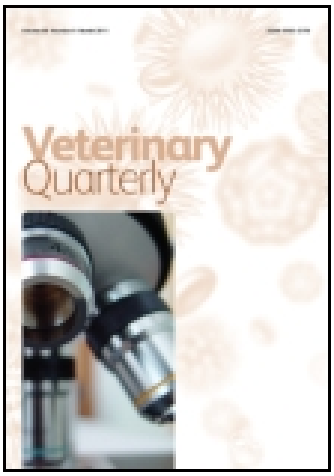

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Mycotoxins: Their implications for human and animal health

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U335. Breast Cancer Res Treatm 1998; 1332: 1-13.
31. Pike MC, Spicer DV, Dahmoush L, and Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol Rev 1993; 15: 17-35.
32. Resnick CE, Fielder PJ, Rosenfeld RG, and Adashi EY. Characterization and hormonal regulation of a rat ovarian insulin-like growth factor binding protein-5 endopeptidase: an FSH-inducible granulosa cell-derived metalloprotease. Endocrinology 1998; 139: 1249-57.
33. Rutteman GR, Willekes-Koolschijn N, Bevers MM, Gugten AA van der, and Misdorp W. Prolactin binding in benign and malignant mammary tissue of female dogs. Anticancer Res 1986; 6: 829-35.
34. Rijnberk A, Eigenmann JE, Belshaw BE, and Hampshire J. Altszuler acromegaly associated with transient overproduction of growth hormone in a dog. N J Am Vet Med Assoc 1980; 177: 534-7.
35. Schedlich LJ, Young TF, Firth SM, and Baxter RC. Insulin-like growth factor-binding protein (IGFBP)-3 and IGFBP-5 share a common nuclear transport pathway in T47D human breast carcinoma cells. J Biol Chem 1998; 17: 18347-52.
36. Selman PJ, Mol JA, Rutteman GR, and Rijnberk A. Progestins and growth hormone excess in the dog. Acta Endocrinol (Copenh)1991; 125, 42-7.
37. Selman PJ, Mol JA, Rutteman GR, and Rijnberk A. Progestin treatment in the dog. I Effects on growth hormone, insulin-like growth factor I and glucose homeostasis. Eur J Endocrinol1994; 131: 413-21.
38. Selman PJ, Mol JA, Rutteman GR, and Rijnberk A. Progestin treatment in the dog II. Effects on the hypothalamic- pituitary-adrenocortical axis. Eur J Endocrinol 1994; 131: 422-30.
39. Selman PJ, Mol JA, Rutteman GR, Garderen E van, and Rijnberk A. Progestin induced growth hormone excess in the dog originates in the mammary gland. Endocrinology 1994; 134: 287-92.
40. Shyamala G, Yang X, Silberstein G, Barcellos-Hoff MH, and Dale E. Transgenic mice carrying an imbalance in the native ratio of A to B forms of progesterone receptor exhibit developmental abnormalities in mammary glands. Proc Natl Acad Sci USA 1998; 95: 696-701.
41. Surmacz E, Guvakova MA, Nolan MK, Nicosia RF, and Sciacca L. Type I insulin-like growth factor receptor function in breast cancer. Breast Cancer Res Treat 1998; 47: 255-67.
42. Syvala H, Pekki A, Blauer M, Pasanen S, Makinen E, Ylikomi T, and Tuohimaa P. Hormone-dependent changes in A and B forms of progesterone receptor. J Steroid Biochem Mol Biol 1996; 58: 517-24.
43. Tonner E, Barber MC, Travers MT, Logan A, and Flint DJ. Hormonal control of insulin-like growth factor-binding protein-5 production in the involuting mammary gland of the rat. Endocrinology 1997; 138: 5101-57.
44. Tuggle CK, and Trenkle A. Control of growth hormone synthesis. Dom Animal Endocrinol 1996; 13: 1-33.
45. Van Garderen E, Wit M de, Voorhout WF, Rutteman GR, Mol JA, Nederbragt H, and Misdorp W. Expression of growth hormone in canine mammary tissue and mammary tumors. Am J Pathol1997; 150: 1037-47.
46. Van Garderen E, Poel H van der, Swennenhuis JF, Wissink EHJ, Rutteman GR, Mol JA, and Schalken JA. Expression and molecular characterization of the growth hormone receptor in canine mammary tissue and mammary tumors. Endocrinology, accepted 1999.
47. Watson ADJ, Rutteman GR, Rijnberk A, and Mol JA. Effect of somatostatin analogue sms 201-995 and antiprogestin agent RU 486 in canine acromegaly. Front Horm Res 1987; 1: 193-8.
48. Wei LL, Hawkins P, Baker C, Norris B, Sheridan PL, and Quinn PG. An amino-terminal truncated progesterone receptor isoform, PRc, enhances progestin-induced transcriptional activity. Mol Endocrinol 1996; 10: 1379- 87.

