Abstracts


This issue of the journal contains 16 papers presented at Mycotox98. The meeting took into account the whole food chain by considering research aspects from biosynthesis to regulation of mycotoxins in food. [These articles are abstracted separately under appropriate headings in this newsletter – Ed.]


This overview with 25 references addresses the questions: what are toxins; when did toxins evolve; where do toxins come from; how can we use toxins and, why do microbes have toxins?


This review with 130 references discusses recent studies on the occurrence, significance and toxicology of important mycotoxins: aflatoxins, fumonisins, ochratoxins and patulin.


A review with 59 references. Mycotoxins may be classified as polyketides, terpenes and nitrogen containing metabolites based on their bio-origin. Progress on the biosynthesis of representative examples of each group is discussed with particular reference to the aflatoxins.


This paper with 146 references reviews the major chemical types of mycotoxins thought to be of importance from the agricultural point of view: aflatoxins, ochratoxin A, patulin, fumonisins, deoxynivalenol and zearalenone, by giving an idea of the levels that tend to be found naturally, and the extent and frequency with which they have been reported worldwide.


The development of new fermented products or fungal derivatives is approached carefully for fear of mycotoxin contamination. This paper with 73 references provides general propositions for the safe management of fungal resources. The main biotic and abiotic factors leading to mycotoxin contamination are considered, and the lessons learned from research on the two main used species, Penicillium camemberti and P. roqueforti, are analysed. Finally, a general strategy in four steps is proposed for obtaining an end product without mycotoxin contamination.


A review with 55 references. Treatments that destroy or inactivate mycotoxins, and the effects of food and feed processing on mycotoxin concentrations and distribution are selectively reviewed. Requirements for technologically and economically feasible decontamination processes are outlined.


In this review with 67 references, currently available data regarding tissue distribution of mycotoxins and their metabolites in animals are considered.


Within the European Union, there is an urgent need for agreement on authorised mycotoxin maximum levels in food to efficiently protect consumers while avoiding unjustified penalisation of producers. This paper presents the current state of the discussions on maximum tolerated levels for aflatoxins, ochratoxin A and patulin in food. The need for validation and harmonisation of statistical tools for sampling plans and analytical methods used to detect these mycotoxins is emphasised.


For the past number of years, the Food Quality and Standards Service of the Food and Nutrition Division of FAO has assisted developing countries in strengthening their capabilities to prevent and control the contamination of foods by mycotoxins. Assistance included the establishment of mycotoxin monitoring and surveillance programmes, the publication of various documents related to mycotoxin prevention and control, the organisation at national and/ or regional levels of training courses on methods of food sampling and analysis for mycotoxin detection, and various programmes and projects for the prevention of mycotoxin contamination at post-harvest level. The rationale for the establishment of methods of sampling and analysis for the...

This paper reports the outcome of a meeting of risk assessors and risk managers on specific aspects of risk analysis and its application to international standard setting for food additives and contaminants. Case studies on aflatoxins and aspartame were used to identify the key steps of the interaction process which ensure scientific justification for risk management decisions. A series of recommendations were proposed in order to enhance the scientific transparency in these critical phases of the standard setting procedure.


A total of 215 apple juice concentrate samples from three producers in Turkey were analysed for patulin using reversed phase HPLC. The detection limit for patulin in single strength apple juice at a sugar content of 11.2 Brix was lower than 5 µg/L. Patulin was detected in all of the samples analysed with concentrations ranging from 7 to 376 µg/L. Of the samples, 43.5% were found to exceed a patulin contamination level of 50 µg/L.


The production of mycotoxins and other secondary metabolites by Penicillium expansum on blackcurrant and cherry juice was studied at 10 and 25°C under storage imitated conditions. P. expansum was able to synthesise extracellular patulin under all conditions, and when unlimited oxygen was available, extracellular chaetoglobosin A was also produced. Patulin, the chaetoglobosin A and C, the communesins A and B and the expansolides A and B could be detected intracellularly depending on the conditions.


The levels of toxigenic moulds and mycotoxins were analysed in 62 samples of medicinal plant material and 11 herbal tea samples. The predominant fungi detected were Aspergillus, Penicillium, Mucor, Rhizopus, Absidia, Alternaria, Chasosporium and Trichoderma. Aspergillus flavus was present in 11 of the 62 medicinal plant samples and in one of the herbal tea samples. Ochratoxin was found in one of 7 medicinal plant samples.


A survey of 32 samples of apple juice concentrates destined for the production of commercial juices was carried out to evaluate the natural occurrence of the Alternaria metabolites alternariol (AOH) and alternariol methyl ether (AME). A HPLC method based on solid-phase extraction columns for extraction and purification of the toxins was used. Both mycotoxins were found as natural contaminants in 50% of the samples analysed. Levels of AOH were in the range 1.35 to 5.42 µg/L.AME was present in most cases only at trace levels, and the highest amount detected was 1.71 µg/L in one sample.


The influence of water activity (a_w) on both Penicillium citrinum growth and citrinin accumulation in wheat was studied. Wheat conditioned at different levels of a_w and inoculated with a citrinin producer strain was incubated at 30°C for 2 months. P. citrinum grew down to a_w 0.775. Citrinin was not detected in the substrate at a_w 0.800 and lower. As a_w increased the toxin was detected earlier and the maximum accumulation increased markedly (65 µg/kg at a_w 0.810, 460 µg/kg at a_w 0.825 and 22 mg/kg at a_w 0.885). Citrinin concentration declined rapidly after reaching the maximum at each a_w level.


The mycophora and mycotoxins in 130 poultry feeds samples from 2 factories in Rio Cuarto, Cordoba, were examined. Isolates belonged predominantly to the genera Aspergillus and Fusarium with A. flavus and F. moniliforme the most frequently isolated species in each genus. Aflatoxin B_1 was found in 48% of the samples with levels ranging from 10 to 123 µg/kg. Zearalenone levels were 327–5,850 µg/kg and deoxynivalenol was not detected in any samples.


In Egypt, commercial roasted salted peanuts (3% NaCl), popcorn (1% NaCl), summer-squash (9% NaCl), sunflower (3% NaCl) and wild-melon (3% NaCl) seeds are contaminated with fungi, mostly Aspergillus flavus, A. niger, Penicillium chrysogenum, P. corylophilum and Rhizopus stolonifer. Contamination of popcorn is about 10 times higher than of the other foods. These fungi were significantly inhibited in seeds treated with 9–21% NaCl and halotolerant A. wentii was the main fungus recovered from the seeds after treatment. NaCl at 9% stimulated emodin production by A. wentii on peanut and citrinin production by P. chrysogenum on popcorn and sunflower. Aflatoxin, citrinin and emodin production on popcorn persisted up to 15% NaCl.


Beauvericin is a cyclohexadepsipeptide mycotoxin which has insecticidal properties and which can induce apoptosis in mammalian cells. Ninety four Fusarium isolates belonging to 25 taxa, 21 in 6 of the 12 sections of the Fusarium genus and 4 that have been described recently, were tested for the ability to produce beauvericin. Beauvericin was produced by Fusarium acuminatum var. acuminatum (1/4 isolates), Fusarium acuminatum var. armeniacum (1/3), F. anthophilum (1/2), F. avenaceum (1/6), F. beemiforme (1/1), F. dianii (2/2), F. equiseti (2/3), F. longipes (1/2), F. nivale (2/2), F. oxysporum (4/7), F. poae (4/4), F. sambucinum (12/14) and F. subglutinans (3/3). These results indicate that beauvericin is produced by many species in the genus Fusarium and that it may be a contaminant of cereals other than maize.

