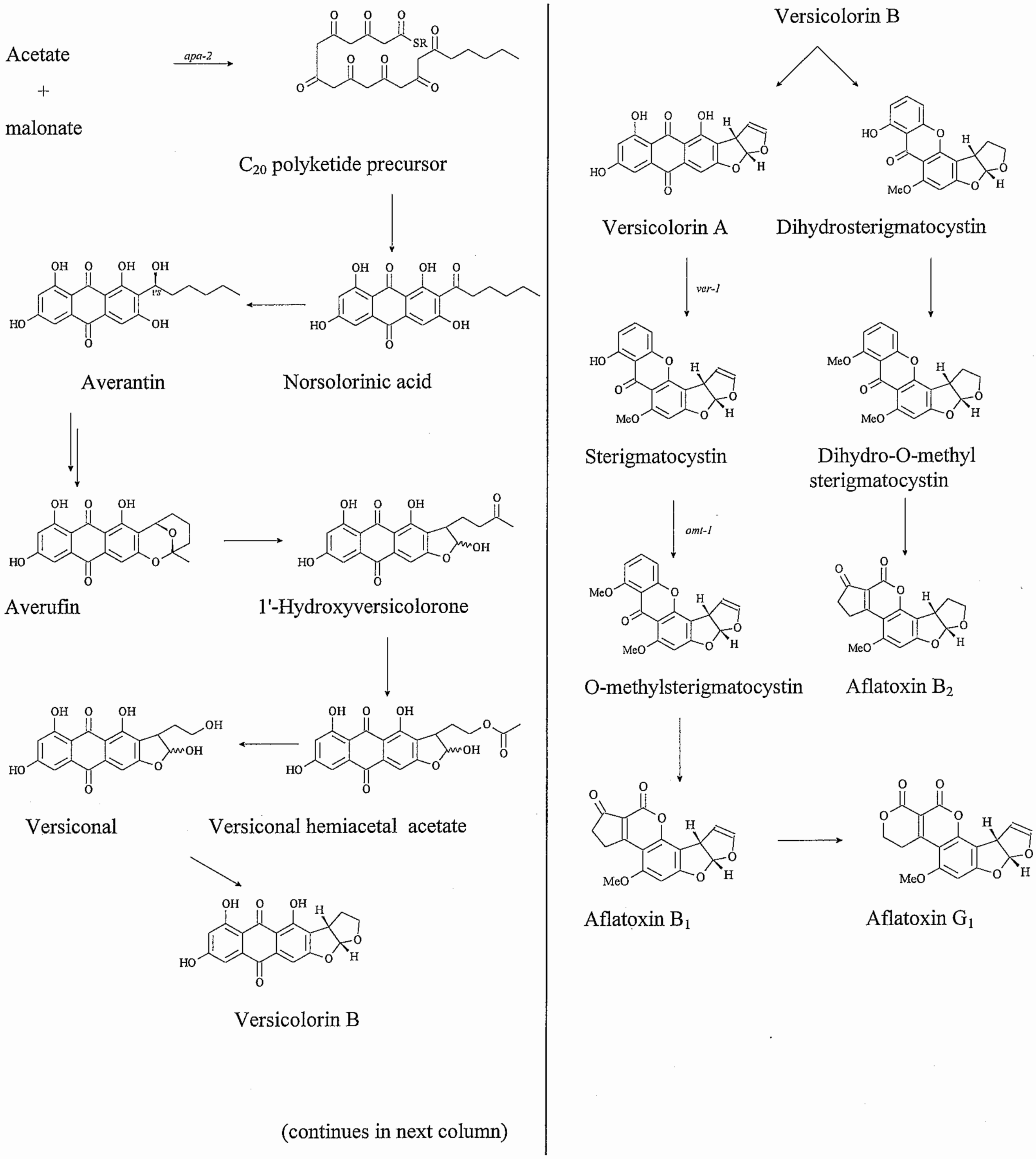


Figure 3: Biosynthesis of aflatoxin. Source: Townsend (1986), Steyn, (1980).

The production yields of the different aflatoxins vary with different growth conditions e.g. enhanced levels of AFB_1 relative to the different aflatoxins vary with different growth conditions e.g. enhanced levels of AFB_1 relative to AFG_1 occur in *A. parasiticus* at elevated temperatures as a result of accelerated catabolism of AFG_1 (Detroy and Ciegler 1971 and references cited). Although 20-38 °C is the optimum temperature range for production, aflatoxin formation can also take place at temperatures as low as 7-12 °C, if an extended incubation period is utilised. Therefore the storage of commodities at reduced temperatures can not be used to prevent aflatoxin production. Aflatoxin production is also affected by trace metals, insecticides, herbal drugs, spices, tricarboxylic acid cycle intermediates and food preservatives and is highly dependent on nutritional factors, e.g. sources of carbohydrates like glycerol (Mateles and Adye, 1965) and glucose.

Determination

At first, the analysis of the aflatoxins involved the grinding of the samples, Soxhlet extraction, solvent partition and clean-up by SiO_2 columns followed by determination by paper chromatography, which was subsequently replaced by SiO_2 TLC. (Shepherd *et al.*, 1987). A number of samples can be analysed simultaneously on one TLC plate and confirmation achieved by derivatisation on the plate followed by a second development. The modern techniques rely on the same principles, although ready-packed clean-up columns are used which contain silica or modified silica (especially C_{18}-bonded phase), e.g. Sep-Pak (Waters, Milford, MA, U.S.A.) and Bond-Elut (Analytichem International, Harbor City, CA, USA); however, the introduction of HPLC is replacing TLC methods in the final quantification step (Shepherd *et al.*, 1987). The problem of fluorescence quenching can be circumvented by pre-column derivatisation with trifluoroacetic acid or post-column reaction with bromine or iodine (Shepherd and Gilbert, 1984; Kok *et al.*, 1986). The latter method has a lower detection limit of 5-30 pg of AFB_1. Cepeda *et al.*, (1996) introduced cyclodextrines to increase the fluorescence of AFB_1 and AFG_1 in the postcolumn excitation of these toxins: substantial improvements (*ca* 30 fold) in detection limits of AFB_1 and AFG_1 were subsequently obtained by the use of heptakis-2,6-β-O-dimethyl-β-cyclodextrin. Gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-electrospray ionisation tandem-mass spectrometry (LC-ESI-MS-MS) can be used for confirmation of aflatoxin B_1 (Kussak *et al.*, 1995). The quest for better and more accurate methods for the determination of the aflatoxins is still continuing. Newer developments include the detection of the aflatoxins with an amperometric detector (Elizalde-González *et al.,* 1998). They separated the four aflatoxins by reversed phase HPLC and used a glassy carbon electrode at a constant potential of 1.4 V as amperometric detector. This method is more sensitive than conventional methods for the detection of the less toxic aflatoxin B_2, which is always present in grains contaminated with aflatoxins. Methods for the determination of the aflatoxins are summarised in **Table 4**.

Substrate	Method	Recoveries, detection limits	
Dust and urine	LC-MS	2 $pg.mg^{-1}$, 50 $pg.ml^{-1}$	Kussak *et al.* (1995)
Milk	Affinity columns and HPLC	85.7 %, 50 $pg.ml^{-1}$	Mortimer *et al.* (1987)
Cheese	Affinity columns and HPLC	75 %, 5 $ng.kg^{-1}$	Sharman *et al.* (1992)
Ground peanuts	ELISA	62-84 %, 5 $\mu g.kg^{-1}$	Li *et al.* (1994)

Table 4: Methods for the determination of aflatoxins.

Immunological methods

Immunological-based screening methods for aflatoxins are rapid: the columns are commercially available and the procedures can be fully automated. Holaday-Velasco minicolumns have been widely used since 1980 in the screening of aflatoxins in corn and peanuts. A disadvantage of this method is that it relies on the characteristic fluorescence of the aflatoxins, which can be very subjective (Gilbert, 1993). There are three types of immunological based assays: batchwise quantitative methods (ELISA), semiquantitative but rapid methods for single samples, and affinity column clean-up. Agri-screen is an ELISA method and Afla-20-cup, EZ-screen and Cite-probe are all commercially available kits for aflatoxin screening, based on the principles of ELISA but using absorbed antibodies in a sandwich format. Aflatest and Oxoid are commercially available affinity columns (Gilbert, 1993). The performance of these different kits has been assessed by Koeltzow and Tanner, (1990) and Dorner and Cole, (1989). Radioimmunoassays (RIA) for AFB_1 have already been reported as far back as 1976 but have not been commercialized (Shepherd *et al.*, 1987).

In commercial double-antibody ELISA kits a microtiter plate with aflatoxin-protein conjugate absorbed to the surface of the wells is supplied. Buffer-diluted methanol extracts of the samples are pipetted separately into the wells of the titer plates, followed by a limited amount of anti-aflatoxin antibodies. There is competition between the bound aflatoxin and the aflatoxin in the sample for antibodies. The titer plate is then washed and a second antibody, with a colour-producing enzyme attached to it, is added, which binds to the anti-aflatoxin antibody bound to the well, and colour is produced with an intensity that is inversely proportional to the aflatoxin concentration in the sample, when the substrate for the colour producing enzyme is added. In single-antibody ELISAs the colour-producing enzyme is conjugated directly to the first anti-body. Although this assay is much quicker it uses more antibodies and is thus more expensive. Commercial ELISA kits have detection limits of about 2 $\mu g.kg^{-1}$ and are best suited for large batches of samples, since up to 93 samples can be assayed on one titer plate, but are not sufficiently reliable to be used as quantitative methods, (Shepherd *et al.*, 1987; Gilbert, 1993). However, recent developments enabled this technology to be utilised with a high level of sensitivity and precision ($ng.kg^{-1}$)(Franco, 1996).

Immunoaffinity columns consist of an anti-aflatoxin antibody bound to a gel material contained in a small plastic cartridge. In practice the crude extract is forced through the column and the aflatoxin is left bound to the recognition site of the immunoglobulin. Extraneous material can be washed off the column with water or aqueous buffer, and the aflatoxin is obtained in a purified form by denaturing the protein gel by elution solvent such as methanol or acetonitrile (Gilbert, 1993). The aflatoxin in this eluate can be quantified with HPLC or with a standard UV spectrometer. The advantages of immunoaffinity columns are that the approach is the same for all matrices (peanuts, milk, nuts etc.), it is much faster and cleaner than conventional silica gel columns (there are no peak interferences when HPLC is used for quantification) and unlike ELISA, all four aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) can be determined individually (HPLC analysis). The disadvantage is that it is still more time-consuming than ELISA (Gilbert, 1993).

The latest development is fiber-optic-based biosensors that uses the high fluorescence of the aflatoxins for detection. These detections require little specialized training and it is very quick to perform analysis with similar detection limits as ELISA's. Aflatoxin was detected using its native fluorescence and also in the competition mode with either FITC (fluorescein isothiocyanate)-labelled antibody or enzyme labelled antibody by Carter *et al.* (1997).

Control and Decontamination

Ammonia has been used to destroy aflatoxins in various feedstuffs either in its gaseous form, or as an ammonium hydroxide solution (Simpson and Pemberton, 1989; Lee *et al.*, 1992; Park, 1992). Addition to animal feeds of sequestering agents, such as activated charcoal, sodium bentonite and hydrated sodium aluminosilicate, which bind to aflatoxin and decrease its bioavailability, has been proposed as a detoxification strategy (Bonna *et al.*, 1991; Harvey *et al.*, 1989; Lindemann *et al.*, 1990). Aflatoxin levels can also be reduced by the process of cooking, the dry roasting of peanuts and the popping of corn; the reduction is, however, modest (Simpson and Pemberton, 1989). Aflatoxin production can be controlled by the isolation or development of atoxigenic strains of *A. flavus* and *A. parasiticus*; these strains are able to competitively exclude toxigenic field strains from the host plant (Cole and Cotty, 1990; Cotty, 1994). Although these strains are not producing aflatoxins, they still have the genes for aflatoxin biosynthesis. Cary *et al.* (1999) isolated and characterized experimentally induced, aflatoxin biosynthetic pathway deletion mutants of *Aspergillus parasiticus* by engineering a plasmid vector (pDEL2) for the purpose of introducing a deletion within the aflatoxin biosynthetic gene cluster. The vector was constructed by PCR amplification of a region of the AF gene cluster from an *A. parasiticus* isolate that had undergone an aberrant recombinational event during transformation with a norA-niaD gene disruption vector. Maas *et al.* (1998) demonstrated that AFB_1 can be degraded up to 50 % by *Aspergillus niger* in liquid culture, the degradation product was, however, aflatoxicol which is also a carcinogenic compound. Similar studies on the ability of microorganisms to degrade aflatoxins has been investigated by several authors including Nakazato *et al.* (1990). Hoogenboom *et al.* (1998) studied, tested and compared three decontamination processes (two based on ammoniation and one based on biological degradation) in an EU project. One of these ammoniation methods, the SOCOFAG procedure was tested to see if the decontamination products are still hazardous.or if they could become hazardous again being metabolized in the rumen or liver of a cow. Kuilman *et al.* (1998) found no aflatoxin metabolites in incubations of cultured bovine hepatocytes with extracts of decontaminated peanut meal.

Occurrence

The aflatoxins are frequent contaminants of commodities such as corn (maize), peanuts, pecan nuts, Brazil nuts, cotton seeds, other energy-rich foodstuffs, and even herbs and spices (Davis *et al.*, 1986; MacDonald and Castle, 1996; Resnik *et al.*, 1996). High levels of aflatoxin contamination are frequently associated with tropical climatic conditions, poor agricultural practices, drought stress, and insect and mechanical damages. Internationally due cognisance is usually given to the standards set by the US FDA (see **Table 5** for the permissible levels of aflatoxin contamination).

Table 5: Maximum levels for aflatoxin contamination set by US Food and Drug Administration.

Substrate	Maximum Level
Food for humans and feed for some animal species	20 ppb
Milk	0.5 ppb
Feed for feedlot cattle	300 ppb
Feed for market hogs	200 ppb
Feed for breeding cattle, breeding hogs and mature poultry	100 ppb

Biological Effects and Mechanism of Action

Toxicologically, the aflatoxins particularly AFB_1 should be regarded as a quadruple threat, i.e. as a potent toxin, a carcinogen, a teratogen and a mutagen. AFB_1 induces liver cancer in all animal species tested so far and has also been linked to liver cancer in humans (Wang *et al.*, 1996). Statistical correlations between contaminated food supplies and high frequencies of human hepatocellular carcinomas (HCC) in Africa and Asia have long implicated aflatoxins as risk factors in human liver cancer (Van Rensburg, 1986). The toxicity of AFB_1 toxicity varies from species to species and differences in susceptibility to aflatoxin are also found amongst individuals and between sexes (See **Table 6**). Numerous biochemical, toxicological and histological analyses have been performed to clarify the fundamental mechanisms of liver injury and hepatoma development. Molecular aetiology has substantiated the previous bio-statistical studies, since AFB_1 causes an activation of the *K1 ras* proto-oncogene and modulates the *p53* tumour suppressor gene. A molecular 'hot spot' in the *p53* gene, a G $\rightarrow$ T transversion at the third base position of codon 249 has been identified in independent studies on HCC patients from Qidong, China (Hsu *et al.*, 1991) and from sub-Saharan Africa (Bressac *et al.*, 1991). The IARC, Lyon, France classified AFB_1 as a human carcinogen in 1987 and confirmed the classification in 1992 and 1999.

Table 6: Acute oral toxicities of aflatoxins

Species	LD_{50} (mg.kg^{-1})						
	B_1	B_2	G_1	G_2	M_1	M_2	B_{2a}
Duckling	0.36	1.68	0.78	1.42	0.32	1.22	24
Rabbit	0.3						
Cat	0.55						
Pig	0.62						
Dog	0.5-1.0						
Sheep	1.0-2.0						
Guinea-pig	1.04						
Monkey	2.2-7.8						
Chicken	6.3						
Rat	7.2 (M)- 6 (F)						
Mouse	9.0						
Hamster	10.2						

Source: Ciegler (1975), Heathcote (1984).

The mutagenic and carcinogenic effects of AFB_1 have been well studied and are believed to arise from metabolic activation of the electron-rich dihydrobisfuran to the corresponding epoxide by the liver P450 isoenzyme CYP 2C and to a lesser extent CYP 1A2 in rats and CYP 3A4 in humans (Gopalkrishnan *et al.*, 1990; Ishii *et al.*, 1986; Forrester *et al.*, 1990). Raney *et al.*, (1992) demonstrated that the AFB_1-epoxide occurs in an *endo* and *exo* form each with different affinities for DNA (see **Figure 4**). The *exo*-epoxide is highly electrophilic and reacts with regions in the DNA helixes which are rich in guanine to form covalent bonds at the *N*-7 of guanine residues, leading to depurination and strand scission events (Essigmann *et al.*, 1982). The *Salomonella* reversion assay indicates that the *exo*-epoxide is at least 500 times more potent as a mutagen than the *endo* stereoisomer (Iyer *et al.*, 1994). AFB_2 on the other hand has no double bond at this position and is practically inactive. AFB_1-epoxide can be metabolised further to 8,9-dihydro-8,9-dihydroxy-aflatoxin B_1 which may bind to cellular proteins, via a Schiff base formation with primary amino groups, inducing cellular injury and eventually cell death (Fink-Gremmels, 1996). In mice virtually all the epoxidation leads to the *exo* isomer, in rats the ratio of *exo* to *endo* is 32 to 1 while in humans the proportion of *endo* to *exo* is higher than in the rat (Neal, 1995)(see **Table 6** for toxicities).

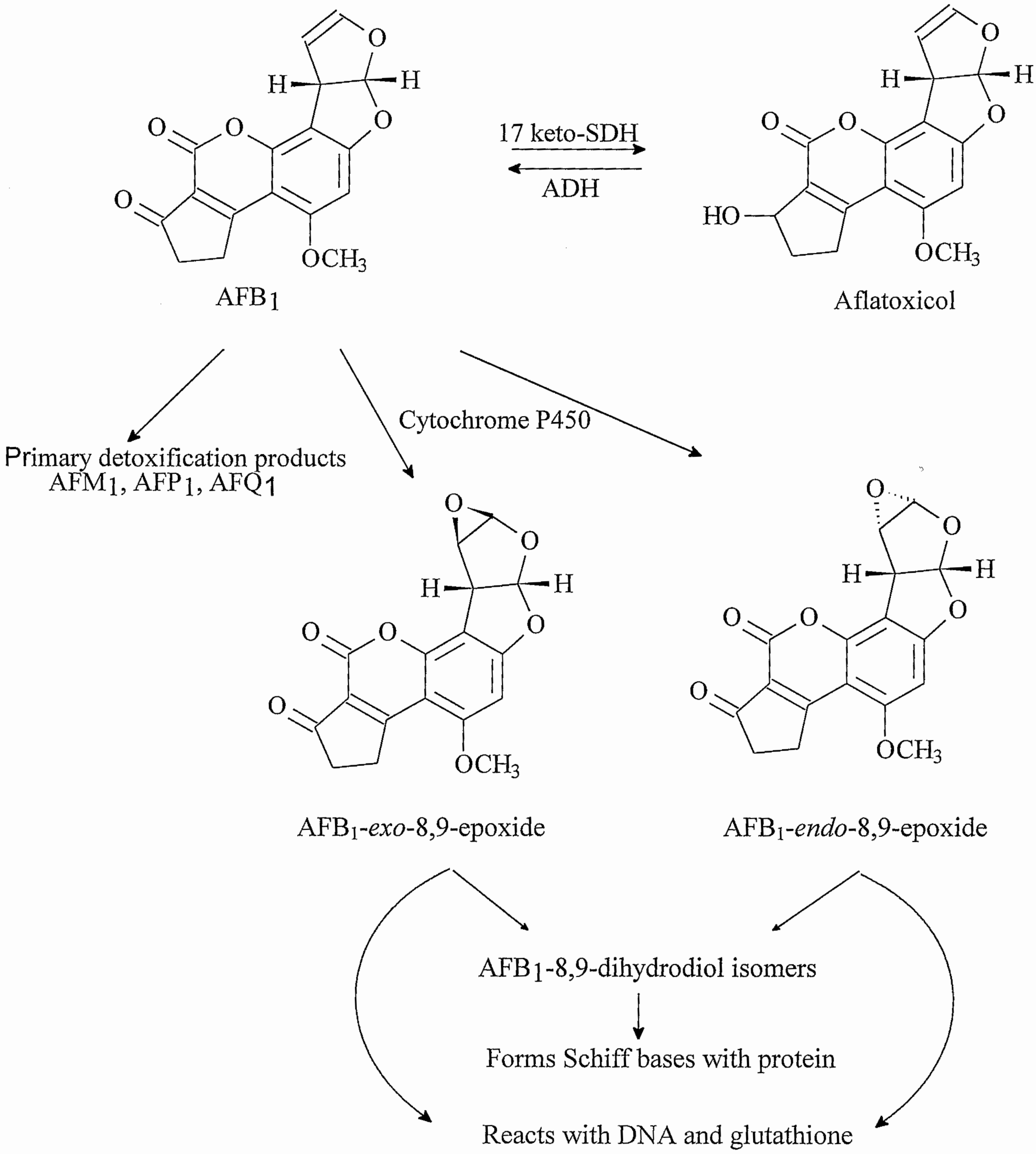


Figure 4: Metabolism of AFB_1 (Neal, 1995).