MYCOTOXINS: THEIR IMPLICATIONS FOR HUMAN AND ANIMAL HEALTH

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ABSTRACT

Mycotoxins contaminate various feed and food commodities, due to the global occurrence of toxinogenic molds. They exert adverse health effects in human and animals. The nature of these toxic effects varies depending on the chemical structure of the toxin. The degree of these adverse effects is not only determined by the toxin concentration present in foods and feeds, but also by the time of exposure. Whilst in animals, next to acute intoxication, losses in productivity, reduced weight gain and immunosuppression are considered as most important feature of mycotoxicoses, genotoxic effects and the involvement of certain mycotoxins such as aflatoxin, ochratoxins and fumonisins in the etiology of human cancers have obtained particular attention. This implies that recent research activities concentrate on mechanistic aspects of mycotoxin-induced pathologies, rather than compiling analytical measures of mycotoxin concentrations in food and feeds.

INTRODUCTION

Mycotoxins comprise a group of chemically diverse compounds originating from secondary metabolism of molds (filamentous fungi). As of yet more than 300 mycotoxins have been identified to induce signs of toxicity in mammalian species. Their global occurrence is considered to be a major risk factor affecting human and animal health as it is estimated that 25% of the world's crop production is contaminated to some extend with mycotoxins (16,28).

The synthesis of mycotoxins by molds is genetically determined and closely related to primary metabolic pathways, such as amino acid and fatty acid metabolism. However, the actual toxin production, and thus the degree of contamination of feed and food commodities is modulated by environmental factors such as substrate composition and texture, humidity and temperature. Plants are more susceptible to mold invasion under conditions of stress such as draught or over-irrigation, insect damage and pesticide exposure. In turn, plant susceptibility determines the fungal growth characteristics and toxin production rate. In daily agricultural practice the consequences of these genetic and environmental influences on toxin production have the following implications:

* fungal invasion of agricultural commodities occurs often already under field conditions (*Fusarium* species, but also *Aspergillus* and *Penicillium spp.*) and shows a considerable seasonal variation.
* fungal growth characteristics can not be correlated to mycotoxin production, in other words, the estimate of cfu (colony forming units) in a certain food/feed commodity is a valid parameter to assess hygienic quality, but fails to predict the actual level of mycotoxin contamination.

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* hygienic measures such as the use of fungistatic and fungicidal agents may effectively interrupt mold invasion but do not affect the mycotoxin level present prior to the application of these compounds.
* the rate of mycotoxin production varies not only between fungal genera but even within strains of one distinct fungal species.
* mycotoxin production can be a 'suicide' mechanism as high concentrations of mycotoxins have fungistatic oreven fungicidal activity - and also antibacterial activity. This implies that plant commodities, which do not show fungal activity at the time of sampling, might have been contaminated at an earlier stage.

In reviewing the complex biology of mold growth and toxin production it becomes evident that no monolithic strategy can be invented to prevent completely fungal invasion and toxin production in daily agricultural practice.

MYCOTOXINS AFFECTING ANIMAL HEALTH AND PRODUCTIVITY

Mycotoxin exposure via feed may result in acute intoxication in animals (8). However, under modern agricultural practice more often subacute intoxication, with a great diversity in symptoms is observed. Moreover, chronic exposure to low doses may even remain undetected but may result in reduced weight gain, diminished productivity and increased susceptibility to infections (7). The occurrence of mycotoxins in animal feed exhibits often a geographic pattern, as for example *Aspergillus* species meet optimal conditions only in tropical and subtropical regions, whereas *Fusarium* and *Penicillium* species are adapted to the moderate climate of North America and Europe. However, worldwide trade with food and feed commodities results in a wide distribution of contaminated material. Typical examples are the **aflatoxins** (Aflatoxin B_1, B_2, G_1, and G2) which are produced by toxinogenic isolates of *Aspergillus flavus* and *A. parasiticus*. Although these molds require subtropical climate for toxin production, the historical case of the Turkey X disease in England in 1961, is the first example of an 'imported' mycotoxicosis (3). At that time, tons of contaminated groundnut meal were unloaded in England and used for the production of formulated feed for turkeys. In poultry aflatoxins cause liver degeneration and impairment of chondrocyte function (the term X refers not only to an at that time unknown disease but also to leg instability observed as a sign of intoxication). As at the same time, intoxicated animals did show signs of neurotoxicity, including opisthotonus. This acute outbreak of a mycotoxicosis might have been a consequence of contamination of ground nut meal with both, aflatoxin B_1 and cyclopiazonic acid, a second mycotoxin synthesized by *Aspergillus flavus* and *Aspergillus parasiticus* (9). The hepatotoxicity of aflatoxin B_1 (AFB_1) finally resulting in liver tumors (hepatocellular carcinoma) has been observed in all animal species including humans (33). In Europe, strong regulations prevent new outbreaks of aflatoxicosis in animals. However, a matter of concern remains the incidental contamination of milk and dairy products with AFM_1, a metabolite of AFB_1, originating from bovine hepatic metabolism (29). Ruminants are unable to fully degrade AFB_1 in their forestomach system (2). This implies that after exposure to contaminated feed commodities, AFM_1 will be excreted with milk by dairy cows.

Whilst aflatoxin contamination of food and feed commodities is regarded as 'imported' problem, and thus may be successfully prevented by regulations, other mycotoxins occur regularly in cereals, grains, corn and silage intended for feed production. Among these, ochratoxin A, the group of trichothecenes and the fumonisins cause greatest concern, as they may effect both, human and animal health.