Heat stability of cyclopiazic acid (CPA) incorporated in milk was assessed under different conditions. Batches of milk containing CPA at 1 mg/L were heated at 60, 80 and 100°C for 15–60 min to simulate heat processing employed in the dairy industry. Only 3–9%, 14–18% and 25–30% of CPA were degraded at each temperature, respectively, and degradation followed a pattern of a first-order reaction. Heating the milk for 2 hr at 60, 80 and 100°C decreased the CPA level by 9–17%, 20–34% and 49–50%, respectively. Storage of the heated milk overnight at 4°C further reduced levels of CPA. Results show that simulation of heat treatments used by the dairy industry induced no significant degradation of CPA in milk.


Interactions between fungi used as starter cultures, Penicillium roqueforti, P. camemberti, P. nalgiovense and Geotrichum candidum, and fungal contaminants associated with cheese were investigated on agar medium at 18 and 25°C. Mutual inhibition on contact was the most common interaction observed. The only other observed interaction was inhibition of the contaminant, while the starter continued to grow, especially in dual cultures involving G. candidum as the starter culture. Dual cultures involving G. candidum showed inhibition of production of the mycotoxins mycophenolic acid, roquefortin C, chaetoglobosin A and CPA produced by the contaminants.


Airborne fungal contaminants in composting facilities are increasingly recognised as important with regard to health hazards for workers. In this study, the exposure to airborne fungal spores in compost plants was investigated. Fungal spores were compared to the spectrum of microbial volatile organic compounds (MVOC) with regard to the physiological properties of each fungal species. The results indicate that the spectrum of MVOCs as well as the production of mycotoxins can be specific for certain species. However, an evaluation of health effects caused by the great variety of MVOCs remains difficult, since information on their toxicological impact is lacking. (In German).


In wild type and mutant strains of Emericella nidulans (=Aspergillus nidulans) sterigmatocystin (STG) biosynthesis is positively correlated with both asexual and sexual sporation. Conditions favouring sporation stimulated STG formation, and vice versa. Both processes were stimulated by light in a veA(+) genetic background and were inhibited by dianimobutanone, an inhibitor of ornithine decarboxylase. Synthesis of the mycotoxin was neither affected in a brlA mutant nor in developmental mutants blocked at later steps in sporulation. As in the wild type strain, dianimobutanone inhibited STG biosynthesis and cleistothecia formation in the brlA mutant. The inhibitor also affected the transcription of brlA.

Results indicate that sporulation and the synthesis of STG are co-regulated at a step previous to the brlA execution point.


Antifungal metabolites produced by Bacillus pumilus in potato dextrose broth were isolated from culture supernatant fluid by precipitation with ammonium sulphate. The metabolites inhibited mycelial growth of many species of Aspergillus, Penicillium and Fusarium and also inhibited production of aflatoxins, CPA, ochratoxin A and patulin. The metabolites were heat-stable and remained active after sterilisation at 121°C for 15 min. Their activity was stable over a wide range of pH (2–10). Chromatographic bioassay revealed only one compound with antifungal activity.


This special issue provides a selection of reviews and specific applications of chromatography in the analysis of foods and other biological materials for mycotoxins. Both cleanup and determination procedures are included. [These articles are abstracted separately under appropriate headings in this newsletter – Ed.]


This paper with 114 references reviews liquid chromatographic–mass spectrometric (LC–MS) procedures for the identification and/or quantification of toxic substances including aflatoxins. LC–MS interface types, mass spectral detection modes, sample preparation procedures and chromatographic systems applied in the reviewed papers are discussed. Basic information about the biosample assayed, work-up, LC column, mobile phase, interface type, mass spectral detection mode and validation data of each procedure is summarised in tables.


This review with 133 references highlights the status of TLC of mycotoxins in various sample matrices. The outstanding merits of TLC in the field of qualitative and quantitative determination of mycotoxins are considered. A comparison between different TLC methods and TLC with HPLC, ELISA and GC methods is made.


Three rapid immunoassays, immuno-filtration, immunochromatography and dipstick, were developed for the detection of sporidesmin A. Each assay format exhibited particular advantages and disadvantages with regard to assay sensitivity, working range and ease of use. The immunofiltration assay proved to be suitable for the detection of sporidesmin A in pasture due to its sensitivity and its potential to be used outside a laboratory environment. Semi-quantitative scores of spiked pasture samples using the immunofiltration assay were in good agreement with the amount of sporidesmin A added to the samples.

The solute–solvent interactions of AOH and AME were studied using spectroscopic and chromatographic techniques with the aim of rationalising the separation methods. The Kamlet and Taft’s solvatochromic comparison method was applied to analyse the UV-visible spectra and to compare with normal and reversed phase HPLC data. It is found that the normal phase chromatographic behaviour of these mycotoxins can be predicted from the spectroscopic studies.


Slaframine was derivatised precolumn with fluorescamine and chromatographed isocratically using HPLC on a Hamilton PRP-1 C-18 polymeric column with fluorescence detection. Using fluorescent derivatisation, sensitivity was increased 100 fold over previously reported GC methods. A liquid–liquid partition was used to extract slaframine from plasma with a 95% recovery. A solid-phase extraction was used to extract slaframine from milk resulting in a 91% recovery.


A rapid HPLC method for the determination of ergoline in ovine plasma is described. Ergotamine was used as an internal standard. A simple extraction procedure with diethyl ether was carried out, before chromatography on a C-8 column, with the excitation and emission wavelengths fixed at 250 and 420 nm respectively, on a fluorimetric detector. The method was linear between 3.5 and 15 µg/L and had good specificity, precision and accuracy. The limit of quantification and the limit of detection were 3.5 and 1.2 µg/L, respectively. Sheep were administered iv with a single dose of ergoline at 17 µg/kg body mass. Plasma ergoline levels decreased very rapidly. The terminal half life and the total clearance of the mycotoxin were found to be 23.6 min and 0.020 L/min/kg body mass, respectively.


An overview with 21 references. The metabolism of some mycotoxins in exposed humans and animals frequently results in a reduction in their toxic potentials and symptoms while other mycotoxins, in particular the heptatotoxic and hepatocarcinogenic aflatoxins, require metabolic activation to produce their potent biological effects. The metabolism involved is complex and is comprised of both toxifying and detoxifying pathways. The sensitivity or otherwise of animal species depends on the balance between these pathways. The nephrotoxicity of ochratoxin A involves not only a direct toxicity, but also a metabolism related genotoxicity.


A review with 62 references. Some mycotoxins alter normal immune function when present in food at levels below observable overt toxicity. Mycotoxin induced immunosuppression may be manifested as depressed T or B lymphocyte activity, suppressed antibody production and impaired macophage/neutrophil-effector functions.


A review with 84 references. In terms of haematotoxicity, mycotoxins can be classified into three categories: those for which haematotoxic effects have not been described in the literature including patulin, verrucosidin, citroviridin and the fumonisins; those able to provoke adverse haematologic effects even though the principal toxicity responsible for the pathology is non-haematologic in nature including ochratoxin, aflatoxin B1 and zearalenone; and those that principally cause adverse haematologic effects associated or not with other minor symptoms including trichothecenes.


This review with 129 references describes the genotoxic effects of several mycotoxins including aflatoxins, ochratoxin A, zearalenone and citrinin, and in particular, the DNA adducts produced and the mutations that might result. The often contradictory results obtained in genotoxicity tests with other mycotoxins such as patulin, trichothecenes, fumonisins, fusarin C and griseofulvin are also discussed.


This paper with 74 references presents the criteria used by the International Agency for Research on Cancer for evaluating the carcinogenicity of substances to animals and humans and defines the four groups that were established for the classification of the substances. The data which contributed to the carcinogenic classification of aflatoxins and ochratoxin A are presented together with the latest summary of the evaluations for 16 other mycotoxins and 3 groups of mycotoxins.


