Abstracts

**Mycotoxins—General**


The feasibility of using atoxigenic strains of *Pithomyces chartarum* for the biological control of toxigenic strains of *P. chartarum* was studied. Pasteure treated with atoxigenic strains of *P. chartarum*, contained up to 80% less sporidesmin than that found in untreated pasture. Maximum sporidesmin levels of 26 µg/kg grass in treated pasture and 113 µg/kg grass in untreated pasture were recorded 14 weeks after treatment.

Torres, N.R. 1998. *Alternaria* alternata, *A. tenuissima*, 2 of *A. radicina*, and 3 of *Alternaria* state of *Pleospora infectoria* isolated from maize were screened to determine their ability to produce alternariol (AOH) and alternariol monomethyl ether (AME) on maize and rice. Only 28 *A. alternata* strains had toxigenic capacity. When maize was used as substrate, 21/28 isolates produced AOH and AME. When grown on rice, 23/28 strains produced AOH and 22/28 produced AME. The level of AOH produced by the isolates ranged front 0.3 to 2.1 mg/kg on maize and from 0.4 to 9.9 mg/kg on rice. AME production ranged between 0.3 and 3.3 mg/kg both on maize and on rice. These results could indicate a low probability of AOH and AME occurring naturally on maize in Argentina.


In Bramley and Cox’s apples inoculated with *Penicillium expansum*, patulin concentration was not significantly different between groups treated with the biocontrol enhancer 2-deoxy-D-glucose (DOG) and those without DOG treatment. Some additional small HPLC peaks were detected from some extracts, one of which corresponded to citrinin.


The inhibition by citrinin of lipid peroxidation of mitochondria, sub-mitochondrial particles and microsomes was studied. Inhibition was reversed in the presence of high concentrations of Fe³⁺ suggesting chelation of the mycotoxin with iron or interference in the reduction of Fe³⁺.


A new cyclospipeptide, designated roseocardin, was isolated from the culture broth of *Trichothecium roseum* TT103. Roseocardin B and destruxins A and B were also isolated during the same procedure. The structure of roseocardin was determined by EI-MS, NMR and X-ray crystallographic analysis. Roseocardin as well as the other cyclospipeptides were shown to produce positive inotropic effects on rat heart muscles.


The syntheses of two 13-membered cyclic peptides, analogues of phomopsin-ustiloxin class of antibiotics, are described. Benzylc hydroxyl group was introduced in desired stereochemistry by lead tetraacetate oxidation followed by methanalysis.

**Mycotoxins—Methodology**


Forty samples of maize gluten and 27 samples of other maize products were examined for 22 different mycotoxins. Aflatoxins were not found above the reporting limit (1–5 µg/kg) in any sample. Ochratoxin A was detected in two samples of maize gluten at 2 µg/kg. Neither sterigmatocystin nor cyclopiazonic acid was detected although the limits of detection for these toxins were poor. Eight maize gluten samples contained zearalenone at up to 500 µg/kg and zearalenone was found in all other maize products with the highest levels occurring in screenings and meal and the lowest amounts occurring in flaked maize and germ. Many samples contained a multi-toxin mixture of trichothecenes, fumonisins and moniliformin. Fumonisins occurred in all except two gluten samples and occurred up to a level of 32 mg/kg in maize screenings, 13 mg/kg in maize and 8 mg/kg in maize germ.

Methods used previously for the determination of aflatoxins, ochratoxins A (OA) and B, cyclopiazonic acid, zearalenone, sterigmatocystin and moniliformin in maize were applied successfully to rice bran. However, recovery of deoxynivalenol and other trichothecene mycotoxins spiked into samples was lower than expected and no citrinin could be recovered. Forty samples of rice bran used in the animal feed industry were examined for the presence of 20 mycotoxins. The level of mycotoxins contamination was low. Aflatoxin B1 was present in 29 samples, usually together with related aflatoxins, up to a total of 28 μg/kg. Most samples also appeared to contain small concentrations of OA.


The development, evaluation and validation of analytical methods for the measurement of mycotoxins in food and feedstuffs are discussed, describing the different links between EC Standards, Measurements and Testing projects and the projects of its predecessors, the Bureau Communautaire de Reference and the Measurements and Testing Programme, the needs of the food and feed industry, EU regulations and the work of the European Standardisation Body in the area of mycotoxins. The preparation of interlaboratory test materials together with their link to certified reference materials (CRMs) is also explained. Finally, the different use of CRMs and the requirements of mycotoxin reference compounds certified for purity is illustrated.


Kernels of corn infected with mycotoxigenic fungi, such as Aspergillus flavus, display FTIR-PAS spectra that differ significantly from spectra of uninfected kernels. Photoacoustic infrared spectral features were identified and an artificial neural network was trained to distinguish contaminated from uncontaminated corn by pattern recognition. Work is in progress to integrate epidemiological information about cereal crop fungal disease into the pattern recognition program to produce a more reliable and specific technique. A model of a hierarchically organised expert system is proposed, using epidemiological factors such as corn variety, plant stress and susceptibility to infection, geographic location, weather, insect vectors and handling and storage conditions, in addition to the analytical data, to predict A. flavus and other kinds of toxigenic fungal contamination that might be present in food grains.


An LC method was developed for simultaneous determination of major secondary metabolites, including cyclopiazonic acid (CPA), O-methylsterigmatocystin (OMST), and versicolorins, produced by Aspergillus species from section Flavi (A. flavus, A. parasiticus, A. tamaris, and A. caelatus). Metabolites were extracted with chloroform and quantified without prior cleanup by means of normal-phase ion-pair partition LC on silica gel with a mobile phase of n-heptane-2-propanol-n-butanol-water-tetraethylammonium hydroxide. Recoveries of CPA and OMST from fungal cultures spiked at 10 mg/L were about 99 and 96%, respectively. Limits of detection for pure standards were 0.25 mg/L for CPA and 0.30 mg/L for OMST.


A review with 72 references. Capillary electrophoresis and micellar electrokinetic chromatography were used for the separation of widely different compounds from natural materials including aflatoxins and other mycotoxins. A discussion of sample extraction and cleanup and the advantages of using CE is presented.

**Mycotoxicoses**


The production of mycotoxins by Alternaria alternata in cellulosic ceiling tiles was examined by TLC and HPLC procedures. AOH and AME were found in ceiling tile extracts, and extracts of control rice cultures of all three isolates produced these mycotoxins plus altenuene and altentoxin 1. Extensive fungal growth and mycotoxin production occurred in the ceiling tiles at relative humidities of 84–89% and 97%, respectively.


This review with 40 references discusses the occurrence of mycotoxins in animal feeds including: aflatoxins in cottonseed cake, groundnut meal, maize and sorghum; fumonisins in maize; ergopeptine and lolitrem alkaloids in tall fescue and perennial ryegrass; and phomopsins in lupin stubbles, pods and seeds.