AFB_1-epoxide forms a conjugate with glutathione by a glutathione-S-transferase (GST) mediated mechanism; glutathione is an alternative for aflatoxin to binding to other nucleophilic centres and is apparently the most important detoxification system (Degen and Neumann, 1978; Neal, 1995). As is evident from **Figure 4** several hydroxylations occur during the metabolism of AFB_1, catalysed by cytochrome P450 enzymes, leading to its secondary metabolism (AFM_1, AFP_1 and AFQ_1). The secondary conjugating processes involve glucuronidation, sulphation and acetylation of primary AFB_1 metabolites (Neal, 1995).

OCHRATOXIN A

The ochratoxins, metabolites of *Aspergillus ochraceus* Wilh. (van der Merwe *et al.*, 1965), are the first group of mycotoxins discovered subsequent to the epoch-making discovery of the aflatoxins. Ochratoxin A (OTA) is a very important mycotoxin owing to its frequent occurrence in nature, its established role in Danish porcine nephropathy and in poultry mycotoxicoses and its implicated role in Balkan endemic nephropathy and urinary system tumours in North Africa (Achour *et al.*, 1993, Bacha *et al.*, 1993, Maaroufi *et al.*, 1995).

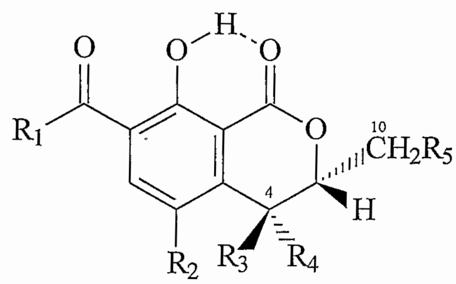
Chemical characteristics and biosynthesis of OTA

Ochratoxin A comprises of a pentaketide-derived dihydroisocoumarin moiety linked via its 12-carboxy group by a peptide bond to *L*-β-phenylalanine (see **Figure 5**). It forms colourless crystals when recrystallized from benzene (mp. 90 ^{o}C with the loss of benzene) and it melts at 169-171 ^{o}C upon crystallization from xylene (Van der Merwe *et al.*, 1965).

The IR spectrum of OTA ($CHCl_3$) displays bands at 1655, 1535 and 3430 cm^{-1} (secondary amide); 1723 cm^{-1} and broad band between 2500 and 2700 cm^{-1} (carboxyl carbonyl group) and a band at 1678 cm^{-1} (lactone carbonyl group). The UV absorption spectra of OTA has λ_{max} 216 nm (ε = 31 500) and 330 nm (ε = 6 400) in MeOH / 0,0005 M H_2SO_4. The most abundant peaks in the mass spectrum of OTA are: *m/z* M^+ 403 (13%), 359 (31%), 358 (18%), 357 (14%), 258 (52%), 257 (97%), 256 (100%), 255 (86%), 242 (49%), 241 (94%), 239 (81%), 238 (99%). IR spectroscopy and X-ray crystallography have demonstrated when OTA exists in solution and in the solid state in the β form; viz. the amide NH is hydrogen-bonded to the phenolic oxygen (Bredenkamp *et al.,* 1989). ^{13}C NMR spectroscopy provided evidence for hydrogen bonding of the phenolic proton to the lactone carbonyl group (Bredenkamp *et al.,* 1989).

The biosynthetic origin of OTA was established employing radioactive precursors, *e.g.*, [1-^{14}C]- and [2-^{14}C]acetate, [2-^{14}C]malonate, DL-[methyl-^{14}C]methionine and chlorine-36 (Steyn *et al.,* 1970) and stable isotope precursors, *e.g.* sodium[^{13}C]formate, [1-^{13}C]- and [1,2-

^{13}C]acetate (De Jesus *et al.,* 1980). OTA is derived from combined pathways, viz., the shikimic acid pathway (phenylalanine) and the polyketide pathway (dihydroisocoumarin); the chlorine atom is probably derived through the action of a chloroperoxidase.



	R_1	R_2	R_3	R_4	R_5
Ochratoxin A	Phenylalanine	Cl	H	H	H
Ochratoxin B	Phenylalanine	H	H	H	H
Ochratoxin C	Phenylalanine ethyl ester	Cl	H	H	H
Ochratoxin A methyl ester	Phenylalanine methyl ester	Cl	H	H	H
Ochratoxin B methyl ester	Phenylalanine methyl ester	H	H	H	H
Ochratoxin B ethyl ester	Phenylalanine ethyl ester	H	H	H	H
Ochratoxin α	OH	Cl	H	H	H
Ochratoxin β	OH	H	H	H	H
4*S*-Hydroxyochratoxin A	Phenylalanine	Cl	OH	H	H
4*R*-Hydroxyochratoxin A	Phenylalanine	Cl	H	OH	H
10-Hydroxyochratoxin A	Phenylalanine	Cl	H	H	OH
Bromo-ochratoxin B	Phenylalanine	Br	H	H	H

Figure 5: Structures of the ochratoxins.

Analogues of OTA

OTB, the natural des-chloro analogue of OTA, is 10 times less toxic than OTA. OTA can be converted into OTB by catalytic dechlorination with palladium-charcoal and ammonium formate (Bredenkamp *et al.* 1989). Steyn and Holzapfel (1967) identified the methyl and ethyl esters of OTA and OTB in a culture of *A. ochraceus* on both sterilised cornmeal and liquid media. The toxicity of the esters of OTA is similar to that of OTA, whereas the OTB derivatives are, as to be expected, non-toxic.

OTA is hydrolysed to the non-toxic OTα (7-carboxy-5-chloro-3,4-dihydro-8-hydroxy-3-methylisocoumarin) in various organs in rats mostly the caecum, duodenum, ileum and the pancreas, whereas the activity in the liver and kidneys is very low or non-existent in rat hepatocytes (Suzuki *et al.*, 1977; Hansen *et al.*, 1982; Stφrmer *et al.*, 1983). OTA is chemically hydrolysed by 6 N HCl and more readily by treatment with α-chymotrypsin or carboxypeptidase A, yielding L-β-phenylalanine and the optically active lactonic acid (OTα). OTβ the des-chloro analogue of OTα, is the hydrolysis product of OTB, and was detected in culture extracts. The less toxic ochratoxin D, 4-hydroxyochratoxin A was isolated by Hutchison *et al.* (1971) from *P. viridicatum.* The (4*R*)-OH-OTA epimer is the major of the two epimers formed from OTA in human and rat liver microsomal systems under the influence of cytochrome P-450's (Stϕrmer *et al.*, 1981, 1983) while the (4*S*)-OH-OTA epimer is more prevalent in pig liver microsomes (Moroi *et al.*, 1985). Oster *et al.* (1991) characterized four cytochrome P-450 fractions in pig liver microsomes; the two predominant forms A_2 and A_3, both with a molecular weight of 54 kDa, and the minor form B_a play an important role in the oxidation of OTA. These two epimers were also found in rat and rabbit liver (Stϕrmer *et al.*, 1981) and rat kidney (Stein *et al.*, 1985). The 10-OH metabolite of OTA was formed from OTA with rabbit liver microsomal system (Stϕrmer *et al.*, 1983). Hadidane *et al.*, (1992) discovered three natural analogues of OTA with the Phe-group replaced with a serine, proline and hydroxyproline group, while Xiao *et al.*, (1996b) reported the isolation of OTα, OTβ, (4*R*)-OH OTA, (4*R*)-OH OTB and 10-OH OTA from a culture of *A. ochraceus*. Five analogs of OTA including the ethylamide of OTA (OE-OTA), the D-phenylalanine form of OTA (d-OTA), the decarboxylated OTA, (DC-OTA), the O-methyl ether of OTA (OM-OTA) and the methyl ester of OTα (M-OTα) were synthesised using OTA or ochratoxin α by Xiao *et al.*, (1995b). The toxicities of these analogues to HeLa cells are shown in **Table 7**. Xiao and coworkers activated OTA to the N-hydroxysuccinimide ester (OTA-NHS) and OTα to acyl chloride (OTα-Cl). They then used nucleophilic substitution reactions with primary amines, amino acids and alcohols to form corresponding amides and esters. OM-OTA was synthesised by the base-hydrolysis of *O*-methylochratoxin methyl ester. An open lactone form of OTA which is much less toxic than OTA is produced at high pH and is relatively stable at physiological pH (Xiao *et al.*, 1996a). Steyn and Payne (1999) synthesised the bromoanalogue of OTA by the treatment of OTB with pyridiniumhydrobromide perbromide. Steyn *et al.*, (1975) prepared 13 new analogues of OTA by substituting L-Phe for L-amino acids Trp, Ala, Tyr, Cys, Pro(4-OH), Glu, Met, Val , Pro, Ser, Asp, Thr and Leu. The typical lesions in cell culture which were associated with OTA toxicity were caused to various extends by all the compounds. In the group of compounds with a higher toxicity rating, four contained an aromatic ring.

Table 7: The toxicity of OTA and its analogs to HeLa cells

Analogs	HeLa cell LC_{50} (mM)
OTA	0.005
OTC	0.009
OTB	0.054
d-OTA	0.163
OTα	0.56
OM-OTA	0.83
DC-OTA	7.6
OE-OTA	10.1

Source: Xiao *et al*., (1995b).

Production of OTA

OTA is produced by a number of both *Aspergillus* and *Penicillium* species, as shown in **Table 8**.

Table 8: Reported OTA-producing species

Aspergillus	***Penicillium***
A. ochraceus (A. alutaceus)	*P. viridicatum (P. verrucosum)*
A. melleus (A. quercins)	
A. alliaceus	
A. ostianus	
A. sclerotiorum	
A. albertensis	
A. wentii	
A. auricomus	
A. niger var. *niger*	
A. awamori	
A. carbonarius	
A. foetidus	
A. sulphureus (A. fresenii)	

Source: Marquardt and Frohlich (1992); Abarca *et al*. (1994); Varga *et al*. (1996); Bragulat *et al*. (1998).

A number of other *Aspergillus* and *Penicillium* species were incorrectly reported to be producers of OTA due to the difficulty associated with the correct identification of the fungi (Pitt, 1987; Samson and Frisvad, 1991).

OTA occurs extensively in many plant and animal products; the contamination is typically associated with grain stored in the temperate climate of Europe and North America. There are substantial annual variations in the OTA content of grains because the production is determined mainly by the temperature and water activity (a_w) of the substrate and the type of substrate, presence of competitive microflora, strains of fungi and the quality of the seed (Marquardt and Frohlich, 1992). The minimum a_w conditions for OTA production by *e.g. A. ochraceus* are 0.83-0.87 and the minimum temperature is 12 °C; the optimal temperature for toxin production is 28 °C and the optimal time depends on the substrate, ranging from 7 to 14 days.

Isolation and Purification

Mouldered substrates are extracted with hot chloroform, ethyl acetate, chloroform-methanol, acidified chloroform or by hexane followed by chloroform-methanol. The preliminary clean-up step is to transfer acidic components of the extract, including OTA and OTB, into a sodium bicarbonate solution, followed by acidification, extraction and column chromatography. The ochratoxins can be separated by chromatography *e.g.* ion-exchange chromatography, partition chromatography on formamide-impregnated cellulose powder, column chromatography on silica gel impregnated with oxalic acid, Sephadex LH20, Sephadex G-25, Florosil, Sephadex chromatography followed by silica gel chromatography, by preparative liquid chromatography or by preparative thin layer chromatography. For final purification OTA is crystallized from benzene, toluene or chloroform (Steyn, 1984). OTA crystallises from toluene and chloroform without solvent of crystallization.

Analysis of OTA

Various methods for the analysis of OTA are based on HPLC, thin layer chromatography (TLC) and ELISA techniques.

TLC analysis is a relative inexpensive way of screening for OTA and was frequently used during the early years of mycotoxin research; it still has many applications. On TLC, OTA displays an intense blue-green fluorescence under long wavelength UV light. It is necessary to use acid modifiers (*e.g.* acetic acid) in TLC mobile phases (*e.g.* chloroform-methanol or toluene) to prevent streaking of OTA on silica gel. Problems with the rapid fading of the fluorescence intensity on the plate can be overcome by exposure of the plate to ammonia vapour, which converts the OTA to its ammonium salt, which displays a more intense blue fluorescence under UV illumination. The limits of detection for OTA on TLC are in the $\mu g.kg^{-1}$ range. Paulsch *et al.* (1982) developed a two-dimensional TLC technique using an acidic and alkaline developing solvent. This method in combination with exposure of the TLC plate to methanol-ammonia, leads to its effective detection. Paulsch *et al.* (1982) used a simple confirmatory test for OTA based on the formation of OTA methyl ester on the TLC plate. There is an increase in the use of reverse phase TLC (RPTLC) and high performance TLC (HPTLC) in mycotoxin analysis. The latter has a much better efficiency of separation and uses less solvent than conventional TLC plates. Frohlich *et al.,* (1988) developed a RPTLC method for sample preparation in which the OTA is extracted from the spot for quantitation by direct spectrofluorimetric testing or by subsequent HPLC analysis. This method has high levels of recovery (94%) and requires smaller amounts of solvents than the standard packed column methods.

HPLC separations are more efficient than those obtained by TLC and have been widely applied to the determination of OTA in various matrices (See **Table 9** for a few examples). Sample clean-up consists usually of extraction with organic solvents together with an acid to suppress the ionization of OTA, followed by further clean-up steps like silica gel, cyano or reversed phase-solid phase extraction (RP-SPE) cartridges, immunoaffinity columns, and liquid-liquid extractions or preparative HPLC techniques. RP columns *e.g.* octadecyl silane (ODS, C18) are usually used in quantitative HPLC separations with acidic aqueous acetonitrile or methanol as mobile phase (Van Egmond, 1991). Detection limits in the $\mu g.kg^{-1}$ range or lower can be obtained by using an HPLC equipped with a fluorescence detector with excitation and emission wavelengths at 330 and 460 nm, respectively (Cohen and Lapointe, 1986).

Improved HPLC detectors such as photodiode-array detectors and improved computer search capabilities have made it possible to monitor the whole spectrum of compounds after separation, permitting further identification (Chu, 1992, Paterson and Kemmelmeier, 1990).

Jiao *et al.* (1991) developed a method for the identification of OTA in food samples by chemical derivatization of OTA to *O*-methyl-OTA ester and GC-MS using negative ion chemical ionization. Thermospray MS has been described by Rajakyla *et al.* (1987) as an expensive alternative to fluorescence detection.

Radioimmunoassay (RIA) is an analytical method that uses radioactivity for quantitative determination of compounds. It usually involves the incubation of a specific antibody with a solution of unknown sample or known standard at a constant amount of labelled toxin followed by the separation of the free and bound toxin and the determination of the radioactivity in the fractions (Chu, 1992). Commercial kits are available for the determination of OTA in various feeds and foodstuffs with RIA.

Table 9: Methods for the determination of OTA in different matrices.

Substrate	Cleanup	Recoveries, detection limits	
Faba beans and wheat	SPE	70%, 0.7 $\mu g.kg^{-1}$	El-Banna and Scott (1984)
Wheat and barley	solvent partition	40 $\mu g.kg^{-1}$	Lepom (1986)
Animal feed, grain	2-stage SPE	90%, 5 $\mu g.kg^{-1}$	Cohen and Lapointe (1986)
Wheat, barley, oats and mixed feed	1.$CHCl_3$ extraction 2.SPE	77-96%, 0.1-0.3 $\mu g.kg^{-1}$	Langseth *et al.* (1989)
Human blood, serum, milk and some foodstuffs	immunoaffinity column	85%, 5-10 $pg.g^{-1}$	Zimmerli and Dick (1995)
Human urine	extraction, column chromatography	60-75%, 5$ng.l^{-1}$	Castegnaro *et al.* (1990)
Beer	1. SPE (C_{18} silica) 2. immunoaffinity column	82-100%, 0,05-0,1 $ng.ml^{-1}$	Scott and Kanhere (1995)

ELISAs involve the use of antibodies generated against conjugates. These conjugates are made by linking OTA to protein (enzymes like horseradish peroxidase) through its carboxylic acid function. Direct ELISA involves the use of a OTA-enzyme conjugate, while indirect ELISA uses a protein-OTA conjugate and a secondary antibody to which an enzyme has been conjugated (Morgan *et al.*, 1986). Commercial kits like RIA are available, but the ELISA technique can be used to measure samples as low as 2.5 pg, which makes ELISA 10 to 100 times more sensitive than RIA (Chu, 1992). Although a lot of work is being done to improve the selectivity of the antisera used in ELISAs (Xiao *et al.*, 1995a), the possibility of cross-reactions cannot be fully ruled out and positive findings obtained by immunoassays need to be confirmed by other techniques.

Antibody technology is used for the clean-up of cereal and animal sample extracts by utilising immunoaffinity columns for the selective isolation of OTA (Sharman *et al.,* 1992). Nakajima *et al.*, (1990), prepared monoclonal antibody affinity columns for the determination of OTA in coffee by binding antibodies specific for OTA to Sepharose 4B. Immunoaffinity columns are commercially available and are used routinely in laboratories for sample clean-up followed by quantitation by HPLC or direct spectrofluorimetric measurement.

Regulations for OTA

OTA contamination is widespread in cereals, coffee, pulses, feedstuffs and other plant products. Raw agricultural products, contaminated with OTA and used as feed, can also contaminate meat and meat products of non-ruminant animals such as poultry and pigs (Van Egmond and Speijers, 1994). This problem does not occur in adult ruminants because OTA is hydrolysed by protozoan and bacterial enzymes in the fore-stomachs of these animals (IPCS, 1990). The detection of OTA in human milk indicates the carry-over of OTA from contaminated food by lactating women. Data on the occurrence of OTA in food and feed are relatively abundant in European countries but scarce for other continents. The levels of ochratoxin A contamination were found at the highest incidences in cereals (corn from 10-500 $\mu g.kg^{-1}$, wheat from 5-135 $\mu g.kg^{-1}$ and barley from 10-500 $\mu g.kg^{-1}$) and for foodstuffs from animal products (kidneys from pigs varying from 2-100 $\mu m.kg^{-1}$) (Van Egmond and Speijers, 1994). The occurrence of OTA is related to the climate and especially the harvest and post-harvest storage conditions (Pohland *et al.*, 1992). There are 77 countries that have mycotoxin regulations and only eight have specific regulations for OTA in one or more commodities (FAO, 1996). The following factors must be taken into consideration before the limits for mycotoxins can be chosen: the availability of toxicological and survey analytical data, the availability of reliable analytical methods, data on the availability on the occurrence of mycotoxins in various commodities, intercountry trade as well as the existence of sufficient food supply (FAO, 1996). The current (proposed) limits are indicated in **Table 10.** The tolerance levels for OTA have been suggested at 1 $\mu g.kg^{-1}$ for infant foods and at 5 $\mu g.kg^{-1}$ for cereals for the EU (Verardi and Rosner, 1995).

The homogeneity of OTA in products is very important, if care is not taken to ensure representative sampling, incorrect estimations of toxin concentrations will be made (FAO, 1996). Analytical quality assurance is essential to guarantee the accuracy of results and the use of reference materials (RMs) plays an important role in checking the performance of methods and proficiencies of laboratories (Van Egmond, 1996). The European Commission's Standards, Measurements and Testing Program has initiated the development of certified RMs for OTA and other mycotoxins (Wood *et al.*, 1995).

Table 10: Limits for Ochratoxin A in the different commodities

Commodity	Limits
Children and infant foods	0.5 - 5 μg.kg^{-1}
Foods	2 - 50 μg.kg^{-1}
Animal feeds	5 - 300 μg.kg^{-1}

Source: FAO (1996)

Ochratoxicosis

OTA is nephrotoxic to all animal species tested so far, and induces experimental liver and kidney tumours (see **Table 11**).

Table 11: Acute oral toxicities of the ochratoxins in different species (mg OTA per kg body weight)

Species	OTA LD_{50}	OTC LD_{50}	OTB LD_{50}	OTα LD_{50}
Pigs	1.0-6.0			
Chickens	3.3	4,8	41.4	22
Dogs	0.2			
Neonatal rats	3.9			
Mature rats	20-30			
Mice	46-58			

Source: Chu (1974); Krogh (1987); Kuiper-Goodman and Scott (1989).