The aneuploidogenic and clastogenic potentials of citrinin and patulin were studied by determining inhibition of microtubule assembly under cell free conditions and by measuring induction of mitotic arrest and micronuclei in cultured Chinese hamster V79 cells. Both toxins inhibited cell free microtubule polymerisation in a concentration dependent manner. Patulin, but not citrinin, bound covalently to reactive thiol groups of microtubule proteins. At concentrations without gross cytotoxicity, mitotic arrest and CREST-positive micronuclei (micronuclei containing whole chromosomes/chromatids) were induced by both toxins. The time course of micronucleus induction and positive CREST staining indicate the aneuploidogenic potential of both toxins. CREST-negative micronuclei (micronuclei containing acentric chromosomal fragments) were induced by patulin but not by citrinin, implying a clastogenic potential of patulin.


Gliotoxin, produced by Aspergillus fumigatus, contains an epipolythiodi oxopiperazine ring that is believed to be involved in redox reactions. The reactive oxygen species produced interact with DNA to form hydroxylated and other altered DNA products. 32P radiolabelling was used to measure DNA adduct formation and, after
enzymatic DNA digestion, adducts were separated by TLC, with ultimate autoradiography and densitometry. There was an increase in 6-hydro-5, 6-dihydroxythymidine (thymine glycol) monophosphate from 0% to 30%, an increase in 8-hydroxy-2′-deoxyguanosine monophosphate from 0% to 4%, an increase in deoxynucleotide diphosphate from zero adducts to six DNA adducts, as well as increases in other as yet unidentified adducts. Time exposure may have a greater effect than concentration based on a 20 hr incubation.


Precision-cut human liver slices were cultured for 24 hr in medium containing coumarin at 0–5 nM or aflatoxin B$_1$ (AFB$_1$) at 0.002 and 0.02 nM using a dynamic organ culture system, and processed for autoradiographic evaluation of unscheduled DNA synthesis (UDS). Coumarin did not induce UDS however treatment with AFB$_1$ produced significant increases in the net grain counts of centrilobular hepatocytes. These results suggest that coumarin is not a genotoxic agent in human liver.

Ochratoxins—General


Ochratoxin A (OA) levels in the plasma of 184 healthy volunteers were surveyed in Tokyo from 1992 to 1996 using a competitive ELISA based on monoclonal antibody and a novel immunoaffinity column-linked HPLC-fluorometry. Eighty five per cent of the cases were positive for OA except in 1994 when 38% were positive. The average value in the positives was estimated as 68 nG/L. This suggests that the population in Tokyo is exposed to OA at high frequency, although the level in plasma is far less than that reported in Europe and Canada.


Blood plasma samples collected from 144 healthy volunteers in 16 locations across Canada in 1994 were analysed for OA. The method of analysis included cleanup by C-18 solid phase extraction and immunoaffinity columns followed by LC with fluorescence detection, which gave 86.5% recovery of OA with a detection limit of 0.15 µg/L. Statistical analysis showed a highly significant effect due to location in Canada but no effect due to age, sex or blood group of donors. The highest mean concentration was found in Winnipeg, and was significantly different by the Student-Neuman-Keuls multiple range test from the lowest levels found in Toronto, Vancouver and Saint John.


Surveys were carried out to estimate the levels of OA in pork, poultry, coffee, beer and pulses in Denmark. A total of 286 samples were analysed. The results show that compared with cereals and cereal products, the contribution from the foods surveyed to the total intake of OA by the Danish population must be considered to be of less importance.


The natural content of OA in grain samples of 6 barley, 2 bread wheat and 1 durum wheat cultivars varied from <0.1 to 0.4 µg/kg grain. Samples of the cultivars were inoculated with Penicillium verrucosum and incubated for up to 23 weeks. With time, all cultivars had increasing OA content, with maximum content in different barley cultivars ranging from 34 to 630 µg/kg grain for $a_w$ 0.75, and 39 to 260,000 µg/kg for $a_w$ 0.85. Corresponding values for the wheat cultivars were 25 to 2,300 µg/kg and 650 to 5,200 µg/kg. Significant varietal differences in OA accumulation were observed for barley, attributable to equilibrum water content, amylose content and natural OA; and for wheat, attributable to protein content and natural OA. Barley SW 1306 95/1203 and SW 906129 Waxy, and wheat SW 39103 accumulated significantly less OA than the other cultivars.


The contribution of different nephron segments to [3H]OA reabsorption was studied together with the possible mechanisms involved. OA was reabsorbed in all nephron segments investigated and under physiological conditions the predominant sites of reabsorption were the proximal straight tubule, ascending limb of Henle’s loop and the terminal collecting duct. Following iv injection of OA into rats it was shown that reabsorption also takes place in vivo.


Morphological changes and associated growth kinetics were correlated to OA production by Aspergillus alutaceus (parent). Two strains (hypo and hyper), isolated following gamma radiation of the parent, which differed in their ability to synthesise OA relative to the parent, were also investigated. There were significant differences between strains in regard to their hyphal growth unit length. The hypo and hyper strains exhibited the highest and lowest hyphal growth unit length, respectively.

Ochratoxins—Methodology


This paper with 133 references reviews chromatographic methods used for the determination of OA in animal and human tissues and fluids. Emphasis is given to HPLC methods. The review includes sampling, sample storage, extraction, spiking procedures, cleanup, detection and determination, and confirmation procedures.


The development of a capillary electrophoresis (CE) method for the quantification of OA in three different
commodities, roasted coffee, corn and sorghum, is reported. The extraction and isolation procedures combine a silica column and an immunoaffinity cleanup column analogous to other chromatographic methods. After separation from interferences by CE, OA was exposed to light from a UV He/Cd laser, and the fluorescence of OA was measured by CE-laser-induced fluorescence. When OA was added to several foods over the range 0.2 to 10 µg/kg, average recoveries were 86% for roasted coffee, 99% for corn and 91% for sorghum. Each instrumental analysis, after extraction and purification, required 13 min, equivalent to HPLC analysis.


An HPLC method is described for the analysis of OA at low µg/kg levels in samples of artificially contaminated cocoa beans. The samples were extracted in a mixture of methanol–water containing ascorbic acid, adjusted to pH and evaporated to dryness. Samples in this state were then placed onto a Benchmate sample preparation workstation where C-18 solid-phase extraction operations were performed. The resulting materials were evaporated to dryness and analysed by reversed phase HPLC with fluorescence detection. The relative standard deviations for multiple injections of sample and standard calculated to be 1.1% and 2.5% for sample and standard, respectively. Recoveries of OA added to cocoa beans ranged from 87 to 106%.


A rapid and reliable procedure has been developed for the determination of OA in wheat and oats. The method consists of extraction of the sample with acidic chloroform, followed by defatting with n-hexane and HPLC determination with fluorometric detection. Mean recoveries for wheat and oats spiked at levels between 1 and 100 µg/kg ranged from 80 to 104%. The limit of determination was 0.8 µg/kg and the relative standard deviation ranged from 3 to 7%. The method was not appropriate for the analysis of barley, rye or trout feed due to a number of false positive results. However, the use of immunoaffinity columns made the analysis of trout feed and rye samples possible, providing excellent cleanup of the extracts with no false positive results and a limit of determination of 0.2 µg/kg.


Patients suffering from Balkan endemic nephropathy and/or urinary tract tumours are found more frequently to have a capacity for rapid debrisoquine (DB) metabolism, a metabolic reaction related mostly to cytochrome P450 (CYP) 2D in humans. Earlier studies, using female DA and Lewis rats phenotyped as poor or extensive DB metabolisers respectively, revealed a parallelism between DB-4 hydroxylation and OA-4 hydroxylation. In this study, OA induced renal carcinogenicity and DNA adducts in DA and Lewis rats of both sexes were compared. OA induced renal adenocarcinoma, DA male rats being most responsive, while DA females were resistant. Lewis rats showed an intermediate renal tumour response. OA also induced malignant transitional cell carcinomas of the bladder in DA male rats only. DNA adducts in the kidney were significantly correlated with renal carcinogenicity of OA, being highest in DA males and lowest in DA females. A parallelism between karyomegalies and tumours of the kidney was observed.