The effects of feeding diets containing moniliformin (MON) at 100 mg/kg of feed and deoxynivalenol (DON) at 16 mg/kg of feed were evaluated in growing broiler chicks from 1 day to 21 days of age. Body weight, body weight gain and feed consumption were decreased by feeding MON and MON plus DON diets. Relative heart weight was increased by the MON diet, whereas relative weights of proventriculus, gizzard and heart were increased by the MON plus DON diet. Effects on serum enzymes and blood parameters are also reported. Histopathological lesions from the MON diet were limited to the kidney and these were moderated in the tissues of the MON plus DON fed chicks. Results indicate additive or less than additive toxicity for most parameters when chicks were fed diets containing MON plus DON.


Five isolates of Fusarium moniliforme and 2 isolates of F. proliferatum were each fermented on rice for 21 days at 25°C. The rice was then dried and mixed into a poultry diet to give 10% by weight feed and fed to baby Pekin ducklings. Acute mortality (death in less than 48 hr) correlated significantly only to the amount of MON in fermented rice but not to the amount of fumonisin B1. These results were confirmed by duckling assay using diets containing the purified mycotoxins.

Grazing on Echinopogon species by livestock in Australia caused symptoms similar to those of perennial ryegrass staggers. An endophytic fungus was observed in the intercellular spaces of the leaves and seeds of New Zealand and Australian specimens of Echinopogon ovatus. Using immunoblotting and an ELISA, the fungus was found to be serologically related to Neotyphodium lolii (the endophyte of perennial ryegrass) and other Epichloë and Neotyphodium species endophytic in pooid grasses. Analogues of the indole-diterpenoid paxilline, thought to be an endophytic in pooid grasses, were detected by ELISA related tremorgens, were detected by ELISA and an ELISA, the fungus was found to be serologically related to Neotyphodium lolii (the endophyte of perennial ryegrass) and other Epichloë and Neotyphodium species endophytic in pooid grasses. Analogues of the indole-diterpenoid paxilline, thought to be a biosynthetic precursor of the lolitremes and related tremorgens, were detected by ELISA and N-formylloline was detected by GC. Endophyte-free specimens of New Zealand E. ovatus did not contain detectable paxilline analogues or lolines. Hynphae similar to those of the E. ovatus endophyte were also found in herbarium specimens of other Echinopogon species. This appears to be the first time that an endophyte Neotyphodium species has been identified in grasses endemic to New Zealand or Australia.


Lolicines A and B, late eluting lolitrem-like compounds, were identified in extracts of perennial ryegrass (Lolium perenne) seed that was infected with the endophytic fungus Neotyphodium lolii. The lolicines were isolated as their 11-O-propionates and their structures determined by MS and NMR spectroscopy. The structures of lolicines A and B were similar to those of paspaline and paspallamine, compounds known to be biosynthetic precursors of several groups of more complex indole-diterpenoids, suggesting that the lolicines might be biosynthetic precursors of the lolitrem group of indole-diterpenoid neotoxins. A third compound, lolitrem N, was isolated as its propionate and identified as 35-epilolitriol.


A review with 81 references. The syntheses of fumitremorgen B, C, verruculogen TR-2 and related compounds, all of which are pentacyclic tremorgenic mycotoxins, are reviewed. Major topics include construction of the pentacyclic ring system, stereoselective formation of 1,3-disubstituted beta-carboline and introduction of a double bond and cis glycol to the ring C of the pentacycle.


Administration by i.p. injection of penitrem A 30 min before a training session in passive avoidance task impaired the performance of rats subjected to a test session 24 hr later. This effect was not antagonised by pretreating administration of physostigmine or bicuculline. Administration of penitrem A 20 min before a training session or 30 min before a test session did not impair performance. In the Morris water maze, doses of penitrem A that induce slight to moderate tremors, but not a lower dose, disrupted place learning. These results suggest that penitrem A disrupts the processes that take place at the time of acquisition, but not those just after acquisition, and does not alter the restitution of information.


Within 10 min of i.p. injection of penitrem A at 3 mg/kg, rats develop severe generalised tremors and ataxia that persist for up to 48 hr. These are accompanied by a three- to fourfold increase in cerebellar cortical blood flow. Changes are described in cerebellar stellate and basket cells, Purkinje cells and astrocytes. Despite widespread loss of Purkinje cells, the animals' behavior becomes almost normal within a week. The affinities of penitrem A for high-conductance calcium-dependent potassium channels and for gamma-aminobutyric acid receptors with the probability of resultant excitotoxicity are considered to be important underlying factors for these changes.


Male rats treated with roardin E at a sub-lethal dose of 2 mg/kg body weight showed a significant increase in glucose-6-phosphatase in the liver. Rats treated with roardin E plus linoleic acid at 300 μM/kg body weight showed a significant decrease in the blood glucose and glutathione levels. The combined effect also resulted in a significant decrease in the levels of superoxide dismutase and glucose-6-phosphatase in the kidney together with a significant increase in the lipid peroxidation in the liver. Results showed that co-administration of linoleic acid with roardin E resulted in increased roardin E toxicity.


A total of 176 isolates of the genus Aspergillus were screened for their ability to produce OA in yeast extract sucrose broth and on moistened corn. Besides being produced by A. ochraceus and A. alliaceus, OA was produced by one isolate of A. fumigatus and one of A. versicolor, species not previously reported to produce this mycotoxin.


The fate of OA along an industrial soluble coffee manufacturing line was investigated. Both the variability and the amount of OA naturally present in a lot of Thai Robusta green coffee was drastically reduced during soluble coffee manufacture. A small proportion of OA was eliminated during green coffee cleaning, but the most significant reduction took place during roasting due to thermal degradation. The roast and ground coffee contained only 16% of the OA originally present in the green coffee. A further reduction in OA levels was observed during soluble coffee manufacture, so that the powder contained only 13% of the OA initially present in the green beans.

The protective effect of vitamins on cytotoxicity induced by OA on red blood cells (RBC) in vitro was investigated. Addition of vitamin C at 10–90 mg/L and OA at 2 mg/L to a RBC suspension significantly retarded OA induced haemolysis. Similarly, inclusion of vitamin A at a concentration of 125–1,000 U/L reduced haemolysis induced by OA.


Macroscopic nephropathy was observed in 506 pigs at slaughter in Bulgaria. Histopathological changes were mainly degenerative and proliferative, and were linked with kidney hypertrophy similar to that of the classical Danish syndrome. Renal cysts formed by dilated tubules, activation or proliferation of capillary and vascular endothelium and the development of neoplastic epithelia. Changes of OA were found in the kidneys of the majority of 96 cases examined and in some feed samples. The dietary OA concentration (100 µg/kg), calculated from serum analyses, closely matched the average of individually analysed feeds.


