The mycoflora of three hybrids of freshly harvested corn grains collected from three regions of the state of Sao Paulo, Brazil, was comprised mainly of Aspergillus species and among the genera Fusarium and Penicillium and Aspergillus species and among the general.

Many of the strains produced patulin and/or citrinin. Citrinin was produced by all strains grown in yeast extract sucrose medium but only one strain was able to produce citrinin in grape juice medium. Patulin was produced in the yeast extract medium by 20 strains and in grape juice medium by 33 strains. No ochratoxin producing fungi were identified.


A total of 1832 fungi belonging to 31 species and 15 genera was isolated from pine nuts. Cladosporium species dominated the mycobiota followed by Phoma macrotrum. Overall, 16 potentially mycotoxigenic species were present on pine nuts.


The mycoflora of three hybrids of freshly harvested corn grains collected from three regions of the state of Sao Paulo, Brazil, was investigated. The fungal population was comprised mainly of Fusarium, Penicillium and Aspergillus species and among the general.

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Fungi were isolated from wine producing grapes and 51 strains were assessed for their potential for producing mycotoxins. Many of the strains produced patulin and/or citrinin. Citrinin was produced by all strains grown in yeast extract sucrose medium but only one strain was able to produce citrinin in grape juice medium. Patulin was produced in the yeast extract medium by 20 strains and in grape juice medium by 33 strains. No ochratoxin producing fungi were identified.


To lessen the effect of mycotoxins on pig performance, mixing contaminated and non-contaminated feedlots, the use of binding agents (e.g., clays and mannanoligosaccharides) and the feeding of higher than normal levels of high molecular weight amino acids have all been used with varying degrees of success. Preventing mould growth and subsequent mycotoxin production during storage of feeds is more successful. This is achieved by storing clean grain at a moisture content less than 14% in clean, preferably insulated bins. Cereals grown in Ireland may be contaminated with deoxynivalenol, zearalenone, fusicaric or ochratoxin. The presence of aflatoxins in animal feeds in Ireland is most likely to be due to the importation of feed ingredients from warmer climates.


The clinical response of pigs to mycotoxins is dependent on the concentration in feed, on the duration of feeding, on the presence or absence of other mycotoxins, and on the species, age, and health status of animal to which the mycotoxin is fed. Deoxynivalenol causes pigs to refuse feed, zearalenone affects the reproductive organs, ochratoxin causes kidney damage and aflatoxins increase susceptibility to disease through their action as immunosuppressants. Aflatoxins can also cause haemorrhages and digestive disorders.


The production of mycotoxins by Alternaria alternata isolated from Chinese weathered wheat kernels was investigated on polished rice and durum wheat grains. These mycotoxins included AOH, AME, alternariol (ALT), alternariol-3′methoxy (ALT-I) and tenuazonic acid (TA). Of 25 isolates tested, all were AOH and AME producers, 21 co-produced ALT and ALT-I, and 8 produced TA in rice culture. TA was the most abundant toxin produced at a level ranging from 1,369 to 3,563 mg/kg. Average concentrations of AOH, AME, ALT and ATX-I were 54, 40, 44 and 8 mg/kg, respectively.

Two new derivatives of patulin, the dimer arising from cyclodeletion at the 3,4-double bond, and 1,4-dihydroxyapatatin acetamide, from attempted epoxidation with dimethylsulphate, have been characterised.


An efficient and enantioselective total synthesis of (+)-equisetin using a diastereoselective Me3Al-mediated intramolecular Diels-Alder reaction as a key reaction step is described.


The absolute configuration of arisugacin F and territrem B were determined via the Kakuewa-Kashman modification of the Mosher NMR method.

Mycotoxins – Methodology


A method for determining AME and AOH in tomato products was developed and evaluated. The method involves extraction with methanol, clarification with ammonium sulphate and partition to chloroform. Quantification was conducted by HPLC with diode array detector. Average recoveries were 98.7% and 84.1% for AME and AOH, respectively. The quantification limits of the method were 2.0 µg/kg for AME and 5.0 µg/kg for AOH.

Mycotoxocoses


The effects of deoxynivalenol (DON), 3-acetylDON, fusaron-X, T-2 toxin, zearalenone, alpha-zearalenol, beta-zearalenol and nivalenol (NIV) on T and B cells in a proliferation assay, antibody-dependent cellular cytotoxicity, and natural killer (NK) cell activity on human peripheral blood mononuclear cells were investigated. The concentrations employed, 0.2–1000 µg/mL, were similar to those which can be found in normal human peripheral blood system. T-2 toxin, fusaron-X, NIV and DON exerted the highest immunosuppressive effect. Mycotoxin induced immunosuppression was manifested as depressed T- and B-lymphocyte activity. Furthermore, by virtue of inhibition of NK cell activity, the protection against tumour development may also be attenuated.


Mice were fed fumonisins (FB, at 10 mg/kg) and AFB, at 10 µg/kg, in the diet, either alone or in combination, for up to 90 days. The animals fed fumonisins or combined toxins showed a significant increase in feed consumption per day compared to control animals. At 90 days, animals fed AFB exhibited a significant decrease in the values of alkaline phosphatase and cholesterol, along with a significant increase in calcium. Mice fed fumonisins showed decreases in triglycerides, cholesterol and calcium. The activity of apurinic transaminase increased significantly in animals fed the combined toxins. Tissue specimens at 60 days showed lesions in the livers of the animals fed AFB or fumonisins. In mice fed AFB, fumonisins and combined toxins, the lesions were intensified in the liver at 60 days in 80, 90 and 100% of the animals, respectively.


Secalecnic acid D (SAD), a cleft palate inducing mycotoxin, reduces palatal cyclic AMP (cAMP) levels. cAMP relays its signal via the transcription factors (TF) such as cAMP response element (CRE) binding protein (CREB), CRE modulatory (CREM) and activator transcription factor-1 (ATF-1) to CRE-containing genes. Electrophoretic mobility shift assays, supershift/doublet assays and Western analyses showed that the cAMP signalling pathway is functional in the palate and that SAD alters CREB phosphorylation and inhibits its binding to CRE, leading to altered expression of genes involved in cell proliferation, an event critical for normal palate development.


The effects of T-2 toxin, diacetoxyscirpenol (DAS) and AFB, at levels up to 1,000 µg/kg diet on performance, health and immune response of enterally and parenterally immunised chicks were examined. T-2 toxin and DAS, fed singly and in combination for 35 days, had no effect on growth or feed efficiency. AFB, at concentrations above 800 µg/kg resulted in decreased growth and feed efficiency after 4 weeks. Feeding T-2 toxin and DAS resulted in oral lesions and mild intestinal inflammation, but no other pathological or histopathological lesions. AFB caused enlargement and discoloration of liver and kidneys and mild intestinal inflammation. No effects of T-2, DAS or AFB were observed on antibody production to antigens administered by enteral or parenteral routes.


In fish fed diets containing FB, at 0, 3.2, 23 or 104 mg/kg for 34 weeks, no tumours were observed in any tissue in the absence of a known initiator. FB, promoted AFB-initiated liver tumours in fish fed FB, greater than or equal to 25 mg/kg for 42 weeks. In N-methyl-N-nitro-nitrosoguanidine-initiated fish, liver tumours were promoted in the 104 mg/kg treatment, but FB, did not promote tumours in any other tissue. The FB, promotional activity in AFB-initiated fish was correlated with disruption of sphingolipid metabolism, suggesting that alterations in sphingolipid signalling pathways are potentially responsible for the promotional activity of FB, in AFB-initiated fish.


The effect of a protracted dry season on the viability of Aedes aegypti (L.) eggs was examined in Townsville, northern Queens- land, Australia. Eggs were placed in several different surface and subterranean larval habitats. After four dry season
months, only 1–10% of eggs remained viable in the surface and subterranean sites, respectively. Protoplanum americana was the most significant cause of egg predation in subterranean breeding sites but fungi, especially Penicillium citrinum, covered egg latches within 15 days. Mycotoxins produced by the spores of *P. citrinum* are believed to have killed embryonating eggs.