OA blocks anion conductance in Madin–Darby canine kidney cells and reduces the potassium concentration gradient in the same cells. In this study OA action on ion channels of rat nerve fibres was investigated. Results point at the paranodal region of myelinated nerve fibres as a target for OA.
consumption of all people in the Netherlands in 1992. A probability distribution was derived to allow estimation of the exposure of the population to FB1 intake in relation to maize intake. It showed that among those in the group considered to be at risk, that is, people with gluten intolerance such as people with celiac or Duhring’s disease, 37% are estimated to be exposed to an intake of FB1 at least 100 µg and 97% to an intake of at least 1 µg/person/day. For all people in the Netherlands these percentages would be 1% and 49%, respectively.


Using the seed-plate technique, 18 different isolates of F. moniliforme were grown on pentachloronitrobenzene agar medium from samples of a local variety of corn from Menia Governorate, Egypt. Isolates were screened for their ability to produce fumonisins on polished rice grains using the solid state fermentation technique. Fourteen of the 18 isolates tested produced FB1 and FB2. Production of FB1 by the 14 isolates ranged from 69 to 4495 µg/kg.


Bulk shipments of maize imported into South Africa from the USA and Argentina during 1992 were sampled at the port of entry to determine fumonisin levels. Of the 79 samples from two US shipments, all were positive for fumonisins, with FB1 constituting approximately 71% of the total fumonisins with an overall mean of 2.35 µg/kg. The maximum FB1 level observed was 3.9 µg/kg. These levels contrast with those obtained from two Argentinean bulk shipments, which also were all positive for fumonisins, but had a mean FB1 level of 0.31 µg/kg and a maximum observed level of 0.7 µg/kg measured over 47 composite samples.


Corn based food products obtained from commercial outlets in Maryland, Nebraska and Arizona, USA, were analysed for total fumonisins by a commercial competitive direct ELISA (CD-ELISA) and for FB1 by HPLC. The highest fumonisin concentrations were found in samples collected in Maryland, where all 18 samples were found positive for fumonisins (200–745 µg/kg food) by CD-ELISA and 15/18 samples were positive for FB1 (<75–5.916 µg/kg) by HPLC. Fumonisins were also detected by CD-ELISA in 14/15 samples collected in Arizona, with concentrations ranging from 200 to 1,450 µg/kg, but analyses by HPLC showed that only 1/15 samples were positive for FB1 (<75–1.565 µg/kg food). Of the 23 samples collected in Nebraska, 20 were positive for fumonisins (200–2,500 µg/kg) by CD-ELISA, but only 10 were positive for FB1 (<75–927 µg/kg) by HPLC. The highest fumonisin and FB1 concentrations were found in cornmeal samples, ranging up to 7,450 µg/kg by CD-ELISA and 5,916 µg/kg by HPLC.


Various commercial maize products intended for human consumption were purchased from several retail supermarkets in the USA and South Africa, and subjected to mycological and chemical analyses. Fungal counts for F. moniliforme and the levels of fumonisin contamination were generally higher in the American maize products. Total fumonisin levels ranged from 0 to 465 µg/kg and from 0 to 3,605 µg/kg in South African and USA products, respectively. ‘Braaiap’ meals contained the highest mean fumonisin levels amongst the South African products (FB1, 177 µg/kg; FB2, 32 µg/kg), whereas the American products with the most contamination were the white meals (FB1, 1,365 µg/kg; FB2, 319 µg/kg).


One strain Fusarium moniliforme that produced high levels of fumonisins and two strains that produced very low levels of fumonisins were applied to maize kernels at planting near Manhattan, Kansas. The distribution of fumonisins in symptomatic and asymptomatic kernels, and the distribution of the three applied strains in the kernels was determined by vegetative compatibility group analysis. Both symptomatic and asymptomatic kernels were extensively colonised with F. moniliforme, but the highest levels of fumonisins were in the symptomatic kernels. A high frequency of ear and kernel infection with a strain that produced little fumonisin in vitro did not consistently decrease the level of fumonisins in field grown corn.


Two Fusarium proliferatum strains isolated from French maize grain, one a high producer of FB1, and the other a low producer of FB1, were tested for FB1 production on maize grain at 1.00 aw and 10, 15 and 30°C. Irrespective of the temperature, both strains had a similar level of conidiogenesis. Conversely, ergosterol grain content depended on temperature and optimal growth was observed at 30°C. Maximum FB1 production occurred at 15°C for both strains while at 30°C FB1 production was low. At 10°C, the strains were notably different in their toxin production kinetics.


The effects of canning, baking and roasting processes on the stability of fumonisins in artificially contaminated and naturally contaminated corn based foods was investigated. Canning of whole kernel corn resulted in a significant decrease in fumonisins by both ELISA (15%) and HPLC (11%) analyses. Canned cream style corn and baked corn bread showed significant decreases in fumonisin levels at an average rate of 9% and 48%, respectively, when analysed by ELISA. Corn muffin mix artificially contaminated with FB1 at 5 mg/kg and naturally contaminated corn muffin mix showed no significant losses of fumonisins after baking. Cornmeal samples artificially contaminated with FB1 at 5 ng/kg and naturally contaminated cornmeal samples roasted at 218°C for 15 min showed almost complete loss of fumonisins.


The effects of temperature and aw on growth and interactions between fumonisin producing isolates of Fusarium moniliforme
and *F. proliferatum* and seven other fungi from maize grain were determined in *vitro*. The type of interaction and index of dominance (I-D) between species were markedly influenced by temperature and *a*<sub>w</sub>. Generally, *F. moniliforme* and *F. proliferatum* were very competitive and dominant against the *Penicillium* species and *Aspergillus flavus*. They were in turn dominated by *A. niger*, but mutually antagonistic when paired with *F. graminearum* and *A. ochraceus*. Using Biolog plates, the effects of *a*<sub>w</sub> and temperature on utilisation patterns of carbon sources in maize were evaluated for the first time. The niche overlap indices (NOIs) for *F. moniliforme* and *F. proliferatum* were >0.90 above 0.96 *a*<sub>w</sub> and 25 and 30°C, indicative of coexistence with other species. Most of species had NOIs >0.90, except in some cases when paired with *F. moniliforme*, where NOIs <0.80 suggested the occupation of different niches. Although there was no significant correlation between the I-D and NOI methods, both suggested that the niche overlap between species was in a state of flux and significantly influenced by both temperature and *a*<sub>w</sub>.


FB<sub>1</sub> was produced in bioreactors using *Fusarium proliferatum* (M5991). Recently, some bioreactor-grown cultures of *F. proliferatum* were found to rapidly hydrolyse FB<sub>1</sub>. Subcultures from stock frozen from these FB<sub>1</sub> degrading variants continued to degrade FB<sub>1</sub> when grown under the same bioreactor conditions. The initial hydrolysis was catalysed by an esterase and retained the FB<sub>1</sub> hydrolytic activity was obtained by extraction of cell paste with 10% Triton X-100. Activity was destroyed by heating to 60°C for 5 min or by the addition of acetonitrile.


As well as FB<sub>1</sub>, certain foods also contain the aminopenitol backbone (AP<sub>1</sub>) that is formed upon base hydrolysis of the ester-linked tricarboxylic acids of FB<sub>1</sub>. Both FB<sub>1</sub> and, to a lesser extent, AP<sub>1</sub> inhibit ceramide synthase due to structural similarities between fumonisins and sphingoid bases. Ceramide synthase acylates AP<sub>1</sub> (but not FB<sub>1</sub> under the conditions tested) to N-palmitoyl-AP<sub>1</sub> (PAP<sub>1</sub>). The toxicity of PAP<sub>1</sub> was evaluated using HT29 cells, a human colonic cell line, and was found to be at least 10 times more toxic than FB<sub>1</sub> or AP<sub>1</sub>. PAP<sub>1</sub> caused sphinganine accumulation by inhibiting ceramide synthase.