The effects of OA on extracellular signal-regulated kinases 1 and 2 (ERK1/2) phosphorylation and activation, as well as on cell morphology were investigated using cloned Madin-Darby canine kidney-C7 (MDCK-C7) and MDCK-C11 cells. Results demonstrate an epithelial dedifferentiation of MDCK-C7 cells, but not of MDCK-C11 cells, after long-term incubation in the presence of OA, a result associated with the ability of OA to stimulate ERK1/2 in MDCK-C7 cells but not in MDCK-C11 cells. OA induced activation of ERK1/2 could be an important intracellular signalling pathway that mediates some of OA effects on renal epithelia.


In rabbits, consumption of a diet containing ochratoxin at 10 mg/kg for 90 days caused significant rises in the intracellular calcium concentration in liver, kidney, skeletal muscle and heart. While the concentration of magnesium increased in liver and kidney, it declined in skeletal muscle and heart. Significant changes occurred only in liver and heart. The calcium/magnesium ratio also increased in all organs studied.
The relation between fumonisin production and the mating population and mating type of *Fusarium* isolates from maize in Argentina at different maturity stages was evaluated. Fifty one isolates of *Fusarium* species belonging to the Liseola section were identified to mating population and tested for their ability to produce fumonisin B₃ (FB₁) and FB₂. Only mating populations associated with maize, *A* (*Fusarium moniliforme*), *D* (*F. proliferatum*) and *E* (*F. subglutinans*) were found. All but two isolates of populations A and D produced high levels of fumonisins ranging from 0.01–3.99 g/kg, whereas isolates of population E yielded less than 0.02 g/kg. Five isolates of *F. proliferatum*, all belonging to mating type D, produced more FB₂ than FB₁. Amounts of FB₃ were similar to FB₂ in cultures of mating population A, but much lower than FB₂ in cultures of mating population D.

Fumonisin production by, and mating populations of, *Fusarium section Liseola* were identified but produced little FB₁. At pH below 5 inbreds and 5 hybrids were tested for the production of FB₁ by *Fusarium moniliforme* and *F. proliferatum* and the mating population and mating type of *Fusarium* were investigated. Baking corn muffins spiked with FB₁ at 5 mg/kg at 175 and 200°C for 20 min resulted in about 84 and 72% retention of FB₁, respectively. At both temperatures, losses of FB₁ were significantly greater at the surface than at the core of the muffins. No significant losses of FB₁ were found when spiked corn masa was fried at 140–170°C for 0–6 min. FB₁ began to degrade at frying temperatures greater than or equal to 180°C and times greater than or equal to 8 min. Frying chips for 15 min at 190°C resulted in a 67% loss of FB₁. These studies suggest that fumonisins are heat stable compounds that survive under most conditions used during baking or frying.

**Fumonisins—General**


**Fumonisins—Toxicoses**


Corn samples collected from agricultural stocks for human consumption in Haimen and Penlai, high and low risk areas for primary liver cancer (PLC) in China, respectively, were analysed for fumonisins, aflatoxins and trichothecenes. Levels and positive rates of fumonisins and DON were significantly higher in Haimen than in Penlai in both 1993 and 1994. Analyses revealed a wide occurrence of AFB₁ and DON, and that the contamination level, as well as levels as well as not significantly different between these areas. Analyses of corn harvested in 1995 revealed that fumonisin contamination in Haimen was significantly higher than in Penlai. These surveys demonstrated that corn harvested in Haimen was highly contaminated with fumonisins and that the contamination level, as well as positive rate in 1993 and 1995, were 10–50 fold higher than those in Penlai, suggesting fumonisins as a risk factor for

**Fumonisins—Methodology**


A total of 10 maize varieties comprising of 5 inbreds and 5 hybrids were tested for the production of FB₁ by *Fusarium moniliforme*. Considerable variation in FB₁ production was found between different maize varieties. Most of the varieties supported good fungal growth but toxin production was not correlated with fungal biomass.

**Fumonisins—Methodology**


Under nitrogen limited conditions both growth of, and production of FB₁ by *Fusarium moniliforme* were significantly greater in cultures of population E when spiked corn masa was fried at 140–170°C for 0–6 min. FB₁ began to degrade at frying temperatures greater than or equal to 180°C and times greater than or equal to 8 min. Frying chips for 15 min at 190°C resulted in a 67% loss of FB₁. These studies suggest that fumonisins are heat stable compounds that survive under most conditions used during baking or frying.

**Fumonisins—Methodology**


This paper reports a sensitive method for the detection and quantitation of FB₁ in human urine. Amberlite XAD-2 non-ionic polymeric adsorbent resin is used prior to strong anion exchange cartridge cleanup. As much as 100 mL of undiluted human urine can be loaded onto the column. Recoveries were 93.6, 95.1 and 94.4% when samples were spiked with 10, 50 and 500 µg/L of FB₁ respectively. This method is highly reproducible and gives good sample cleanup, which is suitable for HPLC analysis.

**Fumonisins—Methodology**


Twenty samples of rough rice (unpolished kernels) collected during the 1995 harvest season from Arkansas and Texas were obtained from rice fields known to include plants with symptoms of *Fusarium* sheath rot putatively caused by *Fusarium proliferatum*. Samples were analysed for FB₁ at three laboratories using three different extracting solvents by HPLC or ELISA methods. Forty percent of the samples were positive for FB₁ at levels less than or equal to 4.3 mg/kg by HPLC. The same samples contained FB₁ at less than or equal to 3.6 mg/kg when measured by an ELISA method. Most samples that were positive for FB₁ were positive for FB₂ and FB₃ by HPLC at levels less than or equal to 1.2 mg/kg. Analysis of shelled unpolished rice showed that the fumonisin level was very high in hulls and low in the remaining brown rice. Milling of brown rice results in bran and white rice fractions and when analysed, fumonisins were found in bran but were below the level of detection in white rice. This is the first report of fumonisins in naturally contaminated rice in the United States.
The effect of exposure to FB₁ in low doses for 4 weeks was examined in weaned piglets. *Fusarium moniliforme* culture was added to the diet so that daily intake of FB₁ was 0, 10, 20 or 40 mg/kg diet. These levels of FB₁ had no significant effect on body weight gain and feed consumption, there was no change in the behaviour of the piglets and no clinical signs. In computer tomographic examinations performed in the second and fourth weeks, mild and more severe pulmonary oedema was diagnosed in the piglets. Dissection revealed mild cases of pulmonary oedema in animals given 10 mg/kg, ranging to severe cases in all animals given 40 mg/kg. (In Hungarian).


The clinical, haematological and pathological responses of adult male and female B6C3F₁ mice to FB₁ were assessed following 14 daily gavage doses of FB₁ ranging from 1 to 75 mg/kg body weight/day. There were no consistent sex related changes. Although all responses were modest, the most notable effects of FB₁ were on the liver, bone marrow, adrenals and kidneys. In the liver, hepatocellular single cell necrosis, mitosis and anisokaryosis were observed, accompanied by elevated serum alanine aminotransferase. In the kidneys, minor histopathological changes were confined to female mice, while mild decreases in ion transport and increases in blood urea nitrogen were seen only in males. In general, the degree of changes observed indicate that mice are not as sensitive a model of FB₁ toxicity as rats.