Ochratoxin A is the causative agent of kidney diseases (degeneration of proximal tubule cells resulting in interstitial nephritis and hyalinization of glomeruli) in pigs generally referred to as porcine nephropathy. Ochratoxin A (OA) is produced by *Aspergillus ochraceus*, but in Northern Europe contamination originates from ochratoxin-producing *Penicillium* species such as *P. verrucosum* and *P. chrysogenum*. Particular in the Scandinavian countries, this disease causes considerable economic losses in pig production. Although OA has been identified as specific nephrotoxin, production losses in animal production are also due to its immuno-suppressive effects, particularly after long term exposure. Levels as low as 200 ppb may induce this effect, whilst typical signs of nephrotoxicity in pigs are observed only at levels exceeding 1400 ppb in feed. In addition, OA was found to impair fertility in boars and to be teratogenic, but only at extremely high concentrations. As OA crosses the placental barrier, fetal growth might be impaired and symptoms such as tail necrosis in piglets have been discussed to be the consequence of OA exposure (32). In poultry, a number of outbreaks of acute ochratoxicosis have been reported from the United States. Feed contamination ranging between 0.3 and 16 ppm OA in the diet resulted in an increased mortality, poor feed conversion, poor growth rates and feed refusal. Post mortem finding revealed edematous, necrotic proximal tubules but also signs of hepatotoxicity (25). In ruminants, the rumen flora degrades OA and signs of intoxication have not been reported (26).

Economic losses attributed to the presence of OA in feeds are not only related to impaired animal health and productivity, but are partly due to the discharge of pig carcasses after slaughter. Due to its high rate of binding to serum proteins, ochratoxin A has a long biological half life and is found frequently as residue in porcine meat and meat products intended for human consumption. As a preventive measure contaminated meat is discharged (measures are included in the meat inspection procedure in Scandinavian countries) to avoid human exposure to residues of OA (see below).

Fusarium species, which may be described as soil fungi, produce a variety of mycotoxins denoted as trichothecenes according to their chemical structure. Prominent examples of **trichothecenes** in animal's feed are deoxynivalenol (DON) and nivalenol (type B trichothecenes), and diacetoxyscirpenol (DAS) and T-2 toxin (type A trichothecenes). As *Fusarium* species (*Fusarium graminearum, Fusarium culmorum*) produce often more than one toxin, co-contamination is common (44). T-2 was the first mycotoxin investigated from this group for its toxicological properties as it may cause severe acute intoxication. It was found to be highly cytotoxic, causing lesions of the mucosa of the gastrointestinal tract resulting in extensive hemorrhage and a general inflammatory response. It inhibits protein synthesis and impairs the maturation of hemopoetic cells in bone marrow leading to pancytopenia and immuno-suppression. The com-

plex syndrome of T-2 toxicosis has often been described as a radiomimetic effect and results in an increased susceptibility of animals to secondary infections. Particularly, T-2 toxin has been shown to increase the susceptibility of animals to *Mycobacterium bovis*, *Salmonella typhimurium*, *Staphylococcus aureus* and experimental herpes virus simplex type I infections in mice. Monogastric animals (swine) are the most sensitive to T-2 toxin, whereas poultry and ruminants (which degrade trichothecenes with the help of the rumen flora) are less sensitive (43). Acute effects of T-2 toxin occur after oral exposure to 0.1 mg/kg BW in pigs; in feed concentration of up to 500 ppb exerted no detrimental effects in fattening pigs, but skin lesion (dust exposure) might occur even at lower concentrations. In addition, experimental studies in monkeys revealed that after exposure to 0.1 µg/kg BW daily for a period of 4-5 weeks most of the animals did show signs of severe immuno-suppression. This indicates, that although T-2 toxin is rapidly metabolized with little or no accumulation in any specific organ, pronounced toxic effects can be observed after long term exposure even to low concentrations, resulting in significant impairment of the immune system. Long term studies in rodents also indicated an increased incidence of pulmonary and hepatic adenomas and a dose-related increase in epithelial hyperplasia in the forestomach of rats and mice.

The concentrations of T-2 toxin in grains, originating from European countries is generally lower than the concentrations of nivalenol and deoxynivalenol (DON). In particular DON may occur at concentrations up to 30 ppm in barley, although considerable variations are know to occur in the level of contamination. Typical years with high levels of contamination were the late 80, (1987, 1989) and recently, 1997 and 1998 whereas in the interim periods the contamination levels varied between 50 and 300 ppb. These low levels are considered to be non-toxic to pigs, although adverse effects in terms of reduced feed conversion and impaired resistance to secondary infections can not be excluded completely. DON, like the other trichothecenes, causes a pronounced immuno-suppression, characterized by a decrease in chemotaxis and phagocytosis in neutrophils and macrophages. In addition, DON depresses serum IgG and/or IgM-levels and antibody response to various antigens. In contrast, serum IgA levels are elevated and exposure to DON mimics many characteristics of the human IgA-nephropathy including glomerular IgA accumulation, increase of IgA immune complexes and hematuria. IgA has a critical role of the immune response of the intestinal mucosa and particularly in the primary defense of the host to exogenous pathogens. This may explain the increased susceptibility of DON-exposed animals to infections. Of practical relevance is that also the acquired immunity through vaccination is impaired by mycotoxin ingestion as demonstrated for *Bordetella bronchiseptica* vaccine and for pseudorabies disease vaccine. The obvious clinical signs of intoxication with DON such as feed refusal and vomiting (DON is also known as vomitoxin) occur only at concentration exceeding 12.5 ppm. It is worthwhile to mention, that very recently these high contamination levels have been observed in food and feed commodities for the first time again, after many years of low level contamination.

