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.
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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.
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Figure 1: Structures of representative mycotoxins
CHAPTER 2: Mycotoxins with Special Reference to the Carcinogenic Mycotoxins

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

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Biological Activity</th>
<th>Producing Genus</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Carcinogenicity, teratogenicity</td>
<td>Aspergillus</td>
<td>Büchi et al. (1966); Van Rensburg (1986), Groopman et al. (1992)</td>
</tr>
<tr>
<td>Citrinin</td>
<td>Nephrotoxicity</td>
<td>Penicillium, Aspergillus</td>
<td>Betina (1984)</td>
</tr>
<tr>
<td>α-Cyclopiazonic acid</td>
<td>Neurotoxicity</td>
<td>Penicillium, Aspergillus</td>
<td>Holzapfel (1968)</td>
</tr>
<tr>
<td>Ergotoxins (ergotamine)</td>
<td>Vasoconstriction, neurotoxicity</td>
<td>Claviceps</td>
<td>Stoll (1952); Scott et al. (1992)</td>
</tr>
<tr>
<td>Fumonisin B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Carcinogenicity, neurotoxicity</td>
<td>Fusarium</td>
<td>Bezuidenhout et al. (1988); Jackson et al. (1996)</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>Carcinogenicity, nephrotoxicity</td>
<td>Aspergillus, Penicillium</td>
<td>Van der Merwe et al. (1965); Pohland et al., (1992); Creppy et al. (1993).</td>
</tr>
<tr>
<td>Penitrem A</td>
<td>Neurotoxicity</td>
<td>Penicillium</td>
<td>De Jesus et al. (1983); Steyn and Vleggaar (1985)</td>
</tr>
<tr>
<td>Phomopsin A</td>
<td>Hepatotoxicity</td>
<td>Phomopsis</td>
<td>Culvenor et al. (1989)</td>
</tr>
<tr>
<td>Sporidesmin A</td>
<td>Hepatotoxicity, photosensitivity</td>
<td>Pithomyces</td>
<td>Mortimer et al. (1978)</td>
</tr>
<tr>
<td>Trichothecenes (T-2 toxin)</td>
<td>Dermotoxicity, hematopoietic</td>
<td>Fusarium</td>
<td>Wannemacher et al. (1991); Plattner et al. (1989); Grove (1996)</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>Estrogenism, reproductive irregularities</td>
<td>Fusarium</td>
<td>Urry et al. (1966)</td>
</tr>
</tbody>
</table>

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)].
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 oblitative 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 trichothecenes has been adequately covered by Grove (1996), who reported that a total of 182 trichothecenes, based on the trichotheccane 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 haemorrhage and exhaustion of the bone marrow, are similar to those induced by T-2 toxin. Ueno et al. (1972) and Vagen 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; Suganata 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 trichotheccenes 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 trichotheccenes, 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 trichotheccenes 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 trichotheccenes 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 B1 (AFB1) has the highest level of occurrence and is the most carcinogenic of the known aflatoxins, while aflatoxin M1 (AFM1) is excreted in the milk of cows and has toxic properties similar to those of AFB1 (Holzapfel et al., 1966).

Chemistry and Metabolism

Aflatoxins B1, B2, G1 and G2 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 (AFB1, AFB2, AFB2a, AFM1, AFM2, AFM2a and aflatoxicol) and the difurocoumarolactone series (AFG1, AFG2, AFG2a, AFGM1, AFGM2, AFGM2a and AFB3) (Heathcote, 1984)(see Figure 2). Aflatoxin B1 (C17H12O6) is a pale-
white 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$_1$**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>269-271 °C</td>
</tr>
<tr>
<td>Optical rotation [α]D</td>
<td>-559 ° (concentration 625 μmol/l in chloroform)</td>
</tr>
<tr>
<td>Infrared</td>
<td>Strong bands at 1770, 1600, 1570, 1390 and 1310 nm.</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>312.3</td>
</tr>
</tbody>
</table>

Source: Pohland et al. (1982).