Groups of male BALB/c mice received s.c. injections of FB₁ at 0–6.25 mg/kg daily for 5 days and the liver and kidneys were sampled 1 day after the last injection. A decrease in kidney weight was observed after fumonisin treatment. Dose dependent FB₁ associated with bone marrow toxicity, starting at doses of 5 mg/kg/day. Hepatotoxicity and changes in adrenal cortex cells were also observed.


The embryotoxicity of AP₁ (hydrolysis product of FB₁) was evaluated in cultured rat embryos. Gestation day 9.5 embryos were exposed to AP₁ at 0–300 µM throughout the entire 45 hr culture period. At 100 µM AP₁, growth and overall development were reduced significantly. There was also a significant increase in the incidence of abnormal embryos: 29% of the embryos had neural tube defects and 36% of the embryos had other abnormalities. At 300 µM AP₁, the incidence of neural tube defects was 15%, and 85% of the embryos had other abnormalities.

associated hepatic and renal lesions were observed in all groups. Terminal uridine triphosphate nick-end labelling in liver and kidney sections confirmed the presence of dose related apoptotic cells at all treatment levels.


Chicken embryos and brine shrimp nauplii were utilised in short-term toxicity bioassays to assess their sensitivity to FB₁. At the cellular level, both bioassays appeared sensitive to FB₁, however, from the standpoint of use as a screening assay, the chicken embryo bioassay is limited by the relatively high dose of FB₁ required per egg. It is anticipated that the design and simplicity of the brine shrimp bioassay will accommodate screening for FB₁ toxicity in contaminated samples.


A disease outbreak characterised by black sticky diarrhoea, severe reduction in food intake, egg production and body weight followed by lameness and death was observed in 2 layer farms in Andhra Pradesh, India. A total of 6700 hens of 64 weeks age and 3000 in 2 layer farms in Andhra Pradesh, India. A total of 6700 hens of 64 weeks age were affected. Around 10% mortality and a 20% reduction in egg production were observed. Postmortem examination showed pale yellow coloured livers with peripheral congestion, mild haemorrhage in the proventriculus and watery ac-cumulations in the intestine. Analysis of the diet indicated contamination with FB₁ up to 8.5 mg/kg and with aflatoxin B₁ up to 0.1 mg/kg. Diarrhoea was induced in day old cockerels by feeding the suspect diet containing FB₁ at 8.5 mg/kg, and in laying hens by feeding a normal diet with FB₁ additions of 8 and 16 mg/kg.


The chronic and acute toxicity of FB₁ to the larvae of the yellow mealworm, Tenebrio molitor, was assessed by administering FB₁ via the diet or injecting directly into the lar-vae. Young larvae fed on a diet containing FB₁ at 450 mg/kg exhibited reduced growth performance but only after consuming the diet for several weeks. The FB₁ contaminated diet also reduced the rate of CO₂ production, food consumption and protein metabolism. FB₁ in the diet did not increase mortality, even when tested at the highest dose of 450 mg/kg diet. Injection of 25 mg FB₁ per larva decreased the CO₂ production, but became significant only 11 days after the injection and was reversible with time.


New Zealand White rabbits were dosed by gavage daily on gestation days (GD) 3–19 with FB₁ at 0.1, 0.5 or 1 mg/kg/day. Maternal lethality occurred at the 0.5 and 10 mg/kg/day doses. When examined on GD 29, there were no differences in maternal body weight, maternal weight gain, maternal organ weights, number of non-live implantations, and number of malformations. Foetal weight was decreased at 0.5 and 1 mg/kg/day (13 and 16%, respectively). Foetal liver and kidney weights were also decreased at these doses. Embryonic sphinganine to sphingosine ratios showed no differences between control and treated embryos on GD 20, although these ratios were increased in maternal urine, serum and kidney. Results suggest that FB₁ did not cross the placenta and that the observed decreased foetal weight was probably the result of maternal toxicity, rather than any developmental toxicity produced by FB₁.


Waste grain, stems and leaves collected from a maize field several months after harvest were analysed by TLC for aflatoxins, DON, OA, sterigmatocystin, T-2 toxin and zearalenone (ZEA). DON and T-2 toxin were found in the grain and ZEA was found in the stem and leaf. No other toxins were detected. As corn stubble is commonly grazed in Argentina and in other countries, these findings identify a further source of mycotoxins that may adversely affect animal health and productivity.


The occurrence of ZEA and ZEA producing fungi in corn, rice and wheat collected in Egypt was surveyed. ZEA was detected in 15/50 corn samples (average concentration 22.3 µg/kg), in 4/45 rice samples (average concentration 15.5 µg/kg) and in 5/40 wheat samples (average concentration 8.8 µg/kg). The efficiency of H₂O₂ at 3, 5 and 10% for destruction of ZEA in contaminated corn was studied. Degradation of ZEA was dependent upon the concentration of H₂O₂, temperature and period of exposure. The highest per cent of degradation was 84%, with 10% H₂O₂ at 80°C for 16 hr, followed by 75% under the same conditions for 8 hr.


Fusarium head blight, induced primarily by Fusarium graminearum, resulted in widespread damage to the Manitoba barley crops of 1993 and 1994, with contamination by DON and other 8-keto-trichothecenes. A commercial enzyme immunoassay for DON gave results that correlated well with GC-MS methods and afforded a rapid and convenient method for screening. In barley samples from 1994, DON, 15-acetylDON, 3-acylDON and 3,15-diacetylDON were detected in the approximate ratio of 47:4:1:1. In view of the higher oral toxicities of 15-acylDON and 3-acetylDON relative to DON, and the unknown oral toxicities of 3,15-diacetylDON, GC-MS assays might be advisable in samples positive for DON from enzyme immunoassay screening.


The viability of Fusarium and Alternaria infesting barley stored for 7 months under different conditions: -20, 4 and 24°C with quiescent air, and 24°C with forced air, was studied. Additionally, the ability of Fusarium to produce DON after storage and during malting was evaluated. All storage conditions reduced the viability of both moulds slightly and significantly for Fusarium. Forced air ventilation at 24°C was the type of storage most effective in reducing the viability of Fusarium, decreasing the percentage of infected kernels to 66%. DON levels did not change after 7 months with respect to stor-
age conditions. However, DON levels were lower in malt produced from barley stored at 24°C with or without aeration.


Phylogenetic analyses of aligned DNA sequences obtained via the polymerase chain reaction from the nuclear 28S ribosomal DNA, nuclear ribosomal internal transcribed spacer region, and beta-tubulin gene exons and introns indicate that the Quorn fungus is Fusarium venenatum rather than F. graminearum as previously reported. Analysis of mycotoxins from rice cultures inoculated with Quorn strain NRRRL 25416 revealed that four type A trichothecenes are produced, but at low levels relative to strain NRRRL 22198 of F. venenatum. No trichothecenes, however, were detected from the analysis of three commercial Quorn products marketed for human consumption in England.