The kidneys are the organs most susceptible to OTA, which can cause both acute and chronic kidney diseases. The renal lesions associated with the diseases include degeneration of the proximal tubules, interstitial fibrosis in the real cortex, hyalinization of the glomeruli and atrophy in the tubular epithelium (Krogh *et al.* 1974, 1977, Krogh 1987). Pigs fed OTA showed reduced feed intake, loss of body weight, increased water consumption followed by polyurea diarrhoea, polydipsia and dehydration (Szczech *et al.*, 1973). Residues of OTA are greatest in the kidney, and in declining order in lean meat, liver and fat (Madsen *et al.*, 1982). Krogh *et al.* (1988) reported nephropathy in pigs fed diets containing 0,2-4 mg.kg^{-1} OTA after four months of exposure; all lesions were confined to the kidney. Gross pathological examination of dogs administered 0.2 to 3 mg of OTA.kg^{-1} BW alone or in two dose combinations for 14 days indicated moderate to severe mucohemorrhargic enteritis of the caecum, colon, and rectum and enlargement of the lymph nodes, which were oedematous, hyperaemic, and focally necrotic. Histopathological examination indicated that renal damage is the main feature of this toxicosis (Szczech *et al.,* 1973). OTA inhibits cell division in these kidney cells and causes apoptotic type morphological lesions in these cells. The nuclear lesions seen in apoptosis are associated with enhanced endonuclease activity, which are responsible for DNA cleavage. OTA causes apoptosis-associated DNA degradation in human lymphocytes (Seegers *et al.*, 1994, and references cited). OTA also causes decreased natural killer cell activity in mice (Luster *et al.*, 1987) and the inhibition of cell division in hematopoietic stem cells (Boorman *et al.*, 1984) and lymphocytes (Creppy *et al.*, 1983b).

Krogh proposed in 1974 that the endemic human interstitial nephropathy diseases in the rural areas of Bulgaria, Romania and Yugoslavia might be related to a high OTA exposure. Balkan endemic nephropathy (BEN) was first identified in the 1950s and is an invariably fatal chronic kidney disease, characterized by contracted kidneys and features changes exclusively in the renal cortex of the kidney. OTA has been found more frequently in food samples and in the serum of people taken from villages with BEN, than in areas where the disease is unknown (Krogh *et al.,* 1977; Pavlovic *et al.*, 1979, Petkova-Bocharova and Castegnaro 1985; and Petkova-Bocharova *et al.*, 1988). The blood of 95% of Tunisian people suffering from urinary system tumours (UST) are OTA positive, with blood concentrations higher than 90 $ng.ml^{-1}$ in several cases (Maaroufi *et al.*, 1999). OTA is also the cause of a nephropathy (Danish porcine nephropathy) affecting many pigs fed mouldy cereal feeds in Scandinavian countries. In Denmark carcasses are condemned if residue levels of OTA in the kidney exceed 25 $ng.g^{-1}$.

Genotoxicity

OTA has been considered from many years as a non-mutagenic carcinogen, since it has been generally negative in a number of gene mutation tests, based on both microorganisms and mammalian cells, and both with and without metabolic activation (Würgler *et al.*, 1991; Bendele *et al.*, 1995; Sakai *et al.*, 1992). However, OTA induced mutations in the modified Ames assay (Dirheimer, 1996), sister chromatid exchange in human peripheral lymphocytes *in vitro* and SOS DNA repair in *E. coli* (cited by Neal 1995; Hennig *et al.*, 1991). OTA-induced single-strand breaks were observed in primary rat hepatocytes (De Groene *et al.*, 1996b) and *in vivo* in rats and mice (Creppy *et al.*, 1985; Kane *et al.*, 1986). Furthermore, chromosomal aberrations could be detected after OTA exposure (Manalowa *et al.*, 1990) and DNA-adducts could be observed *in vivo* in the liver, kidney and spleen of rodents and humans (Pfohl-Leszkowicz *et al.*, 1993a, 1993b) and in cytochrome P450-expressing BEAS-2B cells (Grosse *et al.*, 1994, 1995) and monkey kidney cells (Grosse *et al.*, 1995). These adducts were different for the different organs suggesting different routes of metabolic activation. De Groene *et al.* (1996a) recently demonstrated that OTA mutagenecity requires a cytochrome P450-dependent activation step by using the *lacZ'* gene as reporter gene for mutations in cell lines expressing selected human cytochrome P450 forms.

Teratogenicity and Immunotoxicity

OTA is teratogenic to rats, mice, hamsters and chickens (Brown *et al.*, 1976; Shreeve *et al.*, 1977; Fukui *et al.,* 1987). It causes a marked increase in the number of dead and resorbed foetuses and a decrease in foetal body weight when administered to pregnant rats. Multiple gross, visceral and skeletal anomalies in pups are related to the treatment and dose of OTA administered to rats (Brown *et al.*, 1976). The subchronic exposure of Balb/c mice to OTA suppressed the antibody-production plague forming cells, decreased thymocyte cell counts and the proportion of mature thymic lymphocyte (CD^{4+} or CD^{8+}) cells (Creppy *et al.,* 1982, 1983b). The mitogenic responsiveness of thymocytes and splenocytes to concanavalin A (Con A) is also significantly decreased by OTA exposure. However, interleukin-2 production of Con A-stimulated lymphocytes, natural killer cell activity and humoral antibody titres to a viral antigen are not affected by OTA (Thuvander *et al.*, 1995). Thuvander and coworkers also reported immunosuppression in Balb/c mice (Thuvander *et al.*, 1997) and Sprague-Dawley rats (Thuvander *et al.*, 1996a) after prenatal exposure to OTA. OTA exposure resulted in a decrease in proliferation and antibody, thereby indicating that subchronic, oral exposure to OTA affects certain immune functions in mice but does not suppress immune functions in the offspring (Thuvander *et al.*, 1996b).

Pharmacokinetics of OTA

About 40-65% of OTA orally administered to rats is absorbed in the small intestine, primarily in the proximal part of the jejunum (Kumagai, 1988). OTA has a high binding affinity for plasma constituents and binds to serum-albumin and to an as yet unidentified macromolecule(s) as soon as it reaches the circulation system. This characteristic of OTA retards its elimination by limiting the transfer of OTA from the bloodstream to the hepatic and renal cells and consequently contributes to the prolonged half-life of the toxin (Chu, 1971, Stojkovic *et al.,* 1984; Kumagai, 1985; Hagelberg *et al.*, 1989). OTA also binds more specifically than plasma albumins to a smaller molecular fraction in blood (Stojkovic *et al.*, 1984). There may be a relation between the predominant nephrotoxic affect of OTA in mammals and the binding of OTA to these molecules because such molecules can easily pass through the normal glomular membrane enabling the accumulation of OTA into the kidney (Marquardt and Frohlich, 1992). The pharmacokinetics of OTA and its metabolites in rats were recently reported by Li *et al.*, (1997). The plasma half-life of OTA depends on the degree of absorption and the degree of binding to serum-albumin and a number of other factors. OTA has a very high affinity for this unknown macromolecule (see above) in human serum and may thus have a long plasma half-life. The toxic activity of OTA in monkeys, with an elimination half-life of 35 days and slow absorption from the gastrointestinal tract, seems to be a good model for humans (Hagelberg *et al.*, 1989). Stein *et al.* (1985) reported an efficient reabsorption of OTA by the renal tubules of the kidney that also facilitates reabsorption of OTA into the plasma. This process in the renal proximal tubules which may be responsible for damages to the kidneys of various animal species (Albassam *et al.*, 1987; Szczech *et al.*, 1973; Elling, 1979) was reported by Jung and Endou (1989) to affect only the middle and terminal portions of the nephrons.

Rats excrete OTα, OTA and (4*R*)-OH-OTA mainly in the bile and urine after OTA had been administered intraperitoneally or *per os* (Stϕren *et al*, 1982a, 1982b; Kane *et al.*, 1986; Xiao *et al.*, 1996b). Approximately 1-2% of the OTA was recovered as 4-OH-OTA and a total of 25-40% of the administered OTA was recovered as OTα and 6% as OTA. The relative quantity of OTA eliminated *via* the kidneys and the liver depends partly on the animal species, route of administration and dose, the enterohepatic recirculation and the binding of the toxins to serum macromolecules (Roth *et al.*, 1988). Data on elimination half-lifes and distribution half-lifes of other species are given in **Table 12**.

There are contradictory reports on the sub-chronic and chronic toxicity of OTA: some of the early reports regard OTA as the toxic agent, since its known metabolites are equally or less toxic than OTA, whereas other researchers consider the toxic effects as due to one of its metabolites, since the simultaneous feeding of phenobarbital increases the incidence of liver tumours seen after the feeding of OTA alone (Suzuki *et al.*, 1986) and other more recent findings described below. Phenobarbital is known to induce the activity of various constitutive cytochrome P450 forms in the liver (Soucek and Gut, 1992). Fink-Gremmels *et al.* (1995) provided evidence for a number of unknown metabolites produced by metabolically competent hepatocytes, whereas Malaveille *et al.* (1994) discussed the formation of an OTA phenoxide radical and a thiol derived toxic metabolite as reactive metabolites of OTA.

Distinct possibilities are that OTA might be metabolised yielding both detoxified products and other metabolites responsible for the mutagenic effect described above or OTA may induce DNA base modifications such as alkylation, m5dC and 8-hydroxyguanine due to OTA's induction of oxydative stress (El-Ghissassi *et al.*, 1995). 8-Hydroxyguanine is known to induce G to T and A to C mutations where m5dC could lead to impairment of the regulation (Cheng *et al.*, 1992).

Table 12: Pharmacokinetic data for OTA and some of its derivatives.

Species		OTA	OP-OTA	OTα	OTB	OTC	OTA-OH
Rats (iv)[a]	$t^1/_{2\alpha}$ (min)	160±17	163±5	31±5	14±4	6±1.2	19±4.7
	$t^1/_{2\alpha}$ (min)	126					
Rabbits[b]	$t^1/_{2\alpha}$ (min)	114					
Chickens[c]	$t^1/_{2\alpha}$ (min)	30					
Cattle[e]	$t^1/_{2\alpha}$ (min)	108					
Rats (iv)[a]	$t^1/_{2\beta}$ (h)	103±16	50.5±2.8	9.6±2.3	4.2±1.2	0.6±0.2	6±0.9
Rats (iv)[f]	$t^1/_{2\beta}$ (h)	120					
Rats (p)[f]	$t^1/_{2\beta}$ (h)	170					
Rats (o/iv)[b]	$t^1/_{2\beta}$ (h)	55					
Rats (o/iv)[g]	$t^1/_{2\beta}$ (h)	56					
Pig[c,f,h]	$t^1/_{2\beta}$ (h)	72-150					
Pre-ruminant calf[i]	$t^1/_{2\beta}$ (h)	77					
Mouse[f]	$t^1/_{2\beta}$ (h)	24-48					
Rabbits[c]	$t^1/_{2\beta}$ (h)	8.2					
Chickens[c]	$t^1/_{2\beta}$ (h)	4.1					
Monkeys[f]	$t^1/_{2\beta}$ (h)	840					

$t^1/_{2\alpha}$ = distribution half-life; $t^1/_{2\beta}$ = elimination half-life; p = peripherally, iv = intravenous, o = orally.
Sources: Galtier *et al.*, 1979[b]; Galtier *et al.*, 1981[c]; Mortensen *et al.*, 1983[h]; Ballinger *et al.*, 1986[g]; Fukui *et al.*, 1987[i]; Sreemannarayana *et al.*, 1988[e]; Hagelberg *et al.*, 1989[f]; Marquardt *et al.,* 1996[a];

Prevention of Ochratoxicoses

Superoxide dismutase (SOD) and catalase are enzymes which prevent most OTA-induced nephrotoxic effects and might be used for the prevention of such renal lesions (Baudrimont *et al.*, 1994). Vitamin C significantly reduces the effects of OTA on albino Swiss mice (Bose and Sinha, 1994). Other compounds that are efficient in preventing ochratoxicosis *in vivo* are radical scavengers, vitamins, prostaglandin synthesis inhibitors (*e.g.* indomethacin and aspirin), pH modificators and absorbant resins such as cholestyramine etc. (Madhyastha *et al.*, 1992; Baudrimont *et al.*, 1995; Creppy *et al.*, 1996). Compounds that have a high binding affinity for plasma proteins, such as piroxicam are also promising as potential antidotes for OTA (Creppy *et al.*, 1995). Aspartame, structurally related to OTA prevents OTA binding to plasma proteins and is the best candidate for preventing the OTA-induced subchronic effects (Creppy *et al.*, 1995).

A practical method to prevent ochratoxicosis is to reduce the levels of OTA contamination in foods and feedstuffs by certain cooking processes (Milanez and Leitao, 1996).

Mechanisms of Action of OTA

There appears to be a number of direct and several indirect effects of OTA. The best known effects of OTA are its effect on enzymes involved in the Phenylalanine (Phe) metabolism, its effect on lipid peroxidation and its effect on mitochondrial respiration.

Inhibition of Phe-tRNA Formation

OTA inhibits protein synthesis by competition with Phe in the Phe-tRNA aminoacylation reaction catalysed by phenylalanyl-tRNA synthetase (Creppy *et al.*, 1984). This was shown by Bunge *et al.* (1978), Creppy *et al.* (1979a,b) and Konrad and Röschenthaler (1977) in both bacterial and eukaryotic systems *in vitro*. This inhibition can be reversed by the administration of Phe in hepatoma cells (Creppy *et al.*, 1979a) and *in vivo* in mice (Creppy *et al.*, 1984). In addition to the inhibition of protein synthesis, DNA and RNA synthesis are consequently also inhibited (Crepy *et al.,* 1986). Phe also provides partial prenatal protection from the teratogenic effects of OTA (Mayura *et al.*, 1984) and prevents the immunosuppresive effects of OTA in Balb/c mice (Creppy *et al.* 1983a; 1983b; Haubeck *et al.* 1981). Creppy *et al.,* (1983a) found similar inhibitory effects in the respective tRNA synthetase enzymes, when the Phe was replaced by other amino acids. Roth *et al.* (1993) reported that the inhibitory effect on Phe-tRNA synthetase alone cannot explain the inhibitory effects of OTA on growth in bacteria and that other mechanisms involving OTA-activated substances must be involved. OTA also inhibits the activity of phosphoenolpyruvate carboxykinase (PEPCK) and γ-glutamyl transpeptidase and abolishes the cAMP-mediated increase in the concentration of PEPCK mRNA (Thekkumkara and Patel, 1989). Removal of the Phe moiety from OTA prevents the *in vivo* inhibition of PEPCK activity and protein synthesis (Meisner and Meisner

1981). OTA also inhibits other reactions in which Phe is involved like those catalysed by Phe-hydroxylase (Creppy *et al.*, 1990).

Lipid Peroxidation

Ochratoxin A disrupts hepatic microsomal calcium homeostasis by impairment of the endoplasmic reticulum membrane, probably via lipid peroxidation (Omar *et al.*, 1991). OTA greatly enhances the rate of NADPH- or ascorbate dependent lipid peroxidation both *in vivo* (rats) and *in vitro* (liver or kidney microsomes) as measured by malondialdehyde formation. The efficiency for lipid peroxidation enhancement is related to the presence of the phenolic hydroxyl group of the different ochratoxins and correlates well with their known toxicities (Rahimtula *et al.*, 1988, 1989). OTA stimulates lipid peroxidation primarily by chelating ferric ions (Fe^{3+}) and facilitating their reduction to ferrous ions (Fe^{2+}); the subsequent reoxidation is accompanied by O_2 consumption (Omar *et al.*, 1990). The Fe^{3+}- OTA complex produces the extremely damaging hydroxyl radical in the presence of the NADPH-cytochrome-P-450 reductase system and NADPH (Hasinoff *et al.*, 1990). The OTA-Fe^{2+} complex provides thus the active species which initiates lipid peroxidation in the presence of oxygen. Once this process is initiated, it can be easily propagated in the cellular environment where polyunsaturated fatty acids and oxygen are present. The oxidation of lipids by oxygen continues in a chain of radical reactions. As a consequence of this biochemical process, a wide range of degradation compounds are formed, which are chemically very reactive and produce structural injuries (Baudrimont *et al.*, 1997). There may be a connection between nephropathy caused by OTA, citrinin, iron and lipid peroxidation. Iron is presented to the tubular lumen in proteinuric states because of the glomerular leak of transferrin. Iron would be expected to be dissociated from transferrin in the tubular fluid because of its low pH and bicarbonate content, and exists in a form that could catalyse hydroxyl radical formation. It has been suggested that if iron is available in a form capable of catalysing OH-radical formation, it could result in lipid peroxidation of tubular cell membranes (Stφrmer *et al.*, 1996). Lipid peroxidation caused by the free reactive oxygen species induced by OTA can be prevented in Vero cells by adding superoxide dismutase and catalase, piroxicam or aspartame to the culture medium prior to OTA addition to the medium (Baudrimont *et al.*, 1997). Pfohl-Leszkowicz *et al.*, (1993a,b) linked this ability of OTA to enhance lipid peroxidation, to the genotoxicity expressed by DNA adduct formation. It is also possible that the OTA-induced DNA single strand breaks in mice and rats are produced by reactive oxygen species (Creppy *et al.*, 1985; Kane *et al.*, 1986).

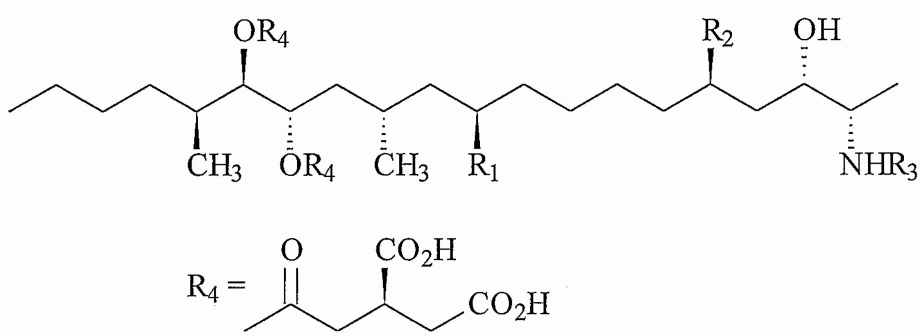
Inhibition of Mitochondrial ATP production

OTA inhibits mitochondrial state 3 and 4 respiration in isolated rat liver mitochondria (Moore and Truelove, 1970) by acting as a competitive inhibitor of mitochondrial transport carrier proteins located in the inner mitochondrial membrane (Meisner and Chan, 1974; Wei *et al.*, 1985; Meisner, 1976). OTA also alters the mitochondrial morphology after *in vivo* administration to rats (Suzuki *et al.*, 1975; Brown *et al.*, 1986). The mitochondrial uptake of OTA is an energy consuming process that results in the depletion of intramitochondrial ATP and the observed ATP decrease was most pronounced in the middle (S2) and the terminal (S3) segment of the proximal tubule (Jung and Endou, 1989). Aleo *et al.*, (1991) suggested that mitochondrial dysfunction is an early event during the development of OTA toxicity and that OTA toxicity to rat proximal tubules in suspension was not related to iron-mediated lipid peroxidation as measured by malondialdehyde production. The importance of the mitochondrial mechanism is not clear because OTα, which is non-toxic, was also able to inhibit mitochondrial respiration more effectively than OTA in rat liver mitochondria (Moore and Truelove, 1970).

FUMONISINS

The fumonisins are a group of mycotoxins, consisting of a 2-amino-12,16-dimethylpolyhydroxyeicosane backbone esterified with propane-1,2,3-tricarboxylic acid side chains on C_{14} and C_{15} (see **Figure 6**). These toxins were first discovered by a South African group led by Marasas (Bezuidenhout *et al.*, 1988; Gelderblom *et al.*, 1988) after an investigation into the cause of equine leukoencephalomalacia (LEM) or better known as 'hole in the head disease' in horses fed feeds contaminated with the fungus *Fusarium moniliforme.* LEM is a well known disease in many countries such as Mexico, the United States of America, Egypt, and South Africa and causes the liquefactive necrosis of the white matter of the brain of horses and donkeys. Corn contaminated with *F. moniliforme* has also been associated with human oesophageal cancer in the Transkei area of South Africa (Rheeder *et al.*, 1992) and China (Yang, 1980; Chu and Li, 1994). Fumonisins have also been reported to cause pulmonary edema syndrome in pigs (Harrison *et al.*, 1990) and a nondescribed poultry disease (ill thrift). Fumonisin A_1 (FA_1) and fumonisin A_2 (FA_2), the *N*-acetyl derivatives of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) respectively, are produced in low yields in cultures of *F. moniliforme* and have the lowest toxicities. These two structural analogues and FB_4 (**Figure 6**) do not occur under natural conditions. The C series of fumonisins (the chemical structures of the C series is identical to the B series except that the C-1 terminal methyl group is missing) was recently discovered in naturally contaminated corn by Seo and Lee (1999) in the presence of the B series, but at much lower concentrations. These compounds were previously found in an isolate of *Fusarium oxysporum* during a screening of fumonisins by the same group in 1996.

FB_1, the most abundant fumonisin in culture and naturally occurring in corn (Rheeder *et al.*,, 1995), has been shown to promote tumour formation in rats and to inhibit ceramide synthetase in neuronal cells, an important enzyme in sphingolipid biosynthesis (Wang *et al.*, 1991).