<table>
<thead>
<tr>
<th>Aflatoxin</th>
<th>LD$_{50}$ (μg per day-old duckling)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_1$</td>
<td>18</td>
</tr>
<tr>
<td>$G_1$</td>
<td>39</td>
</tr>
<tr>
<td>$B_2$</td>
<td>84</td>
</tr>
<tr>
<td>$G_2$</td>
<td>173</td>
</tr>
<tr>
<td>$M_1$</td>
<td>17</td>
</tr>
<tr>
<td>$M_2$</td>
<td>62</td>
</tr>
</tbody>
</table>

**Table 3: Toxicities of the principal aflatoxins** (Carnaghan et al., 1963; Heathcote, 1984).
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**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: AFB1 and AFB2 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 C20 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 C1-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-I) → O-methylsterigmatocystin (omt-I). 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 parasiticus* 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.
Acetate + malonate → \[ \text{C}_{20} \text{ polyketide precursor} \]

Averantin → Norsolorinic acid

\[ \text{Averufin} \rightarrow 1'-\text{Hydroxyversicolorone} \]

\[ \text{Versiconal} \rightarrow \text{Versiconal hemiacetal acetate} \]

\[ \text{Versicolorin B} \]

(continues in next column)

\[ \begin{align*}
\text{Versicolorin A} & \quad \text{Dihydrosterigmatocystin} \\
\text{Sterigmatocystin} & \quad \text{Dihydro-O-methyl sterigmatocystin} \\
\text{O-methylsterigmatocystin} & \quad \text{Aflatoxin B}_2 \\
\text{Aflatoxin B}_1 & \quad \text{Aflatoxin G}_1
\end{align*} \]

**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\textsubscript{1} relative to the different aflatoxins vary with different growth conditions e.g. enhanced levels of AFB\textsubscript{1} relative to AFG\textsubscript{1} occur in \textit{A. parasiticus} at elevated temperatures as a result of accelerated catabolism of AFG\textsubscript{1} (Detry 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\textsubscript{2} columns followed by determination by paper chromatography, which was subsequently replaced by SiO\textsubscript{2} TLC. (Shepherd \textit{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\textsubscript{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 \textit{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 \textit{et al.}, 1986). The latter method has a lower detection limit of 5-30 pg of AFB\textsubscript{1}. Cepeda \textit{et al.}, (1996) introduced cyclodextrines to increase the fluorescence of AFB\textsubscript{1} and AFG\textsubscript{1} in the postcolumn excitation of these toxins: substantial improvements (ca 30 fold) in detection limits of AFB\textsubscript{1} and AFG\textsubscript{1} were subsequently obtained by the use of heptakis-2,6-ß-O-dimethyl-ß-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\textsubscript{1} (Kussak \textit{et al.}, 1995). The quest for better and more accurate methods for the determination of the aflatoxins...
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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 B₂, which is always present in grains contaminated with aflatoxins. Methods for the determination of the aflatoxins are summarised in Table 4.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Method</th>
<th>Recoveries, detection limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust and urine</td>
<td>LC-MS</td>
<td>2 pg mg⁻¹, 50 pg ml⁻¹</td>
</tr>
<tr>
<td>Milk</td>
<td>Affinity columns and HPLC</td>
<td>85.7 %, 50 pg ml⁻¹</td>
</tr>
<tr>
<td>Cheese</td>
<td>Affinity columns and HPLC</td>
<td>75 %, 5 ng kg⁻¹</td>
</tr>
<tr>
<td>Ground peanuts</td>
<td>ELISA</td>
<td>62-84 %, 5 µg kg⁻¹</td>
</tr>
</tbody>
</table>

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-Vetasco 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 Afle-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 Domer and Cole, (1989). Radioimmunocassays (RIA) for AFB₁ 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 ELISA the colour-producing enzyme is conjugated directly to the first antibody. Although this assay is much quicker it uses more antibodies and is thus more expensive. Commercial ELISA kits have detection limits of about 2 μg.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).