### Ochratoxins – General


Human milk samples were collected from 80 Norwegian women. The usual food intake during the last year was recorded using a quantitative food frequency questionnaire. Seventeen out of 80 human milk samples contained ochratoxin A (OA) in the range 10–182 ng/L. Women who a high dietary intake of liver paste (liverwurst, liver pate) and cakes (cookies, fruitcakes, chocolate cakes, etc.) were more likely to have OA contaminated milk. The risk of OA contamination was also increased by the intake of juice (all kinds). In addition, the results indicate that breakfast cereals, processed meat products and cheese could be important contributors to dietary OA intake.

Ochratoxin contamination was also unrelated to smoking, age, parity and anthropometric data other than body weight.


In a nation-wide evaluation of data collected by German Food Control 1995–1999, a total of 613 samples of coffee were analyzed for OA. The median concentrations for green coffee, roasted coffee, defatted roasted coffee together with low-acid defatted roasted coffee, and for soluble coffee were 0.4, 0.6, 0.4 and 0.7 µg/kg, respectively. The result is a mean daily total intake per consumer of 9 µg OA.


Robusta coffee cherries collected before and during sun drying from two coffee farms in Thailand were examined for moulds producing OA. Aspergillus ochra-

### Ochratoxins – Methodology


A method is described for the determination of OA in red wine and vinegar using an acidic chloroform extraction, an immunosaffinity cleanup step, and HPLC determination with fluorescence detection. The detection limit was estimated 0.2 µg/mL. The mean recovery factors were found at 91.3 and 96.6% for wine and vinegar, respectively. Thirty-one samples of red wine originating from Mediterranean Sea countries and 15 samples of vinegar were examined for the presence of OA. All red wine samples contained OA. Seventy-two percent of these samples were found to be contaminated with more than 0.1 µg/mL. All 15 vinegar samples showed the presence of OA. The most contaminated ones were three balsam-vinegar samples containing 0.0.156, 0.102 and 0.252 µg/L.


A collaborative study was conducted to evaluate an LC method for OA using a sequential phenyl silane and immunosaffinity column cleanup. The test portion was extracted with methanol and sodium bicarbonate by shaking. The extract was filtered, centrifuged and then cleared up on a phenyl silane column before being eluted from the washed column with methanol-water. The eluate was diluted with phosphate buffered saline and applied to an OA immunosaffinity column, which was washed with water. OA was eluted with methanol, the solvent was evaporated and the residue was redissolved in injection solvent and applied to a reversed phase LC apparatus followed by fluorescence detection. In samples spiked with OA at 4 µg/kg recoveries ranged from 65 to 97%. The relative standard deviation for repeatability (RSD) ranged from 2 to 22% and the relative standard deviation for reproducibility (RSD) ranged from 14 to 26%.

The method showed acceptable within and between laboratory precision, as evidenced by HORRAT values, at the low level of determination for OA in roasted coffee.


OA levels in human serum samples collected from 40 healthy individuals and 93 individuals suffering from different urinary disorders in Isparta, Turkey, are presented. Four different kinds of urinary disorders were represented: chronic renal failure treated by haemodialysis, chronic renal failure treated by peritoneal dialysis, patients with bladder cancer and patients with renal stones. The mean concentration of OA in the healthy group was 0.4 ± 0.28 µg/L. The highest mean concentration was found in the group of patients treated by haemodialysis, 2.1 ± 1.2 µg/L. The mean concentrations of OA in all patients groups were higher compared to the control group. A higher level of OA in dialysis groups compared to the control, renal stones and bladder cancer groups could probably be explained by the reduced glomerular filtration rate of these patients.


Age related differences, especially in nephro- and immunotoxicity of OA, were investigated in young adult (aged 12 weeks) and old (aged 27–30 months) female SPF rats treated by gavage with OA at 0.07, 0.34 or 1.68 mg/kg body weight for 4 weeks. In both age groups, survival was significantly decreased in the highest dose group. OA induced primarily nephropathy. Old rats were more sensitive to induction of tubular karyomegaly and vacuolization/reco- sion. In young rats, OA induced a dose related thickening of the basement membrane and reduction in splenic T-cell fraction. Decreased IgG levels were seen at 0.34 mg/kg (young and old rats) and 1.68 mg/kg (young rats). Vacuolation of the white brain matter (cerebellar medulla and ventral parts.
of the brain stem) was significantly increased in young rats at 0.34 and 1.68 mg/kg and in old rats at 0.07 and 0.34 mg/kg.


Several chemical and biological markers associated with oxidative stress were measured in rats to determine if this process is involved in OA mediated toxicity. Male rats dosed with OA at up to 2 mg/kg (24 le exposure) did not increase the formation of biomarkers of oxidative damage such as the lipid peroxidation marker malondialdehyde in rat plasma, kidney and liver, or the DNA damage marker 8-oxo-7,8-dihydro-2'-deoxyguanosine in kidney DNA. However, OA treatment at 1 mg/kg did result in a 2.25 decrease in alpha-tocopherol plasma levels and a 5-fold increase in the expression of the oxidative stress responsive protein haem oxygenase-1, specifically in the kidney.

**Fumonisins – General**


This article describes the events leading to the discovery of the fumonisins in South Africa in 1988 and highlights the first 10 years (1988–1998) of fumonisin research. The predominant fungus isolated from mouldy corn implicated in a field outbreak of equine leukoencephalomalacia (ELEM) in the Transkei region of South Africa was Fusarium verticilloides (F. moniliforme). This fungus was also prevalent in mouldy home-grown corn consumed by people in high-incidence areas of oesophageal cancer in the Transkei region of South Africa. Culture material of F. verticilloides strain MRC 826, which was isolated from mouldy corn in Transkei, was shown to cause ELEM in horses, porcine pulmonary oedema syndrome in pigs and liver cancer in rats. A short-term cancer initiation/promotion assay in rat liver was used to purify the carcinogenic(s) in the culture material and FB1 and FB2 were finally isolated from culture material of F. verticilloides MRC 826.


The absolute stereochemical description of FB1 and presumably of its congeners is now secure. This article summarises studies leading to this conclusion and outline the biosynthetic and synthetic studies of FB1.


Abstracts of 42 papers given at the Fumonisin Risk Assessment Workshop are presented. Topics covered include occurrence, analytical methods, toxicity and risk assessment of fumonisins and control of fumonisin production.


The presence of FB1 and FB2 in corn based food on the Danish retail market was surveyed. A total of 70 samples were analysed and 37% contained FB1, and 21% contained FB2. No fumonisins were found in sweet corn (canned or frozen), corn-on-the-cob, corn starch or ground powder for babies. FB1 was found in about half of the corn flake, corn snack and popcorn samples, whereas FB2 was seen to a lesser extent. Both FB1 and FB2 were found in 75% or more of the corn flour, tacos and polenta samples. In general, the content of FB1 was in the range 1–1000 µg/kg and the content of FB2 was in the range 4–250 µg/kg.


Households in rural and urban areas of KwaZulu Natal, South Africa, were surveyed to assess the exposure of the inhabitants to FB1. Of the 50 rural maize samples examined, 32% had levels of FB1 ranging from 0.1 to 22.2 mg/kg, whereas 29% of the 28 cooked maize (pilu) samples contained FB1 ranging from 0.1 to 0.4 mg/kg. The incidence and levels of FB2 in faces were 33% and 0.5–39.0 mg/kg, respectively. Of the 49 urban maize samples analysed 6.1% contained FB2 in the range 0.2–0.5 mg/kg, whereas 34% faecal samples contained FB2 in the range 0.6–16.2 mg/kg. No FB1 was detected in urban faecal samples.


Samples of maize imported into Taiwan during 1997–1998 were collected and analysed for FB1. Eight of 118 samples were found to contain FB1 and values ranged from 334 to 1614 µg/kg. The frequency of FB1 found in maize samples imported from Australia was 29%, followed by Thailand (10%) and USA (5%). Only four samples contained FB2 in excess of 300 µg/kg.


Samples of corn belonging to 19 cultivars with distinct types of germplasm, endosperm and length of vegetative cycle, were analysed for FB1 and FB2. The cultivars were grown in experimental fields in three locations within the State of Sao Paulo, Brazil, during the 1997/1998 crop. All 23 samples were contaminated with fumonisins, with concentrations of FB1 ranging from 1.63 to 25.69 µg/kg with a mean of 6.15 µg/kg and of FB2 from 0.38 to 8.60 µg/kg with an average of 1.86 µg/kg. In terms of fumonisins, these high levels put the corn cultivated in Sao Paulo among the most contaminated in the world reported to date.