Another Fusarium toxin with a different structure is **zearalenone** (ZEN) produced by *Fusarium graminearum* and other *Fusarium* species. ZEN is a macrocyclic lactone with high binding affinity to estrogen receptors. It is regularly found in corn and corn products. ZEN causes an estrogen- like syndrome with enlargement of mammae and nipples in both sexes and vulva swelling and vulvovaginitis progressing to vaginal prolaps particularly in young piglets. In adult animals fertility is impaired (cystic ovaria). These signs are observed predominantly in pigs, which are considered to be the most sensitive species (adverse effects have been observed at concentrations in feeds below 0.05 ppm), but also incidentally in bovines as ZEN is not completely degraded by the rumen flora. Higher concentrations of zearalenone induce constant estrus, a pseudo-pregnancy syndrome and complete infertility (11).

The most recently discovered *Fusarium* toxin is **Fumonisin B_1** (FB_1), a representative of the fumonisin group produced predominantly by *Fusarium moniliforme*. Unlike all other mycotoxins it has a low lipid solubility which led to its discovery not earlier that 1988 (31). Fumonisins (6 different derivatives have been described of which FB_1 is recognized as the most toxic) resemble the structure of cellular sphingolipids and thus impair ceramide synthesis by inhibiting sphinganine-N-acetyl-transferase (ceramide synthetase). The complete inhibition of ceramide synthetase by fumonisin B_1 causes a disproportional increase of the intracellular free sphinganine concentration. Although the fate of the accumulated sphinganine is unclear, pathologic findings seem to be related to this primary mechanism. However, the clinical symptoms resulting from exposure to FB_1 in feed show remarkable variation across species. In horses (and other equines) FB_1 causes encephalomalacia (denoted ELEM: equine leukoencephalomalacia), a disease characterized by the degeneration of neurons (historically: hole-in-the-head disease). In pigs, the main symptom of FB_1 exposure is pleural edema (PPE - porcine pulmonary edema) impairing respiratory and hart function (10). In ruminants, FB_1 is not degraded by the rumen flora, but like in other species poorly absorbed from the intestinal lumen. However, despite the low circulating levels of FB_1, accumulation of free sphinganine bases have been found in proximal tubular cells, accompanied by an impairment of renal function. In laboratory animal species including rats, mice and rabbits, FB_1 was found to induce hepatotoxicity and nephrotoxicity and exerts tumor-promoting activity (see below).

Finally, it should be noted, that other toxins as for example *Penicillium roqueforti* toxins (bovine ketosis and mastitis), *Monascus* metabolites (liver cirrhosis in pigs) may cause health problems on individual farms. Other toxins including *Neotyphodium* toxins (ergopeptine alkaloids in tall fescue, *Rhizoctonia leguminicola* toxins (slaframine in slobber's disease) and *Acremonium* toxins (lolitrems causing stagger's disease) are of equal (economic) importance in certain areas in the world (8).

In conclusion, according to their chemical structure, mycotoxins exert a broad variety of biological effects. The nature and intensity of these effects depend on the actual concentration of an individual mycotoxin and the time of exposure. In addition, under practical conditions, feed commodities are often contaminated with more than one mycotoxin, as mold species produce different mycotoxins at the same time. These co-occurring mycotoxins can exert additive effects, as

for example demonstrated for various trichothecenes, but may also act antagonistic, as for example observed with feeds containing trichothecenes but at the same time zearalenone, and commodities, containing aflatoxins and at the same time cyclopiazonic acid.

PREVENTIVE MEASURE TO ELIMINATE MYCOTOXIN CONTAMINATION IN FEEDS

As mentioned above, prevention of mold infestation of plants, remains an ambitious project, and all strategies including the use of fungicidal compounds or competitor mould strains remained as of yet unsuccessful under practical conditions. Subsequently, various approaches have been undertaken to decontaminate feed commodities, by means of chemical agents to prevent animal diseases as well as economic losses (7). The most prominent example is the application of ammonia treatment of aflatoxin contaminated feed commodities. Although this technology is widely applied, the quality of treated feed is reduced and decontaminated material can be restrictedly used in diets of ruminants and to a lesser percentage in diets for pigs and poultry, as decontamination results in an adverse taste and a reduction of the nutritional value. Another approach is the use of adsorbents (for example silica clays), reducing the bioavailability of mycotoxins from the gastrointestinal tract of animals and thus preventing toxicity (40). Again, numerous approaches have been tested, comprising the use of silica clays, which very successfully prevent aflatoxin absorption, but bind other toxins only moderately. In Europe, the best results were as of yet achieved with a product, which is based on silica clay equipped with microbial enzymes such as epoxidase and esterases which are able to inactivate certain mycotoxins such as trichothecenes and zearalenone by means of enzymatic cleavage (36).