Immunofaffinity 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 immunofaffinity 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 (AFB₁, AFB₂, AFG₁ and AFG₂) 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).
CHAPTER 2: Mycotoxins with Special Reference to the Carcinogenic Mycotoxins

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 alumino-silicate, 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 AFB1 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.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Maximum Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food for humans and feed for some animal species</td>
<td>20 ppb</td>
</tr>
<tr>
<td>Milk</td>
<td>0.5 ppb</td>
</tr>
<tr>
<td>Feed for feedlot cattle</td>
<td>300 ppb</td>
</tr>
<tr>
<td>Feed for market hogs</td>
<td>200 ppb</td>
</tr>
<tr>
<td>Feed for breeding cattle, breeding hogs and mature poultry</td>
<td>100 ppb</td>
</tr>
</tbody>
</table>

**Biological Effects and Mechanism of Action**

Toxicologically, the aflatoxins particularly AFB₁ should be regarded as a quadruple threat, i.e. as a potent toxin, a carcinogen, a teratogen and a mutagen. AFB₁ 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 AFB₁ 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 biostatistical studies, since AFB₁ causes an activation of the \( K\text{I} \) 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 AFB₁ as a human carcinogen in 1987 and confirmed the classification in 1992 and 1999.
CHAPTER 2: Mycotoxins with Special Reference to the Carcinogenic Mycotoxins

Table 6: Acute oral toxicities of aflatoxins

<table>
<thead>
<tr>
<th>Species</th>
<th>B1</th>
<th>B2</th>
<th>LD50 (mg.kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duckling</td>
<td>0.36</td>
<td>1.68</td>
<td>0.78</td>
</tr>
<tr>
<td>Cat</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>0.5-1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>1.0-2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>1.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>2.2-7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>7.2 (M)- 6 (F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


The mutagenic and carcinogenic effects of AFB1 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 AFB1-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). AFB2 on the other hand has no double bond at this position and is practically inactive. AFB1-epoxide can be metabolised further to 8,9-dihydro-8,9-dihydroxy-aflatoxin B1 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).
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 (AFM₁, AFP₁ and AFQ₁). The secondary conjugating processes involve glucuronidation, sulphation and acetylation of primary AFB₁ 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 °C with the loss of benzene) and it melts at 169-171 °C upon crystallization from xylene (Van der Merwe *et al.*, 1965). The IR spectrum of OTA (CHCl₃) 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 \( \lambda_{\text{max}} \) 216 nm (ε = 31 500) and 330 nm (ε = 6 400) in MeOH / 0.0005 M H₂SO₄. 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). ¹³C 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., [1-¹⁴C]- and [2-¹⁴C]acetate, [2-¹⁴C]malonate, DL-[methyl-¹⁴C]methionine and chlorine-36 (Steyn *et al.*, 1970) and stable isotope precursors, e.g. sodium[¹³C]formate, [1-¹³C]- and [1,2-
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.

![Structure of OTA]

**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; Størmer *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 (4R)-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 (Størmer et al., 1981, 1983) while the (4S)-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 A2 and A3, both with a molecular weight of 54 kDa, and the minor form Bα play an important role in the oxidation of OTA. These two epimers were also found in rat and rabbit liver (Størmer 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 (Størmer 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, (4R)-OH OTA, (4R)-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