	R_1	R_2	R_3
Fumonisin A_1	OH	OH	CH_3CO
Fumonisin A_2	H	OH	CH_3CO
Fumonisin B_1	OH	OH	H
Fumonisin B_2	H	OH	H
Fumonisin B_3	OH	H	H
Fumonisin B_4	H	H	H

Figure 6: Structures of the fumonisins.

Chemical characteristics of the fumonisins

FB_1 is a stable compound that persists through most normal food processing procedures (see **Table 13** for the physical data of FB_1).

Table 13: Physical and spectroscopic data of FB_1.

Melting point:	103-105 °C
Optical rotation $[\alpha]_D$:	-28° (concentration 2 mg/ml)
Infrared (KBr):	3450, 2934, 1729 and 1632 cm^{-1}

Fumonisins are soluble in most polar solvents including water and are not soluble in non-polar solvents. There is no known detoxification process for fumonisin-contaminated foods and feeds. Bezuidenhout *et al.* (1988) elucidated the structure of the fumonisins by employing NMR and mass spectroscopic techniques, while the absolute configuration (see **Figure 6**) was determined by contributions from a number of authors including Blackwell *et al.* (1994a), ApSimon *et al.*, (1994) and Shier *et al.*, (1995) using a combination of NMR and chiral GC methods. Laurent *et al.* (1990), prepared the ammonium salt of FB_1, while the acetylated and methylated derivatives of FB_1 were prepared by Bezuidenhout *et al.*, (1988) and Laurent *et al.*, (1990) respectively. FB_1 and FB_2 can be hydrolysed by heating with hydrochloric acid or potassium hydroxide to yield the aminopentol of FB_1 and the aminotetraol of FB_2 (Gelderblom *et al.*, 1993).

Production of the fumonisins

F. moniliforme is the most important producer of the fumonisins, however, a number of other *Fusarium* species are known to be producers of these toxins as shown in **Table 14**.

Table 14: Fungal producers of fumonisins

Fusarium moniliforme	*Fusarium napiforme*
Fusarium dlamini	*Fusarium proliferatum*
Fusarium nygamai	*Fusarium anthophilum*
Fusarium subglutinans	

Source: Thiel *et al.* (1991); Marassas (1995) and references cited.

Optimum conditions for the production of fumonisins are: *F. moniliforme* is grown on wet sterilized corn and incubated at 20°C for 11-13 weeks (Alberts *et al.*, 1990 and Le Bars *et al.*, 1992). The mouldy material is extracted with aqueous methanol, and the fumonisin-containing extract purified by liquid-liquid partition; followed by cleanup with XAD-2, silica gel and reverse phase chromatography (Cawood *et al.*, 1991; Vesonder *et al.*, 1990). Although corn cultures are still the best way of producing large quantities of unlabeled fumonisins, liquid media are ideal for the production of unlabelled and ^{14}C labelled fumonisins (Miller, 1994). Liquid media usually consists of a carbon source like glucose or sucrose, a phosphate buffer (pH ± 4) and salts like $MgSO_4$, CaCl, NaCl, NH_4Cl, Na_2SO_4 and $MnSO_4$. Alberts *et al.* (1993), developed a technique for the production of [^{14}C]FB_1 by *F. moniliforme* MRC 826 in 'patty' corn cultures by using *L*-[methyl-^{14}C]methionine as the precursor (Blackwell *et al.*, 1994b).

Determination and occurrence of the fumonisins

A wide variety of extraction and clean-up methods exist for the determination of fumonisins. In general the feeds or foods are extracted with an organic solvent mixture, cleanup by liquid-liquid partition, solid phase extraction, column chromatography or immunoaffinity columns. Quantitation is done by TLC (Shelby *et al.*, 1994), HPLC (Thiel *et al.*, 1993), post-hydrolysis gas chromatography (Sydenham *et al.*, 1990), GC-MS (Plattner *et al.*, 1990), liquid secondary ion mass spectrometry (LCIMS), FAB/MS (Korfmacher *et al.*, 1991) or MS/MS (Plattner *et al.*, 1990). In many cases fumonisins are reacted with naphthalene-2,3-dicarboxyaldehyde - potassium cyanide; *o*-phthalaldehyde - mercapto-ethanol (Shephard *et al.*, 1990); FMOC (Holcomb *et al.*, 1993) or 4-fluoro-7-nitrobenzofurazan (Scott and Lawrence, 1992) to yield fluorescent derivatives which can easily be detected by HPLC systems equipped with fluorescent detectors. Several immunochemical methods for the determination of FB_1 have also been developed including enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies (Azcona-Olivera *et al.*, 1992a); polyclonal antibodies (Azcona-Olivera *et al.*, 1992b, Usleber *et al.*, 1994) and anti-idiotype/anti-anti-idiotype antibodies (Chu *et al.*, 1995). Cross-reaction with FB_2 and FB_3 is very low and immunochemical methods can thus underestimate the total concentration of fumonisins in a commodity; no cross-reaction occurs with the hydrolysed fumonisins. Schneider *et al.* (1995) developed a competitive direct dipstick enzyme immunoassay (EIA) and an enzyme-linked immunofiltration assay (ELIFA) for the detection of FB_1. A nylon membrane was coated with anti-FB_1 antibodies and with anti-horseradish peroxidase (HRP) antibodies. An FB_1-HRP conjugate was used both as the labelled antigen for competitive assay of FB_1 and for non-competitive binding to the anti-HRP antibodies (negative control). Immunoaffinity columns which use monoclonal antibodies are commercially available (FumoniTest, Vicam, Watertown, MA) and have detection limits at ppm levels. Detection limits are reported to be 100 ppb for TLC, 50 ppb for LC-MS and 200 ppb for ELISA (Thiel *et al*., 1996 and references cited).

Fumonisin contamination of corn and corn-based products has been reported on all continents. **Table 15** contains suggested safety limits for fumonisins in animal feeds (Thiel *et al.,* 1996 and references cited).

Table 15: Suggested safety limits for fumonisins

Species	Limit
cattle and poultry	50 000 ppb
Pigs	10 000 ppb
Horses	5 000 ppb

Source: Thiel *et al*. (1996) and references cited.

Table 16 contains some information regarding fumonisin contamination found in different countries. Corn (maize) is a staple food of many people living in Southern Africa; FB_1 contamination of corn may, therefore, pose a serious threat to human health in these regions.

Table 16: References to fumonisin contamination found in different countries.

Country	Commodity	Level	Author
Italy	Corn and corn based foods	<5310 ppb for FB_1 & <1480 ppb for FB_2	Doko and Visconti (1994)
Argentina	Corn	combined fumonisin levels of 1585 - 9990 ppb	Sydenham *et al*. (1993a)
Kenya	Corn	<1 ppm FB_1 (mostly)	Kedera *et al.* (1999)
Brazil	Corn and corn based feeds	0,2 - 38,5 ppm FB_1 & 0,1 - 12,0 ppm FB_2	Sydenham *et al*. (1994)
U.S.A.	Corn and feeds	1,3 - 27,0 ppm FB_1 & 0,1 - 12,6 ppm FB_2	Thiel *et al*. (1991)
India	Corn		Chatterjee and Mukherjee (1994)
India	Corn kernels	100-4740	Shetty and Bhat (1997)
Switzerland	Corn-based products		Pittet *et al*. (1992)
China	Corn	18-155 ppm FB_1	Chu and Li (1994)
New Zealand	Forage grass	1 and 9 ppm of FB_1	Mirocha *et al*. (1992)

Decontamination

The fine particulate matter (< 3 mm) in corn contains the highest levels of fumonisins. By removing the 'fines' from bulk shipments of corn the fumonisin contamination is reduced significantly (Sydenham *et al*., 1994). Sydenham *et al*., (1993b) developed a chemical method for the reduction of fumonisin levels in corn by treating it with a slurry of 0.1 M calcium hydroxide for 24 hours at 25 °C. The process of milling and the ammoniation treatment of contaminated corn also reduce the levels of fumonisins in corn products (Norred *et al.*, 1991). All these different treatments suggest that the fumonisins are concentrated on the outer pericarp layer of corn kernels (Sydenham *et al.*, 1993b).

Biological Effects and Mechanism of Action of the Fumonisins

FB_1 has been established to induce completely different toxic effects in different animal species [LEM in horses, hepatotoxic and hepatocarcinogenic to rats and pulmonary oedema syndrome in pigs (Marasas *et al.*, 1988; Harrison *et al.*, 1990)]. In rats the liver is the main target for toxicity, characterised by cirrhosis and cholangiofibrosis. FB_1 is also foetotoxic to rats, suppressing both growth and foetal bone development (Lebepe-Mazur *et al.,* 1995). FB_1, FB_2 and FB_3 also affect the kidneys of rats after prolonged exposure, but are, according to Gelderblom *et al.* (1988, 1992), not very acutely toxic to rats. Toxic response to fumonisins by rat hepatoma cell line H4TG was visible within 48 h with an IC_{50} value of 4 $\mu g.ml^{-1}$ for FB_1 and 2 $\mu g.ml^{-1}$ for FB_2 (Shier *et al.*, 1992). IC_{50} values were not affected by the density of the cell cultures, indicating that fumonisins are not metabolically activated to express their toxic effects. This was confirmed by the absence of metabolites of fumonisins in the urine, bile and blood of rats fed FB_1 and FB_2 (Shephard *et al.*, 1993, 1995) and in primary rat hepatocytes (Cawood *et al.*, 1994). FA_1 and FA_2 are less cytotoxic, but PA_1 and PA_2 the hydrolysis products have similar or greater toxicity than the parent compounds (Abbas *et al.,* 1993; Gelderblom *et al.*, 1993). Elimination half-times of 18 min and 40 min were found in toxicokinetic studies of FB_1 in blood plasma of rats and monkeys respectively (Shephard *et al.*, 1992; 1993). Fumonisins are non-mutagenic according to the *Salmonella* test and non-genotoxic according to the DNA-repair assay with *Eschericia coli* (Gelderblom *et al.*, 1996b) and do not induce unscheduled DNA synthesis in primary rat hepatocytes (Gelderblom *et al.*, 1989; Norred *et al.*, 1990). FB_1 mimics genotoxic carcinogens, both in cancer initiation and promotion (Gelderblom *et al.*, 1992, 1994a,b) and also with respect to the induction of resistant hepatocytes in rat liver. FB_1 induces gamma glutamyltranspeptidase (GGT) and the placental form of glutathione-S-transferase (GSTP). These enzymes are histological markers for putative preneoplastic lesions which are initiated by genotoxic carcinogens (Gelderblom *et al.*, 1996b). Cancer initiation is affected by the induction of 'resistant' hepatocytes, whose multiplication can be stimulated selectively by a cell proliferation stimulus in the presence of a 2-acetylaminofluorene induced mitoinhibitory effect (Gelderblom *et al.,* 1992 and 1993). When diethylnitrosamine is used as a cancer initiator, FB_1 acts as a cancer promoter, as indicated by the formation of γ-glutamyltranspeptidase and GSTP positive foci (Gelderblom *et al.*, 1988, 1996c). Fumonisins differ from genotoxic carcinogens in the sense that their cancer initiation step requires prolonged exposure of the fumonisin while in the case of genotoxic carcinogens this step is normally completed within a few hours or days (Gelderblom *et al.*, 1992). Gelderblom and co-workers, (1994b), for example, found that administration of a dose of 30.8 mg FB_1 per 100 g body weight to rats over a period of 21 days initiated cancer while the administration of a similar dosage over 7 days did not initiate cancer.

Of all the possible mechanisms of action of fumonisin toxicity in mammals, the one most studied is the inhibition of sphingosine and sphinganine *N*-acyltransferase. The disruption of sphingolipid biosynthesis, by the inhibition of the conversion of sphinganine to *N*-acyl-sphinganines (dihydroceramides) which precedes the introduction of the double bond of sphingosine, is reported to be connected with the diseases associated with fumonisins (Wang *et al.*, 1991). The disruption of the mechanism of sphingolipids (free long chain bases which are important components of cell membranes) could have serious effects on cell growth, differentiation and behaviour (Merrill, 1991). This action of fumonisins has been studied in rat liver hepatocytes (Wang *et al.*, 1991), mouse cerebellar neurons *in situ* (Merrill *et al.*, 1993) and *in vivo* in ponies (Wang *et al.*, 1992) and pigs (Riley *et al.*, 1993) and leads to the accumulation of sphingoid bases, which according to Schroeder *et al.*, (1994) is more likely to cause fumonisin mitogenicity than the inhibition of complex sphingolipid biosynthesis *per se*. Mitogens often affect cell transformations and this effect may explain the carcinogenicity of fumonisins. FB_1 and FB_2 are cytotoxic to renal epithelial (LLC-PK_1) cells and inhibit proliferation after a lag period of at least 24 h in which the cells appear to function normally. Inhibition of sphingolipid biosynthesis, with an EC_{50} of 10-15 μM for FB_1, occurred before cell proliferation and cell death thus supporting the hypothesis that this inhibition is an early event in the toxicity of fumonisins (Yoo *et al.*, 1992). Yoo *et al.* (1992), also found that the sphinganine levels increased greatly after only 6 hr exposure to 35 μM FB_1 in LLC-PK_1 cells, and that these cells are much less sensitive than primary rat hepatocytes to fumonisin inhibition of *de novo* sphingolipid biosynthesis. Riley and co-workers (1993) found a relationship between the lower toxicity of FB_1 to the kidneys than to the liver of Sprague-Dawley rats, and the degree of disruption of the sphingolipid metabolism of these two organs.

The significantly higher elevation of the levels of free sphingosine, free sphinganine and the free sphinganine:sphingosine ratios found in the kidney when compared to the liver were also closely reflected in the urine of the rats. Free long chain bases and lysosphingolipids modulate intracellular signalling systems [e.g. protein kinase C, enzymes of diacylglycerol and phosphatidic acid metabolism and the tyrosine kinase activity of the epidermal growth factor (EGF) receptor] are cytotoxic to some cells and affect protein translocation, ATPases and calcium homeostasis (Riley *et al.*, 1993 and references cited). Gelderblom *et al.*, (1995) demonstrated that FB_1 inhibits the mitogenic response of the EGF *in vitro* in primary hepatocytes. Very little is known about the mechanisms involved in the inhibition of growth-related responses in hepatocytes, although the disruption of fatty acid metabolism has been implicated as playing a role (Gelderblom *et al.*, 1996a; 1997).

Apart from the inhibition of sphingolipid biosynthesis, FB_1 has also been found to affect the synthesis of cellular lipids by altering the incorporation of palmitic acid (Gelderblom *et al.*, 1996a). Gelderblom and co-workers (1996a) monitored the fatty acid levels of the major phospholipids (phosphatidylethanolamine and phosphatidylcholine) and neutral lipid triacylglycerides *in vitro* in rat hepatocytes and *in vivo* in rats and found alterations in the n-6 fatty acid profiles and decreases in the free cholesterol (membrane associated) levels, which resulted in a higher phosphatidylcholine: cholesterol ratio; this suggested a more rigid membrane structure. A significant increase in the serum and the total cholesterol content of the liver was found when the highest level of 250 mg $FB_1.kg^{-1}$ was administered to rats. Fumonisins may thus have important effects on membrane components, the fatty acid storage pool and the accumulation of long chain fatty acids within the cell which could eventually lead to the disintegration of membrane structures and eventually result in cell death (Gelderblom *et al.*, 1996b; 1997).

Cell proliferation, an important factor in cancer initiation and promotion, is controlled by long chain fatty acids *via* their control of prostaglandin levels (Cornwell and Morisaki, 1984). Prostaglandins can either inhibit or stimulate cell proliferation depending on the cell type. *In vitro* studies using Balb/c 3T3 cells, have shown that arachidonic acid metabolism is required for the mitogenic response of the EGF (Nolan *et al.*, 1988; Handler *et al.*, 1990).

Primary hepatocytes exposed to FB_1 showed an accumulation of polyunsaturated fatty acids. Gavino and co-workers (1981) demonstrated that increased levels of polyunsaturated fatty acids are associated with lipid peroxidation in normal and cancer cells, which implies that FB_1

can indirectly cause lipid peroxidation (See Mechanisms of Action of Ochratoxin A) (Gelderblom *et al.,* 1996b, 1998).

FB_1 causes lesions in the liver, kidneys, heart and lungs and subsequent death of broiler chicks (Javed *et al.*, 1992). Qureshi and Hagler, (1991) found that FB_1 can effect the macrophage dependent immune system of chickens. These findings have been confirmed by Chatterjee and Mukherjee (1994) who found a significant reduction in the viability and phagocytic potential of macrophages from chicken peritoneal exudate cells. Fumonisin consumption may thus result in a decreased immune response, which results from FB_1-induced depressed macrophages and consequently leads to infections.

The short half-life of fumonisins in monkey plasma (only trace levels are left after 4 hr) indicates that the direct measurement of fumonisins in blood is not be suitable for the determination of fumonisin exposures in animals and humans. Methods have, therefore, been developed to monitor fumonisin toxicosis in monkeys by measuring the sphingamine:sphingosine ratio (Shephard *et al.*, 1993,1996).

FB_1 was found to be a powerful inducer of malondialdehyde (one of the secondary products formed during lipid peroxidation) and to inhibit both protein and DNA synthesis (Ennamany *et al.*, 1995; Mobio *et al.*, 1998; Abado-Becognee *et al.*, 1998).

FB_1 and FB_2 are phytotoxic and damage weed and crop cultivars like soybean and tomato. The primary site of fumonisin toxicity in jimson weed is the plasmalemma or tonoplast. It causes rapid, light-dependent cytoplasmic degeneration and chloroplast disruption (Abbas *et al.*, 1992). FB_1 and the TA-toxin produced by *Alternaria alternata* f.sp. *lycopersici* have similar structures and produce identical genotype-specific necrotic symptoms on detached leaves of resistant and susceptible tomato lines (Marasas *et al.,* 1996 and references cited).

FB_1 and TA-toxin (see **Figure 7**) are more phytotoxic to corn and tomato seedlings than FB_2 and FB_3 and cause reductions in shoot and root length (Lamprecht *et al.*, 1994). The mechanism of action in plants is not yet known.

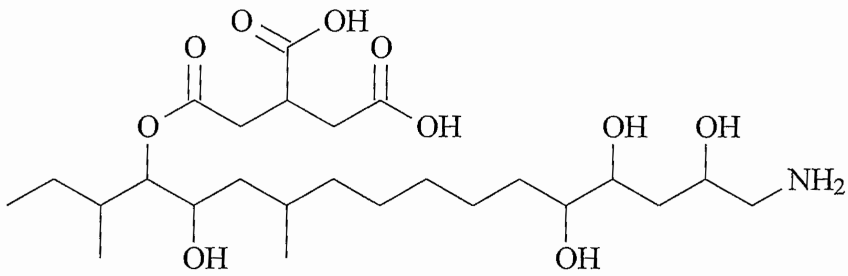


Figure 7: Structure of TA-toxin.

Conclusion

Sterling research is currently being done on mycotoxins. These efforts are focused on the molecular genetics of toxinogenic filamentous fungi (Bennett, 1994; O'Donnell, 1997; Bennet and Keller, 1997), a molecular understanding of the basic mechanism of their action; species differences in metabolism and pharmacokinetics; immunobased and physicochemical techniques for the quantification of mycotoxins; analysis of the risk involved in the exposure of man and domestic animals to mycotoxins (Kuiper-Goodman, 1989; Kuiper-Goodman and Scott, 1989; Kuiper-Goodman, 1996) and the associated regulations for the control of mycotoxin contamination (Kuiper-Goodman, 1995) and applying plant molecular biotechnological techniques to breed mycotoxin-resistant cereal- and nut-producing cultivars.

REFERENCES

Abado-Becognee, K., Mobio, T.A., Ennamany, R., Fleurat-Lessard, F., Shier, W.T., Badria, F., and Creppy, E.E. (1998). *Arch. Toxicol.*, **72**, 233-236.

Abarca, M.L., Bragulat, M.R., Castella, G. and Cabanes, F.J. (1994). Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. *Appl. Environ. Microbiol.*, **60**, 2650-2652.

Abbas, H.K., Paul, R.N., Boyette, C.D. and Duke, S.O. (1992). Physiological and ultrastructural effects of fumonisin on jimsonweed leaves. *Can. J. Bot.*, **70**, 1824-1833.

Abbas, H.K., Gelderblom, W.C.A., Cawood, M.E. and Shier, W.T. (1993). Biological activities of fumonisins, mycotoxins from *Fusarium moniliforme*, in jimsonweed (*Datura stramonium* L.) and mammalian cell cultures. *Toxicon*, **31**, 345-353.

Achour, A., El-May, M., Bacha, H., Hamammi, M., Maaroufi, K., and Creppy, E.E. (1993). Néphropathies interstitielles chroniques. Approches cliniques et étiologiques; Ochratoxine A. dans Human Ochratoxicosis and its pathologies (E.E. Creppy, M. Castegnaro, G. Dirheimer, eds) John Libbey Eurotext INSERM 231, 227-234.