In addition, a few reports have been published on a specific treatment of acute mycotoxicoses, but these were restricted to neurotoxic compounds, such as the penitrem A intoxication in dogs (application of barbiturates to reduce tremor), and lolitrem B intoxication (application of sedatives) in horses. The most general advice is, to remove contaminated material from the animals. Supportive treatment is recommended to correct the effects of diarrhoea, dehydration, coagulopathia, and oral and dermal necrosis occurring as a consequence of trichothecene exposure. In the case of exposure to zearalenone, resulting in persistent estrus or retained corpora lutea, administration of prostaglandin $F_2\alpha$ has been recommended. Vaginal or rectal prolaps and physical damage to the external genitalia must be treated individually. Supportive therapy to improve organ function is advised in individual cases of ochratoxicosis (improve diuresis) and aflatoxicosis (liver protection diet and administration of vitamin E and selenium).

MYCOTOXINS IN HUMAN HEALTH

Like animals humans are susceptible to mycotoxins. Exposure originates from the consumption of contaminated plant commodities, but might occur also via a secondary route following the consumption of meat, milk and eggs, containing residual amounts of mycotoxins ingested by food-producing animals (14). This carry over - linking animal production to public health - has been observed for virtually all mycotoxins and is based on their high lipophilicity. However, the concentrations found in animal tissues are considerable lower than those found in plant commodities and thus residues in edible tissues of animals contribute only to a small percentage of mycotoxin exposure to humans. An exception might be the exposure of young children (babies and infants) consuming higher amount of milk and dairy products in relation to their body weight than adults (28)

Despite the fact that human mycotoxicoses have been reported since the Middle Ages, when ergot alkaloid intoxication was a common disease, the causal relationship between mycotoxin exposure and diseases in humans remains a matter of controversial discussion. As experimental and epidemiological evidence suggests that certain mycotoxins are associated with different forms of human cancer, mechanistic research has focused primarily on mutagenic and carcinogenic fungal toxins (15). In particular, the mechanisms involved in the experimentally recognized carcinogenicity of aflatoxins, ochratoxins and fumonisins have gained worldwide attention.

Aflatoxin B_1 carcinogenicity: The first step to assess the role of aflatoxin B_1 and other mycotoxins in human cancers is the elucidation of mechanisms leading to mutagenicity, the primary event in tumor initiation. Mutagenicity has been assessed previously by the *Salmonella typhimurium* assay (the AMES Test). The results obtained from various *Salmonella* type strains indicated that AFB_1 requires metabolic conversion to induce point as well as frame shift mutations. It is now generally accepted that AFB_1 exerts these mutagenic effects by a cytochrome P450 mediated reaction yielding the 8,9-epoxide. This nucleophilic intermediate reacts with DNA, forming persistent adducts (primarily the AFB-N^7-guanine), which in turn induce mutations in somatic cells. Further metabolic processing of the AFB_1- epoxide molecule involves the formation of the dihydro-diol (8,9-dihydro-8,9 dihydroxy-aflatoxin B_1) which may bind to cellular proteins, thus inducing cellular injury and eventually cell death. Finally, the aflatoxin-epoxide will react with glutathione either spontaneously or by a glutathione-S-transferase mediated mechanism. Glutathione-conjugation is generally considered to be the most important detoxification step competing with other biotransformation steps yielding less toxic metabolites of AFB_1 including aflatoxicol, and the aflatoxin-derivatives M_1, P_1 and Q_1 (20).

The comparison of activation and detoxification of the reactive aflatoxin epoxide in different species has provided the mechanistic basis for the understanding, why animal species vary in their sensitivity to toxic and carcinogenic effects induced by AFB_1. In contrast to experiments in rodents, evidence is accumulating that human CYP3A4 exhibits a low affinity for AFB_1 whereas human CYP1A2 was found to be the high affinity form. This implies, that the low concentration of AFB_1 encountered via food commodities will be metabolically activated by CYP1A2, whilst CYP3A4 mediated biotransformation processes yield predominantly the detoxification products of AFB_1 (17). Furthermore, it has been shown that the nucleophilic epoxide occurs in two isomeric forms, the endo-8,9-epoxide as well as its exo form. These isomeric forms have different affinities to DNA, providing evidence for an intercalative transition state between the AFB_1-exo-8,9-epoxide and the B-from of the double helix of the DNA (41). Isomeric 8,9-epoxides are expected to yield isomeric glutathione conjugates. This reaction is supported by glutathione-S-transferases (GST). It has been

shown that the low sensitivity of mice towards aflatoxin induced hepatocarcinoma is apparently related to the relatively high constitutive expression of a hepatic GST isoenzyme in this animal species. In addition, rats which are highly sensitive to aflatoxin-induced hepatocarcinogenesis express related enzymes (GST Yc2) only in very low levels. In contrast to these results obtained with two rodent species, there is no evidence suggesting the existence of a GST isoenzyme with significant conjugating capacity in human liver (45). In particular, constitutively expressed GST enzymes capable of effectively conjugating the most reactive exo-stereoisomer of AFB_1 are apparently lacking in normal human liver (4).

The insight into the metabolic processing of AFB_1 can be applied in strategies towards risk management. Induction of detoxification pathways by dithionides, like oltipraz, seems to offer the possibility to reduce the incidence of human hepatocellular carcinoma (HCC) (4). In addition, in human risk assessment strategies, attention should be paid to co-exposure to other mycotoxins, such as cyclopiazonic acid which is produced by the same mold species; this might result in reduced AFB_1 activation and mutagenicity and thus explain the geographic differences in aflatoxin-related diseases (30).