This paper with 74 references reviews the development of sensitive chromatographic analytical methods for the determination of fumonisins in a range of mainly maize or maize based food matrices. The most widely used method involves strong anion exchange solid phase extraction and the use of o-phthalaldehyde as derivatising agent. By contrast, the development of TLC methods enables large numbers of samples to be screened economically. The recent advances in LC-MS have resulted in the development of suitable methods for fumonisin analysis without the need for derivatisation.


A method is described for the quick, precise and accurate detection of fumonisins in a two step chromatographic process. In the first step, fumonisins are extracted from a sample and isolated on an immunoaffinity column. In the second step, fumonisins are converted to fluorescent derivatives and quantified by either HPLC or fluorometer. These methods offer significant improvements in performance compared to earlier methods, including limits of detection as low as 16 µg/kg with HPLC-based detection and 250 µg/kg for fluorometer-based detection, greater assay linearity, larger immunoaffinity column capacity, and extended assay range for both methods. As a result of the efficient separation, the improved HPLC method offers the advantage of precise individual quantification of FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>.


An LC method for determining FB<sub>1</sub> and the total hydrolysis product of FB<sub>1</sub>, HB<sub>1</sub>, in tortillas is described. The method uses acetonitrile-phosphate buffer extraction, solid-phase C-18 cleanup, o-phthalaldehyde and 2-mercaptoethanol derivatisation, and reversed-phase LC. Average recoveries from tortillas spiked with FB<sub>1</sub> and HB<sub>1</sub> at 250, 500 and 1000 µg/kg were 86.5% for FB<sub>1</sub> and 82.6% for HB<sub>1</sub>. Tortillas and masa from the Texas–Mexico border were analysed for FB<sub>1</sub> and HB<sub>1</sub>. Average amounts of FB<sub>1</sub> and HB<sub>1</sub> in tortillas were 187 and 82 µg/kg, respectively, and in masas 262 and 64 µg/kg, respectively.


FB<sub>1</sub> and FB<sub>2</sub> were isocratically separated on a fluorocarbon column without using an ion pair reagent and nonvolatile buffer during the HPLC and were detected by an o-phthalaldehyde postcolumn derivatisation system using a fluorescence detector. The minimum detectable concentrations of FB<sub>1</sub> and FB<sub>2</sub> in corn by this system were 10 and 10 µg/kg, respectively.


A new HPLC method for the determination of the sphingoid bases, sphinganine and sphingosine, in serum of animals exposed to fumonisins has been developed involving lipid extraction, cleanup on a silica minicolumn and alkaline hydrolysis prior to pre-column o-phthalaldehyde derivatisation and HPLC separation, and quantification by fluorescence detection. Based on serum from both normal and fumonisin exposed vervet monkeys, the method was shown to be reproducible with a relative standard deviation of 10%.


The sequences of the ribosomal ITS1 regions of different fumonisin producing *Fusarium* species were determined and compared to the sequences of fumonisin non-producing species. In general, the ITS1 sequences were highly homologous. However, some minor sequence polymorphisms were
detected, which differentiates potential fumonisin producing *Fusarium* species from nonproducing species. By using these sequence differences, a PCR-ELISA for potential fumonisin producing *Fusarium* species was developed. All other ubiquitously occurring foodborne fungi tested showed negative results with this test.


Use of a direct competitive ELISA as a postcolumn monitoring system after LC is described for analysis of different fumonisin analogues. Without cleanup and derivatization, sample extracts were directly injected into a C-18 reversed-phase column and then subjected to LC. Fractions were analysed by ELISA. LC using a water–methanol gradient separated the 3 major fumonisins, FB1, FB2 and FB3, and FB3 as low as 0.1 ng could be detected. Recovery of FB1 at 100–1000 μg/kg added to ground corn was 78.8%.


An improved *Tenebrio* test is described for the bioassay of *Fusarium* mycotoxins. The improved test uses microtitration plates where individual *Tenebrio* larvae are put on a small wheat flour disk containing a mycotoxin. After incubation for 7 days at 30°C, larval growth and diet consumption were determined and the cellular energy allocation (CEA) used as a biomarker of physiological disturbance. Where the toxin impaired the growth rate of the larvae, the CEA index was significantly different when compared to controls.


FB1 was added to the semipurified Oregon test diet (OTD) used in rainbow trout feeding studies. The diet was then assayed for FB1 by high pressure LC and the biological availability of FB1 was determined by measuring the change in free sphingoid bases in liver, kidney and serum as a mechanism-based biomarker. Fumonisin was not easily quantified in the OTD with recoveries ranging from 12 to 81%. However, FB1 in the diet was readily absorbed and biologically active as evidenced by marked increases in free sphinganine in liver, kidney and serum. The magnitude of the increase in free sphinganine at 100 μg/kg in the OTD was comparable to that known to be associated with liver toxicity in rats, pigs and ponies.


This article with 94 references reviews the various mechanistic hypotheses for fumonisin induced animal diseases, including porcine pulmonary oedema, equine leukoencephalomalacia, nephrotoxicity and hepatotoxicity in several animal species, and liver cancer promotion activity in rats. The onset and progression of *Fusarium moniliforme* associated diseases in pigs, horses, rabbits, mice and rats in vivo, are closely correlated with evidence for disruption of sphingolipid metabolism. Fumonisins also affect other aspects of lipid metabolism and sites of cellular regulation that are reportedly independent of the disruption of sphingolipid metabolism.


This is the first report of FB1 being associated with an outbreak of equine leukoencephalomalacia (ELEM) at Oaxaca, Mexico. ELEM was reported to be the cause of death of 100 donkeys following 3 postmortem examinations in which macroscopic and microscopic cerebral white matter liquefactive necrosis were observed in the donkeys and FB1 was determined in 14 corn feed samples using HPLC and TLC. FB1 concentration in the samples ranged from 0.67 to 13.3 mg/kg.


Pregnant Charles River rats were dosed orally with FB1 at 0–50 mg/kg body weight on gestation days 3–16. At 50 mg/kg, maternal toxicity including inappetence, emaciation, lethargy, death and resorption of entire litters, and foetal toxicity including increased number of late deaths, decreased foetal body weight, decreased crown rump length, increased incidence of hydrocephalus and increased incidence of skeletal anomalies, were seen. Histopathological evaluation of tissues from dams revealed dose related toxic changes in kidney and liver tissues. Dose related increases in Sa/So ratios were seen in maternal liver, kidney, serum and brain, but there was no effect on foetal liver, kidney and brain. These data suggest that FB1 does not cross the placenta and further suggest that the observed foetal toxicity is a secondary response to maternal toxicity.


The *in vivo* effects of diets containing FB2 or FB3 were compared with FB1 in male rats. Animals were fed culture material of FB1 nonproducing *Fusarium moniliforme* isolates to provide low (4.6–6.7 mg/kg), mid...
Pregnant Syrian hamsters were dosed with FB1 at 0–18 mg/kg by gavage on days 8–12 of gestation and euthanised on day 15. Live foetuses were weighed and examined for gross external and internal abnormalities and skeletal anomalies. FB1 was shown to cause dose-dependent foetal death and delayed foetal development without causing foetal abnormalities.


A short and convenient route to the synthesis of an A-ring aromatic trichothecene analogue is described, employing a cyclobutyl carbonyl rearrangement as the key step.


In this overview with 34 references the efficiency of modern sample preparation techniques are discussed and compared to well-established techniques with respect to the determination of zearalenone in corn and type B trichothecenes in wheat. This includes the use of immune-affinity columns and of multifunctional Mycosep columns as well as the use of extraction for the trace analysis of these trichothecenes in cereals, foodstuffs and solid cultures. Methods based on mass spectrometry are especially emphasised.