Albassam, M.A., Yong, S.I., Bhatnagar, R., Sharma, A.K. and Prior, M.G. (1987). Histopathologic and electron microscopic studies on the acute toxicity of ochratoxin A in rats. *Vet. Pathol.*, **24**, 427.

Alberts, J.F., Gelderblom, W.C.A., Thiel, P.G., Marasas, W.F.O., van Schalkwyk, D.J. and Behrend, Y. (1990). Effects of temperature and incubation period on the production of fumonisin B_1, by *Fusarium moniliforme. Appl. Environ. Microbiol.,* **56**, 1729-1733.

Alberts, J.F., Gelderblom, W.C.A., Marasas, W.F.O. and Rheeder, J.P. (1993). Evaluation of liquid media for fumonisin production by *Fusarium moniliforme* MCR 826. *Mycotoxin Research,* **10**, 107-115.

Aleo, M.D., Wyatt, R.D. and Schnellmann R.G. (1991). Mitochondrial dysfunction is an early event in ochratoxin A but not oosporein toxicity to rat renal proximal tubules. *Toxicol. Appl. Pharmacol.*, **107**, 73-80.

ApSimon, J.W., Blackwell, B.A., Blais, L., Fielder, D.A., Greenhalgh, R., Kasitu, G., Miller, J.D. and Savard, M. (1990). Mycotoxins from *Fusarium* species: detection, determination and variety. *Pure Appl. Chem.*, **62** , 1339-1346.

ApSimon, J.W., Blackwell, B.A., Edwards, O.E. and Fruchier, A. (1994). Relative configuration of the C-1 to C-5 fragment of fumonisin B_1, *Tetrahedron Lett.*, **35** (42), 7703-7706.

Azcona-Olivera, J.I., Abouzied, M.M., Plattner, R.D., and Pestka, J.J. (1992a). Production of monoclonal antibodies to mycotoxins fumonisin B_1, B_2 and B_3. *J. Agric. Food Chem,* **40**, 531-534.

Azcona-Olivera, J.I., Abouzied, M.M., Plattner, R.D., Norred, W.P. and Pestka, J.J. (1992b). Generation of antibodies reactive with fumonisins B_1, B_2 and B_3 by using cholera toxin as the carrier-adjuvant. *Appl. Environ. Microbiol.* **58** , 169-173.

Bacha, H., Maaroufi, K., Achour, A., Hamammi, M., Ellouz, F., and Creppy, E.E. (1993). Ochratoxines et ochratoxicoses humaines en Tunisie. . dans Human Ochratoxicosis and its pathologies (E.E. Creppy, M. Castegnaro, G. Dirheimer, eds) John Libbey Eurotext INSERM 231, 111-121.

Ballinger, M.B., Phillips, T.D. and Kubena, L.F. (1986). Assessment of the distribution and elimination of ochratoxin A in the pregnant rat. *J. Food Safety*, **8**, 1-24.

Baudrimont, I., Betbeder, A.M., Gharbi, A., Pfohl-Leszkowicz, A., Dirheimer, G. and Creppy, E.E. (1994). Effect of superoxide dismutase and catalase on the nephrotoxicity induced by subchronical administration of ochratoxin A in rats. *Toxicology*, **89**, 101-111.

Baudrimont, I., Murn, M., Betbeder, A.M., Guilcher, J., and Creppy, E.E. (1995). Effect of piroxicam on the nephrtoxicity induced by ochratoxin A in rats. *Toxicology*, **95**, 147-154.

Baudrimont, I., Ahouandjivo, R. and Creppy, E.E. (1997). Prevention of lipid peroxidation induced by ochratoxin A in Vero cells in culture by several agents. *Chemico Biol. Int.,* **104**, 29.

Beardall, J.M. and Miller, J.D. (1994). Diseases in humans with mycotoxins as possible causes. In: Miller, J.D. and Trenholm, H.L. (Eds.), *Mycotoxins in grain: Compounds other than aflatoxin*. Eagan Press, St Paul, MN. pp. 487-539

Bendele, A.M., Neal, S.B., Oberly, T.J., Thompson, C.Z., Bewsey, B.J., Rexroat, L.E., Carlton, W.W. and Probst, G. (1995). Evaluation of ochratoxin A for mutagenecity in a battery of bacterial and mammalian cell assays. *Food Chem. Toxicol.*, **23**, 911-918.

Bennett, J.W. (1994). From molecular genetics and secondary metabolism to molecular metabolites and secondary genetics. *Can. J. Bot.*, **73,** Suppl. 1, S917-S924.

Bennett, J.W. and Keller, N.P. (1997). Mycotoxins and their prevention. In: Anke, T. (Ed.), *Fungal Biotechnology,* Chapman and Hall, Weinheim, pp. 265-273.

Bennett, J.W., Bhatnagar, D. and Chang, P.K. (1994). The molecular genetics of aflatoxin biosynthesis. In: Powell, K.A. *et al.,* (Eds.), *The Genus Aspergillus*. Plenum Press, New York, pp. 51-58.

Betina, V. (1984). Citrinin and related substances. In: Betina, V. (Ed.), *Mycotoxins: Production, Isolation, Separation andPurification*. Elsevier, Amsterdam, pp. 217-236.

Bezuidenhout, S.C., Gelderblom, W.C.A., Gorst-Allman, C.P., Horak, R.M., Marasas, W.F.O., Spiteller, G. and Vleggaar, R. (1988). Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme. J. Chem. Soc., Chem. Commun.*, 743-745.

Blackwell, B.A., Edwards, O.E., ApSimon, J.W. and Fruchier, A. (1994a). Relative configuration of the C-10 to C-16 fragment of fumonisin B_1. *Tetrahedron Lett.*, **36** (12), 1973-1976.

Blackwell, B.A., Miller, J.D. and Savard, M.E. (1994b). Production of Carbon-14-labeled fumonisins in liquid culture. *J. AOAC Int.*, **77**, 507-511.

Bonna, R.J., Aulerich, R.J., Bursian, S.J., Poppenga, R.H., Braselton, W.E. and Watson, G.L. (1991). Efficacy of hydrated sodium calcium aluminosilicate and activated charcoal in reducing the toxicity of dietary aflatoxin to mink. *Arch. Environ. Contam. Toxicol.*, **20**, 441-447.

Boorman, G.A., Hong, H.L., Dieter, M.P., Hayes, H.T., Pohland, A.E., Stack, M. and Luster, M.I. (1984). Myelotoxicity and macrophage alteration in mice exposed to ochratoxin A. *Toxicol. Appl. Pharmacol.,* **72**, 304-312.

Bose, S. and Sinha, S.P. (1994). Modulation of ochratoxin-produced genotoxicity in mice by vitamin C. *Food Chem. Toxicol.*, **32** (6), 533-537.

Bragulat, M.R., Abarca, M.L., Accensi, F., and Cabanes, F.J. (1998). New screening method for ochratoxigenic molds in pure cultures. *Revue Méd. Vét.*, **149**, 515.

Bredenkamp, M.W., Dillen, J.L.M., Van Rooyen, P.H. and Steyn, P.S. (1989). Crystal structures and conformational analysis of ochratoxin A and B: Probing the chemical structure causing toxicity. *J. Chem. Soc. Perkin Trans.* II, 1835-1839.

Bressac, B., Kew, M., Wands, J. and Ozturk, M. (1991). Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature,* **350**, 429-431.

Brown, M.H., Szczech, G.M. and Purmalis, B.P. (1976). Teratogenic and toxic effects of ochratoxin A in Rats. *Toxicol. Appl. Pharmacol.*, **37**, 331.

Brown, T.P., Manning, R.O. and Fletcher, O.J. (1986). The individual and combined effects of citrinin and ochratoxin A on renal ultrastructure in layer chicks. *Avian Dis.*, **30** (1), 191-198.

Büchi, G., Foulkes, D.M., Kurono, H. and Mitchell, G.F. (1966). Total synthesis of racemic aflatoxin B_1. *J. Amer. Chem. Soc.*, **87**, 882.

Bunge, I., Dirheimer, G. and Röschenthaler, R. (1978). *In vivo* and *in vitro* inhibition of protein synthesis in *Bacillus stearothermophilus* by ochratoxin A. *Biochem. Biophys. Res. Commun.*, **83**, 398.

Carnaghan, R.B.A., Hartley, R.D. and O'Kelly, J. (1963). Toxicity and fluorescence properties of the aflatoxins. *Nature*, **200**, 1101.

Carter, R.M., Jacobs, M.B., Lubrano, G.J. and Guilbault, G.G. (1997). Rapid detection of aflatoxin B_1 with immunochemical optrodes. *Analytical Letters*, **30,** 1465-1482.

Cary, J.W., Barnaby, N., Ehrlich, K.C. and Bhatnagar, D. (1999). Isolation and characterization of experimentally induced, aflatoxin biosynthetic pathway deletion mutants of *Aspergillus parasiticus. Appl. Microbiol. Biotechnol.*, **51**, 808-812.

Castegnaro, M., Maru, V., Maru, G. and Ruiz-Lopez, M-D. (1990). High-performance liquid chromatographic determination of ochratoxin A and its 4*R*-4-Hydroxy metabolite in human urine. *Analyst,* **115**, 129-131.

Cawood, M.E., Gelderblom, W.C.A., Vleggaar, R., Behrend, Y., Thiel, P.G. and Marasas, W.F.O. (1991). Isolation of the fumonisin mycotoxins: a quantitative approach. *J. Agric. Food Chem.*, **39**, 1958-1962.

Cawood, M.E., Gelderblom, W.C.A., Alberts, J.F. and Snyman, S.D. (1994). Interaction of ^{14}C-labelled fumonisin B mycotoxins with primary hepatocyte cultures. *Food Chem. Toxicol.*, **32**, 627-632.

Cepeda, A., Franco, C.M., Fente, C.A., Vazquez, B.I., Rodriquez, J.L., Prognon, P. and Mahuzier, G., (1996). Postcolumn excitation of aflatoxins using cyclodextrins in liquid chromatography for food analysis. *J. Chromatogr. A,* **721**, 59-68.

Chatterjee, D. and Mukherjee, S.K. (1994). Contamination of Indian maize with fumonisin B_1 and its effects on chicken macrophage. *Lett. Appl. Microbiol.*, **18**, 251-253.

Chatterjee, M. and Townsend, C.A. (1994). Evidence for the probable final steps in aflatoxin biosynthesis. *J. Org. Chem.*, **59**, 4424-4429.

Cheng, K.C., Cahill, D.S., Kasai, H., Nishimura, S., and Loeb, L.A. (1992). 8-hydroxyguanine, an abundant form of oxidative DNA damage, causes G to T and A to C substitutions. *J. Biol. Chem.* **267**, 166-172.

Chu, F.S. (1971). Interaction of ochratoxin A with bovine serum albumin. *Arch. Biochem. Biophys.*, **147**, 359-366.

Chu, F.S. (1974). Studies in ochratoxin. *CRC Crit. Rev. Toxicol.*, **2**, 499.

Chu, F.S. (1992). Recent progress on analytical techniques for mycotoxins in feedstuffs. *J. Anim. Sci.,* **70**, 3950-3963.

Chu, F.S. and Li, G.Y. (1994). Simultaneous occurrence of fumonisin B_1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. *Appl. Environ. Microbiol.*, **60**, 847-852.

Chu, F.S., Huang, X. and Maragos, C.M. (1995). Production and characterization of anti-idiotype and anti-anti-idiotype antibodies against fumonisin B_1. *JAOAC.*, **43**, 261-267.

Ciegler, A. (1975). Mycotoxins. Occurrence, chemistry and biological activity. *Lloydia,* **38**, 21- 35.

Cohen, H. and Lapointe, M. (1986). Determination of ochratoxin A in animal feed and cereal grains by liquid chromatography with fluorescence detection. *JAOAC.*, **69**, 957-959.

Cole, R.J. (1986). Modern methods in the analysis and structural elucidation of mycotoxins. Academic Press, New York.

Cole, R.J. and Cotty, P.J. (1990). Biocontrol of aflatoxin production by using biocompetitive agents. In: Robens, J.F. (Ed.), *A perspective on aflatoxin in field crops and animal food products in the United States*. Agricultural Research Service, Beltsville, MD. pp .62-68.

Cornwell, D.G. and Morisaki, N. (1984). FA paradoxes in the control of cell proliferation: prostaglandins, lipid peroxides and cooxidation reactions. In: Free Radicals in Biology VI. Proyor W. A. (Ed.) Academic Press, Orlando, FL. pp. 95-148.

Cotty, P.J. (1994). Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the populations of *A. flavus* infecting cotton bolls and on the aflatoxin content of cottonseed. *Phytopathology*, **84**, 1270-1277.

Creppy, E.E., Lugnier, A.A.J., Beck, G., Röschenthaler, R. and Dirheimer, G. (1979a). Action of ochratoxin A on cultured heptoma cells, revision of inhibition by phenylalanine. *FEBS Lett.,* **104**, 287-290.

Creppy, E.E., Lugnier, A.A.J., Fasiolo, F., Heller, K., Röschenthaler, R. and Dirheimer, G. (1979b). *In vitro* inhibition of yeast phenylalanyl-tRNA synthetase by ochratoxin A. *Chem. Biol. Int.,* **24**, 257.

Creppy, E.E., Schlegel, M., Röschenthaler, R. and Dirheimer, G. (1980). Phenylalanine prevents acute poisoning by ochratoxin A in mice. *Toxicol. Lett.,* **6**, 77-80.

Creppy, E.E., Lorkowski, G., Rösenthaler, R., and Dirheimer, G. (1982). Kinetics of immunosuppresive action of ochratoxin A on mice. Mycotoxins and Phytotoxins, Technical University Vienna Ed., 289-292.

Creppy, E.E., Kern, D., Steyn, P.S., Vleggaar, R., Röschenthaler, R. and Dirheimer, G. (1983a). Comparative study of the effect of ochratoxin A analogues on yeast aminoacyl-tRNA synthetase and on the growth and protein synthesis of hepatoma cells, *Toxicol. Lett.*, **19**, 217-224.

Creppy, E. E., Stφrmer, F.C., Röschenthaler, R. and Dirheimer, G. (1983b). Effects of two metabolites of ochratoxin A, (4*R*)-hydroxyochratoxin A and ochratoxin α on the immune response in mice., *Infect. Immun.* **39**, 1015-1018.

Creppy, E. E., Röschenthaler, R. and Dirheimer, G. (1984). Inhibition of protein synthesis in mice by ochratoxin A and its prevention by phenylalanine. *Food Chem. Toxicol.,* **22**, 883-886.

Creppy, E. E., Kane, A., Dirheimer, G., Lafarge-Fraysinet, C., Mousset, S. and Fraysinet, C. (1985). Genotoxicity of ochratoxin A in mice: DNA single-strand break evaluation in spleen, liver and kidney. *Toxicol. Lett.*, **28**, 29-35.

Creppy, E. E., Giessen-Crouse, E., Roth, A., Röschenthaler, R. and Dirheimer, G. (1986). Effect of ochratoxin A on enzyme activities and macromolecules synthesis in MDCK cells. *Arch. Toxicol. Suppl.* **9**, 310-314.

Creppy, E.E., Chakor, K., Fisher, M.J. and Dirheimer, G. (1990). The mycotoxin ochratoxin A is a substrate for phenylalanine hydroxylase in isolated rat hepatocytes and *in vivo. Arch. Toxicol.*, **64**, 279-284.

Creppy, E.E., Castegnaro, M. and Dirheimer, G. (Eds.) (1993). Human ochratoxicosis and its pathologies. John Libbey Eurotext Ltd., Vol 231.

Creppy, E.E., Baudrimont, I. and Betbeder, A-M. (1995). Prevention of nephrotoxicity of ochratoxin A, a food contaminant. *Toxicol. Lett.*, **82/83**, 869-877.

Creppy, E.E., Baudrimont, I., Belmadoni, A. and Betbeder, A-M. (1996). Aspartame as a preventive agent of chronic toxic effects of ochratoxin A in experimental animals. *J. Toxicol.,* **15**, 207-221.

Culvenor, C.C.J., Edgar, J.A., Mackay, M.F., Gorst-Allman, C.P., Marasas, W.F.O., Steyn, P.S., Vleggaar, R. and Wessels, P.L. (1989). Structure elucidation and absolute configuration of phomopsin A, a hexapeptide mycotoxin produced by *Phomopsis leptostromiformis*. *Tetrahedron*, **45**, 2351-2372.

Davis, N.D., Currier, G.C. and Diener, U.L. (1986). Aflatoxin contamination of corn hybrids in Alabama. *Cereal Chemistry*, **63**, 467-470.

Degen, G.H. and Neumann, H. G. (1978). The major metabolite of aflatoxin B_1 in the rat is a glutathione conjugate. *Carcinogenesis*, **11**, 823-827.

De Groene, E.M., Hassing, G.I.A., Blom, M.J., Seinen, W., Fink-Gremmels, J. and Horbach, G.J. (1996a). Development of human cytochrome P450 expressing cell lines; application in mutagenicity testing of ochratoxin A. *Cancer Res.*, **56**, 299-304.

De Groene, E.M., Jahn, A., Horbach, G.J. and Fink-Gremmels, J. (1996b). Mutagenecity and genotoxicity of the mycotoxin ochratoxin A. *Environ. Toxicol. Pharmacol.*, **1**, 21-26.

De Jesus, A.E., Steyn, P.S., Vleggaar, R. and Wessels, P.L. (1980). Carbon-13 nuclear magnetic resonance assignments and biosynthesis of the mycotoxin ochratoxin A. *J. Chem. Soc. Perkin Trans.*, **I**, 52-54.

De Jesus, A.E., Steyn, P.S., Van Heerden, F.R., Vleggaar, R., Wessels, P.L. and Hull, W.E. (1983). Tremorgenic mycotoxins from *Penicillium crustosum.* Isolation of penitrems A-F and the structure elucidation and absolute configuration of penitrem A. *J. Chem. Soc., Perkin Trans. I*, 1847-1856.

Detroy, R.W. and Ciegler, A. (1971). Aflatoxin biosynthesis in *Aspergillus parasiticus*: effect of methionine analogs. *Can. J. Microbiol.*, **17**, 569-574.

Dirheimer, G. (1996), (personal communication).

Doko, M.B. and Visconti, A. (1994). Occurrence of fumonisins B_1 and B_2 in corn and corn-based human foodstuffs in Italy. *Food Addit. Contam.*, **11**, 433-439.

Dorner, J.W. and Cole, R.J. (1989). Comparison of two ELISA screening tests with liquid chromatography for determination of aflatoxins in raw peanuts. *JAOAC.*, **72**, 962-964.

El-Banna, A.A. and Scott, P.M. (1986). Fate of mycotoxins during processing of foodstuffs III. Ochratoxin A during cooking of faba beans (*Vicia faba*) and polished wheat. *J. Food Prot.*, **47** (3),189-192.

El-Ghissassi, F., Barbin, A., Nair, A., and Bartsch, H. (1995). Formation of 1,N^6-ethenoadenine and 3, N^4-ethenocytosine by lipid peroxidation products and nucleic acid bases. Chem. Res. Toxicol., 8, 279-283.

Elizalde-González, M.P., Mattusch, J., and Wennrich, R. (1998). Stability and determination of aflatoxins by high-performance liquid chromatography with amperometric detection. *Journal of Chromatography A*, **828**, 439-444.

Elling, F. (1979). Ochratoxin A-induced mycotoxic porcine nephropathy: Alterations in enzyme activity in tubular cells. *Acta Pathol. Microbiol. Scand.*, **87**, 237.

Ennamany, R., Marzetto, S., Saboureau, D., and Creppy, E.E. (1995). *Cell Biol. Toxicol.*, **11**, 347-354.

Engel, G. and Teuber, M. (1984). Patulin and other small lactones. In: Betina, V. (Ed.), Mycotoxins: Production, isolation, separation and purification. 291-314 (Elsevier, Amsterdam).

Engler, K.H., Coker, R.D. and Evans, I.H. (1999). A colorimetrtic technique for detecting trichothecenes and assessing relative potencies. *Appl. Environ. Microbio*l. **65,** 1854-1857.

Essigmann, J.M, Green, C.L., Croy, R.G., Fowler, K., Büchi, G. and Wogan, G.N. (1982). Interactions of aflatoxin B_1 and alkylating agents with DNA: structural and functional studies. *Cold Spring Harbor Sympos. Quant. Biol.*, **47**, 327-337.

FAO (1996). *Worldwide Regulations for Mycotoxins, 1995. A Compendium*. FAO Food and Nutrition Paper. : Food and Agriculture Organization, Rome.

Fink-Gremmels, J., Jahn, A. and Blom, M.J. (1995). Toxicity and metabolism of ochratoxin A. *Natural Toxins*, **3**, 214-220.

Fink-Gremmels, J. (1996). Personal communication.