<table>
<thead>
<tr>
<th>Analogs</th>
<th>HeLa cell LC₅₀ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTA</td>
<td>0.005</td>
</tr>
<tr>
<td>OTC</td>
<td>0.009</td>
</tr>
<tr>
<td>OTB</td>
<td>0.054</td>
</tr>
<tr>
<td>d-OTA</td>
<td>0.163</td>
</tr>
<tr>
<td>OTα</td>
<td>0.56</td>
</tr>
<tr>
<td>OM-OTA</td>
<td>0.83</td>
</tr>
<tr>
<td>DC-OTA</td>
<td>7.6</td>
</tr>
<tr>
<td>OE-OTA</td>
<td>10.1</td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Aspergillus</th>
<th>Penicillium</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ochraceus (A. alutaceus)</td>
<td>P. viridicatum (P. verrucosum)</td>
</tr>
<tr>
<td>A. melleus (A. quercins)</td>
<td></td>
</tr>
<tr>
<td>A. alliaceus</td>
<td></td>
</tr>
<tr>
<td>A. ostianus</td>
<td></td>
</tr>
<tr>
<td>A. sclerotiorum</td>
<td></td>
</tr>
<tr>
<td>A. albertensis</td>
<td></td>
</tr>
<tr>
<td>A. wentii</td>
<td></td>
</tr>
<tr>
<td>A. auricomus</td>
<td></td>
</tr>
<tr>
<td>A. niger var. niger</td>
<td></td>
</tr>
<tr>
<td>A. awamori</td>
<td></td>
</tr>
<tr>
<td>A. carbonarius</td>
<td></td>
</tr>
<tr>
<td>A. foetidus</td>
<td></td>
</tr>
<tr>
<td>A. sulphureus (A. fresenii)</td>
<td></td>
</tr>
</tbody>
</table>

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 μg.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.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cleanup</th>
<th>Recoveries, detection limits</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faba beans and wheat</td>
<td>SPE</td>
<td>70%, 0.7 μg.kg⁻¹</td>
<td>El-Banna and Scott (1984)</td>
</tr>
<tr>
<td>Wheat and barley</td>
<td>solvent partition</td>
<td>40 μg.kg⁻¹</td>
<td>Lepom (1986)</td>
</tr>
<tr>
<td>Animal feed, grain</td>
<td>2-stage SPE</td>
<td>90%, 5 μg.kg⁻¹</td>
<td>Cohen and Lapointe (1986)</td>
</tr>
<tr>
<td>Wheat, barley, oats and mixed feed</td>
<td>1.CHCl₃ extraction</td>
<td>77-96%,</td>
<td>Langseth et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>2.SPE</td>
<td>0.1-0.3 μg.kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>Human blood, serum, milk and some foodstuffs</td>
<td>immunoaffinity column</td>
<td>85%, 5-10 μg.g⁻¹</td>
<td>Zimmerli and Dick (1995)</td>
</tr>
<tr>
<td>Human urine</td>
<td>extraction, column chromatography</td>
<td>60-75%, 5ng.l⁻¹</td>
<td>Castegnaro et al. (1990)</td>
</tr>
<tr>
<td>Beer</td>
<td>1. SPE (C₁₈ silica)</td>
<td>82-100%,</td>
<td>Scott and Kanhere (1995)</td>
</tr>
<tr>
<td></td>
<td>2. immunoaffinity column</td>
<td>0.05-0.1 ng.ml⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

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 μg.kg⁻¹, wheat from 5-135 μg.kg⁻¹ and barley from 10-500 μg.kg⁻¹) and for foodstuffs from animal products (kidneys from pigs varying from 2-100 μm.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 μg.kg⁻¹ for infant foods and at 5 μg.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).
CHAPTER 2: Mycotoxins with Special Reference to the Carcinogenic Mycotoxins

Table 10: Limits for Ochratoxin A in the different commodities

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children and infant foods</td>
<td>0.5 - 5 μg.kg⁻¹</td>
</tr>
<tr>
<td>Foods</td>
<td>2 - 50 μg.kg⁻¹</td>
</tr>
<tr>
<td>Animal feeds</td>
<td>5 - 300 μg.kg⁻¹</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Species</th>
<th>OTA LD₅₀</th>
<th>OTC LD₅₀</th>
<th>OTB LD₅₀</th>
<th>OT₅₃ LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs</td>
<td>1.0-6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chickens</td>
<td>3.5</td>
<td>4.8</td>
<td>41.4</td>
<td>22</td>
</tr>
<tr>
<td>Dogs</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonatal rats</td>
<td>3.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature rats</td>
<td>20-30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>46-58</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


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 mucohemorrhagic 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.m1 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.g1.