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An LC method for determining DON in white flour, whole wheat flour and bran at or above the US Food and Drug Administration advisory level of 1 mg/kg was evaluated by an interlaboratory study. Samples of processed wheat (flour and bran) were extracted by blending with acetonitrile–water (84 + 16). Extracts were filtered and passed through a solid phase extraction (SPE) column then the eluate was chromatographed on a reversed phase LC column with a water-methanol solvent. DON was measured at 220 nm. Average recoveries of DON from white flour, whole wheat flour and bran spiked at 0.5, 1.0 and 2.0 mg/kg were 94, 87 and 97%, respectively. Within laboratory relative standard deviation (repeatability) ranged from 3.1–21.7% and between laboratory relative standard deviation (reproducibility) ranged from 10.8–38.7%. On the basis of the results of this study, the SPE/LC method for DON in white flour, whole wheat flour and bran was adopted as a peer verified method by AOAC International.


A rapid and sensitive method was developed for simultaneous detection of NIV, DON, 3-acetylDON and 15-acetylDON in wheat flour. Samples were extracted with acetonitrile–water (84 + 16) and the extract was filtered and purified by a column containing a combination of charcoal, celite and other adsorbants. For screening analysis, the column eluate was only extracted with ethyl acetate. After evaporation of the solvent, the dried residue was redissolved in acetonitrile–water (2 + 8) and then analysed by reversed phase LC with diode array detection. Recoveries of NIV, DON, 3-acetylDON, and 15-acetylDON from spiked whole wheat flour, were 45–52, 91–103, 81–85 and 84–92%, respectively. GC–ECD detection limits for all mycotoxins tested were <30 µg/kg.


A rapid and accurate method to quantify zearalenone (ZEA) in corn is described. Samples were extracted with acetonitrile–water (90:10 v/v) and the extract was diluted with water and applied to a Vicam Zearala Test immunoaffinity column. The column was washed with water and ZEA was eluted with methanol and quantified by reversed phase HPLC with fluorometric detection using acetonitrile–water–methanol (46:46:8 v/v) as mobile phase. Average recoveries of ZEA from corn spiked at levels of 0.1–10 mg/kg ranged from 93 to 99.5%, with relative standard deviations of <6%. The detection limit was 3 µg/kg.


A method for qualitative and quantitative analysis of DON, 3-acetylDON, fusarenon X, DAS, 15-monoacetylcircispenol, T-2 toxin, acroconitriol and ZEA by GC/MS without chemical derivatisation by means of the on-column injection technique is described. Chromatographic separation of the toxins extracted from barley was achieved as a single peak, and the specific EI mass spectra of each toxin were obtained. The fatty acids in the extract that interfere with measurements of the toxins on the gas chromatogram were removed by precipitation as an insoluble metal soap with zinc acetate. Additional cleanup was accomplished using a Bond Elut Florisil cartridge. The quantitative detection limit in barley ranged from 0.1 to 0.5 mg/kg.


A method is described for the determination of eight trichothecenes of type A and B in a variety of complex matrices including heavily moulded and pigmented cereals, whole cereal ears, cereal-based foods, mixed feeds and faeces from swine. Trichothecenes were determined as their trifluoroacetyl derivatives by GC with ion trap MS detection operating in chemical ionisation mode. The reactant gas, isobutane, was used, and optimum parameters of measurement were determined. For sample preparation, a cleanup procedure was developed using a combination of Florisil and cation exchange cartridges for solid phase extraction.


The effects of fusarenon X (FX) on mouse thymus and T-cell subpopulations were studied. In mice receiving ip injections of FX, the thymus showed severe atrophy, the thymic cortex almost completely disappeared and the total number of thymocytes decreased to 2.2% of that of normal mice. CD4(+)CD8(+) thymocytes were almost completely depleted by this treatment while CD4(+)CD8(-), CD4(-)CD8(+) and CD4(-)CD8(-) thymocytes were not reduced to such an extent. In spleen, CD4(+) or CD8(+) lymphocytes and CD4(-)CD8(-) non-T cells remained unchanged. The lymphocyte nuclei were fragmented and positive for TUNEL (TdT-mediated dUTP nick-end labelling) staining in the thymic cortex 20 hr after FX injection. By electron microscopy, apoptotic lymphocytes with condensed nuclei and stroma cells ingesting many nuclear fragments were frequently observed in the thymic cortex. Interenucleosomal DNA fragmentation was apparent in the thymocytes treated with FX both in vivo and in vitro.


The genotoxicity of NIV was tested in cultured CHO cells and in several mouse organs and tissues using the alkaline single-cell gel electrophoresis (SCG, or Comet) assay. NIV at 50 and 100 mg/L damaged the nuclear DNA of CHO cells in the absence of S9 mix. In an in vivo study, mice were sacrificed 2, 4 and 8 hr after either oral (20 mg/kg) or ip (3.7 mg/kg) administration of NIV and DNA damage was measured by
the modified SCG assay. After oral dosing, DNA damage appeared in the kidney and bone marrow at 2 hr, and in the stomach, jejunum and colon at 2, 4 and 8 hr, respectively. Liver and thymus DNA were not damaged. After ip injection, DNA damage was seen only in the colon where there was extensive damage, as in the oral study, at 8 hr.


A review with 34 references. Zearalenones cause a variety of adverse responses in domesticated animals, particularly swine, and may contribute to the overall load of environmental estrogens, which are believed to adverse impact on sensitive biological processes. Studies on structure-activity relationships among zearalenones provide clues about how estrogen receptors.


Adult mink were given diets containing moniliformin (MON) at concentrations of 10–240 mg/kg provided by *Fusarium fujikuroi* culture material. Mink fed diets that contained moniliformin at more than 40 mg/kg refused to eat significant quantities of feed. Feeding adult mink diets that contained moniliformin at 8 or 17 mg/kg in a 30 day subacute trial showed no significant adverse effects on feed consumption, body weights, haematologic parameters or serum chemical values, or any notable histologic changes in tissues. Consumption of the 17 mg/kg diet by female minks from 2 weeks prior to the breeding season resulted in significant neonatal mortality and reduced kit body weights at birth and at 8 weeks of age.


Lipid peroxidation may be one of the main manifestations of cellular damage in the toxicity of several mycotoxins. *Kluyveromyces marxianus* was used in this study to determine the oxidative damage induced by T-2 toxin. Malondialdehyde produced from the decomposition of lipid peroxides, was monitored using the thiobarbituric acid reaction. Electron paramagnetic resonance spin trapping technique showed that free radical production was promoted by T-2 toxin. The spin trapping data strongly suggest that initially generated hydroxyl radicals react with ethanol that is present in the samples and the alpha-hydroxyethyl radical formed in this process are then trapped. These data demonstrate that T-2 toxin stimulates lipid peroxidation in a biological system due to an increased generation of hydroxyl radicals.


During 1995, 159 samples of milk, 97 samples of dry milk for infant formula and 114 samples of yoghurt were randomly collected in supermarkets in four large Italian cities and checked for AFM$_1$ by immunoaffinity column extraction and HPLC. AFM$_1$ was detected in 136 of the milk samples in amounts ranging from 1 to 108.5 ng/L, in 81 of the dry milk samples in amounts ranging from 1 to 101.3 ng/kg and in 91 of the yoghurt samples in amounts ranging from 1 to 496.5 ng/L. Only two samples of milk, two samples of yoghurt, and one sample of dry milk had levels of AFM$_1$ exceeding the Swiss legal limits. AFM$_1$, contamination levels in milk and yoghurt samples collected in the period of November to April were about four times as high as those in samples collected in the period of May to October.