The ability of Fusarium strains, belonging to five different species, to produce volatile compounds and trichothecenes was monitored by GC/MS. Isolates were grown for 21 days on autoclaved wheat kernels. The volatiles were collected after 3 and 21 days on Tenax GR traps using a dynamic headspace method, and immediately after volatiles collection the cultures were checked for trichothecenes. Of nine analysed strains, seven were toxigenic and produced both types A and B trichothecenes. Among volatile metabolites, sesquiterpene hydrocarbon trichodiene was produced exclusively by toxigenic strains representing four species and was detected in all toxigenic strains after 3 days incubation. Trichodiene presence can serve as an indication of fungicide treatment the cultures were checked for trichothecene nonproducing members of the genus.


Several genes in the trichothecene biosynthetic pathway of Fusarium sporotrichioides have been shown to reside in a gene cluster. Sequence analysis of a cloned DNA fragment located 3.8 kb downstream from Tril 5 has led to the identification of the Tril 6 gene. Disruption of Tril 6 results in an altered trichothecene production phenotype characterised by the accumulation of isotrichodermin, a trichothecene pathway intermediate. The evidence suggests that Tril 6 encodes a C-15 hydroxylase involved in trichothecene biosynthesis.


The sequences and relative locations of the MRRTril 5, MRRTril 6, and MRRTril 4 genes in the biosynthetic pathway for macrocyclic trichothecenes in Myrothecium roridum are reported. Based on sequence comparisons, MRRTril 5 encodes the enzyme trichodiene synthase, which has been shown to catalyse the first step in the trichothecene pathways of Fusarium and Trichothecium species. MRRTril 6 encodes a transcription factor required for pathway gene expression, and the predicted MRRTril 4 product is a cytochrome P450 monooxygenase responsible for the initial oxygenation step in the pathway. The sizes of the predicted products of MRRTril 5 and MRRTril 4 show good agreement with their apparent counterparts in the Fusarium pathway; however, the protein specified by MRRTril 6 is almost twice the size of its putative homologue in F. sporotrichioides.


The HPLC method adopted by the AOAC for the quantitative analysis of ZEA has been modified for application to maize, with quantification by HPTLC. The method has been validated by spiking uncontaminated extracts of maize with ZEA over the range 10–320 µg/kg. A linear relationship was found between 10 and 80 µg/kg, but at higher levels, the observed values were below the fitted line.


The performance characteristics of immunoaffinity chromatography as a new cleanup technique for the determination of ZEA in corn is reported for the first time and compared to a conventional cleanup procedure. Despite a constant error of about 4 µg/kg in the examined working range of 10–200 µg/kg, analytical data obtained from the analysis of spiked and naturally contaminated samples showed good correspondence for the compared methods.


The extraction behaviour of DON and some related type B trichothecenes from spiked and naturally contaminated wheat flour with modified supercritical CO2 has been investigated and optimised under several conditions. The extraction fluid was decompressed over a solid-phase trap and the amount of deposited analytes was determined by HPLC-diode array detection or GC-electron capture detection without any further cleanup. Recovery rates of around 90% were achieved for spiked wheat samples and 53% for naturally contaminated samples. The performance of the optimised supercritical fluid extraction method was compared with an already well established...
analytical method employing extraction on a rotary shaker in combination with Mycosep cleanup.

**Trichothecenes—Toxicses**


To study the effects of trichothecenes on haematopoietic progenitors, a culture model of human erythroblastic progenitors (BFU-E) optimised for toxicological studies was used to determine the effects of T-2, HT-2, diacetoxyscirpenol (DAS) and DON on red blood cell precursor proliferation and differentiation. Results showed that human BFU-E are as sensitive to trichothecenes as human CFU-GM, except for DON, in the range of concentrations tested. Differentiation of erythroblastic progenitors could be perturbed by these mycotoxins.


The effect of feeding diets containing DON at 0.6, 1.8 or 4.7 mg/kg on the immune response and performance of growing pigs was evaluated. Both restricted and *ad libitum* feeding were used. Performance was recorded as weight gain, feed intake, efficiency of feed utilisation and carcass quality. Immune response parameters recorded included primary and secondary antibody titres after injections of five different antigens. A significant DON dose-dependent reduction in secondary antibody response to tetanus toxoid was observed. No other indication of dose-dependent immune response inhibition or stimulation was found. Significantly reduced feed intake with increased levels of DON was observed in groups fed restricted rations according to weight, but not in animals fed *ad libitum*.


Molecular mechanisms by which DON superinduces cytokine gene expression were investigated by looking at the post transcriptional effects of this mycotoxin on IL-2 gene expression in murine EL-4 thymoma cells. Northern analysis revealed that doses of 50 to 500 µg/L DON superinduced IL-2 mRNA expression in a dose and time dependent manner. In accordance with the mRNA levels, IL-2 production was significantly elevated in the presence of 50 to 250 µg/L DON. To assess the effects of DON at 500 µg/L on IL-2 mRNA half-life, three transcriptional inhibitors were used. Results suggested that DON can superinduce IL-2 at both the mRNA and the protein level and that this superinduction can be explained, in part, by post transcriptional mechanisms such as enhanced mRNA stability.


Oral DON exposure in mice results in elevated cytokine gene expression, increased production of IgA and IgA nephropathy. The potential role of macrophages in these effects were determined in an *ex vivo* model whereby Peyer’s patch (PP) and spleen cells were prepared from mice 2 hr after oral exposure to DON at 0 or 25 mg/kg body wt cultured and then evaluated for IgA and cytokine IL-6 production. Both PP and to a lesser extent spleen cells from treatment mice produced more IgA over a 7 day period than did corresponding control cells. The DON effect was completely removed in PP cultures that were depleted of macrophages but not in macrophage depleted spleen cultures. DON exposure similarly increased production of IL-6, an important helper factor for IgA secretion. In LPS stimulated PP and spleen cell cultures, IL-6 production was also removed by macrophage depletion. A potential co-stimulatory role for macrophages was further suggested because both IgA and IL-6 production increased when macrophage depleted PP cells from DON treated animals were co-cultured with peritoneal macrophages from DON-treated animals. Taken together these and previous results suggest that macrophages may play a key mechanistic role in elevated IgA production and IgA nephropathy in DON exposed mice.


A single i.p. injection of T-2 toxin at 0.35, 1.75 or 3.5 mg/kg body weight induced time and dose dependent thymic atrophy in young female BALB/c mice. T-2 toxin at 1.75 mg/kg induced maximal atrophy by day 3 with complete recovery by day 7. Flow cytometric analysis showed that the CD4+CD8+ double positive thymocyte population decreased markedly. The *in vivo* effects of T-2 toxin included the induction of DNA fragmentation of similar to 200 base pairs in ladder form and cell death in thymocytes. Furthermore, flow cytometric analysis of PI stained thymocytes from animals dosed with T-2 toxin revealed the formation of apoptotic cells. Of nine different trichothecenes tested, T-2 toxin appeared to be the most potent agent to induce apoptosis in the thymus.