The two important Fusarium ear rots of corn, Gibberella car rot (Fusarium graminearum, formally F. moniliforme and related species) and Fusarium ear rot (F. verticilloides and allied species) grow under different environmental conditions. F. graminearum grows well only between 26 and 28°C and requires rain both at silking and during disease progression. F. verticilloides grows well at higher temperatures, and ear rot and fumonisin accumulation are associated with drought and insect stress and growing hybrids outside their areas of adaptation. The best available strategies for reducing the risk of fumonisin contents of maize are to ensure that hybrids are adapted to the environment and to limit drought stress and insect herbivory. It may also be necessary to make use of alternative strategies such as producing hybrids that contain enzymes to degrade fumonisin as it is produced.
Fusarium moniliforme is a facultative fungal endophyte and during the biotrophic endophytic association with maize, as well as during saprophytic growth, it produces fumonisins. The fungus is transmitted vertically and horizontally to the next generation of plants via clonal infection of seeds and plant debris. A biological control system using an endophytic bacterium, Bacillus subtilis, has been developed that shows great promise for reducing mycotoxin accumulation during the endophytic (vertical transmission) growth phase. Because this bacterium occupies the identical ecological niche within the plant, it is considered an ecological homologue to F. moniliforme, and the inhibitory mechanism, regardless of the mode of action, operates on the competitive exclusion principle. In addition to this bacterium, an isolate of a species of the fungus Trichoderma shows promise in the post-harvest control of the growth and toxin accumulation from F. moniliforme on corn in storage.

Regulation of fumonisin biosynthesis has been generated. A mutant of Fusarium verticillioides, FT536, carrying a disrupted gene named FCC1, was grown on corn kernels or the mammary barrier into the milk but in these cases, fumonisins were not detectable or quite low in dry milled products. Fumonisin residues in the starting material for high-fructose corn syrup and most other wet-milled food ingredients. Similar effects were noted for the dry-milling process. Fumonisin residues were not detected or quite low in dry flaking gits and corn flour, higher in corn germ, and highest in corn bran. Extrusion of dry-milled products reduced fumonisin concentrations by 30-90% for mixing-type extruders and 20-50% for nonmixing extruders. Cooking and canning generally had little effect on fumonisin content. In the mass process, measurable fumonisin was reduced following the cooking, soaking and washing steps, with little conversion of fumonisin to the hydrolysed form. Shredding, baking and frying at commercial times and temperatures generally had no effect. Studies have shown no fumonisin residues present in food products or ingredients.

Secondary metabolism (fumonisin biosynthesis) and fungal development (conidiation) in F. verticillioides.

The naturally contaminated raw material (corn flour), intermediate product (extruded corn flakes) and final product (roasted corn flakes). Only one method, using immunoaffinity column cleanup, provided reliable results in the determination of fumonisins in corn flake samples at the intermediate and final stages of processing. About 60-70% of the initial amount of fumonisins were lost during the entire cycle of corn flake processing, with less than 30% losses occurring during the intermediate extension step (70-170°C for 2 to 5 min). The effect of different additives commonly present in commercial products (sodium chloride, sucrose and ferrous sulphate heptadecylate) on the reliability of fumonisin analysis was also investigated. The presence of sodium chloride strongly reduced fumonisin recovery when strong anion-exchange columns were used for the cleanup step, whereas the other additives appeared to have little or no effect on the accuracy of fumonisin analysis.


Studies on the effects of wet-milling on fumonisin residues in corn found these residues were not detectable in comstack, the starting material for high-fructose corn syrup, and most other wet-milled food ingredients. Similar effects were noted for the dry-milling process. Fumonisin residues were not detectable or quite low in dry flaking gits and corn flour, higher in corn germ, and highest in corn bran. Extrusion of dry-milled products reduced fumonisin concentrations by 30-90% for mixing-type extruders and 20-50% for nonmixing extruders. Cooking and canning generally had little effect on fumonisin content. In the mass process, measurable fumonisin was reduced following the cooking, soaking and washing steps, with little conversion of fumonisin to the hydrolysed form. Shredding, baking and frying at commercial times and temperatures generally had no effect. Studies have shown no fumonisin residues present in food products or ingredients.


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samples produced at 180°C. Low concentrations of NCM-FB1 were achieved with this method was 10 µg/kg. Samples contained NCM-FB1, FB1, and hydrolysed FB1. All samples were contaminated with FB1, (22–194 µg/kg) and hydrolysed FB1, (5–247 µg/kg), whereas those spiked with FB1 and sucrose showed only NCM-FB1, in samples produced at 180°C. Various corn containing food samples from the German market were analysed for the presence of NCM-FB1, FB1, and hydrolysed FB1. All samples were contaminated with FB1, (22–194 µg/kg) and hydrolysed FB1, (5–247 µg/kg). An LC method sufficiently sensitive to detect FB1 in low concentrations ranging from 10 to 76 µg/kg.


As an HPLC method for the determination of fumonisin in milk was modified to include semiautomatic equipment in the purification step by means of immunosaffinity columns, and in the derivatisation and LC injection steps. The modified method provided high reproducibility, short process time and the analysis of several samples in the same time. The detector response was linear in the range 0.001–0.100 mg/L and the detection limit was 0.010 mg/L. Recoveries from milk spiked with FB1, at 0.025, 0.05 and 0.1 mg/L, were 83.1, 79.7 and 73.5%, respectively. (In Italian).


A modified method has been used for the determination of the sphingamine (Sa)/sphingosine (So) ratio in human serum and urine of healthy subjects and endemic nephropathy patients from Brodski Posavina, Croatia. Free sphingoid bases, Sa and So, were obtained by base hydrolysis. Afterwards, precolumn α-phthalaldehydehydri derivatisation, HPLC separation and quantification by fluorescence detection were performed. The results obtained pointed to a sphingolipid metabolism impairment which may have been induced by fumonisins or fumonisin-like mycotoxins. As statistically significant differences were recorded in the subjects not yet affected with endemic nephropathy, an impairment in the metabolism of sphingolipids might be considered as an early indicator of endemic nephropathy.


Sa concentrations in the livers of mice and in the livers and kidneys of rats were measured in conjunction with a tumour bioassay. The ability of Sa levels in this role was initially questioned because they were highly variable when compared across time points. However, a conceptual framework and data are presented that support the use of Sa as a biomarker for a dose response of FB1, on cell death. This framework is reasonably consistent with observed Sa concentrations in the examined tissues, the literature on fumonisin effects on sphingolipid synthesis and an hypothesised mechanism through which FB1 increases age-specific tumour incidence.

ROLES IN SIGNAL TRANSDUCTION AND DISRUPTION BY FUMONISINS. Environmental Health Perspectives 109: 283–289.

An overview with 59 references. The accumulation of sphingoid bases is a primary cause of the toxicity of fumonisin. Nonetheless, the full effects of fumonisins probably involve many biochemical events. The elevations in sphingoid bases also affect the amounts of other lipids, including the l- and di-acyl phosphatides and N-acetyl derivatives of Sa. Furthermore, the aminopentyl backbone of FB1 (AAP) is both an inhibitor and a substrate for ceramide synthase, and the resultant N-palmitoyl-AAP is an even more potent inhibitor of ceramide synthesis.


Genes have been identified that inhibit FB1-induced apoptosis in African green monkey kidney fibroblasts (CV-1) cells and two mouse embryo fibroblasts (MEFs). A baculovirus gene, inhibitor of apoptosis (CpIAP), protected these cells from apoptosis. CpIAP blocks apoptosis induced by the tumour necrosis factor (TNF) pathway as well as other mechanisms. Further support for the involvement of the TNF signal transduction pathway in FB1-induced apoptosis was the cleavage of caspase 8. Inhibition of caspases by the baculovirus gene p35 also inhibited FB1-induced apoptosis.


In order to identify genes that are induced by FB1, a PCR-based subtraction approach was employed. Eight genes that showed high similarity (>90%) to known mammalian genes were identified. These genes included tumour necrosis factor type 1 receptor associated protein 2 (TRAP2), human leukaemia virus receptor (GLVR1), human Scaffold attachment factor A (SAAF-A) also called heterogeneous nuclear ribonucleoprotein U (hknpu), human protein kinase C-binding protein (RACK7), human oligoacetyltransferase STT3 subunit, mouse WW-domain binding protein 2 (WBWP2), human fibronectin, and an unknown human clone. The ability of FB1 to alter gene expression and signal transduction pathways may be necessary for its carcinogenic and toxic effects.