Previously it has been shown that some probiotic bacteria are able to tolerate and remove AFB$_1$ from media. Two strains of bacteria, *Lactobacillus rhamnosus* 1/3 and *L. helveticus* Åki 4 were tested for their ability to bind AFB$_1$. The bacteria were pelleted and suspended in PBS containing AFB$_1$ at 5 mg/L and incubated for up to 72 hr at 37°C and 5% CO$_2$. *L. rhamnosus* 1/3 binds AFB$_1$ rapidly and in a similar way to the most effective removers found so far, and was able to bind 75% of the aflatoxin. *L. helveticus* Åki 4 was able to bind only 30–40% of the toxin.


The efficacy of typical sorting methods which have been practically employed in industry for excluding aflatoxin contaminated materials from peanuts was evaluated by determining aflatoxin concentration in the peanuts before and after sorting treatments. The effectiveness of aflatoxin removal varied from almost 0 to 100% depending on the methods used. Aflatoxin was most effectively reduced by the manual exclusion of deteriorated peanuts, such as mouldy or worm-eaten nuts, based on visual inspection. The effective removal of aflatoxin by using a colour sorter required precise tuning of the operating conditions. Sifting and air-fan sorting had no significant effect. In some cases, these sorting procedures, even in combined use, could not reduce the aflatoxin concentration in peanuts to below 10 µg/kg, which is the legal concentration limit under Japanese customs regulations. (In Japanese).


Studies were conducted over 2 years to determine the effect of different inoculum rates of the biological control agents, nontoxicigenic colour mutants of *Aspergillus flavus* and *A. parasiticus*, on preharvest aflatoxin contamination of Florunner peanuts. Replicate plots were treated with 0, 2, 10 and 50 g/m of row of an equal mixture of the colour mutant infested rice in 1994, and the same plots were retreated in 1995. Aflatoxin concentrations were determined by HPLC. Aflatoxin concentration means for total kernels in 1994 were 337.6, 73.7, 34.8 and 33.3 µg/kg for the 0, 2, 10 and 50 g/m treatments, respectively. For the same repeated treatments in 1995, aflatoxin concentrations in total kernels averaged 718.3, 184.4, 35.9 and 0.4 µg/kg, respectively. Regression analysis revealed a stronger relationship between inoculum rate and aflatoxin concentrations in the second year of treatment. Compared with controls, treatments produced respective reductions in aflatoxins of 74.3, 95 and 99.9% in the second year.

The previously reported channel image sorter has been used to re-sort colour-sorted and hand-sort rejects and to sort mainstream (pre-hand-sort) USA pistachios. Sorting was carried out at commercial speeds of up to 163 kg/channel/hr. Recoveries of good nuts of 39–67% on re-sorted product and 97.8% on mainstream nuts were achieved. Aflatoxin levels were reduced from 8.6–4.8 to 0.04–2.5 µg/kg on colour-sort rejects and from 22 to 15 µg/kg on hand-sort rejects. For mainstream product, aflatoxin levels were reduced from 0.12 to 0 µg/kg by image sorting, compared to reduction to 0.04 µg/kg by hand sorting. Quality for mainstream sorting improved significantly, particularly for other damage, serious insect damage, gross defects and loose kernels. Re-sort quality improved as well, but recovered product will still require drying.

KUILMAN, M.E.M., MAAS, R.F.M., JUDAH, D.J. and FINKGREMMELS, J. 1998. Bovine hepatic metabolism of aflatoxin B1. Journal of Agricultural and Food Chemistry 46: 2707–2713. Bovine hepatocyte cultures were used to study the biotransformation of AFB1. Hepatocytes were cultured in monolayers and their metabolic function was assessed. The principal metabolites of AFB1 were AFM1 and AFB1-dihydriodiol. Minor amounts of AFB1-glutathione conjugate and a polar metabolite were also detected. No AFP1, AFQ1, AFB2a or aflatoxicol was detected. No polar metabolite were also detected. No AFP1, AFQ1, AFB2a or aflatoxicol was detected. The HPLC method developed provided the simultaneous detection of AFB1 and the metabolites.


Laboratory assays were performed with detached milk stage maize ears and dusky sap beetles (Carpophilus lugubris Murray) carrying the Kodiak Concentrate formulation of Bacillus subtilis (Ehrenberg) Cohn. After 1 day of exposure to the B. subtilis-contaminated C. lugubris, the colonisation of mechanically damaged kernels by Aspergillus flavus was reduced from 82% when the A. flavus was inoculated first, to 41% when B. subtilis was added by C. lugubris before the A. flavus. In field cage studies C. lugubris-dispersed B. subtilis reduced visible A. flavus colonisation by 97% when the A. flavus was added to purposely damaged maize ears 4 days after C. lugubris. These studies indicate that dispersal of B. subtilis by natural populations of C. lugubris is a potentially useful means for reducing A. flavus and aflatoxin in maize.


The influence of water activity (a_w) on the kinetics of aflatoxin and ZEA production in amaranth grains at 25°C was studied. Minimum a_w for aflatoxin production was 0.825. Accumulation of AFB1, B2, G1 and G3 was similar at a_w 0.825 and 0.868. Maximum accumulation of total aflatoxins at a_w 0.902 was detected after 21 days, with an appreciable increment in the concentration of AFB1, and G2. Amounts of aflatoxins produced were lower than those reported for aflatoxin production on other cereals and legumes. ZEA was not detected at a_w 0.902. Maximum accumulation of ZEA was after 35 days at a_w 0.925 and after 49 days at a_w 0.950.


Studies of the activity levels of the fungal cell wall degradative enzyme, beta-1, 3-glucanase, were carried out with Zea mays L. embryogenic callus cultures and kernels of genotypes with different abilities to resist aflatoxin formation on the ears. Dual culture experiments of Aspergillus flavus with maize callus demonstrated that the growth of A. flavus was inhibited more by callus of a resistant genotype (Tex 6 x Mo17 denoted TxM) than by a sensitive genotype (Pa91). The inhibition correlated with the activity levels of beta-1,3-glucanase in the callus and in the culture medium. The presence of the fungus caused an increase in enzyme activity in TxM but not in Pa91 callus.


The water insoluble residues of some carotenoid rich fruits and vegetables, such as apricots, oranges, brussels sprouts, carrots, yellow/red peppers and tomatoes, were sequentially extracted with n-hexane, dichloromethane, acetone and 2-propanol, and solvent extracted materials were tested for inhibition of mutagenicities induced by several substances including AFB1 in Salmonella typhimurium. Antimutagenic activities were found in many extracts, but especially in the n-hexane extracts. In the case of oranges, for example, 100 µg of this extract reduced the bacterial mutagenicity of AFB1 by 72%.


Six isolates of Bacillus pumilus were tested for their ability to inhibit aflatoxin production by Aspergillus parasiticus YES broth. Aflatoxin production was inhibited in both simultaneous and deferred antagonism assays, suggesting that the inhibitory activity was due to extracellular metabolite(s). A range of media was tested and all media supported bacterial growth and production of the metabolite(s). The metabolite(s) were produced over a wide range of temperature (25 to 37°C) and pH (4 to 9) of growth of B. pumilus. They were stable over a wide range of pH (4 to 10) and were not inactivated after autoclaving at 121°C for 30 min.


Detoxification of AFB1 by Armillariella tabescens multienzyme, isolated from mycelium pellets of A. tabescens, was confirmed by TLC and rat assay. The results of toxicology and pathology studies showed that the toxicity of AFB1 was minimised after treatment with A. tabescens multienzyme. The result of the Ames test indicated that the mutagenic activity of multienzyme-treated AFB1 was greatly reduced (or inactivated) compared with that of untreated controls. The IR spectrum suggests that the multienzyme is responsible for opening the difuran ring of AFB1.