Forrester, L.M., Neal, G.E., Judah, D.J., Glancey, M.J. and Wolf, C.R. (1990). Evidence for involvement of multiple forms of cytochrome P450 in aflatoxin B_1 metabolism in human liver. *Proc. Natl. Acad. Sci. USA*, **87**, 8306-8310.

Franco, J., (1996). Mycotoxins in nuts. Proceedings of the IX International IUPAC Symposium on Mycotoxins and Phycotoxins, Rome, 334-335.

Frohlich, A.A., Marquardt, R.R. and Bernatsky, A. (1988). Quantitation of ochratoxin A: use of reverse phase thin-layer chromatography for sample cleanup followed by liquid chromatography or direct fluorescence measurement. *JAOAC.*, **71**, 949-953.

Fukui, Y., Hoshino, K., Kameyama, Y., Yasui, T., Toda, C. and Nagano, H. (1987). Placental transfer of ochratoxin A and its cytotoxic effect on the mouse embryonic brain. *Food Chem. Toxicol.*, **25**, 17.

Galtier, P., Charpenteau, J.L., Alvinerie, M. and Labouche, C. (1979). The pharmacokinetic profile of ochratoxin A in the rat after oral and intravenous administration. *Drug Metabol. Dispos.*, **7**, 429-434.

Galtier, P., Alvinerie, M. and Charpenteau, J.L. (1981). The pharmacokinetic profiles of ochratoxin A in pigs, rabbits and chickens. *Fd. Cosmet. Toxicol.* **19**, 735-738.

Gavino, V.C., Miller, J.S., Ikharebha, S.O., Milo, G.E. and Maizewell, D.G. (1981). Effect of polyunsaturated fatty acid and antioxidants on lipid peroxidation in tissue cultures. *J. Lipid Res.*, **22**, 763-769.

Gelderblom, W.C.A., Jaskiewicz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vleggaar, R. and Kriek, N.P.J. (1988). Fumonisins novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.*, **54**, 1806-1811.

Gelderblom, W.C.A., Marasas, W.F.O., Thiel, P.G., Semple, E. and Farber, E. (1989). Possible non-genotoxic effects of active carcinogenic components produced by *Fusarium moniliforme. Proc. Am. Assoc. Cancer Res.*, **30**, 144.

Gelderblom, W.C.A., Marasas, W.F.O., Vleggaar, R., Thiel, P.G. and Cawood, M.E. (1992). Fumonisins: Isolation, chemical characterization and biological effects. *Mycopathologia*, **117**, 11-16.

Gelderblom, W.C.A., Cawood, M.E., Snyman, D., Vleggaar, R. and Marasas, W.F.O. (1993). Structure-activity relationships of fumonisins in short-term carcinogenesis and cytotoxicity assays. *Food Chem. Toxicol.* , **31**, 407-414.

Gelderblom, W.C.A., Cawood, M.E., Snyman, D. and Marasas, W.F.O. (1994a). Fumonisin B_1 dosimetry in relation to cancer initiation in rat liver. *Carcinogenesis*, **15**, 209-214.

Gelderblom, W.C.A., Kriek, N.P.J., Marasas, W.F.O. and Thiel, P.G. (1994b). Toxicity and carcinogenecity of the *Fusarium moniliforme* metabolite, fumonisin B_1 in rats. *Carcinogenesis*, **12**, 1247-1251.

Gelderblom, W.C.A., Snyman, S.D., Van der Westhuizen, L. and Marasas, W.F.O. (1995). Mitoinhibitory effect of fumonisin B_1 on the EGF-induced mitogenic response in primary rat hepatocytes. *Carcinogenesis*, **16**, 625-631.

Gelderblom, W.C.A., Smuts, C.M., Abel, S., Snyman, S.D., Cawood, M.E., van der Westhuizen, L. and Swanevelder, S. (1996a). Effects of fumonisin B_1 on protein and lipid synthesis in primary rat hepatocytes. *Food Chem. Toxicol.*, **34**, 361-369.

Gelderblom, W.C.A., Snyman, S.D., Abel, S., Lebepe-Mazur, S., Smuts, C.M., van der Westhuizen, L., Marasas, W.F.O., Victor, T.C., Knasmulle, R.S. and Huber, W. (1996b). Hepatotoxicity - and carcinogenecity of the fumonisins in rats: a review regarding mechanistic implications for establishing risk in humans. In: Jackson, L.S., De Vries, I.W. and Bullerman L.D. (Eds.), *Fumonisins in Food*, Plenum Press, New York, pp. 279-296.

Gelderblom, W.C.A., Snyman, S.D., Lebepe-Masur, S., Van der Westhuizen, L., Kriek, N.P.J. and Marasas W.F.O. (1996c). The cancer promoting potencial of fumonisin B_1 in rat liver using diethyl nitrosamine as a cancer initiator. *Cancer Lett.*, **109**, 101-108.

Gelderblom, W.C.A., Smuts, C.M., Abel, S., Snyman, S.D., Van der Westhuizen, L., Huber, W.W. and Swanevelder, S. (1997). Effects of fumonisin B_1 on the levels and fatty acid composition of selected lipids in rat liver *in vivo. Food Chem. Toxicol.*, 35, 647-656.

Gilbert, J .(1993). Recent advances in analytical methods for mycotoxins. *Food Addit. Contam.*, **10**, 37-48.

Gilbert, J. (1995). Analysis of mycotoxins in food and feed: Certification of DON in wheat and maize. *Nat. Toxins*, **3**, 263-268.

Gopalakrishnan, S., Harris, T.M. and Stone, M.P. (1990). Intercalation of aflatoxin B_1 in two oligodeoxynucleotide adducts, comparative proton NMR analysis of d(ATC^{AFB}GAT).d(ATCGAT) and d(AT^{AFB}GCAT)2. *Biochemistry*, **29**, 10438-10448.

Goto, T., Wicklow, D.T. and Ito, Y. (1996). Aflatoxin and cyclopiazonic acid production by a sclerotium-producing *Aspergillus tamarii* strain. *Appl. Environ. Microbiol.*, **62**, 4036-4038.

Groopman, J.D., Hall, A.J. and Whittle, H. (1992). Molecular dosimetry of aflatoxin-N7-guanine in human urine obtained in Gambia, West Africa. *Cancer Epidemiol. Biomarkers Prevention,* **1,** 221-227.

Grosse, Y., Pfeifer, A., Mace, K., Harris, C.C., Dirheimer, G. and Pfohl-Leszkowicz, A. (1994). Biotransformation of ochratoxin A by human broncial epithelial cells (BEAS-2B) expressing human cytochrome P450s and implication of glutathione conjugation, *Toxicol. Lett.*, **74S**, 33.

Grosse, Y., Baudrimont, I., Castegnaro, M., Betbeder, A-M., Creppy, E.E., Dirheimer, G. and Pfohl-Leszkowicz, A. (1995). Formation of ochratoxin A metabolites and DNA-adducts in monkey kidney cells. *Chem.-Biol. Int.*, **95**, 175-187.

Grove, J.F. (1996). Non-macrocyclic trichothecenes, Part (2), *Prog. Chem. Org. Nat. Prod.*, **69**, 1-70 and references cited therein.

Hadidane, R., Bacha, H., Creppy, E.E., Hammami, M., Ellouze, F. and Dirheimer, G. (1992). Isolation and structure determination of natural analogues of the mycotoxin ochratoxin A produced by *Aspergillus ochraceus. Toxicology*, **76**, 233-243.

Hagelberg, S., Hult, K. and Fuchs, R. (1989). Toxicokinetics of ochratoxin A in several species and its plasma binding properties. *J. Appl. Toxicol.*, **9**, 91-96.

Handler, J.A., Danilowics, M. and Eling, T.E. (1990). Mitogenic signalling by epidermal growth factor requires arachidonic metabolism in BALB/c 3T3 cells. *J. Biol. Chem.,* **265**, 3669-3673.

Hansen, C.E., Dueland, S., Drevon, C.A. and Stфrmer, F.C. (1982). Metabolism of ochratoxin A by primary cultures of rat hepatocytes. *Appl. Environ. Microbiol.*, **43**, 1267-1271.

Harrison, L.R., Colvin, B.M., Greene, J.T., Newman, L.E. and Cole, J.R. (1990). Pulmonary edema and hydrothorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.*, **2**, 217-221.

Harvey, R.B., Kubena, L.F., Phillips, T.D., Huff, W.E. and Corrier, D.E. (1989). Prevention of aflatoxicosis by addition of hydrated sodium calcium aluminosilicate to the diets of growing barrows. *Am. J. Vet. Res.*, **50**, 416-420.

Hasinoff, B.B., Rahimtula, A.D. and Omar, R.F. (1990). NADPH-cytochrome-P-450 reductase promoted hydroxyl radical production by the iron(III)-ochratoxin A complex. *Biochemica et Biophysica Acta,* **1036**, 78.

Haubeck, H.-D., Lorkowski, G., Kölsch, E. and Röschenthaler, R. (1981). Immunosuppression by ochratoxin A and its prevention by phenylalanine. *Appl. Environ. Microbiol.*, **41**, 1040.

Heathcote, J.G. (1984). Aflatoxins and related toxins. In: Betina, V. (Ed.), *Mycotoxins: Production, Isolation, Separation and Purification*, Elsevier, Amsterdam, pp. 89-130.

Hennig, A., Fink-Gremmels, J. and Leistner, L. (1991). Mutagenicity and effects of ochratoxin A on the frequency of sister chromatid exchanges after metabolic activation. In: Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, I.N. and Bartsch, H. (Eds.), *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours*. International Agency for Research in Cancer, Lyon. pp. 255-260.

Holcomb, M., Thompson, H.C., Jr., and Hankins, L.J. (1993). Analysis of fumonisin B_1 in rodent feed by gradient elution HPLC using precolumn derivatization with FMOC and fluorescence detection. *J. Agric. Food Chem.*, **41**, 764.

Holzapfel, C.W., Steyn, P.S. and Purchase, I.F.H. (1966). Isolation and structure of aflatoxins M_1 and M_2. *Tetrahedron Lett.*, **25**, 2799-2803.

Holzapfel, C.W. (1968). The isolation and structure of cyclopiazonic acid, a toxic metabolite of *Penicillium cyclopium* Westling. *Tetrahedron,* **24,** 2101-2109.

Hoogenboom, L.A.P., Fink-Gremmels, J., Bol, J., Smith, J.E., Delort-Laval, J., Tulliez, J., Guyomard, C., Gautier, J.-P., Neal, G.E., Coker, R.D., Heidenreich, E., Gareis, M., and Bauer, J. (1998). Development of strategies and methods for testing the safety of decontaminated products; results from an EU-project. *Revue Méd. Vét.*, **149**,6, 564.

Hsieh, D.P.H. (1990). Health risks posed by mycotoxins in foods. *Korean J. Toxicol.*, **6**, 159-166.

Hsu, I.C., Metcalf, R.A., Sun, T., Welsch, J.A., Wang, N.J. and Harris, C.C. (1991). Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature*, **350**, 427-428.

Hutchison, R.D., Steyn, P.S. and Thompson, D.L. (1971). The isolation and structure of 4-hydroxy-ochratoxin A and 7-carboxy-3,4-dihydro-3-methylisocoumarin from *Penicillium viridicatum*. *Tetrahedron Lett.*, **43**, 4033-4036.

IPCS, International Programme on Chemical Safety (1990). *Environmental Health Criteria No. 105*. World Health Organization, Geneva, pp. 15.

Ishii, K., Maeda, K., Kamataki, T. and Kato, R. (1986). Mutagenic activation of aflatoxin B_1 by several forms of purified cytochrome P450. *Mutat. Res.*, **174**, 85-88.

Iyer, R.S., Coles, B.F., Thier, K.D., Guengerich, F.P. and Harris, T.M. (1994). DNA adduction by the potent carcinogen aflatoxin B_1: Mechanistic studies. *J. Am. Chem. Soc.*, **116**, 1603-1609.

Jackson, L.S., De Vries, J. and Bullerman, L.B. (Eds.) (1996). *Fumonisins in food.* Plenum Press, New York.

Jago, M.V., Peterson, J.E., Payne, A.L. and Campbell, D.G. (1982). Lupinosis: response of sheep to different doses of phomopsin. *Aust. J. Exp. Biol. Med. Sci.*, **60**, 239-251.

Javed, T., Bunte, R.M., Bennett, G.A., Richard, J.L., Dombrink-Kurtzman, M.A., Côté, L.M. and Buck W.B. (1992). Comparative pathologic changes in broiler chicks on feed amended with *Fusarium proliferatum* culture material or purified fumonisin B_1 and moniliformin. In *Abstracts of 106th AOAC International Annual Meeting, August 31-September 3, 1992,* Cincinatti, OH, pp. 30.

Jiao, Y., Blaas, W., Rühl, C. and Weber, R. (1991). Identification of ochratoxin A in food samples by chemical derivatization and gas chromatography-mass spectrometry. *J. Chromatogr.*, **595**, 364-367.

Jung, K.Y. and Endou, H. (1989). Nephrotoxicity assessment by measuring cellular ATP content. II. Intranephron site to ochratoxin A nephrotoxicity. *Toxicol. Appl. Pharmacol.*, **100**, 383.

Kane, A., Creppy, E.E., Roth, A., Röschenthaler, R. and Dirheimer, G. (1986). Distribution of the [^{3}H]-label from low doses of radioactive ochratoxin A ingested by rats, and evidence for DNA single-strand breaks caused in liver and kidneys. *Arch. Toxicol.*, **58**, 219-224.

Kedera, C.J., Plattner, R.D., and Desjardins, A.E. (1999). Incidence of *Fusarium* spp. and levels of fumonisin B_1 in maize in Western Kenya. *Appl. Environ. Microbiol.* **65,** 41-44.

King, B. (1979). Outbreak of ergotism in Wollo, Ethiopia. *The Lancet,* 1411.

Koeltzow, D.E. and Tanner, S.N. (1990). Comparative evaluation of commercially available aflatoxin test methods. *J. Assoc. Off. Anal. Chem.*, **73**, 584-589.

Kok, W.Th., Van Neer, C.H.Th., Traag, W.H. and Tuinstra, L.G.M.Th. (1986). Determination of aflatoxins in cattle feed by liquid chromatography and post-column derivatization with electrochemically generated bromide. *J. Chromatogr.*, **367**, 231-236.

Konrad, I. and Röschenthaler, R. (1977). Inhibition of phenylalanyl-tRNA synthetase of *Bacillus subtilus* by ochratoxin A. *FEBS Lett.,* **83**, 341.

Korfmacher, W.A., Chiarelli, M.P., Lay, J.O., Jr., Bloom, J., Holcomb, M. and McManus, K.T. (1991). Characterization of the mycotoxin fumonisin B_1: comparison of thermospray, fast atom bombardment, and electrospray mass spectrometry. *Rapid Commun. Mass Spectrom.*, **5**, 463-468.

Krogh, P. (1987). Ochratoxin in foods. In: Krogh, P. (Ed.), *Mycotoxins in food.* 97-121. (Academic Press, London).

Krogh, P., Axelson, N.H., Elling, F., Gyrd-Hansen, N., Hald, B., Hyldgard-Henson, J., Larsen, A.E., Madsen, A., Mortenson, H.P., Moller, T., Peterson, O.K., Ravnskov, U., Rostgaard, M., and Aalund, O. (1974). Experimental porcine nephropathy. Changes in renal function and structure induced by ochratoxin A contaminated feed. *Acta Pathol. Microbiol. Scand. Sect. A*, **246**, (Suppl), 21.

Krogh, P., Hald, B., Plestina, R. and Ceovic, S. (1977). Balkan (endemic) nephropathy and foodborne ochratoxin A: Preliminary results and foodstuffs. *Acta Pathol. Microbiol. Scand. Sect. B*, **85**, 238-240.

Krogh, P., Gyrd-Hansen, N., Hald, B., Larsen, S., Nielsen, J.P., Smith, M., Ivanoff, C. and Meisner, H. (1988). Renal enzyme activities in experimental ochratoxin A-induced porcine nephropathy: Diagnostic potential of phosphoenolpyruvate carboxykinase and gamma-glutamyl transpeptidase activity. *J. Toxicol. Environ. Health,* **23**, 1.

Kuilman, M.E.M., Maas, R.F.M., Judah, D.J. and Fink-Gremmels, J. (1998). Bovine hepatic metabolism of AFB_1. *Revue Méd. Vét.*, **6**, 577.

Kuiper-Goodman, T. (1989). Risk Assessment of Mycotoxins. In: Natori, S., Hashimoto, K. and Ueno, Y. (Eds.), *Mycotoxins* and *Phycotoxins '88.* 257-264 (Elsevier, Amsterdam).

Kuiper-Goodman, T. (1995). Mycotoxins: Risk assessment and legislation. *Toxicol. Lett.*, **82/83**, 853-859.

Kuiper-Goodman, T. (1996). Risk assessment of ochratoxin A: an update. *Food Addit. Contam.*, **13** (Suppl), 53-57.

Kuiper-Goodman, T. and Scott, P.M. (1989). Review: Risk assessment of the mycotoxin ochratoxin A. *Biomed. Environ. Sci.*, **2**, 179-248.

Kuiper-Goodman, T., Scott, P.M., McEwen, N.P., Lombaert, G.A. and Ng, W. (1996). Approaches to risk assessment of fumonisins in corn-based foods in Canada. In: Jackson, L.S., De Vries, J.W. and Bullerman, L.B. (Eds.), *Fumonisins in Food,* Plenum Press, New York, pp. 369-393.

Kumagai, S. (1985). Ochratoxin A: plasma concentration and excretion into bile and urine in albumin deficient rats. *Food Chem. Toxicol.*, **23**, 941-943.

Kumagai, S. (1988). Effects of plasma ochratoxin A and luminal pH on the jejunal absorption of ochratoxin A in rats. *Food Chem. Toxicol.*, **26**, 753-758.

Kussak, A., Nilsson, C-A. and Andersson, B. (1995). Determination of aflatoxins in dust and urine by liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.*, **9**, 1234-1237.

Lacey, J. (Ed.) (1985). *Trichothecenes and other mycotoxins.* John Wiley and Sons, New York.

Lacey, J. (1991). Natural occurrence of mycotoxins in growing and conserved forage crops. In Smith, J.E. and Henderson, R.S. (Eds.), *Mycotoxins and animal foods.* CRC Press, Boca Raton. FL, pp. 363-397.

Lamprecht, S.C., Marasas, W.F.O., Alberts, J.F., Cawood, M.E., Gelderblom, W.C.A., Shephard, G.S., Thiel, P.G. and Calitz, F.J. (1994). Phytotoxicity of fumonisins and TA-toxin to corn and tomato. *Phytopathology*, **84**, 383-391.

Langseth, W., Ellingsen, Y., Nymoen, U. and Okland, E.M. (1989), High-performance liquid chromatographic determination of zearalenone and ochratoxin A in cereals and feed. *J. Chromatogr.*, **478**, 269-274.

Laurent, D., Lanson, M., Goasdoué, N., Kohler, F., Pellegrin, F., and Platzer, N. (1990). Étude en RMN ^{1}H et ^{13}C de la macrofusine, toxine isolée de maïs infesté par *Fusarium moniliforme* Sheld. *Analysis*, **18**, 172-179.

Le Bars, J., Le Bars, P., Dupuy, J., Baudra, H. and Cassini, R. (1992). Biotic and abiotic factors in fumonisin and accumulation. In *Abstracts of 106th AOAC International Annual Meeting, August 31- September 3, 1992,* Cincinatti, OH, pp. 106.

Lebepe-Mazur, S., Bal, H., Hopmans, E., Murphy, P. and Henrich, S. (1995). Fumonisin B_1 is fetotoxic in rats. *Vet. Hum. Toxicol.*, **37**, 126-130.

Lee, L.S., Bayman, P. and Bennett, J.W. (1992). Mycotoxins. In: Finkelstein, B. and Ball,C. (Eds.), *Biotechnology of filamentous fungi. Technology and Products.* Butterworth-Heinemann, Boston, pp. 463-502.

Lepom, P. (1986). Simultaneous determination of the mycotoxins citrinin and ochratoxin A in wheat and barley by high-performance liquid chromatography. *J. Chromatogr.*, **35**, 335-559.

Li, S., Marquardt, R.R., Frohlich, A.A., and Vitti, T.G. (1997). Pharmacokinetics of ochratoxin A and its metabolites in rats. *Toxicol. Appl. Pharmacol.*, **145**, 82-90.

Li, S., Marquardt, R.R., Frohlich, A.A., Xiao, H. and Clarke, J.R. (1994). The development of a quantitative ELISA for aflatoxin B_1 in ground peanuts using antibodies isolated from the yolk of a laying hen. *J. Food Protec.*, **57** (11), 1022-1024.

Lindemann, M.D., Blodgett, D.J. and Kornegay, E.T. (1990). Further evaluation of aflatoxicosis in weaning/growing swine and its amelioration by dietary additives. *J. Anim. Sci.* **68**, Suppl. 1, 39.