The disruption in Sa and So concentrations in plasma and urine of vervet monkeys (Cercopithecus aethiops) was measured following a single gavage dose of FB₁ at either 1 or 10 mg/kg body weight. In the low dose monkeys, none of the parameters measured increased significantly above the control values. In the high dose monkeys the plasma Sa/So ratios were significantly increased above the corresponding control ratios after 3 days and continued to be significantly raised for another 27 days, whereafter the ratios declined to control values after 51 days. The plasma aspartate aminotransferase activities increased significantly above their control values from day 5 to day 23 and the gamma-glutamyl transferase activities from day 7 until the end of the study period. The plasma Sa/So ratio, plasma creatinine and urea values in both groups of monkeys did not increase above the control values.


Vervet monkeys (Cercopithecus aethiops) were dosed with repeated gavages of FB₁ at 1 mg/kg body weight three times/week continuously over a 51-day period. The plasma Sa/So ratio reached a maximum after 30 days with a 3-fold increase above the ratio of the control monkeys and then declined slowly to double the value in control after 51 days. The lack of a clear elevation in urinary Sa/So ratios after 51 days of multiple exposure in the dosed monkeys indicates that the plasma ratio is more sensitive than urinary changes in monkeys. This is confirmed by the plasma levels of liver function enzymes of which aspartate transami-nase, glutamyl-transferase and lactate dehydrogenase were increased in the dosed monkeys, while the plasma indicators of renal function were not increased above the levels in the control monkeys.


Lung, liver, heart and kidney tissues from fumonisin-exposed pigs, sheep, rabbits, and rats were examined ultrastructurally. Endothelial alterations were present in the pulmonary capillary endothelial cells of rats, but at doses that did not induce pulmo-nary oedema. These alterations were present only in pigs and not in other species. In addition, these endothelial alterations were not present in any other organs of pigs.


An overview with 69 references. Porcine pulmonary oedema has been reproduced experimentally in pigs by feeding of natu-rally contaminated corn. F. verticilloides culture material and by iv administration of FB₁. Hepatic lesions consisting of apoptosis, necrosis and hepatocyte proliferation also are observed. Fumonisin has been shown to induce an accumulation of membranous material in pulmonary capillary endothelial cells and this change appears specific to this cell type and to swine. In short-term cardio-vascular studies, fumonisin decreased left ventricular dp/dtmax (an index of cardiac contractility), mean systemic arterial pres-sure, heart rate and cardiac output, and increased mean pulmonary artery pressure and pulmonary artery wedge pressure. These changes are compatible with the inhibi-tion of L-type calcium channels by increased Sa and/or So concentration. Therefore, fumonisin-induced pulmonary oedema in swine appears to result from acute left-sided heart failure mediated by altered sphingolipid biosynthesis.


An overview with 124 references. FB₁ is poorly absorbed and rapidly eliminated in faeces of rodents. Minor amounts are retained in liver and kidneys. FB₁ induces apoptosis of hepatocytes and of proximal tubule epithelial cells. More advanced lesions in both organs are characterised by simultaneous cell loss (apoptosis and necro-sis) and proliferation (mitosis). Micro-scopically and other findings suggest that an imbalance between cell loss and replace-ment develops, a condition favourable for carcinogenesis. On the molecular level, fumonisins inhibit ceramide synthase and disrupt sphingolipid metabolism and, theo-retically, sphingolipid-mediated regulatory processes that influence apoptosis and mito-sis. Liver sphingolipid effects and toxicity are correlated, and ceramide synthesis inhibi-tion occurs in liver and kidney at doses below their respective no-observed-effect levels.


Data from the National Toxicology Pro-gram’s carcinogenesis study of FB₁ in B6C3F₁ mice, were used to fit the Mool-gavkar-Venzon-Knudson (MVK) two-stage, clonal-expansion model of carcinogenesis. In addition to tumour data from the conven-tional 2-year bioassay, the study included data on tissue weights, cell proliferation, cell death and sphingolipid metabolism in primary target organs. The model was used to predict 2-year liver tumour rates in mice. The model was able to reproduce reasonably well the observed tumour rates in both female and male mice, predicting substan-tially increased rates above background only at the highest doses of FB₁ in females.


In a recent National Toxicology Program study in Fischer rats and B6C3F₁ mice, FB₁ caused renal carcinomas in male rats and liver cancer in female mice. In an earlier study in male ED-IX rats, FB₁ caused hepatic toxicity and hepatocellular carci-no genesis. An early effect of FB₁ exposure in these target organs is apoptosis. However, there is also some evidence of oncotic necrosis following FB₁ administration, espe-cially in the liver. Induction of apoptosis may be a consequence of ceramide synthase inhibition and disruption of sphingolipid metabolism by FB₁. FB₁ is not genotoxic in bacterial mutagenesis screens or in the rat liver unscheduled DNA-synthesis assay. FB₁ may be the first example of an appar-ently nongenotoxic agent producing tumours through a mode of action involving apoptotic necrosis, atrophy and consequent regeneration.
amdc12-3.fm  Page 8  Tuesday, August 28, 2001  10:36 AM

B6C3F1 mice FB1 in the diets of male rats induced renal gentic in female F344 rats up to 100 mg/kg. Intracellular adenomas and carcinomas were containing FB 1 for 2 years: female rats received 0–100 mg/kg, male rats, 0–150 mg/kg. Female mice, 0–80 mg/kg, and, male mice, 0–150 mg/kg. FB1 was not tumorigenic in female F344 rats up to 100 mg/kg. FB1, in the diets of male rats induced renal tubule adenobroms and carcinomas in 0, 0, 19 and 31% rats at 0, 5, 15, 50 and 150 mg/kg, respectively. FB1 in the diet of male mice did not affect tumour incidence. Hepatocellular adenomas and carcinomas were induced by FB1 in the female mice, occurring in 11, 6, 2, 40 and 83% of female mice that consumed diets containing 6, 15, 50 and 80 mg/kg, respectively.


A review with 79 references of the hepatocarcinogenic effects of fungal cultures of Fusarium verticillioides (= Fusarium moniliforme) in B6C3F1 mice fed diets containing fumonisin. Fumonisin intake levels of between 0.8 and 1.6 mg/kg body weight/day over approximately 2 years produce liver cancer in male ED IX rats. Fumonisin-induced liver cancer is significantly lower than with the feeding of 0.8 mg/kg body weight/day in rats. A review of 79 references of the hepatocarcinogenic effects of fumonisin-contaminated diets in male ED IX rats. Fumonisin intake levels of between 0.8 and 1.6 mg/kg body weight/day over approximately 2 years produce liver cancer in male ED IX rats. Exposure levels of less than 0.8 mg/kg body weight/day fail to induce cancer, although mild toxic and premalignant lesions are induced. These studies supported the findings of long-term investigations indicating that a cytotoxic/proliferative response is required for cancer induction and that a no-effect threshold exists for cancer induction. The mechanisms proposed for cancer induction are highlighted and include the possible role of oxidative damage during initiation and the disruption of lipid metabolism, integrity of cellular membranes, and altered growth regulatory responses as important events during promotion.


An overview with 116 references. The biochemical consequences of fumonisin disrup-
tion of sphingolipid metabolism most likely to alter cell regulation are increased free sphingoid bases and their-phosphates, alterations in complex sphingolipids, and decreased ceramide biosynthesis. Because free sphingoid bases and ceramide can induce cell death, the functional inhibition of ceramide synthase can inhibit cell death induced by ceramide but promote free sphingoid base-induced cell death. Theoretically, at any time the balance between the intracellular concentration of effectors that protect cells from apoptosis (decreased ceramide, increased sphingosine 1-phosphate) and those that induce apoptosis (increased ceramide, free sphingoid bases, altered fatty acids) will determine the cellular response.


In studies with F344/N/Nct BR rats consuming diets containing FB1 at up to 448 mg/kg for 28 days, female rats demonstrated more sensitivity than male rats in the induction of hepatocellular apoptosis and mitosis. Conversely, induction of renal tubule apoptosis and regeneration were more pronounced in male than in female rats. Induction of renal tubule apoptosis and hyperplasia correlated with the incidence of renal tubule carcinomas that developed in the 2-year feeding study with FB1 in the F344/Nct BR rats. The data are consistent with the hypothesis that the induction of renal tubule carcinomas in male rats could be partly due to the continuous compensatory regeneration of renal tubule epithelial cells in response to the induction of apoptosis by FB1.