Lymphoid organs of 4 strains of male and female mice were examined histologically and biochemically at 24 hr after oral dosing of T-2 toxin at 0–10 mg/kg body weight. The results showed that T-2 toxin could induce apoptotic cell death in the lymphoid organs of mice. These changes were more prominent in female BALB/c and C57BL/6 mice.


The characteristics of T-2 toxin induced cell damage in the intestinal crypt epithelia was investigated in mice. Following T-2 toxin inoculation at 0–10 mg/kg body weight, dead cells showing pyknosis were sporadically observed in the crypt epithelia, and the nuclei of these cells contained fragmented DNA. Morphological changes consistent with those of apoptosis were observed. The mitotic index in the crypt epithelia drastically decreased at 6 hr after T-2 toxin inoculation, but thereafter it recovered to almost the same value as controls. However, the apoptotic index in the crypt epithelia increased with time.


Chickens were fed ZEA at 0.187–1.5 mg/kg or T-2 toxin at 0.187–6.0 mg/kg then orally infected with *Cryptosporidium baileyi* oocysts at 1 week of age. In chickens kept on the lower doses of ZEA and T-2 toxin,
the infection ran a similar course to that in the control groups and the animals became resistant to re-infection. However, at higher doses of 2.0–6.0 mg/kg T-2 toxin, there was reduced weight gain and some other physiological parameters were affected. Although there were certain modifications of the immune response, the chickens became resistant to re-infection. Only early (1 week of age) parasite infection and 6 mg/kg T-2 toxin in the feed significantly depressed body weight gain and immunity.

**Aflatoxins—General**


An evaluation was made of the genotypic diversity (DNA fingerprinting) of 269 Aspergillus flavus strains, including subpopulations isolated from grain sampled at harvest from a maize field near Kilbourne, Illinois. Eight A. flavus genotypes were isolated from grain samples harvested in different years (1988–1991). Ninety-eight percent of the A. flavus genotypes produced sclerotia and 53 % produced aflatoxin. The high genotypic diversity recorded for each subpopulation (grain, maize insects, soil, air), in addition to a limited sample size, precluded any assessment of the relative importance of these subpopulations as sources of A. flavus infective inoculum.


In a survey of aflatoxin M1 (AFM1) contamination in raw milk at farm level and bulk milk at dairies, a total of 187 milk samples were collected during 1993/94 and analysed for AFM1 by ELISA. At farm level an average of 20% of the samples was positive (greater than 3 ng/kg) with concentrations between 3, 6 and 10.6 ng/kg milk. Of the 157 bulk milk samples, 37 were positive at levels between 10 and 50 ng/kg. None of the milks exceeded the EU tolerance of 50 ng/kg.


The incidence of AFM1 in milk in and around Chennai city, Madras, India was surveyed. Of 325 milk samples collected, 36 samples contained AFM1. The range of contamination was 0.1–1 µg/L.


Mechanisms of resistance to infection by Aspergillus flavus and accumulation of aflatoxin were studied in kernels of resistant (GF-MAS.gk, Mp420) and susceptible (Pioneer 3154, Deltapine G-4666) corn genotypes. Proteins from kernel extracts of corn genotypes were analysed and consistent differences in protein profiles were detected among genotypes. Several proteins were unique to or present in greater concentration in resistant genotypes, whereas others were present only in susceptible genotypes. Extracts of resistant kernels showed markedly greater antifungal activity against A. flavus than did susceptible kernel extracts suggesting a role for kernel proteins in resistance to A. flavus infection and aflatoxin contamination.


The rate of Aspergillus flavus growth and production of aflatoxin in yeast extract sucrose medium generally decreased as the concentrations of NaCl increased from 2 to 12%. AFB1 production was decreased by 78% at an irradiation dose level of 2.0 kGy. Viable gamma irradiated conidia (2.0 kGy) of A. flavus showed increased sensitivity to NaCl concentrations and levels of aflatoxin produced by the irradiated conidia decreased in the presence of 2, 4 or 6% NaCl.


Aspergillus parasiticus var. globosus was able to grow in presence of different concentrations of sodium selenite at 0.052–4.0% or concentrations of potassium tellurite up to 2.0%. Growth of the fungus was decreased greatly by the increase of metals concentrations. Selenite slightly stimulated aflatoxin formation at lower concentrations and highly inhibited it at higher concentrations. Aflatoxin production was decreased greatly by increasing tellurite concentrations.


Propolis ethanolic extract (PEE) at 3 and 4 g/L and ultragriseofulvin at 0.75 and 1 g/L reduced the percentage of conidia germination in two Aspergillus flavus isolates. PEE at 1–4 g/L decreased the mycelial dry mass of A. flavus isolates by 11–80 %, and AFB1 production by 34–100 %. Ultragriseofulvin concentrations of 0.25–1 g/L reduced the growth and AFB1 production of the isolates by 16–88 and 48–98 %, respectively.


One of the sterigmatocystin (STG) Cluster (stc) genes, aflR, functions as a pathway specific transcriptional regulator for activation of other genes in the STG pathway. It is shown here that one important level for control of stc gene expression requires genes that were first identified as early acting regulators of asexual sporulation. Results are consistent with a model in which both asexual sporulation and STG production require inactivation of proliferative growth through inhibition of fadA dependent signalling. This regulatory mechanism is conserved in aflatoxin producing fungi and could therefore provide a means of controlling aflatoxin contamination.


A method is described for the purification of O-methyltransferase I, which catalyses conversions both of demethylsterigmatocystin (DMST) to STG and of dihydro-demethylsterigmatocystin (DHDMST) to dihydrosterigmatocystin during aflatoxin biosynthesis to apparent homo- geneity from the cytosol fraction of the mycelia of Aspergillus parasiticus. Results indicate that DMST and DHDMST commonly serve as substrates for the enzyme. MT-I kinet- ics deviated substantially from standard Michaelis-Menten kinetics, demonstrating substrate inhibition at a higher substrate concentra-

Aflatoxins—Methodology


A method for the determination of aflatoxin in urine is described. The urine samples were cleaned up by an automated procedure using immunofluorescence columns before analysis by HPLC and fluorescence de- tection. Postcolumn derivatization with bro- mine allowed the simultaneous determina- tion of aflatoxin and AFB1, B2, G1, G2, M1 and Q1. Average recovery of aflatoxin was 99% in the range 4–40 µg/L of spiked urine samples. The relative standard deviations were all between 1% and 3%. The limit of detection was 1 µg/L.