The final question to be addressed in a mechanistic approach towards the understanding of the role of aflatoxins in human hepatocellular carcinomas is the extent of promotion yielding a clone of neoplastic cells which might progress into pre-neoplastic nodules leading to cancer. It has been demonstrated that AFB_1 causes an activation of the K1 *ras* proto-oncogene in rats and modulates the *p53* tumor suppresser gene. Modifications of the *p53* gene have been found in a variety of human carcinomas (39). In general, the *p53* gene encodes for a transcription factor and functional alterations due to missense mutations and loss of the p53 protein by nonsense or frameshift mutations provide a selective advantage for clonal expansion of (pre) neoplastic cells (5). The mutation spectra of p53 in human hepatocellular carcinomas show an almost equal incidence of transitions and transversions with a high frequency of frameshift mutations. Preferential clustering of the mutations occurs at two hot spots: the domain IV at codon 234-258 and domain V involving codon 270-286. There is increasing evidence that the G-->T transversion at the third base of codon 249 is a specific feature of AFB_1 induced endemic HCC (1). However, no correlation could be established between aflatoxin-DNA-adducts and incidence of *p53* mutations. Thus, in tumor patients other factors including hepatitis virus infections might contribute to the observed mutations of the codon 249.

Ochratoxin A carcinogenicity: Already in 1978 it was proposed by Krogh (27) that an endemic disease in certain Balkan regions might be related to a high exposure to OA. Striking similarities in clinical symptoms and pathological alterations in human patients with those well known from the porcine nephropathy were noted. In endemic areas of Balkan Nephropathy, a high prevalence of epithelial tumors of the upper urinary tract could be observed, initiating the question about the role of ochratoxins in the etiology of these diseases (6). Chronic experiments in Fisher 344/N rats had indicated that OA could induce renal as well as liver tumors. Despite the experimental and epidemiological evidence of OA mediated carcinogenicity, the underlying mechanisms are incompletely understood: OA was nonmutagenic in the *Salmonella* mutation assay with and without metabolic activation by S9-mix. However, intact hepatocytes were able to transform OA into mutagenic metabolites (24). Furthermore, DNA- adducts in liver, kidney and spleen could be observed in *in vivo* experiments in rodents and in human patients using the [^{32}P]post-labeling technique (37,38). Recently, we reported that OA mutagenicity requires a cytochrome P450 dependent activation step as demonstrated in cell lines expressing selected human cytochrome P450 forms and equipped with the bacterial *lacZ'* gene as reporter for mutations (21). CYP1A1, 1A2, 2C10 and 3A4 all increased the mutation frequency of OA. The analysis of these mutations revealed that predominantly large deletions could be found and only to a minor extent other small alterations could be detected. In addition, using the conventional DNA alkaline elution assay, a large percentage of OA-induced DNA single strand breaks could be observed in primary cultures of rat hepatocytes (22).

Primary hepatocytes can convert OA to a number of different metabolites (13). Previously it was reported that rat hepatocytes may convert OA to metabolites, which could increase the mutation frequency in the *Salmonella* mutation assay conducted with the type strains TA1535, TA1538 and TA100 but not in strains TA1537 and TA98 (24). As intact cells seem to metabolize OA in a different or more complex way than microsomes (12,22) it can be assumed that apart from oxidative/microsomal pathways further processing of metabolites is required for the mutagenic response. Moreover, we could demonstrate that apart from cytochrome P450 activation of OA, conjugation to glutathione is essential for the induction of mutagenicity. In glutathione-depleted CYP450-expressing cell lines no mutagenicity was observed (23). However, it remains to be elucidated whether OA is a direct mutagen or exerts its DNA-damaging effects via quinone redox-cycling, a process accompanied by radical formation resulting in aspecific DNA damage and somatic mutations.

Fumonisin Carcinogenicity: Fumonisins show a remarkable structural similarity to free sphingoid bases, in particular to sphinganine as mentioned above. Sphinganine is an intermediate in the biosyntheses of other long-chain sphingoid bases and complex sphingolipids. Based on the general features of sphingolipid function in any cell, the reduction in complex sphingolipids and the accumulation of intermediates and breakdown products originating from the biosynthetic pathway of ceramides, do not only seem to explain the cytotoxicity but also the impairment of other essential cellular functions. This includes the control of membrane integrity and functionality, intracellular calcium-sequestration as well as cell proliferation, differentiation and apoptosis (42).

Fumonisin B_1 (FB_1) is non-mutagenic and non-genotoxic when tested according to the standard protocols for genotoxic carcinogens (19). However, *in vivo* studies provided evidence for fumonisin-induced carcinogenicity in rodents (18,35). In contrast to other even weak genotoxic carcinogens, DNA synthesis is stimulated by fumonisins (*in vitro*). Together with the above-mentioned alterations in cellular functions, including proliferation, differentiation and apoptosis, it can be assumed that fumonisin carcinogenicity is based on an epigenetic mechanism. The finding that - at least at very high exposure levels - FB_1 may act as a complete carcinogen is not detrimental to this hypothesis as it is known from other non-genotoxic mutagens and carcinogens that extensive cytotoxi-

city will be followed by preneoplastic lesions (34).