The toxicity of low dietary levels of FB1 (3, 10 and 25 mg/kg diet) were monitored in rats over a period of 24 months. Mild toxic effects, including single cell necrosis (apop-
tosis), proliferation of bile duct epithelial cells, and early signs of fibrosis, bile duct hyperplasia and in one case, adenofibrosis, were noticed in the liver of the rats fed the 25 mg/kg diet. A significant increase in the level of oxidative damage was also noticed in the liver of the rats in this group. Hepato-
cyte nodules, staining positively for glu-
tathione-S-transferase plasmatic form, were observed macroscopically in the 25 mg/kg group and to a lesser extent in the 10 mg/kg group. The most prominent toxic lesions caused by FB1 in the kidneys were restricted to the tubular epithelium manifesting as granular cast, necrosis, apoptosis, calcifica-
tion and the presence of regenerative foci in the proximal convoluted tubules.

sin-contaminated diets. Food and Chemical Toxicology 39: 507–511.

Rats were fed fumonisins (FB1 + FB2) in the diet at 4 mg/kg with and without the addition of activated charcoal at 20 mg/kg diet for 1 week. In rats fed the fumonisin con-
taminated diet, the Sa concentration and Sa/So ratio increased significantly and revers-
ibly in kidney, while urine and liver did not show a significant increase of Sa/So ratio. The addition of activated charcoal to the fumonisin diet did not alter the change of Sa/So biomarker for fumonisin exposure.


Ten milk-fed male Holstein calves were administered purified FB1 at 1 mg/kg iv, daily for 7 days. Calves were euthanised on day 7. In treated calves, serum Sa concentra-
tion increased from day 3 onward, whereas, serum So concentration was unchanged. Other parameters including heart rate, car-
diac output, stroke volume, mean arterial pressure and mean pulmonary artery pres-
sure were unchanged in treated and control calves. Fumonisin treated calves developed metabolic acidosis but all survived for 7 days.

MATHUR, S., CONSTABLE, P.D., EPP-
LEY, R.M., WAGGONER, A.L., TUM-

Ten milk-fed male Holstein calves aged 7 to 14 days were administered FB1 at 1 mg/ kg, iv, daily until euthanised on day 7. FB1 treated calves were lethargic and had...
decreased appetite from day 4 onward, serum biochemical evidence of severe liver and bile duct injury, and impaired hepatic function. Treated calves also had biochemical evidence of renal injury that functionally involved the proximal convoluted tubules. Sa and So concentrations in liver, kidney, lung, heart and skeletal muscle were increased in treated calves. Sa, but not So, concentration was increased in brains of treated calves. This is the first report of FB1 induced renal injury and organ sphingolipid alterations in cattle.


The toxicities of purified FB1, FB2, and FB3, individually and in combination (3:1:1 ratio), were evaluated with regard to their embryo toxicity by injection of the toxins into the air cell of chicken eggs at 72 hr of incubation. The 50% lethal dose for FB1 was 18.73 µg/egg. A comparison of the toxicity of FB1, FB2, and FB3, individually and in combination found that FB1 was the most toxic. Microscopic examination of chicken embryos exposed to fumonisins did not reveal any gross developmental abnormalities; however, severe haemorrhages of the heart, neck and thoracic area of the dead embryos were evident.


FB1 elevated the intracellular free Sa concentration in both LLC-PK1 and Chinese hamster ovary (CHO) cells. However, CHO cells are resistant to fumonisin cytotoxicity at 50 µM, while LLC-PK1 cells are sensitive at concentrations greater than 55 µM. The intracellular concentration of free Sa in LLC-PK1 cells treated at 50 µM FB1 for 72 hr was approximately 1450 pmol/mg protein, indicating that the mass amount of elevated free Sa in CHO cells was about 32% of that in LLC-PK1 cells. Adding monounsaturated fatty acids to CHO cells along with 50 µM FB1 treatment for 72 hr caused both necrosis and apoptosis.


A review with 10 references. Zeara- lone (ZEA) is resistant to most food processing treatments. When administered orally it is well absorbed and is able to reach intracellular targets. Its metabolism is complex. Urinary and biliary excretion of the mycotoxin and metabolites occur, with a possible entero-hepatic cycle. Milk excretion is also observed. Acute toxicity of ZEA is weak. It provides reproductive disorders after competitive fixation to the intracellular receptors of oestrogens. Although it is not genotoxic, ZEA is carcinogenic in animals. For lack of epidemiological data, no evaluation of its carcinogenicity in humans has been proposed. (In French).


Sixty-six isolates of Fusarium graminearum associated with Fusarium head blight were collected in North Carolina and tested for in vitro growth rate, in vitro production of DON and ZEA, and pathogene- nicity on three cultivars of soft red winter wheat. Significant differences among isolates were found for all three traits. Randomly Amplified Polyphomorphic DNA (RAPD) analysis revealed high levels of genotypic diversity among isolates. There were no significant differences between levels of DON produced by the five isolates associated with the highest levels of disease.


An international collection of 116 wheat lines was evaluated for Fusarium head blight (FHB) resistance and concentration of DON in grain. Plants were inoculated with mixed isolates of F. graminearum in the greenhouse by injecting conidia into a single spikelet of each spike, and in the field by scattering F. graminearum-infected wheat kernels on the soil surface. Significant dif- ferences in FHB ratings and DON levels were observed among cultivars. Correlation coefficients were significant between FHB symptom ratings, seed quality traits and DON levels. Thus, the percentage of scabbed spikelets and kernels can be gener- ally used to predict DON levels in harvested wheat grain.


The effects of different environments on maize resistance to Gibberella ear rot, dis- ease symptoms, DON concentration and grain yield were measured in three maize inbred lines and five hybrids at six locations in eastern Canada. All genotypes were inoc- ulated with a three-isolate macroconidial mix of Fusarium graminearum using a ker- nel stab inoculation technique. Results show that year to year variation is more important than variation associated with multiple loca- tions in testing for genotypic resistance to Gibberella ear rot, according to disease symptoms and DON content. Regression models indicated that higher ear rot severity and DON concentration were associated with an increase in the total number of days from July to September with relative humid- ity equal to or greater than 80%.


T-2 toxin biosynthesis by Fusarium sporotrichioides and DON biosynthesis by F. graminearum were studied by comparing the nucleotide sequence of the 25-kb core trichothecene gene cluster from each organism. This comparative genetic analysis allowed the prediction of proteins encoded by two trichothecene genes, TR19 and TR10, that had not previously been described from either Fusarium species. Differences in gene struc- ture also were correlated with differences in the types of trichothecenes that the two spe- cies produce. Gene disruption experiments showed that F. sporotrichioides TR17 (FtTR17) is required for acetylation of the oxygen on C-4 of T-2 toxin. Sequence analy- sis indicated that F. graminearum TRl7 (FgTR17) is nonfunctional.

Edwards, S.G., Pirozoi, S.R., Hare, M.C. and Jenkinson, P. 2001. Quantification of trichothecene-producing Fusarium in harvested grain by competitive PCR to determine efficacies of fungicides against Fusar-
A PCR based assay to quantitatively determine the levels of deoxynivalenol (DON) and zearalenone (ZEA) was developed. The assay was based on the amplification of specific primer pairs using polymerase chain reaction (PCR) technology. The amplified DNA was then analyzed by agarose gel electrophoresis and the band intensity was proportional to the concentration of the mycotoxin in the sample.

**References:**


Twenty-eight laboratories from 12 different countries participated in an interlaboratory study for the determination of ZEA in maize and DON in maize and wheat using their usual in-house methods. For the final separation and quantification either GC, HPLC, TLC or ELISA were employed. Coefficients of variation (CV) between laboratory mean results ranged from 28 to 41% for ZEA and from 32 to 38% for DON. A good trueness was obtained for the wheat samples spiked with DON at 475 µg/kg. However, a significant deviation from the respective target value was observed for maize samples spiked with ZEA at 102 µg/kg. The high CVs can be traced back to problems occurring by the determination of the concentration of the participants’ own calibrant solutions. Additionally, the variability of the results is strongly influenced by the use of different separation and quantification procedures.