Two indirect ELISA have been investi- gated for the determination of AFB1, employ- ing only reagents commercially available whose composition is not exactly known. In both cases the antigen (AB1-BSA) was coated to the solid phase. In one procedure the specific antibody was a conjugate with peroxidase, while in the other one it was not conjugated, and a second antibody labelled with alkaline phosphatase was used. The equilibria in solution were studied by compe- tition ELISA, obtaining K, the affinity con- stant of the antigen-antibody complex in solu- tion. Similar results were obtained with the two procedures. A procedure for the determi- nation of AFB1 in food samples was devel- oped.


The clear characterisation of aflatoxin in- festation in tree or ground nuts requires a knowledge of the distribution of such infes-
tation among the nuts comprising the population under study. This paper discusses relations between measured sample means and the confidence that can be applied to such results.


The results of the black light test for aflatoxin contaminated maize carried out in a large food factory in the State of Sao Paulo, Brazil, was evaluated against bi-directional TLC analysis. All 286 samples were accepted by the black light test (7 fluorescent points), however, the results from TLC analysis showed that 96 samples were contaminated and 14 showed AFB$_1$ contamination levels higher than 20 µg/kg. If the rejection criterion of one or more fluorescent points were applied then 95 samples would be rejected and 87 results would be false positives because they did not have contamination levels over 20 µg/kg which is the acceptance limit of the black light test. The results indicate that the black light test, as utilised by this factory, was not able to indicate lots with possible contamination and the black light test, as recommended in the literature, would produce a high number of false positives.


The AOAC Methods (with slight modification) for the recovery of aflatoxins were applied to dates (Phoenix dactylifera). The Romer minicolumn method failed to detect any aflatoxin in contaminated date fruits. Using the HPLC and postcolumn derivatization procedure, the contaminants branch (CB) method was found to give average recoveries of 76 and 84% for date variants Lulu and Naghal, respectively. The recovery of total aflatoxins by the Best Food (BF) extraction and purification method was about 35% less than with the CB method.


Test samples were taken from farmers’ stock peanut lots contaminated with aflatoxin and kernels were divided into the following grade components: sound mature kernels plus sound splats (SMKSS), other kernels (OK), loose shelled kernels (LSK), and damaged kernels (DAM). Aflatoxins in SMKSS, OK, LSK and DAM components represented 7, 8, 33, and 52% of the total aflatoxin mass, respectively. Cumulatively, three aflatoxin risk components OK, LSK, and DAM accounted for 93% of total aflatoxin, but only 18% percent of test sample mass. Correlation analysis suggests that the most accurate predictor of aflatoxin concentration in the lot is the cumulative aflatoxin mass in the high three risk components OK + LSK + DAM. Linear regression equations relating aflatoxin in OK + LSK + DAM to aflatoxin concentration in the lot were developed. The cumulative aflatoxin in the OK + LSK + DAM components was not an accurate predictor of aflatoxin in the SMKSS component.


Transmittance near infra red (NIR) spectrums (500–15000 nm) of individual peanuts was measured to detect the internally moldy nuts. The mouldy nuts, which usually showed little difference from the sound nuts by visual observations, could be distinguished from each other by comparing the transmittance ratio of 700 nm to 1100 nm. The fungal hydrolysis of the triglycerides that were contained in the nut seemed to account for these differences on the NIR spectra.


A simple test tube screening procedure using fresh coconut milk agar medium for identifying toxigenic strains of Aspergillus, based on UV fluorescence (365 nm) and visual detection is proposed and evaluated.


Aflatoxins were determined at ng/L levels in beer by immunoaffinity column cleanup and reversed phase LC with fluorescence detection after trifluoroacetic acid derivatization. Silanised vials were necessary for the evaporation step in order to obtain good recoveries of aflatoxins from spiked beer samples. Recoveries for AFB$_1$, B$_2$, G$_1$ and G$_2$ averaged 90–104%, 94%, 84–87% and 89%, respectively. Detection limits were 19–20 ng/L for AFB$_1$, B$_2$, and G$_1$ and 15–16 ng/L for AFB$_2$ and G$_2$ obtained by using an excitation wavelength of 360 nm. At 340 nm these detection limits were lowered to about 2 ng/L. Analysis of 24 beer samples, the majority from the United States and Mexico, showed natural contamination of 5 Mexican samples and one Brazilian sample.


A comprehensive summary of the data from this laboratory and the literature and based on more than 1000 individual cases of hepatocellular carcinoma (HCC) is presented here and shows: 1) A high level and high prevalence of exposure to aflatoxins occur in West Africa, Mozambique and some regions of China; 2) a high prevalence of the 249 (ser) p53 mutation is detected in these countries; and 3) HCCs from countries with low or no exposure to aflatoxins show a very low prevalence of the 249 (ser) p53 mutation and distinctly different p53 mutation spectra, probably indicating different etiologies. Experimental and epidemiologic studies demonstrate an interaction between hepatitis B virus (HBV) infection and aflatoxins in hepatocarcinogenesis. The relevance of the biochemical/molecular markers of aflatoxin exposure, HBV vaccination and the reduction of aflatoxin exposure, in addition to the interaction between HBV infection and other risk factors in liver carcinogenesis, are discussed with regard to the implementation of measures for primary prevention.


p53 Protein expression in HCCs from a high incidence area of Guangxi, Southern China, where HBV infection and dietary intake of AFB$_1$ are high, was examined. Immunohistochemical staining of p53 protein was carried out using a polyclonal rabbit antibody (CM-1). p53 Protein expression was detected in 13/30 HCCs. Expression of p53 was found in 25% of the less than or equal to 5.0 cm diameter HCCs, in 37% of 5.1–10.0 cm diameter HCCs and in 71% of the greater than 10.0 cm diameter HCCs. Expression of p53 was observed more in moderately and poorly differentiated than in the well differentiated HCCs and more frequently seen in HCCs from younger patients. No significant association between p53 expression and sex, HBV infection, cirrhosis or alpha-fetoprotein has been found.

In 1995, 234 adults from Qidong, People’s Republic of China, were enrolled and followed in an oltipraz chemoprevention trial. Residents of this area are at high risk for development of HCC, in part due to consumption of aflatoxin contaminated foods. Healthy eligible individuals were randomised into three arms to receive p.o. 125 mg of oltipraz daily, 500 mg of oltipraz weekly or placebo for 8 weeks. There were no consistent changes in biomarker levels in the placebo arm over the 16 week observation period, nor was any apparent effect observed in the arm receiving 125 mg of oltipraz each day. However, individuals receiving 500 mg of oltipraz once a week for 8 weeks showed a triphasic response to oltipraz. No effect was observed during the 1st month of the intervention, whereas a significant diminution in adduct levels was observed during the 2nd month of active intervention and during the 1st month of follow-up. A partial rebound in adduct levels toward baseline values was observed during the 2nd month postintervention. Linear regression models up to week 13 confirmed a significant weekly decline of biomarker levels in the group receiving 500 mg of oltipraz once a week. However, despite these effects relative to baseline values within the 500 mg weekly arm, there were no statistically significant differences in biomarker trajectories between treatment arms.