Luster, M.I., Germolec, D.R., Burleson, G.R., Jameson, C.W., Ackermann, M.F., Lamm, K.R. and Hayes, H.T. (1987). Selective immunosuppression in mice and natural killer cell activity by ochratoxin A. *Cancer Res.*, **47**, 2259-2263.

Maaroufi, K., Achour, A., Hamammi, M., El-May, M., Betbeder, A.M., Ellouz, F., Creppy, E.E., and Bacha, H. (1995). Ochratoxin A in human blood in relation to nephropathy in Tunisia. *Human and Experimental Toxicology*, **14**, 609-615.

Maaroufi, K., Abid, S., Cherif, A., Achour, A., Zakhama, A., Creppy, E.E., and Bacha, H. (1999). Molecular aspects of human ochratoxicosis in Tunisia. *J.Toxicol.-Toxin Reviews*, **18**, 263-276.

Maas, R.F.M., Georgiou, N.A., Polman, T.H.G., Bol, J., and Fink-Gremmels, J. (1998). Aflatoxin B_1 bioconversion by Aspergillus niger. *Revue Méd. Vét.*, 6, 572.

MacDonald, S. and Castle, L. (1996). A UK retail survey of aflatoxins in herbs and spices and their fate during cooking. *Food Addit. Contam.*, **13**, 121-128.

Madhyastha, M.S., Frohlich, A.A. and Marquardt, R.R. (1992). Effect of dietary cholestyramine on the elimination pattern of ochratoxin A in rats. *Food Chem. Toxicol.*, **30**, 709.

Madhyastha, M.S., Marquardt, R.R. and Abramson, D. (1994). Structure-activity relationships and interactions among trichothecene mycotoxins as assessed by yeast bioassay. *Toxicon.*, **32**, 1147-1152.

Madsen, A., Hald, B. and Mortensen, H.P. (1982). Feeding experiments with ochratoxin contaminated barley for bacon pigs. 1.Influence on pig performance and residues. *Acta Agric. Scand.*, **32**, 225.

Malaveille, C., Brun, G. and Bartsch, H. (1994). Structure-activity studies in *E.coli* strains on ochratoxin A (OTA) and its analogues implicate a genotoxic free radical and acytotoxic thiol derivative as reactive metabolites. *Mut. Res.*, **307**, 141-147.

Manolova, Y., Manolov, G., Parvanova, L., Petkova-Bocharova, T., Castegnaro, M. and Chernozemsky, I.N. (1990). Induction of characteristic chromosomal aberrations, particularly X-trisomy, in cultured human lymphocytes treated by ochratoxin A, a mycotoxin implicated in Balkan endemic nephropathy. *Mutat. Res.*, **292**, 143-149.

Mantle, P.G. (1991). Miscellaneous toxigenic fungi. In: Smith, J.E. and Henderson, R.S. (Eds.), *Mycotoxins and Animal Foods.* CRC Press, Boca Raton, F.L. pp. 141-152.

Marasas, W.F.O., Kellerman, T.S., Gelderblom, W.C.A., Coetzer, J.A.W., Thiel, P.G. and Van der Lugt, J.J. (1988). Leukoencephalomalacia in a horse induced by fumonisin B_1; isolated from *Fusarium moniliforme. Onderstepoort J. Vet. Res.*, **55**, 197-203.

Marasas, W.F.O. (1996). Fumonisins: History, World-Wide Occurrence and Impact. In: Jackson, L. (Ed.), *Fumonisins in Food.* Plenum Press, New York. pp. 1-17.

Marquardt, R.R. and Frohlich, A.A. (1992). A review of recent advances in understanding ochratoxicosis. *J. Anim. Sci.*, **70**, 3968-3988.

Mateles, R.I. and Adye, J.C. (1965). Production of aflatoxin in submerged cultures. *Applied Microbiology*, **13**, 208-211.

Mayura, K., Parker, R., Berndt, W.O. and Phillips, T.D. (1984). Ochratoxin A-induced teratogenesis in rats: partial protection by phenylalanine. *Appl. Environ. Microbiol.,* **48**, 1186.

Meisner, H. (1976). Energy-dependent uptake of ochratoxin A by mitochondria. *Arch. Biochem. Biophys.*,**173**, 132-140.

Meisner, H. and Chan, S. (1974). Ochratoxin A an inhibitor of mitochondrial transport. *Biochem.*, **13**, 2759.

Meisner, H. and Meisner, P. (1981). Ochratoxin A, an *in vivo* inhibitor of renal phosphoenolpyruvate carboxykinase. *Arch. Biochem. Biophys.*, **208**, 146.

Merrill, A.H. (1991). Cell regulation by spingosine and more complex sphingolipids. *J. Bioenerg. Biomembr.*, **23**, 83-104.

Merrill, A.H., van Echten, G., Wang, E. and Sandhoff, K. (1993). Fumonisin B_1 inhibits sphingosine (Sphinganine) *N*-acyltransferase and *de novo* sphingolipid biosynthesis in cultured neurons *in situ*. *J. Biol. Chem.*, **268**, 27299-27306.

Milanez, T.V. and Leitao, M.F. (1996). The effect of cooking on ochratoxin A content of beans, variety 'Carioca'. *Food Addit. Contam.*, **13** (1), 89-93.

Miller, J.D. (1994). Production and purification of fumonisins from a stirred jar fermenter. *Natural Toxins,* **2**, 354-359.

Miller, J.D. and Trenholm, H.L. (1994). *Mycotoxins in grain: Compounds Other than Aflatoxin*. Eagan Press, St. Paul, MN.

Mirocha, C.J., Gilchrist, D.G., Shier, W.T., Abbas, H.K., Wen, Y. and Vesonder, R.F. (1992). AAL toxins, fumonisins (biology and chemistry) and host specificity concepts. *Mycopathologia*, **117**, 47-56.

Mobio, T.A., Abado-Becognee, K., Ennamany, R., Fleurat-Lessard, F., Shier, W.T., Badria, F. and Creppy, E.E. (1998). Implication of lipid peroxidation in the cytotoxicity and genotoxicity of fumonisin B_1. *Revue Méd. Vét.*, **6**, 646.

Moore, J.H. and Truelove, B. (1970). Ochratoxin A, inhibition of mitochondrial respiration. *Science*, **168**, 1102.

Morgan, M.R.A. (1989). Mycotoxin immunoassays (with special reference to ELISAs). *Tetrahedron,* **45**, 2237-2249.

Morgan, M.R.A., McNerney, R., Chan, H.W-S. and Anderson, P.H. (1986). Ochratoxin A in pig kidney determined by enzyme-linked immunosorbent assay (ELISA). *J. Sci. Food Agric.*, **37**, 475-480.

Moroi, K., Suzuki, S., Kuga, T., Yamazaki, M. and Kanisawa, M. (1985). Reduction of ochratoxin A toxicity in mice treated with phenylalanine and phenobarbital. *Toxicol. Lett.,* **25**, 1-5.

Mortensen, H.P., Hald, B. and Madsen, A. (1983). Feeding experiments with ochratoxin A contaminated barley for bacon pigs. 5. Ochratoxin A in pig blood. *Acta Agric. Scand.*, **33**, 235-239.

Mortimer, P.H., di Menna, M.E. and White, E.P. (1978). Pithomycotoxicosis "facial eczema" in cattle. In: Wylie, T.D. and Morehouse, L.G. (Eds.), *Mycotoxic fungi, mycotoxins, mycotoxicoses - an encyclopeadic handbook.* Marcel Dekker, New York, Vol. 2. pp. 63-72.

Mortimer, D.N., Gilbert, J. and Shepherd, M.J. (1987). Rapid and highly sensitive analysis of aflatoxin M_1 in liquid and powdered milks using an affinity column cleanup. *J. Chromatogr.*, **407**, 393-398.

Nakajima, M., Terada, H., Hisada, K., Tsubouchi, H., Yamamoto, K., Uda, T., Kawamura, O. and Ueno, Y. (1990). *Food Agric. Immunol.*, **2**, 189-195.

Nakazato, M., Morozumi, S., Saito, K., Fujinuma, K., Nishima, T., and Kasai, N.(1990). *Appl. Environ. Microbiol.*, **56**, 1459-1470.

Natori, S., Hashimoto, K. and Ueno, Y. (1989). *Mycotoxins and Phycotoxins '88*. Elsevier, Amsterdam.

Neal, G.E. (1995). Genetic implications in the metabolism and toxicity of mycotoxins. *Toxicol. Lett.*, **82/83**, 861-867.

Nolan, R.D., Danilowicz, R.M., Eling, T.E. (1988). Role of arachidonic acid metabolism in the mitogenic response of Balb/c 3T3 fibroblasts to epidermal growth factors. *Molecular Pharmacol.*, **33**, 650-656.

Norred, W.P., Plattner, R.D., Vesonder, R.F., Hayes, R.F., Bacon, C.W. and Voss, K.A. (1990). Effect of *Fusarium moniliforme* metabolites on unscheduled DNA synthesis (UDS) in rat primary hepatocytes. *Toxicologist*, **10**, 165.

Norred, W.P., Voss, K.A., Bacon, C.W. and Riley, R.T. (1991). Effectiveness of ammonia treatment on detoxification of fumonisin-contaminated corn. *Food Chem.Toxicol.*, **29**, 815-819.

O'Donnell, K. (1997). Phylogenetic evidence indicates the important mycotoxigenic strains Fn-2, Fn-3, Fn-2B and Fn-M represent a new species of *Fusarium. Mycotoxins*, **45**, 1-10.

Omar, R.F., Hasinoff, B.B., Mejilla, F. and Rahimtula, A.D. (1990). Mechanism of ochratoxin A stimulated lipid peroxidation. *Biochem. Pharmacol.*, **40** (6), 1183.

Omar, R.F., Rahimtula, A.D. and Bartsch, H. (1991). Role of cytochrome P-450 in ochratoxin A-stimulated lipid peroxidation. *J. Biochem. Toxicol.*, **6**, 203-209.

Oster, T., Jayyosi, Z., Creppy, E.E., Souhaili El Amri, H. and Batt, A-M. (1991). Characterization of pig liver purified cytochrome P-450 isoenzymes for ochratoxin A metabolism studies. *Toxicol. Lett.*, **57**, 203-.

Park, D.L. (1992). Perspectives on mycotoxin decontamination procedures. *Microb. Hyg. All.*, **4**, 21-27.

Paterson, R.R. and Kemmelmeier, C. (1990). Neutral, alkaline and difference ultraviolet spectra of secondary metabolites from *Penicillium* and other fungi, and comparisons to published maxima from gradient high performance liquid chromatograph with diode-array detection. *J. Chromatogr.*, **511**, 195.

Paulsch, W.E., Van Egmond, H.P. and Schuller, P.L. (1982). Thin layer chromatographic method for analysis and chemical confirmation of ochratoxin A in kidneys of pigs. In: *Proceedings, V International IUPAC Symposium Mycotoxins and Phycotoxins. September 1-3, 1982, Vienna, Austria.* Austrian Chem. Soc., Vienna, pp. 40-43.

Pavlovic, M., Plèstina, R. and Krogh, P. (1979). Ochratoxin A contamination of foodstuffs in an area with Balkan (endemic) nephropathy. *Acta Pathol. Microbiol. Scand.*, Sect B, **87**, 243-246.

Petkova-Bocharova, T. and Castegnaro, M. (1985). Ochratoxin contamination of cereals in an area of high incidence of Balkan endemic nephropathy in Bulgaria. *Food Addit. Contam.*, **2**, 267-270.

Petkova-Bocharova, T., Chernozemsky, I.N. and Castegnaro, M. (1988). Ochratoxin A in human blood in relation to Balkan endemic nephropathy and urinary system tumours in Bulgaria. *Food Addit. Contam.,* **5,** *299-301.*

Pfohl-Leszkowicz, A., Grosse, Y., Kane, A., Creppy, E.E. and Dirheimer, G. (1993a). Differential DNA adduct formation and disappearance in three mouse tissues after treatment with the mycotoxin ochratoxin A. *Mutat. Res.*, **289**, 265-273.

Pfohl-Leszkowicz, A., Grosse, Y., Kane, A., Gharbi, A., Beaudrimont, I., Obtrecht, S., Creppy, E.E. and Dirheimer, G. (1993b). Is the oxidation pathway implicated in the genotoxicity of ochratoxin A. In: E.E. Creppy, M. Castegnaro and G. Dirheimer (Eds.), *Human Ochratoxicosis and its Pathologies*. Vol. 231, John Libbey Eurotext Ltd, London, pp. 177-187.

Pitt, J.I. (1987). *Penicillium viridicatum*, *Penicillium verrucosum* and production of ochratoxin A. *Appl. Environ. Microbiol.*, **53**, 266-269.

Pittet, A., Parisod, V. and Schellenberg, M. (1992). Occurrence of fumonisins B_1 and B_2 in corn-based products from the Swiss market. *J. Agric. Food Chem.*, **40**, 1352-1354.

Plattner, R.D., Beremand, M.N. and Powell, R.G. (1989). Analysis of trichothecene mycotoxins by mass spectrometry and tandem mass spectrometry. *Tetrahedron*, **45**, 2241-2262.

Plattner, R.D., Norred, W.P., Bacon, C.W., Voss, K.A., Peterson, R., Shackelford, D.D. and Weisleder, D. (1990). A method of detection of fumonisins in corn samples associated with field cases of equine leukoencephalomalcia. *Mycologia,* **82** (6), *698-702.*

Pohland, A.E., Schuller, P.L., Steyn, P.S. and van Egmond, H.P. (1982). Physicochemical data for some selected mycotoxins. *Pure Appl. Chem.*, **54**, 2219-2284.

Pohland, A.E., Nesheim, S. and Friedman, L. (1992). Ochratoxin A: a review. *Pure Appl. Chem.*, **64**, 1029-1046.

Qureshi, M.A. and Hagler, W.M. (1991). Effect of fumonisin B_1 exposure on chicken macrophage functions *in vitro. Poult. Sci.,,* **71**, 104-112.

Rabie, C.J., Sydenham, E.W., Thiel, .G., Lübben, A. and Marasas, W.F.O. (1986). T-2 Toxin production by *Fusarium acuminatum* isolated from oats and barley. *Appl. Environ. Microbiol.*, **52**, 594-596.

Rahimtula, A.D., Bereziat, J.C., Bussachini-Griot, V. and Bartsch, H. (1988). Lipid peroxidation as a possible cause of ochratoxin A toxicity. *Biochem. Pharmacol.,* **37**, 4469.

Rahimtula, A.D., Castegnaro, M., Bereziat, J.C., Bussachini-Griot, V., Broussole, L., Michelon, J. and Bartsch, H. (1989). In Bach, P.H. and Lock, E.A. (Eds.), *Nephrotoxicity*, Plenum Press, New York, pp. 617.

Rajakyla, E., Laasasenabo, K. and Sakkers, P.J. (1987). Determination of mycotoxins in grain by high-performance liquid chromatography and thermospray liquid chromatography-mass spectrometry. *J. Chromatogr.*, **384**, 391-402.

Raney, K.D., Coles, B., Guengrich, F.P. and Harris, T.M. (1992). The endo-8,9-epoxide of aflatoxin B_1: a new metabolite. *Chem. Res. Toxicol.*, **5**, 333-335.

Resnik, S., Neira, S., Pacin, A., Martinez, E., Apro, N. and Latreite, S. (1996). A survey of the natural occurrence of aflatoxins and zearalenone in Argentine field maize:1983-1994. *Food Addit. Contam.*, **13** (1), 115-120.

Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S and van Schalkwyk, D.J. (1992). *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology*, **82** (3), 353-357.

Rheeder, J.P., Sydenham, E.W., Marasas, P.G., Thiel, P.G., Shepard, G.S., Schlechter, M., Stockenström, S., Cronje, D.W., Viljoen, J.H., (1995). Fungal infestation and mycotoxin contamination of South African commercial maize harvested in 1989 and 1990. *S.A. J. Science*, **91**, 127-132.

Riley, R.T., An, N-H., Showker, J.L., Yoo, H-S., Norred, W.P., Chamberlain, W.J., Wang, E., Merrill, A.H., Jr., Motelin, G., Beasley, V.R. and Haschek, W.M. (1993). Alteration of tissue and serum sphinganine to sphingosine ratio: an early biomarker of exposure to fumonisin-containing feeds in pigs. *Toxicol. Appl. Pharmacol.*, **118**, 105-112.

Roth, A., Chakor, K., Creppy, E.E., Kane, A., Rösenthaler R., and Dirheimer, G. (1988). Evidence of enterohepatic circulation of ochratoxin A in rats. *Food Chem. Toxicol.* **26**, 753-758.

Roth, A., Eriani, G., Dirheimer, G. and Gangloff, J. (1993). Kinetic properties of pure overproduced *Bacillus subtilis* phenylalanyl-tRNA synthetase do not favour its *in vivo* inhibition by ochratoxin A. *FEBS Lett.*, **326**, 87.

Roux, C. (1986). *Leptosphaerulina chartarum* sp. nov., the teleomorph of *Pithomyces chartarum. Trans. Br. Mycol. Soc.*, **86**, 319.

Sakai, M., Abe, K., Okumura, H., Kawamura, O., Sugiura, Y., Horie, Y. and Ueno, Y. (1992). Genotoxicity and fungi evaluated by SOS microplate assay. *Nat. Toxins*, **1**, 27-34.

Samson, R.A. and Frisvad, J.C. (1991). Current taxonomic concepts on *Penicillium* and *Aspergillus*. In: Chelkowski, J. (Ed.), *Cereal Grain Mycotoxins, Fungi and Quality in Drying and Storage*. Elsevier, Amsterdam. pp. 405-439.

Schneider, E., Usleber, E. and Märtlbauer, E. (1995). Rapid detection of fumonisin B_1 in corn-based food by competitive direct dipstick enzyme immunoassay/enzyme-linked immunofiltration assay with integrated negative control reaction. *J. Agric. Food Chem.*, **43**, 2548-2552.

Schreeve, B.J., Patterson, D.S.P., Pepin, G.A., Roberts, B.A. and Wrathall, A.E (1977). Effect of feeding ochratoxin to pigs during early pregnancy. *Brit. Vet. J.*, 412-417.

Schroeder, J.J., Crane, H.M., Xia, J., Liotta, D.C. and Merrill, A.H. (1994). Disruption of sphingolipid metabolism and stimulation of DNA synthesis by fumonisin B_1. *J. Biol. Chem.*, **269** (5), 3475-3481.

Scott, P.M. (1997). Multi-year monitoring of Canadian grains and grain-based foods for trichothecenes and zearalenone. *Food Addit. Contam.*, **14** (4), 333-339.

Scott, P.M. and Kanhere, S.R. (1995). Determination of ochratoxin A in beer. *Food. Addit. Contam.*, **12** (4), 591-598.

Scott, P.M. and Lawrence, G.A. (1992). Liquid chromatographic determination of fumonisins with 4-fluoro-7-nitrobenzofuran. *J. AOAC. Int.*, **75**, 829-834.

Scott, P.M., Lombaert, G.A., Pellaers, P., Bacler, S. and Lapp, J. (1992). Ergot alkaloids in grain foods sold in Canada. *J. AOAC Int.*, **75**, 773-779.

Seegers, J.C., Lottering, M-L. and Garlinski, P.J. (1994). The mycotoxin ochratoxin A causes apoptosis-associated DNA degradation in human lymphocytes. *Med. Sci. Res.*, **22**, 417.

Seo, J-A, and Lee, Y-W. (1999). Natural occurrence of the C series of fumonisins in moldy corn. *Appl. Environ. Microbiol.* **65**, 1331-1334.

Shapira, R., Paster, N., Eyal, O., Menasherov, M., Mett, A. and Salomon, R. (1996). Detection of aflatoxigenic molds in grains by PCR. *Appl. Environ. Microbiol.*, **62**, 3270-3273.

Sharman, M., MacDonald, S. and Gilbert, J. (1992). Automated liquid chromatographic determination of ochratoxin A in cereals and animal products using immunoaffinity column clean-up. *J. Chromatogr.*, **603**, 285-289.

Shelby, R.A., Rottinghaus, G.E. and Minor, H.C. (1994). Comparison of thin-layer chromatography and competitive immunoassay methods for detecting fumonisins on maize. *J. Agric. Food Chem.*, **42**, 2064-2067.

Shephard, G.S., Thiel, P.G., Sydenham, E.W., Alberts, J.F. and Gelderblom, W.C.A. (1992). Fate of a single dose of the ^{14}C- labelled mycotoxin, fumonisin B_1 in rats. *Toxicon.*, **30**, 768-770.

Shephard, G.S., Thiel, P.G., Sydenham, E.W., Alberts, J.F. and Cawood, M.E. (1993). Distribution and excretion of a single dose of the mycotoxin fumonisin B_1 in a non-human primate. *Toxicon.*, **32**, 735-741.

Shephard, G.S., Thiel, P.G., Sydenham, E.W. and Snijman, P.W. (1995). Toxicokinetics of the mycotoxin fumonisin B_2 in rats. *Food Chem. Toxicol.*, **33**, 591-595.