**References:**


A method for the determination of DON, NIV, 3-acetylDON, fusarenon X, HT-2 toxin, Das and neosolaniol in wheat, based on capillary GC with flame ionisation detection (FID) has been developed and validated. The trichotheccenes were extracted from the sample by acetone/toluene (4:16, v/v) followed by liquid/liquid extraction. The extract was evaporated to dryness and the trichotheccenes were derivatised to trimethylsilyl ethers. The residue was dissolved in iso-octane and washed with water. The final extract was analysed by GC with FID. The average recoveries were 84% in the range 0.2–0.67 mg/kg cereal products. The average RSD was 6.9% and the limit of quantification was 0.2 mg/kg.
posed DON tolerance limit of 120 µg/kg. Thirteen of the 22 wheat samples exceeded this limit. A survey was carried out in the Netherlands for DON in cleaned wheat. A temporary tolerance limit of 500 µg/kg for each of the individual trichothecenes.


Various analytical methods used in the analysis of type B trichothecenes (DON, NIV, 3- and 15-acetyl-DON) in cereals were compared and optimised. The extraction solvent of choice was a mixture of acetonitrile–water (84:16, v/v). The MycoSep 225 column was chosen as the best alternative for cleanup of grain samples. For GC–electron capture detection analysis, derivatisation of analytes with heptfluorobutyric anhydride prior to the final determination was chosen as the most suitable procedure. HPLC–photodiode array (at 221 nm) analysis was more suitable than HPLC of the fluorescent coumarin-3-carboxylic derivatives. Recoveries obtained in spiked corn, rice and wheat are reported.


Four trichothecenes, T-2 toxin, HT-2 toxin, DON and other trichothecenes, were used as a culture model of CFU-MK for toxicological studies. At low concentrations, trichothecenes cause cytotoxic effects in megakaryocyte progenitors, which could induce thrombocytopenia. Sensitivity of human CFU-MK is compared to respective sensitivities of human red blood cell progenitors (BFU-E) and white blood cell progenitors (CFU-U-GM) that were described in previous works.


A review with 89 references. The ingestion of feed contaminated with ZEA leads to reproductive disorders in a great number of species. Pigs are the most sensitive, especially young females. Poultry are considered to be resistant to ZEA. Ruminants are only very rarely affected, but excrete the mycotoxin and its metabolites in milk. (In French).


ZEA was administered to Albino male and pregnant female mice at 5 or 10 µg/kg and genotoxic effects were evaluated. Chromosomal aberrations in bone marrow and spermatocytes of adult male mice and chromosomal analysis and teratological effects of mice embryos assessed. ZEA was found to reduce the mitotic activity in treated males and embryos. In treated males and females, ZEA induced some chromosomal abnormalities but with no significant increase over controls.


ZEA administration starting 10 days after ovulation was studied in 6-cycling-trot-ter mares. After an entire estrous cycle (Cycle 1), mares were given 7 mg purified ZEA per os daily beginning on day 10 of Cycle 2. Toxin exposure was continued until the subsequent ovulation. The toxin had no effect on the length of the interovulatory intervals, luteal and follicular phases. It did not influence significantly the plasma progesterone profiles, follicular activity and uterine oedema.


In a farrow-to-finish pig unit, an induc- tion-of-parturition program was applied in gilts and sows with PGF2 alpha 113 days post service, followed by oxtocin 24 hr later. This program resulted in a high proportion of animals farrowing within the working hours of the day. When spay-legs and oedematous swelling and reddening of the vulva started to be observed in newborn piglets, a concurre- nt decline or parameters related to a partu- rition also was noticed. Mycotoxicological analysis of the feeds revealed a co-occurring contamination with DON and ZEA. For a 4-week period, sows were divided into two groups: an induction-of-parturition group and a non-induction-of-parturition group. Signifi- cant differences were found between the two groups relating to prevalence of dystocia and pregnancy duration. Moreover, it was found that prevalence of spay-legs and swelling of the vulva were highly correlated with reduc- tion of percentage of sow farrowing within the working day and increase of pre-warming mortality.


Post-transcriptional effects of DON on TNF-alpha and IL-6 gene expression were studied in lipopolysaccharide (LPS)-stimu- lated macrophage RAW 264.7 cells. DON was found to enhance both TNF-alpha and IL-6 protein secretion in the presence of LPS. Upon addition of the transcriptional inhibitor, 5,6-dichloro-1-beta-D-ribofuranosyl benzimidazole (DRB), secretion of both cytokines was inhibited. Using Northern analysis, the mRNA stabilities of TNF- alpha and IL-6 were studied in DRB-treated cells exposed to DON and LPS in both ayn- chronicus and delayed synchronous modes. T2 toxin at 10 µg/ml mRNA were rapidly stabilised by DON in both models. These results suggest that post-transcriptional con- trol via enhancement of mRNA stability is likely to contribute to proinflammatory cytokine superinduction in macrophages by DON and other trichotheces.


T-2 toxin at 2 mg/kg body weight was orally inoculated to pregnant mice at differ- ent days of gestation and the fetuses were examined 24 hr later. The number and region of pyknotic or karyorrhectic cells varied according to inoculation date. In the gestation day 13.5 subgroup, a moderate to high number of pyknotic or karyorrhectic neuronal cells were observed in the central nervous system, peri-ventricular zone to subventricular zone, and pykrosis or kary- orrhesis were also observed in a small num- ber of chondroblasts and chondrocytes. In the gestation day 16.5 subgroup, a moderate to high number of pyknotic or karyorrhectic cells were observed in the thymus and renal subcapsular parenchyma. T-2 toxin is known to readily cross the rat placenta and it seems that these effects might be a direct effect of T-2 toxin on fetuses.
ponents of whole meals marketed in 12 Australian Mycotoxin Newsletter Vol. 12, No. 3 (September 2001)

B1, B2, G1 and G 2 in cooked food com-

MIDIO, A.F., CAMPOS, R.R. and SAB-
portion of the amounts of AFM1 lost from
of the ripening/storage period but only a
in brine started low and increased by the end
3.9 and 4. 4 times higher than those in milk,
whereas concentrations in whey were lower
than those in curd and milk. AFM 1 was
present in cheese at higher concentrations at
the beginning than at the end of the ripen-
storage period. Concentrations of AFM 1
in brine started low and increased by the end
of the ripening/storage period but only a
portion of the amounts of AFM 1, lost from
cheese was found in the brine.

fast food outlets of the city of Sao
Paulo, SP, Brazil. Food Additives and
Contaminants 18: 445–448.

Samples of cooked components of regu-
lar meals served at fast food outlets of the
city of Sao Paulo, Brazil were analysed for
AFB1, B1, G1 and G2. The 322 samples
were composed of prepared traditional Brazilian
and ethnic foods in which aflatoxins might
be present. Aflatoxins were detected in 30
samples in the range 2.80–1332 µg/kg.
AFB1 was detected in all contaminated sam-
ple.
The contamination levels and fre-
quency of AFB1 and G1 in positive samples
above 20 µg/kg were high.

MARTINS, M.L., MARTINS, H.M. and
BERNAUDO, F. 2001. Aflatoxins in
spices marketed in Portugal. Food

Seventy-nine prepackaged samples of 12
different types of spice powders were
selected from supermarkets and ethnic shops
in Lisbon, Portugal, and analysed for afla-
toxins. AFB1 was detected in 34 samples.
All of the cayenne pepper samples were contam-
inated with AFB1; levels ranging from 2 to
32 µg/kg. Eight of 10 nutmeg samples con-
tained AFB1; levels in the range 1–58 µg/
kg. Paprika contained AFB1; levels ranging
from 1 to 20 µg/kg. Chili, cumin, curry
powder, saffron and white pepper samples
had levels ranging from 1 to 5 µg/kg. Afla-
toxins were not detected in cardamom,
cloves, ginger and mustard. None of the
samples analysed contained AFB2, G2, or
AFB2.

BROWN, R.L., CHEN, Z.Y., MENKIR,
LANEY, E., KELLER, N. and BEN-

A recent report of an aflatoxin producing

A new aflatoxin produc-
ing species of Aspergillus tamauri prompted a taxonomic re-examination of aflatoxico-
and non-aflatoxicous isolates identified as
A. tamauri as well as the closely related A.
cereus. Because of genetic, morphological
and mycotoxicological differences, the aflatoxin
producing isolates of A. tamauri are given
species rank as Aspergillus pseudotamarii
sp. nov.