The major risk factors for human liver cancer: HBV related liver injury, male gender, aflatoxin exposure and p53 expression, were evaluated and compared in experiment-

NEWS, NOTES, AND MISCELLANEA

The following items were gleaned from the April 1998 issue of the Mycotoxicology Newsletter, an international forum for mycotoxins, edited by Dr Angelo Visconti, Institute of Toxins and Mycotoxins, CNR, Viale Einaudi 51, 70125 Bari, Italy (fax: +39 80 548 6063; email <visconti@area.ba.cnr.it>). Entry to the mailing list for the newsletter, which is sponsored by U.S. company VICAM, is free.

Forthcoming meetings

The 1st International Symposium on Mycotoxins in the Food Chain (Processing and Toxicological Aspects), MYCOTOX 98, will be held in Toulouse, France, from 2–4 July 1998. The following sessions will comprise the scientific program: 1. Sources of mycotoxins in food, including “food starters”; 2. Technological and biological transformation of mycotoxins; 3. Toxicological properties of mycotoxins; and 4. Risk assessment and regulations. Contact: Carte Blanche, 19 rue Mahuziès, 81100 Castres - France. Fax: +33-56-372-3032.

The 8th International Fusarium Workshop will be held by IMI from 17–21 August 1998. These dates fall between the 7th International Congress of Plant Pathology, Edinburgh, UK, and the 6th International Mycological Congress, Jerusalem, Israel. The Fusarium Workshop is held under the auspices of the ISPP Fusarium Committee. Workshop facilitator is Dr David Brayford: fax +44-78-447-0909; email <dbrayford@cabi.org>.

The Argentinian Microbiology Society is having a 50th anniversary meeting from 6–9 September 1998 at the Sheraton Hotel in Buenos Aires. There will be a round table on “Mycotoxins and their economic and trade aspects”. Contact: Dr Horacio Frade, Departamento Microbiologia e Immunologia, Ministerio de Salud y Accion Social, Buenos Aires, Argentina: fax +54-1-340-0853; email HFrade@anmat.gov.ar.

The 3rd International Conference on Bioaerosols, Fungi, and Mycotoxins will be held in Saratoga Spring, New York, 23–25 September 1998. Contact: Christine Grosse, ENY Occupational & Environmental Health Center, 155 Washington Avenue, Albany, NY 12210, USA.

New journal

The Journal of Food Mycology, which was scheduled to start in March 1998, will publish high standard basic and applied papers on all aspects of the field of food mycology, including food-borne fungi, biochemical properties of fungi, taxonomy, physiology, and potential mycotoxin formation. It will provide a forum for research papers, notes, reviews, short communications, and letters on the control of fungal food spoilage and potential or actual mycotoxin production, and on use of fungi for production of fermented foods. The editor is John I. Pitt, Food Science Australia, PO Box 52, North Ryde, NSW 2113, Australia: email <john.pitt@dfst.csiro.au>.

Free mycotoxin posters

VICAM LP, a company which prides itself on its willingness to be an educational resource for both science and industry, has announced that it has created a new, informative mycotoxin poster. This poster serves as a handy reference tool providing useful information on mycotoxins while being exceptionally eye-catching in its full-colour display of various foods. It is available in English or Spanish versions. Contact Ms Linda Poirier at VICAM (fax +1 617 926 7045) if you would like a copy of the poster.

Proceedings

The 19th German Mycotoxin Workshop was held in Munich in July last year. The proceedings of the workshop, containing some 50 contributions, are now available from Prof. E. Maerklbauer, University of Munich at a cost of 50 DM plus postage. Fax Prof. Maerklbauer on +49 892 180 2106 for further details. ☎
Australian Mycotoxin Newsletter

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In dogs, acute and sub-acute aflatoxicosis are the most frequently observed forms of the disease. They are usually fatal and characterised by hepatitis. Diagnosis is based on both anatomo-pathological examination of the liver and aflatoxin quantitative analysis in food. This paper reports aflatoxicosis in five dogs resulting from mouldy bread consumption. The clinical signs observed are compared with the previously reported ones in the literature. (In French).


Feeding of aflatoxin to ducks produces extensive oval cell proliferation in the liver associated with a prolonged elevation of serum alpha-fetoprotein. Short term feeding of 0.075–0.6 mg/kg of aflatoxin to young male Pekin ducks results in rapid and massive dose-related proliferation of “oval” cells, which extend from the portal zone across the hepatic lobule within three to five weeks. Longer term feeding of 0.15 mg/kg and 0.3 mg/kg results in prolonged elevations of serum alpha-fetoprotein. Prolonged elevation of serum alpha-fetoprotein serves as a marker of oval cell proliferation preceding HCC development.


Young male adult guinea pigs were fed a diet without supplemental ascorbic acid (AA) or the same diet supplemented with 0.1 or 2.5% AA for four weeks. The animals were then euthanised and Phase I and Phase II drug metabolising components in the liver were determined. The Phase I components increased in response to increased intake of AA from 0 to 0.1%, but were unaffected by further increase. However, the Phase II components increased with increased intake of AA except for glutathione S transferase. In vitro metabolism of AFBl using liver microsomes showed tendency towards increased production of AFM with increased AA intake. The production of AFBl was not affected by AA intake. AFBl-DNA production was increased when AA intake was increased to 0.1%. However, it was lowered with further increase in AA intake to 2.5%.


The influence of AFBl incubated in vitro with rabbit liver microsomes on some cytochrome P450-dependent monooxygenases activities was studied. A strong competitive inhibition on aniline hydroxylation was observed. In contrast, only weak inhibitions of both pentoxyresorufin (PROD) and ethoxyresorufin O-dealkylases (EROD) activities were obtained. The inhibition was non-competitive for PROD activity and mixed for EROD.


One day old guinea pigs were fed diets containing AFBl at 0.037 mg/kg/day, copper at 200 mg/L drinking water, or a combination of both for 6 months. In the copper group there were no pathomorphological changes. For the AFBl group, liver damage was established. In the combined group, liver injury was more frequent and more severe compared to the AFBl group and biliary copper excretion was diminished compared with the copper group. Histologically, only the livers of this group exhibited degeneration, atrophy and steatosis of liver cells, inflammatory processes and a more or less prominent fibrosis. ©

Trout were initiated by a 30 min exposure to AFBl at 10 µg/kg. At 3 months post initiation, animals were started on either control diet or a diet containing 444 mg/kg dehydroepiandrosterone (DHEA). Tumours were not detected in initiated controls until 7 months after initiation. In initiated trout fed DHEA, the first tumour was detected 5 months after initiation (after 2 months of dietary DHEA). In a second experiment, trout were fed DHEA at 888 or 1776 mg/kg either prior to and during a 4 week initiation period of dietary AFBl administration, or for 8 weeks following initiation with AFBl. Neither exposure protocol provided protection towards AFBl hepatocarcinogenesis.

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