Shephard, G.S., van der Westhuizen, L., Thiel, P.G., Gelderblom, W.C.A., Marasas, W.F.O. and van Schalkwyk, D.J. (1996). Disruption of sphingolipid metabolism in non-human primates consuming diets of fumonisin-containing *Fusarium moniliforme*. *Toxicon.*, **32**, 735-741.

Shepherd, M.J. and Gilbert, J. (1984). An investigation of HPLC post-column iodination conditions for the enhancement of aflatoxin B_1 fluorescence. *Food Addit. Contam.*, **1**, 325-335.

Shepherd, M.J., Mortimer, D.N. and Gilbert, J. (1987). A review of approaches to the rapid analysis of aflatoxins in foods. *J. Assoc. Publ. Analysts*, **25**, 129-142.

Shetty, B.H. and Bhat, R.V. (1997). Natural occurrence of fumonisin B_1 and its co-occurrence with aflatoxin B_1 in Indian sorghum, maize and poultry feeds. *J. Agric. Food Chem.*, **45**, 2170-2173.

Shier, W.T. and Abbas, H.K. (1992). A simple procedure for the preparation of aminopentols (fumonisin hydrolysis products AP_1 and AP_2) from *Fusarium moniliforme* on solid media. *Abstr. 106th AOAC Int. Ann. Mtg.*, Aug. 31- Sept. 3, 1992, Cincinatti, OH, 237.

Shier, W.T., Abbas, H.K. and Badria, F.A. (1995). Complete structures of the sphingosine analog mycotoxins fumonisin B_1 and AAL toxin: absolute configuration of the side chains. *Tetrahedron Lett.*, **36**, 1571-1574.

Simpson, T.J. and Pemberton, A.D. (1989). High field ^1H NMR studies on the ammoniation of aflatoxin B_1. *Tetrahedron*, **45**, 2451-2464.

Smith, B.L. (1985). Recent advances in facial eczema (pithomycotoxicosis) research. In: Lacey, J. (Ed.). *Trichothecenes and other mycotoxins*. 325-329 (John Wiley and Sons, Chicester).

Smith, J.E. and Henderson, R.S. (Eds.) (1991). Mycotoxins and Animal Foods. CRC Press, Boca Raton, F.L.

Soucek, S. and Gut, I. (1992), Cytochrome P450 in rats: structures, functions, properties and relevant human forms. *Xenobiotica,* **22**, 83-103.

Sreemannarayana, O.A., Frohlich, A.A., Vitti, T.G., Marquardt, R.R. and Abramson, D. (1988). Studies of the tolerance and disposition of ochratoxin A in young calves. *J. Anim. Sci.*, **66**, 1703.

Stein, A.F., Phillips, T.D., Kubena, L.F. and Harvey, R.B. (1985). Renal tubular secretion and readsorption as factors in ochratoxicosis: Effects of probenecid on nephrotoxicity. *J. Toxicol. Environ. Health*, **16**, 593.

Steyn, P.S. (1980). The biosynthesis of mycotoxins: A study in secondary metabolism. Academic Press, New York.

Steyn, P.S. (1984). Ochratoxin and related dihydroisocoumarins. In: Betina V. (Ed.), *Mycotoxins - Production, Separation and Purification.* 183-215 (Elsevier, Amsterdam).

Steyn, P.S. (Ed.) (1989). Mycotoxins. Tetrahedron Symposia in Print, No 37, *Tetrahedron,* **45** (8).

Steyn, P.S. (1993). Mycotoxins of human health concern. In: Creppy, E.E., Castegnaro, M. and Dirheimer, G. (Eds), *Human ochratoxicosis and its pathologies.* ***231,*** John Libbey Eurotext London, pp. 3-31.

Steyn, P.S. (1995). Mycotoxins, general view, chemistry and structure. *Toxicol. Lett.*, **82/83**, 843-851.

Steyn, P.S. and Holzapfel, C.W. (1967). The isolation of the methyl and ethyl esters of ochratoxins A and B, metabolites of *Aspergillus ochraceus* Wilh. *J. S. Afr. Chem. Inst.*, **20**, 186-189.

Steyn, P.S. and Vleggaar, R. (1985). Tremorgenic mycotoxins. *Fortschr. Chem. Org. Naturst.*, **48**, 1-80 and references cited.

Steyn, P.S., Holzapfel, C.W. and Ferreira, N.P. (1970). The biosynthesis of the ochratoxins, metabolites of *Aspergillus ochraceus*. *Phytochemistry,* **9**, 1977-1983.

Steyn, P.S., Vleggaar, R., Du Preez, N.P., Blyth, A.A. and Seegers, J.J. (1975). The *in vitro* toxicity of ochratoxin A in monkey kidney epithelial cells. *Toxicol. Appl. Pharmacol.,* **32**, 198-203.

Steyn, P.S., Thiel, P.G. and Trinder, D.W. (1991). Detection and quantification of mycotoxins by chemical analysis. In: Smith, J.E. and Henderson, R.S. (Eds). *Mycotoxins and Animal Foods*. CRC Press, Boca Raton, FL, pp. 165-221.

Steyn, P.S. and Payne, B.E. (1999). The synthesis of bromo- and iodo-ochratoxin B. *S.Afr.J.Chem.* **52**, 69-70

Stojkovic, R., Hult, K., Gamulin, S. and Plestina, R. (1984). High affinity binding of ochratoxin A to plasma constituents. *Biochem. Int.*, **9**, 33-38.

Stoll, A. (1952). Recent investigations on ergot alkaloids. *Fortsch. Chem. Org. Naturs.*, **9**, 114-174.

Stϕren, O., Helgerud, P., Holm, H. and Stϕrmer, F.C. (1982a). Formation of (4*R*)-4-hydroxyochratoxin A and ochratoxin α from ochratoxin A by rats. In: *Proceedings, V International IUPAC Symposium Mycotoxins and Phycotoxins. September 1-3, 1982, Vienna, Austria*. Austrian Chemical Society, Vienna, pp. 321-324.

Stϕren, O., Holm, H. and Stϕrmer, F.C. (1982b). Metabolism of ochratoxin A by rats. *Appl. Environ. Microbiol.*, **44**, 785-789.

Stϕrmer, F.C., Hansen, C.E., Pederson, J.I., Hvistendahl, G. and Aasen, A.J. (1981). Formation of (4*R*)- and (4*S*)-4-hydroxyochratoxin A by liver microsomes from various species. *Appl. Environ. Microbiol.*, **42**, 1051-1056.

Stϕrmer, F.C., Stϕren, O., Hansen, C.E., Pederson, J.I., and Aasen, A.J. (1983). Formation of (4*R*)- and (4*S*)-4-hydroxyochratoxin A and 10-hydroxyochratoxin A by rabbit liver microsomes. *Appl. Environ. Microbiol.*, **45**, 1183-1187.

Stϕrmer, F.C., Myhre, C. and Wiger, R. (1996). Ochratoxin A induced apoptosis in HL 60 cells is enhanced by antioxidants. In: Miraglia, M., Brera, C. and Onori, R. (Eds.), *Proceedings of the IX International IUPAC Symposium on Mycotoxins and Phycotoxins*, p.226.

Sugamata, M., Ihara, T., Todate, A., Okumura, H., Yoshino, N., Hamaguchi, M., Nakamura, K. and Ueno, Y. (1997). Apoptic cellular damages in thymus, spleen and liver after nivalenol-induced acute toxicosis in mice: a ultrastructural study. *Mycotoxins*, **45**, pp. 39-43 and reference cited.

Suzuki, S., Kozuka, Y., Satoh, T. and Yamazaki, M. (1975). Studies on the nephrotoxicity of ochratoxin A in rats. *Bull. Environ. Contam. Toxicol.*, **43**, 180.

Suzuki, S., Satoh, T. and Yamazaki, M. (1977). The pharmacokinetics of ochratoxin A in rats. *Japan J. Pharmacol.*, **27**, 735-744.

Suzuki, S., Moroi, K., Kanisawa, M. and Satoh, T. (1986). Effects of drug metabolizing enzyme inducers on carcinogenesis and toxicity of ochratoxin A in mice (abstract). *Toxicol. Lett.*, **31** (Suppl.), 206.

Sydenham, E.W., Thiel, P.G., Marasas, W.F.O., Shephard, G.S., van Schalkwyk, D.J. and Koch, K.R. (1990). Evidence for the natural occurrence of fumonisin B_1, a mycotoxin produced by *Fusarium moniliforme. J. Agric. Food Chem.*, **38**, 1900-1903.

Sydenham, E.W., Shephard, G.S., Thiel, P.G., Marasas, W.F.O., Rheeder, J.P., Peralta Sanhueza, C.E.P, González, H.H.L. and Resnik, S.L. (1993a). Fumonisins in Argentinian Field-Trial Corn. *J. Agric. Food Chem.*, **41**, 891-895.

Sydenham, E.W., Thiel, P.G., Marasas, W.F.O., Stockenström, S., van der Westhuizen, L. and Shephard, G.S. (1993b). Physical and chemical procedures for the removal of fumonisins from maise.In *Proceedings of the UK Workshop on Mycotoxins*, 21-23 April 1993.

Sydenham, E.W., van der Westhuizen, L., Stockenström, S., Shephard, G.S. and Thiel, P.G. (1994). Fumonisin-contaminated maize: physical treatment for the partial decontamination of bulk shipments. *Food Addit. Contam.*, **11** (1), 25-32.

Szczech, G.M., Carlton, W.W., Tuite, J. and Caldwell, R. (1973). Ochratoxin A toxicosis in swine. *Vet. Pathol.*, **10**, 347.

Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U-S., Sugiura, Y. and Ueno, Y. (1988). Worldwide contamination of cereals by the *Fusarium* mycotoxins nivalenol, deoxynivalenol, and zearalenone. 1. Survey of 19 countries. *J. Agric. Food Chem.*, **36**, 979-983.

Tatsuno, T. (1997). Scabby wheat intoxication and the discovery of nivalenol (a review). *Mycotoxins*, **45**, 11-12.

Thekkumkara, T.J. and Patel, M.S. (1989). Ochratoxin A decreases the activity of phosphoenolpyruvate carboxykinase and its mRNA content in primary cultures of rat kidney proximal convoluted tubular cells. *Biochem. Biophys. Res. Commun.*, **162**, 916-920.

Thiel, P.G., Marasas, W.F.O., Sydenham, E.W., Shephard, G.S., Gelderblom, W.C.A. and Nieuwenhuis, J.J. (1991). Survey of fumonisin production by *Fusarium* species. *Appl. Environ. Microbiol.*, **57** (4), 1089-1093.

Thiel, P.G., Sydenham, E.W., Shephard, G.S. and van Schalkwyk, D.J. (1993). Study of the reproducibility characteristics of a liquid chromatographic method for the determination of fumonisins B_1 and B_2 in corn: IUPAC collaborative study. *J. AOAC Int.*, **76** (2), 361-366.

Thiel, P.G., Sydenham, E.W. and Shephard, G.S. (1996). The reliability and significance of analytical data on the natural occurrence of fumonisins in food. In: Jackson, L., De Vries, J.W. and Bullerman, L.B., (Eds.), *Fumonisins in Food*, Plenum Press, New York, pp. 145-151.

Thuvander, A., Breitholtz-Emanuelson, A. and Olsen, M. (1995). Effects of ochratoxin A on the mouse immune system after subchronic exposure. *Food Chem. Toxicol.*, **33**, 1005-1011.

Thuvander, A., Breitholtz-Emanuelson, A. Brabencova, D. and Gadhasson, I. (1996a). Influence of perinatal ochratoxin A exposure on the immune system in mice. *Food Chem. Toxicol.*, **34**, 547-554.

Thuvander, A., Funseth, E., Breitholtz-Emanuelson, A., Hallen, I.P. and Oskarsson, A. (1996b). Effects of ochratoxin A on the rat immune system after perinatal exposure. *Nat. Toxins*, **4**, 141-147.

Thuvander, A., Dahl, P. and Breitholtz-Emanuelson, A. (1997). Prenatal exposure of Balb/c mice to ochratoxin A: effects on the immune system in the offspring. *Nat. Toxins*, **4**, 174-180.

Tönsing, E.M., Steyn, P.S., Osborn, M. and Weber, K. (1984). Phomopsin A, the causative agent of lupinosis, interacts with microtubules *in vivo* and *in vitro*. *Eur. J. Cell. Biol.*, **35**, 156-164.

Townsend, C.A., (1986). Progress towards a biosynthetic rationale of the aflatoxin pathway. *Pure Appl. Chem.*, **58**, 227-238.

Trail, F., Mahanti, N. and Linz, J. (1995). Molecular biology of aflatoxin biosynthesis. *Microbiology*, **141**, 755-765.

Ueno, Y., Sato, N., Ishii, K., Saiha, K. and Enomoto, M. (1972). Toxicological approaches to the metabolites of the *Fusaria V.* Neosolaneol, T-2 toxin and butenolide, toxic metabolites of *Fusarium sporotrichioides* NRRL 3510 and *Fusarium poae* 3287. *Jpn. J. Exp. Med.*, **42**, 461-472.

Uraguchi, K. and Yamazaki, M. (Eds.) (1978). *Toxicology - Biochemistry and Pathology of Mycotoxin*s. Halsted Press, New York.

Urry, W.H., Wehrmeister, H.L., Hodge, E.B. and Hidy, P.H. (1966). The structure of zearalenone. *Tetrahedron Letters*, **27**, 3109-3114.

Usleber, E., Straka, M. and Terplan, G. (1994). Enzyme immunoassay for fumonisin B_1 applied to corn-based food. J. *Agric. Food Chem.*, **42**, 1392-1396.

van der Merwe, K.J., Steyn, P.S. and Fourie, L. (1965). Mycotoxins. Part II. The constitution of ochratoxins A, B and C, metabolites of *Aspergillus ochraceus* Wilh. *J. Chem. Soc.*, 7083-7088.

van Egmond, H.P. (1991). Methods for determining ochratoxin A and other nephrotoxic mycotoxins. Mycotoxins, In: Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, I.N., and Bartsch, H. (Eds.). *Endemic Nephropathy and Urinary Tract Tumours.*, International Agency for Research on Cancer, Lyon, pp. 55-70.

van Egmond, H.P. (1996). Analytical methodology and regulations for ochratoxin A. *Food Addit. Contam.*, **13**, 11-13.

van Egmond, H.P. and Speijers, G.J.A. (1994). Survey of data on the incidence and levels of ochratoxin A in food and animal feed worldwide. *J. Nat. Toxins,* **3**, 125-143.

van Rensburg, S.J. (1986). Role of mycotoxins in endemic liver cancer and oesophageal cancer. In: Steyn, P.S. and Vleggaar, R. (Eds.). *Mycotoxins and Phycotoxins.* Elsevier, Amsterdam, pp. 483-494.

van Rensburg, S.J. and Altenkirk, B. (1974). *Claviceps purpurea* - ergotism. In: Purchase, I.F.H. (Ed.). *Mycotoxins*. Elsevier, Amsterdam, p.. 69.

Varga, J., Kevei, E., Rinyu, E., Teren, J. and Kozakiewicz, Z. (1996). Ochratoxin production by *Aspergillus* species. *Appl. Environ. Microbiol.*, **62** (12), 4461-4464.

Verardi, G. and Rosner, H. (1995). Some reflections on establishing United Community legislation on mycotoxins. *Natural Toxins,* **3**, 337-340.

Vesonder, R., Peterson, R., Plattner, R. and Weisleder, D. (1990). Fumonisin B_1: isolation from corn culture and purification by high performance liquid chromatography. *Mycotoxin Res.*, **6**, 85-88.

Wang, E., Norred, W.P., Bacon, C.W., Riley, R.T. and Merrill, A.H. (1991). Inhibition of sphingolipid biosynthesis by fumonisins: implications for diseases associated with *Fusarium moniliforme*. *J. Biol. Chem.*, **266**, 14486-14490.

Wang, E., Ross, F., Wilson, T.M., Riley, R.T. and Merrill, A.H. (1992). Increases in serum sphingosine and sphinganine and decreases in complex sphingolipids in ponies given feed containing fumonisins, mycotoxins produced by *Fusarium moniliforme*. *J. Nutr.*, **122**, 1706-1715.

Wang, L-Y., Hatch, M., Chen, C-J., Levin, B., You, S-L., Lu, S-N., Wu, M-H., Wu, W-P., Wang, L-W., Wang, Q., Huang, G-Y., Yang, P-M., Lee, H-S. and Santella, R.M. (1996). Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan. *Inter. J. Cancer*, **67** (5), 620-625.

Wannemacher, R.W., Bunner, D.L. and Neufeld, H.A. (1991). Toxicity of trichothecenes and other related mycotoxins in laboratory animals. In: Smith, J.E. and Henderson, R.S. (Eds.). *Mycotoxins and Animal Foods*. CRC Press, Boca Raton, FL, pp. 499-552.

Watson, A.J., Fuller, L.J., Jeenes, D.J. and Archer, D.B. (1999). Homologs of aflatoxin biosynthesis genes and sequence of aflR in *Aspergillus oryzae* and *Aspergillus sojae*. *Appl. Environ. Microbiol.*, **65**, 307-310.

Wei, Y.-H., Lu, C.-Y., Lin, T.-N. and Wei, R.D. (1985). Effect of ochratoxin A on rat liver mitochondrial respiration and oxidative phosphorylation. *Toxicology*, **36**, 119.

Wood, G.M, Entwisle, A.C., Farnell, P.J., Patel, S. and Boenke, A. (1995). *The certification of the ochratoxin A mass fraction of two wheat reference materials (CRMs 471 and 472),* Document BCR/25/95 (Brussels: Commission of the European Communities).

Würgler, F.E., Friedrich, U. and Schlatter, J. (1991). Lack of mutagenecity of ochratoxin A and B, citrinin, patulin and cnestine in *Salmonella typhimurium* TA 102. *Mutat. Res.* 261, 209-216.

Xiao, H., Clarke, J.R., Marquardt, R.R. and Frohlich, A.A. (1995a). Improved methods for conjugating selected mycotoxins to carrier proteins and dextran for immunoassays. *J. Agric. Food Chem.,* **43**, 2092-2097.

Xiao, H., Marquard, R.R., Frohlich, A.A. and Ling, Y.Z. (1995b). Synthesis and structure elucidation of analogs of ochratoxin A. *J. Agric. Food Chem.*, **43** (2), 524-530.

Xiao, H., Madhyastha, S., Marquardt, R.R., Li, S., Vodela, J.K., Frohlich, A.A. and Kemppainen, B.W. (1996a). Toxicity of ochratoxin A, its opened lactone form and several analogs: structure-activity relationships. *Toxicol. Appl. Pharmacol.*, **137**, 182.

Xiao, H., Marquardt, R.R., Abramson, D. and Frohlich, A.A. (1996b). Metabolites of ochratoxins in rat urine and in a culture of *Aspergillus ochraceus*. *Appl. Environ. Microbiol.*, **62** (2), 648.

Yagen, B., Joffe, A.Z., Horn, P., Mor, N. and Lutsky, I.I. (1977). Toxins from a strain involved in ATA. In: Rodricks, J., Hesseltine, C.W. and Mehlman, M.A. (Eds.). *Mycotoxins in human and animal health.* 327-336 (Pathotox Publishers, ILL).

Yang, C.S. (1980). Research on esophageal cancer in China: A review. *Cancer Res.*, **40**, 2633-2644.

Yoo, H-S., Norred, W.P., Wang, E., Merrill Jr,. A.H. and Riley, R.T. (1992). Fumonisin inhibition of *de novo* sphingolipid biosynthesis and cytotoxicity are correlated in LLC-PK1 cells. *Toxicol. Appl. Pharmacol.*, **114**, 9-15.

Yoshino, N., Takizawa, M., Akiba, H., Okumura, H., Tashiro, F., Honda, M. and Ueno, Y. (1996). Transient elevation of intracellular calcium ion levels as an early event in T-2 toxin-induced apoptosis in human promyelotic cell line HL-60. *Natural Toxins*, **4**, 234-241.

Yoshino, N., Takizawa, M., Okumura, H., Ihara, T., Sugamata, M., Tashiro, F., Honda, M., Nakamura, K. and Ueno, Y. (1997a). Nivalenol induced apoptosis in human peripheral blood lymphocytes *in vitro* and mouse peripheral blood lymphocytes *in vivo. Mycotoxins*, **45,** 33-37.

Yoshino, N., Takizawa, M., Tashiro, F., Honda, M. and Ueno, Y. (1997b). T-2 Toxin induced apoptosis in human peripheral blood lymphocytes *in vitro. Res. Com. Biochem. Cell. Biol.*, **1** (2/3), 218-228.

Zimmerli, B. and Dick, R. (1995). Determination of ochratoxin A at the ppt level in human blood, serum, milk and some foodstuffs by high-performance liquid chromatography with enhanced fluorescence detection and immunoaffinity column cleanup: methodology and Swiss data. *J. Chromatogr.*, **666**, 85-99.