KICH, M., MENDOZA, C., MUL-
LANEY, E., KELLER, N. and BEN-
stigmatocystin-producing Emericella
variant from agricultural desert soils. Systemic and Applied Microbiology 24:
131–138.
An unusual, sterigmatocystin-producing taxon with characteristics of both Emericella nidulans and E. rugulosa was isolated repeatedly during a mycorrhizal survey of desert cotton field soils where aflatoxin is a chronic problem. Members of this taxon had ascospores with smooth convex walls like *E. nidulans* but grew slowly like *E. rugulosa*. Traditional morphological characters, secondary metabolite profiles of mycelial extracts and Southern blot analysis of genomic DNA indicate that these isolates constitute a new non-rugulose variant of *E. rugulosa*.


Some *Aspergillus* *nidulans* (E-20) multienzymes have previously been reported to present detoxifying activities against aflatoxins. The isolation and purification of an intracellular enzyme, named aflatoxin-deoxylzyme, which exhibited detoxification activity on AFB1, is described. The enzyme exhibited a specific activity of 7.09 nmol min/mg at pH 6.0 and 28°C. The activity of the purified enzyme was confirmed by Ames test.


The concentration of acetosyringone has been shown to increase about 10-fold when certain metabolically active plant tissues are wounded. Two GUS (beta-glucuronidase) reporter constructs, nor1-GUS (pGAP12) and ver1-GUS (pGAP13), were used to study the effect of acetosyringone on expression of aflatoxin biosynthetic genes, nor1 and ver1. GUS activities of these two reporter constructs were inhibited by 80% in the presence of acetosyringone at 2 mmol/L. Aflatoxin production in a toxigenic strain was also shown to be inhibited by acetosyringone to the same level.


Echinocandin B (ECB), a lipopolypeptide used as a starting material for chemical manufacture of the anti-Candida agent LY303366, is produced by fermentation using a strain of *Aspergillus nidulans*. In addition to ECB, the wild-type strain also produces a significant level of sterigmatocystin (STG). Characterization of a mutant strain, which is blocked in STG biosynthesis, was the result of a chromosomal translocation. The chromosomal regions containing the breakpoints of the translocation were isolated and DNA sequencing and PCR analysis of the chromosomal breakpoints demonstrated the translocation occurred within the stcW gene of the STG biosynthetic pathway, resulting in disruption of the open reading frame for this gene.


The aflR gene product is the main transcriptional regulator of aflatoxin biosynthesis in *Aspergillus* *sojae* and *A. flavus*. Although *A. sojae* strains do not produce aflatoxins, they do have an aflR homologue. When compared with the aflR of *A. parasiticus*, the *A. sojae* gene contains two mutations: an HAHA motif and a premature stop codon. To investigate the functionality of the *A. sojae* aflR gene product, a GAL4 one-hybrid system in yeast was used. Results indicate that the premature stop codon of the *A. sojae* aflR is the key to its functionality and leads to prevention of aflatoxin biosynthesis through loss of the transcription of aflatoxin-biosynthesis-related genes.

**Aflatoxins – Methodology**


Phage-displayed random peptide libraries were tested as sources of peptides that mimic the binding of AFB1 to monoclonal antibodies (Mabs) raised against the toxin. For two of the three Mabs tested, clones were obtained by panning, producing phage that bound specifically to MAb 13D1-1D9 (MAb 24; specific for AFB1 and G1) and MAb 6E12-1E9 (MAb 13; specific for AFB1, G1, and B1) in ELISA. The amino acid sequences of the binding peptides varied. Those binding to MAb 24 contained the sequence ‘...YMD...', and those that bound to MAb 13 contained the dipeptide ‘PW’. Mimotope phage was used in a competition ELISA format for assaying aflatoxin concentrations. The results show that mimotope preparations are effective substitutes for pure toxin in these ELISA procedures.


Mouse Mabs were developed against a synthetic AFB1-lysine-cationised bovine serum albumin conjugate. The isotype of one of these antibodies, IIA4B3, has been classified as immunoglobulin G(H). The affinity and specificity of IIA4B3 were further characterised by a competitive radioimmunoassay. IIA4B3 had about a 10-fold higher affinity for binding to AFB1-lysine adduct than to AFB1, when [3H]AFB1-lysine was used as the tracer. The concentration for 50% inhibition for AFB1-lysine was 0.61 pmol; that for AFB1 was 6.85 pmol. An analytical method based on a competitive radioimmunoassay with IIA4B3 and [3H]AFB1-lysine was validated with a limit of detection of 10 fmol of AFB1-lysine adduct. The method has been successfully applied to the measurement of AFB1-lysine adduct levels in human serum samples collected from the residents of areas at high risk for liver cancer.


An ELISA was developed to detect moulds producing aflatoxins in maize and peanuts by an antibody produced to extra-cellular antigen from *Aspergillus parasiticus*. This antibody recognised species with phenotypic similarities to *A. parasiticus*, *A. flavus* and the domesticated species *A. sojae* and *A. oryzae*. Maize and peanuts inoculated with spores of *A. parasiticus* and incubated at 15°C for 18 days or 21°C for 7 days were analysed for mould antigens and aflatoxin levels. At 15°C, mould antigens were detected by day 4 in maize when 0.16 μg/kg of aflatoxin was detected by ELISA but not by TLC. Antigens were detected in peanuts by day 4 before aflatoxin was found. Likewise, at 21°C, antigens were detected by day 4 in maize when less than 1 μg/kg of aflatoxin was detected by ELISA but not by TLC. But by day 2 in peanuts when no aflatoxin was detected, *A. parasiticus* could be detected before it could produce aflatoxins.

PAPP, E., FARKAS, A., OTTA, K.H. and MÉNSSÖLYICS, E. 2001. Validation and robustness testing of an OPLC method for the determination of afla-
A collaborative study was conducted to evaluate the effectiveness of an immunoassay cleanup column LC method for the determination of aflatoxin M1. In milk at proposed European regulatory limits. Liquid milk was centrifuged, filtered and applied to an immunoaffinity column. The column was washed with water and aflatoxin was eluted with pure acetonitrile. AFM1 was separated from aflatoxin B1 by immunoaffinity column cleanup LC method for the determination of aflatoxin B1, B2, G1 and G2. These results indicate that polyclonal antibody based EIA and IAC methods for aflatoxin analysis offer a suitable alternative to the more expensive monoclonal antibody based methods.

In 1992, the European Union set up a network of National Reference Laboratories and charged the Community Reference Laboratory with the responsibility to design a proficiency testing scheme for assessing the analytical ability of laboratories involved in the official control of APM, in milk. Since 1996, two exercises of proficiency testing have been performed on samples of milk powder and liquid milk. The trials were conducted according to ISO Guide 43. The interlaboratory RSD, obtained for both 1996 and 1998 exercises were in the range 15.7–33.9%. Compared with other published studies, this indicates a very good precision for the performance of this laboratory network in the analysis of traces of APM, in milk.

A new method based on the use of florisil and C8 solid phase extraction columns has been developed for the preparation of extracts from aflatoxin-contaminated peanut meal, following chemical decontamination. Food Additives and Contaminants 18: 329–341.

The coffee-specific diterpenes cafestol and kahweol have been reported to be anti-carcinogenic in several animal models. Cafestol and kahweol added to rat primary hepatocytes reduced the expression of cytochrome P450 CYP2C11 and CYP3A2, the key enzymes responsible for AFB1 activation to AFB1-8,9 epoxide. In addition, these diterpenes induced significantly GST Yc1, the most efficient rat GST subunit involved in AFB1-8,9 epoxide detoxification. These effects of cafestol and kahweol resulted in a marked dose dependent inhibition of AFB1 mediated DNA binding in this rat in vitro culture system. In human liver epithelial cell lines (THLE) stably transfected to express AFB1, the liver tumor promotion of one hotspot GC to TA transition in the p53 gene of affected individuals.
and kahweol also produced a significant inhibition of AFB1 DNA adduct formation linked with induction of the human glutathione S-transferase GST-mu.