**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 (CD4+ or CD8+) 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 (4R)-OH-OTA mainly in the bile and urine after OTA had been administered intraperitoneally or per os (Ståren 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.**

<table>
<thead>
<tr>
<th>Species</th>
<th>OTA</th>
<th>OP-OTA</th>
<th>OTα</th>
<th>OTB</th>
<th>OTC</th>
<th>OTA-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats (iv)</td>
<td>160±17</td>
<td>163±5</td>
<td>31±5</td>
<td>14±4</td>
<td>6±1.2</td>
<td>19±4.7</td>
</tr>
<tr>
<td>Rabbits</td>
<td>126</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chickens</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>108</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats (iv)</td>
<td>103±16</td>
<td>50.5±2.8</td>
<td>9.6±2.3</td>
<td>4.2±1.2</td>
<td>0.6±0.2</td>
<td>6±0.9</td>
</tr>
<tr>
<td>Rats (p/v)</td>
<td>120</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats (o/iv)</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>72-150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ruminant calf</td>
<td>77</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>24-48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbits</td>
<td>8.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chickens</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkeys</td>
<td>840</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$t^{1/2a}$ = distribution half-life; $t^{1/2b}$ = elimination half-life; p = peripherally, iv = intravenous, o = orally.

Sources: Galtier et al., 1979b; Galtier et al., 1981c; Mortensen et al., 1983h; Ballinger et al., 1986a; Fukui et al., 1987f; Sreemannarayana et al., 1988e; Hagelberg et al., 1989f; Marquardt et al., 1996a;

**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 modifiers 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 (Størmer 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 C₁₄ and C₁₅ (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 A₁ (FA₁) and fumonisin A₂ (FA₂), the N-acetyl derivatives of fumonisin B₁ (FB₁) and fumonisin B₂ (FB₂) respectively, are produced in low yields in cultures of \textit{F. moniliforme} and have the lowest toxicities. These two structural analogues and FB₄ (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 \textit{Fusarium oxysporum} during a screening of fumonisins by the same group in 1996.

FB₁, the most abundant fumonisin in culture and naturally occurring in corn (Rheeder \textit{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 \textit{et al.}, 1991).

\begin{center}
\begin{tikzpicture}
  \node at (-4,0) {\textbf{Figure 6:} Structures of the fumonisins.}
  \begin{table}[h]
    \begin{tabular}{|c|c|c|c|}
      \hline
      & R₁ & R₂ & R₃ \\
      \hline
      Fumonisin A₁ & OH & OH & CH₃CO \\
      Fumonisin A₂ & H & OH & CH₃CO \\
      Fumonisin B₁ & OH & OH & H \\
      Fumonisin B₂ & H & OH & H \\
      Fumonisin B₃ & OH & H & H \\
      Fumonisin B₄ & H & H & H \\
      \hline
    \end{tabular}
  \end{table}
\end{tikzpicture}
\end{center}

\textbf{Chemical characteristics of the fumonisins}

FB₁ is a stable compound that persists through most normal food processing procedures (see Table 13 for the physical data of FB₁).
Table 13: Physical and spectroscopic data of FB1.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>103-105 °C</td>
</tr>
<tr>
<td>Optical rotation $[\alpha]_D$</td>
<td>-28° (concentration 2 mg/ml)</td>
</tr>
<tr>
<td>Infrared (KBr)</td>
<td>3450, 2934, 1729 and 1632 cm$^{-1}$</td>
</tr>
</tbody>
</table>

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 FB1, while the acetylated and methylated derivatives of FB1 were prepared by Bezuidenhout et al., (1988) and Laurent et al., (1990) respectively. FB1 and FB2 can be hydrolysed by heating with hydrochloric acid or potassium hydroxide to yield the aminopentol of FB1 and the aminotetraol of FB2 (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

<table>
<thead>
<tr>
<th><em>Fusarium moniliforme</em></th>
<th><em>Fusarium napiforme</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fusarium dlamini</em></td>
<td><em>Fusarium proliferatum</em></td>
</tr>
<tr>
<td><em>Fusarium nygamai</em></td>
<td><em>Fusarium anthophilum</em></td>
</tr>
<tr>
<td><em>Fusarium subglutinans</em></td>
<td></td>
</tr>
</tbody>
</table>