In conclusion, the variety of chemical structures and subsequently broad diversity of biological effects induced by mycotoxins, require a substance-by-substance evaluation. Beside typical organ-specific lesions principal toxic mechanisms affecting cellular functions such as cell membrane integrity, cellular energy metabolism and macromolecular syntheses, and intercellular communication in terms of cell proliferation and differentiation deserve attention. As a consequence, research on detrimental health effects induced by mycotoxins move from descriptive toxicology toward mechanism based research including molecular epidemiology.

REFERENCES

1. Aguilar F, Hussain SP, and Cerutti P. Aflatoxin B_1 induces the transversion of G → T in codon 249 of the *p53* tumor suppressor gene in human hepatocytes. Proc Natl Acad Sci USA 1993; 90: 8586-90.
2. Auerbach H, Maas RF, Op den Camp HJM, Pol A, and Fink-Gremmels J. Biodegradation of aflatoxin B1 by rumen micro-organisms in vitro and its effect on rumen fermentation. J Dairy Sci 1999; *submitted*.
3. Blount WP. Turkey 'X'disease. Turkey 1961; 9: 52.
4. Buetler TM, Bammler TK, Hayes JD, and Eaton DL. Oltipraz-mediated changes in aflatoxin B_1 biotransformation in rat liver: implications for human chemointervention. Cancer Res 1996; 56: 2306-13.
5. Carson DA, and Lois A. Cancer progression and *p53*. Lancet 1995; 346:1009-11.
6. Castegnaro M, Bartsch H, and Chernozemsky I. Endemic nephropathy and urinary tract tumors in the Balkans. Cancer Res 1987; 47: 3608-9.
7. Charmley LL, Trenholm HL, Prelusky DB, and Rosenberg A. Economic looses and decontamination. Nat Toxins 1995; 3: 199-203, 221.
8. Cheeke PR. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. J Anim Sci 1995; 73: 909-18.
9. Cole RJ. Etiology of Turkey 'X'disease in retrospect: A case for the involvement of cyclopiazonic acid. Mycotoxin Res 1986; 2: 3-7.
10. Diaz GJ, and Boermans HJ. Fumonisin toxicosis in domestic animals: a review. Vet Hum Toxicol 1994; 36: 548-55.
11. Diekman MA, and Green ML. Mycotoxins and reproduction in domestic livestock. J Anim Sci 1992; 70: 1615-27.
12. Fink-Gremmels J, Blom MJ, and Woutersen-van Nijnanten FMA. Comparative aspects of ochratoxin A metabolism. In: Scudamore KA, ed. Proceedings of the UK Workshop on Occurrence and Significance of Mycotoxins, 1993; 124-30.
13. Fink-Gremmels J. Toxicity and metabolism of ochratoxin A. Natural Toxins 1995; 3: 214-20.
14. Fink-Gremmels J. Schimmelpilze in und auf Lebensmitteln. In: Ernährungsbericht 1996; Deutsche Gesellschaft für Ernährung, D-Frankfurt.
15. Fink-Gremmels J. Mycotoxins: Mechanism of action at the molecular level. In: Miraglia M, van Egmond H, Brera C, Gilbert J, eds. Mycotoxins and Phycotoxins - Developments in Chemistry, Toxicology and Food Safety, 1998; 247-62.
16. Fink-Gremmels J, and Georgiou NA. Risk assesment of mycotoxins for the consumer. In: Ennen G, Kuiper HA, Valentin A, eds. Residues of Veterinary Drugs and Mycotoxins in Animal Products. NL-Wageningen Press 1996; 159-74.
17. Gallagher EP, Wienkers LC, Stapleton PL, Kunze KL, and Eaton DL. Role of human microsomal and human complementary DNA-expressed cytochrome P450 1A2 and cytochrome P450 3A1 in the bioactivation of aflatoxin B_1. Cancer Res 1994; 54: 101-8.
18. Gelderblom WCA, Kriek NPJ, Marasas WFO, and Thiel PG. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1 in rats. Carcinogenesis 1991;12: 1247-51.
19. Gelderblom WCA, and Snyman SD. Mutagenicity of potentially carcinogenic mycotoxins produced by *Fusarium moniliforme*. Mycotoxin Res 1991; 7: 46-52.
20. Gorelick NJ. Risk assessment for aflatoxin: I. Metabolism of aflatoxin B1 by different species. Risk Analysis 1990; 10: 539-59.
21. Groene EM de, Hassing GIA, Blom MJ, Seinen W, Fink-Gremmels J, and Horbach GJ. Development of human cytochrome P450 expressing cell lines; application in mutagenicity testing of ochratoxin A. Cancer Res 1996; 56: 299-304.
22. Groene EM de, Jahn A, Horbach GJ, and Fink-Gremmels J. Mutagenicity and genotoxicity of the mycotoxin ochratoxin A. Environmental Toxicology and Pharmacology 1996; 1: 21-6.
23. Groene EM de, Schaaf GJ, Horbach GJ, and Fink-Gremmels J. The role of glutathione in the mutagenicity of ochratoxin A. ISSX proceedings Food Toxins & Host Mechanisms Conditioning Toxic Responses, Sitges, Spain, 1996; *abstract* pp 23.