Aflatoxin-induced haemolysis in erythrocytes was found to be significantly reduced by the addition of vitamin A at 125–1250 IU/ml to the incubation medium. The decrease in haemolysis was almost dose dependent.


AFB1 exposure of duck hepatitis B virus (DHBV) infected Pekin ducks induced a significant increase in viral replication associated with an intense biliary ductular cells proliferation. Extremely high levels of AFB1-DNA adducts and AFB1 albumin adducts were detected in duck liver and serum respectively, as compared to other animal species exposed to a similar AFB1 dose. DHBV infection was found to induce a non-significant increase in AFB1-albumin adduct levels in duck serum. During the treatment duration there was no effect on formation of oxidative base damage within DNA and no effect on oxidative lipid peroxidation following either viral infection or AFB1 exposure.


The in vitro effects of AFB1 on bovine (bov in italics) hepatic mitochondrial respiratory complexes, 2: α-ketoglutarate cytochrome c and succinate cytochrome c reductases were examined. Although the observed inhibitory and stimulatory effects of AFB1 were consistent with the changes in the kinetic parameters (Km and Vmax values), these parameters were not consistent with the observed effects of the toxin at certain concentrations. These observations are discussed in terms of the relative locations of the enzymes in the mitochondria, and the previously reported inhibitory and uncoupling effects of the toxin on cow liver mitochondrial respiration.


Murine peritoneal macrophages stimulated with lipopolysaccharide (LPS) after AFB1 pretreatment show a decreased production of nitric oxide (NO). The percentage of NO production in AFB1-pretreated macrophages was inversely increased by the addition of cholera toxin, phorbol 12-myristate 13-acetate and isoximycin. This suggests that AFB1 affects the function of signalling constituents, including guanine nucleotide binding protein, protein kinase C (PKC) and the calcium ion. AFB1 pretreatment significantly decreased P38 activity and tyrosine phosphorylation after LPS stimulation. Taken together, these data propose that in murine peritoneal macrophages the inhibition of LPS stimulated NO production by AFB1 is related to the suppression of kinase-mediated intracellular signal transduction.


The role of TNF alpha in the enhancement of hepatotoxicity of AFB1 by lipopolysaccharide (LPS) was investigated in rats. Male Sprague-Dawley rats were treated ip with AFB1 at 1 mg/kg and 4 hr later with Escherichia coli LPS. LPS administration resulted in a marked rise in TNF alpha levels at 6 hr, which preceded the onset of liver injury. When the increase in TNF alpha was attenuated by administration of either pentoxifylline or anti-TNF alpha serum, liver injury was prevented. LPS treatment resulted in the upregulation of gene transcription for cyclooxygenase-2 (COX-2). However, administration of the selective COX-2 inhibitor NS-398 did not decrease injury. TNF alpha and COX-2 inhibitors did not affect hepatic sequestration of neutrophils. Furthermore, it did not appear that TNF-alpha contributed to injury through inhibition of tissue repair. These data support the hypothesis that LPS induced expression of TNF alpha underlies the potentiation of AFB1-induced hepatotoxicity.


Ebselen, an organic selenium compound, protects against the cytotoxicity of AFB1 through its antioxidant capability. Fischer 344 rats were first treated with ebselen at 5 mg/kg, 5 days/week via gavage for 4 weeks, then given AFB1 at 0.4 mg/kg, via gavage once a week, or AFB1 plus ebselen for another 24 weeks. The results showed that the hepatocarcinogenicity of AFB1, in rats was significantly reduced by ebselen treatment. Ebselen treatment significantly reduced the formation of hepatic AFB1-DNA adducts and 8-hydroxydeoxyguanosine caused by AFB1 exposure.


The effects of methyl-deficiency and dietary restriction (DR) on hepatic cell proliferation and telomerase activity was studied in male Fischer 344 rats pretreated with AFB1. Rats were gavaged 5 days per week for 3 weeks with AFB1, at 25 µg/rat/day. Rats were then fed a methyl-sufficient (MS) diet or a methyl-deficient (MD) diet with or without DR. DR decreased hepatic cell proliferation, while the MD diet and AFB1 pretreatment increased cell proliferation. Telomerase activity was decreased by DR and increased by the MD diet and AFB1 pretreatment. The same trend was observed with GST-positive foci: AFB1 pretreated rats, methyl deficiency increased the number of foci, while DR decreased the number. These results are consistent with a role of telomerase in hepatocarcinogenesis.


The administration of AFB1, to rats at 2 mg/kg ip caused significant increase in the activities of gamma-glutamyl transpeptidase, 5-nucleotidase, acid phosphatase, acid ribonuclease as well as content of lipid peroxides in liver after six weeks. However, the activities of succinate dehydrogenase, glucose-6-phosphatase, catalase, superoxide dismutase, glutathione S-transferase, glutathione peroxidase and glutathione reductase in liver were decreased. The levels of glycogen and reduced glutathione were also decreased: There were significant elevations in the levels of serum transaminases, phosphatases, dehydrogenases and bilirubin following AFB1 administration. Picroliv at 25 mg/kg/day orally for six weeks significantly prevented the biochemical changes induced by AFB1.

Single doses of AFB1, at 2 mg/kg, ip caused significant biochemical changes in liver and serum of rats. Oral administration of picroliv at 25 mg/kg/day for 15 days, 6 weeks after AFB1 treatment, significantly prevented the biochemical changes induced in liver and serum of AFB1 treated rats. The hepatocurative effect of picroliv and silymarin, a plant based standard hepatoprotective, are comparable.


The tree shrew (Tupaia belangeri chinensis) is a unique species that can be infected with human HBV, is susceptible to AFB1-induced liver cancer, and shows a synergistic interaction between HBV and AFB1, for liver cancer. Two groups of tree shrews were fed AFB1, at 400 µg/kg body weight for 4 weeks. One week prior to AFB1 administration, one group also received oltipraz at 0.5 mmol/kg, po daily for 5 weeks. Aflatoxin-albumin adducts were determined in serum and urine. Adducts increased rapidly in 2 weeks to plateau then they diminished after cessation of AFB1 exposure. Oltipraz significantly attenuated the overall burden of aflatoxin-albumin adducts throughout the exposure period with a median reduction of 80%. In a single cross-sectional analysis at the end of AFB1 dosing, oltipraz treatment decreased urinary aflatoxin-N7-guanine by 93%.


Sodium bentonite from southern Argentina was evaluated for its ability to reduce the effects of total aflatoxins (AFB1, 5 mg/kg) in the diet of growing broiler chickens. Body weight gains were significantly lower for broilers fed diets containing aflatoxins alone. No differences were found between the body weight gains of chickens fed diets without aflatoxins and those of chickens fed aflatoxin plus sodium bentonite at 0.3% of diet. Alterations in the levels of serum total protein, albumin and globulins were observed for aflatoxin diets, and moderate protection was provided by the sorbent. However, the histopathological findings in liver sections of broilers fed diets with aflatoxins plus sodium bentonite indicated a non-protective effect of this adsorbent, because a moderate hepatic steatosis was observed.


The density of CD3+ cells was evaluated in the lamina propria of the intestines of chickens following application of aflatoxin and two kinds of zeolites for 30 days. Analysis of CD3+ cells showed a significantly increased number of T-lymphocytes after the application of both sorbents. There was no increase in the number of examined cells after the application of AFB1 alone. The possible role of damage to the bacterial biofilm by sorbents is discussed.


Mule ducklings were fed diets containing AFB1 at 200 µg/kg with or without the addition of beta-carotene (BC) at 200 or 400 mg/kg, or astaxanthin (AS) at 200 mg/kg. AFB1 alone or with the addition of BC or AS resulted in a significantly lower daily feed intake than for the control group. There were no significant differences in relative organ weights among treatment groups. Blood biochemical parameters and antibody titers were also evaluated. AFB1 treatment had the highest activities of aspartate aminotransferase and alanine aminotransferase (ALT) in the serum. The addition of BC at 400 mg/kg significantly reduced ALT activity as compared with AFB1 alone.


Two hundred species of Solanaceae were tested for their sensitivity to AAL toxins T-A and T-B. Twenty-five species were found to be sensitive to AAL toxins at a concentration of 0.2 µM used for distinguishing sensitive and insensitive tomato plants. Three species were as sensitive as the sensitive tomato line, indicating that AAL toxins effectively act on a broader range of plant species within the Solanaceae.