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$_4$Cl, Na$_2$SO$_4$ and MnSO$_4$. Alberts et al. (1993), developed a technique for the production of $[^{14}$C]$\text{FB}_1$ 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**

<table>
<thead>
<tr>
<th>Species</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>cattle and poultry</td>
<td>50 000 ppb</td>
</tr>
<tr>
<td>Pigs</td>
<td>10 000 ppb</td>
</tr>
<tr>
<td>Horses</td>
<td>5 000 ppb</td>
</tr>
</tbody>
</table>

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; FB₁ 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.**

<table>
<thead>
<tr>
<th>Country</th>
<th>Commodity</th>
<th>Level</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>Corn and corn based foods</td>
<td>&lt;5310 ppb for FB₁ &amp; &lt;1480 ppb for FB₂</td>
<td>Doke and Visconti (1994)</td>
</tr>
<tr>
<td>Argentina</td>
<td>Corn</td>
<td>combined fumonisin levels of 1585 - 9990 ppb</td>
<td>Sydenham et al. (1993a)</td>
</tr>
<tr>
<td>Kenya</td>
<td>Corn</td>
<td>&lt;1 ppm FB₁ (mostly)</td>
<td>Kedera et al. (1999)</td>
</tr>
<tr>
<td>Brazil</td>
<td>Corn and corn based feeds</td>
<td>0,2 - 38,5 ppm FB₁ &amp; 0,1 - 12,0 ppm FB₂</td>
<td>Sydenham et al. (1994)</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>Corn and feeds</td>
<td>1,3 - 27,0 ppm FB₁ &amp; 0,1 - 12,6 ppm FB₂</td>
<td>Thiel et al. (1991)</td>
</tr>
<tr>
<td>India</td>
<td>Corn</td>
<td>100-4740</td>
<td>Chatterjee and Mukherjee (1994)</td>
</tr>
<tr>
<td>India</td>
<td>Corn kernels</td>
<td>18-155 ppm FB₁</td>
<td>Shetty and Bhat (1997)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Corn-based products</td>
<td>1 and 9 ppm of FB₁</td>
<td>Pittet et al. (1992)</td>
</tr>
<tr>
<td>China</td>
<td>Corn</td>
<td></td>
<td>Chu and Li (1994)</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Forage grass</td>
<td></td>
<td>Mirocha et al. (1992)</td>
</tr>
</tbody>
</table>

**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**

FB₁ 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. FB₂ is also foetotoxic to rats, suppressing both growth and foetal bone development (Lebepe-Mazur et al., 1995). FB₁, FB₂ and FB₃ 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 IC₅₀ value of 4 μg.ml⁻¹ for FB₁ and 2 μg.ml⁻¹ for FB₂ (Shier et al., 1992). IC₅₀ 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 FB₁ and FB₂ (Shephard et al., 1993, 1995) and in primary rat hepatocytes (Cawood et al., 1994). FA₁ and FA₂ are less cytotoxic, but PA₁ and PA₂ 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 FB₁ 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). FB₁ 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. FB₁ 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, FB₁ 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 FB₁ 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-acylsphinganines (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. FB₁ and FB₂ are cytotoxic to renal epithelial (LLC-PK₁) 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 EC₅₀ of 10-15 µM for FB₁, 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 FB₁ in LLC-PK₁ 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 FB₁ 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 FB1 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, FB1 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 FB1 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 FB1 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 FB1
can indirectly cause lipid peroxidation (See Mechanisms of Action of Ochratoxin A) (Gelderblom et al., 1996b, 1998).

FB₁ 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 FB₁ 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 FB₁-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).

FB₁ 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).

FB₁ and FB₂ 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). FB₁ 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).

FB₁ and TA-toxin (see Figure 7) are more phytotoxic to corn and tomato seedlings than FB₂ and FB₃ and cause reductions in shoot and root length (Lamprecht et al., 1994). The mechanism of action in plants is not yet known.
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; Bennett 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


CHAPTER 2: Mycotoxins with Special Reference to the Carcinogenic Mycotoxins


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