24. Hennig A, Fink-Gremmels J, and Leistner L. Mutagenicity and effects of ochratoxin A on the frequency of sister chromatid exchanges after metabolic activation. In: Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours. Casteganaro M, Plestina R, Dirheimer G, Chernozemsky IN and Bartsch H (eds) International Agency for Research in Cancer, Lyon, France, 1991: pp 255-60.
25. Huff WE, Kubena LF, and Harvey RB. Progression of ochratoxicosis in broiler chicks. Poultry Sci 1988; 67: 1139-46.
26. Kiessling K, Petterson H, Sandholm K, and Olson M. Metabolism of aflatoxin, ochratoxin, zearalenone and three trichothecenes by intact rumen fluid, rumen protozoa and rumen bacteria. Appl Environm Microbiology 1984; 1070-3.
27. Krogh P. Causal association of mycotoxic nephropathy. Acta Pathologica et Microbiologica Scandinavica 1978; 269 (Suppl): 1-28.
28. Kuiper-Goodman T. Food Safety: Mycotoxins and Phytotoxins in Perspective. In: Miraglia M, van Egmond H, Brera C, Gilbert J, eds. Mycotoxins and Phycotoxins - Developments in Chemistry, Toxicology and Food Safety 1998; 25-48.
29. Kuilman MEM, Maas RF, Judah DJ, and Fink-Gremmels J. Bovine metabolism of aflatoxin B_1. Food Chemistry 1998; 46: 2707-13.
30. Kuilman MEM, Sabater Vilar M, Peters WHM, and Fink-Gremmels J. Aflatoxin B1 mutagenicity is reduced in the presence of CPA in the Ames test using human and rat microsomes for metabolic activation. In: Kuilman MEM. Thesis Utrecht University 1999; Chapter 6.
31. Marasas WF. Fumonisins: their implications for human and animal health. Nat Toxins 1995; 3: 193-8.
32. Marquardt RR, and Frohlich AA. A review of recent advances in understanding ochratoxicosis, J Anim Sci 1992; 70: 2968-3988.
33. Massey TE, Stewart RK, Daniels J, and Liu L. Biochemical and molecular aspect of mammalian susceptibility to aflatoxin B1 carcinogenicity. Proc Soc Exp Biol Med 1995; 208: 231-27.
34. Merrill AH, Jr Schmelz EM, Wang E, Schroeder JJ, Dillehay DL, and Riley RT. Role of dietary sphingolipids and inhibitors of sphingolipid metabolism in cancer and other diseases. J Nutr 1995; 125 (Suppl): 1677S-1682S.
35. NIH, 1999: Two years chronic toxicity assay in rodents exposed to dietary fumonisin B_1. National Institute of Health, Washington DC, USA, in press.
36. Pasteiner S. Efficacy tests with Mycofix plus. In: Mycotoxins in Animal Husbandry. Erber, Austria 1998; 83-154.
37. Pfohl-Leszkowicz A, Grosse Y, Kane A, Creppy EE, and Dirheimer G. Differential DNA adduct formation and disappearance in three mouse tissues after treatment with the mycotoxin ochratoxin A Mutat Res 1993; 289: 265-73.
38. Pfohl-Leszkowicz A, Grosse Y, Castegnaro M, Petkova-Bocharova T, Nicolov IG, Chernozemsky IN, Bartsch H, Betbeder AM, Creppy EE, and Dirheimer G. Ochratoxin A related DNA adducts in urinary tract tumors of Bulgarian subjects. In: Post-labeling methods for detection of DNA adducts. IARC Scientific Publication, Lyon, France, 1993; 124: 141-8.
39. Puisieux A, Lim S, Groopman J, and Ozturk M. Selective targeting p53 gene mutational hotspots in human cancers by etiologically defined carcinogens. Cancer Res 1991; 51: 6185-9.
40. Ramon AJ, Fink-Gremmels J, and Hernandez E. Prevention of toxic effects of mycotoxins by means of non-nutritive absorbent compound. J Food Protect 1996; 59: 631-41.
41. Raney VM, Harris TM, and Stone MP. DNA conformation mediates aflatoxin B1-DNA binding and the formation of N^7 adducts by aflatoxin B1 8,9-exo-epoxide. Chem Res Toxicol 1993; 6: 64-88.
42. Riley RT, Wang E, Schroeder JJ, Smith ER, Plattner RD, Abbas H, Yoo H-S, and Merrill AH. Evidence for disruption of sphingolipid metabolism as a contributing factor in the toxicity and carcinogenicity of fumonisins. Nat Toxins 1996; 4: 3-15.
43. Rotter BA, Thompson BK, Lessard M, Trenholm HL, and Tryphonas H. Influence of low level exposure to Fusarium mycotoxins on selected immunological and hematological parameters in young swine. Fundam Appl Toxicol 1994; 23: 117-24.
44. Smith TK. Recent advances in the understanding of Fusarium trichothecene mycotoxicosis. J Anim Sci 1992; 70: 3989-93.
45. Slone DH, Gallagher EP, Ramsdell HS, Rettie AE, Stapelton PL, Berlad LG, and Eaton DL. Human variability in hepatic glutathione S-transferase mediated conjugation of aflatoxin B1 epoxide and other substrates. Arch Biochem Biophys 1995; 301: 345-54.