This paper reports that Aspergillus nidulans AflR (AnAflR) is a 45 kDa protein that binds to the palindromic sequence 5'-TCG(N-5)CGA-3' found in the promoter regions of several aflatoxin and STG cluster genes (ste genes). The in vivo relevance of this AnAflR binding site was assessed and it was found that ste gene activation required both AnAflR and at least one TCG(NF5)CGA AnAflR binding site.
There are approximately 15 chemical steps in the biosynthesis of AFB₁ from norsolorinic acid. A new protocol of cell-free enzyme preparation has been developed from Aspergillus parasiticus which carries out all of these transformations for the first time. The key experimental step involves rapid concentration and efficient dialysis by membrane filtration to remove primary and secondary metabolites, cofactors and small biomolecules. All enzymes of the aflatoxin biosynthetic pathway have been dramatically stabilised by this procedure, and the effects of added substrates and cofactors can be assayed against virtually no background reactions. The overall pathway from norsolorinic acid to AFB₁ has been investigated, cofactor requirements defined for each step and a time-course run in which only versicolorin A and STG were observed to accumulate.

Part of the nucleotide sequence of the ver1 homologue in each of two strains of Aspergillus oryzae, A. sojae and A. flavus were compared with two homologues in A. parasiticus. The homologues in A. oryzae and A. sojae (non-aflatoxin-producers) exhibited an extremely high degree of sequence identity with that of A. flavus and A. parasiticus. No sequence fingerprint was found to distinguish between A. oryzae and A. flavus, or between A. sojae and A. parasiticus. By Southern analysis, a total of 46 strains of A. oryzae were examined for the presence of the ver1 homologue. The homologue was detected in 38 strains. Morphologically, strains with and without the ver1 homologue were indistinguishable.

DNA polymorphisms were used to infer relationships of a morphologically distinct strain of Aspergillus nomius. Mycologia 90: 618–623.

The interactions of AFM₁ with self-assembled metal-supported bilayer lipid membranes (s-BLMs) composed of egg phosphatidylcholine produced ion current increases which reproducibly appeared within about 8–10 s after exposure of the lipid membranes to the toxin. The magnitudes of the current signals were related to the toxin concentration, which could be determined within the range of 1.9–20.9 nM. In another series of experiments, AFM₁ was found to affect the kinetics and time of signal generation due to DNA hybridisation, which was electrochemically monitored by using s-BLMs. The electrochemical ion current across s-BLMs was found to increase due to the presence of single stranded DNA and decrease due to the formation of double stranded DNA. The toxin reduced the initial rate of signal change and increased the time to reach equilibrium. This provided a means for the rapid (less than 1 min) and sensitive (detection limit 0.5 nM) detection of AFM₁ based on measurements of the initial rate of hybridisation.


An extractionless method for determining AFM₁ in human urine is described. The biological fluid is injected directly into the chromatographic system after simple dilution and centrifugation. A precolumn, packed with a cation exchange phase and coupled online to a column-switching LC system, is used for sample pretreatment and concentration. The analytes are non-selectively desorbed with the LC eluent and cleaned by means of a column-switching procedure. Average AFM₁ recovery reached 97% in the 10–100 ng/L range of urine. The detection limit of AFM₁ in urine and milk was 2.5 ng/L. The method was applied to determine AFM₁ in the urine of AFB₁ gavaged rats, in the urine of potentially exposed workers and in milk.


An overview with 56 references. Carcinogen DNA and protein adducts promise to provide a more objective measure of human exposure to environmental carcinogens than can be obtained by questionnaire data or environmental measurements. However, there are a number of issues that must be addressed, including sensitivity, specificity, temporal relationship between exposure and disease, and their mechanistic role in the process of carcinogenesis.


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DNA polymorphisms were used to infer relationships of a morphologically distinct new strain of aflatoxin producing Aspergillus to other members of Aspergillus sect. Flavi. The new strain produced both B and G aflatoxins and differed both morphologically and physiologically from other aflatoxin producers. Three isolates of the new fungus were compared with representatives of three known aflatoxin producing species. Regions of four genes were amplified using PCR and digested with restriction enzymes. Restriction fragment length polymorphism analysis of all four gene regions indicated that the new Aspergillus isolates are closely related to A. nomius, but are diverged from other aflatoxin producing species.


interaction between cyclodextrins and aflatoxins $Q_1$, $M_1$ and $P_1$. Fluorescence and chromatographic studies. Journal of Chromatography A 815: 21–29.

The fluorescence properties of AFM$_1$, $Q_1$ and $P_1$ in solution and the effect of various cyclodextrins on their fluorescence emission were studied. A substantial enhancement of the fluorescence emission of AFQ$_1$ in the presence of the cyclodextrins, excepting gamma-cyclodextrin, was observed. No important fluorescence enhancement was found for AFP$_1$ or AFM$_1$.


A number of probability distributions that have been used to model the occurrence of aflatoxin in peanuts are compared. Two distributions, the compound gamma and the negative binomial, are shown to have special appeal in that both can be justified by reasoning from the fundamental biological and stochastic processes that generate the aflatoxin. Both models fit the data well, appreciably better than other models examined. An attractive aspect of the compound gamma and the negative binomial distributions is that, as a consequence of their theoretical underpinnings, both involve parameters that have meaningful interpretations.

Aflatoxins


Although the pathogenesis of hepatocellular carcinoma (HCC) remains poorly understood, hepatitis B virus and dietary aflatoxin exposures are established etiological factors for this disease. A pilot study was conducted to determine whether constitutional genetic instability, based on the quantification of mutagen induced chromatid breaks in cultured lymphocytes, modifies an individual’s risk of HCC development. Preliminary findings suggest that differences in host factors related to the predisposition to chromosome breakage, the capacity for DNA repair, or both, may be involved in HCC development by influencing the predisposition of hepatitis B virus integration into human DNA or that the carcinogens induced DNA damage susceptibility.


The first case of human systemic infection by an Aspergillus flavus isolate demonstrated to produce aflatoxins in vitro and in vivo is described. The patient, a 41 year old man with acute myelogenous leukaemia, developed a complication of suspected pulmonary Aspergillus infection during remission induction therapy. The autopsy revealed lesions in the lungs, myocardium, kidneys, brain, thyroid gland and skin due to a suspected Aspergillus species. A fungus isolated from the right lung and the skin lesions was identified as $A$. flavus. AFB$_1$, $B_2$ and $M_1$ were detected in culture filtrates of the isolate and in an extract of lung lesions. These aflatoxins are considered to have played an important role in damaging the immune system of the patient through their toxic effects.


The metabolism of AFM$_1$ and AFB$_1$ were studied in vitro using human liver microsomes. Formation of primary metabolites associated with metabolic activation to the respective epoxides reflected the differences between the carcinogenic potentials of the two toxins and, similar to AFB$_1$, the conjugation of AFM$_1$-epoxide with reduced GSH was catalysed by mouse, but not human liver cytosol. Although the majority of the binding of [$^{3}$H]AFB$_1$ to microsomal protein was dependent on metabolic activation, a high level of retention of [$^{3}$H]AFM$_1$ by microsomes was observed. Experiments using human cell line cells either expressing or not expressing human cytochrome P450 enzymes in assays of acute toxicity (MTT assays) have demonstrated a directly toxic potential of AFM$_1$ in the absence of metabolic activation, in contrast to AFB$_1$. Caution therefore needs to be exercised in designating the formation of AFM$_1$ as essentially detoxification when considering a biological response in which cytotoxicity may play a significant role, such as immunotoxicity.


AFB$_1$ is activated to AFB$_1$ exo-8, 9-epoxide primarily by cytochrome P450 enzymes, particularly P450 3A4. However, P450 3A4 and other P450s also oxidise AFB$_1$ to less dangerous products. The exo-epoxide is unstable in H$_2$O at 25°C and the diol product undergoes base-catalysed rearrangement to a dialdehyde that reacts with protein lysine residues. AFB$_1$ exo-8, 9-epoxide reacts with DNA to give adducts in high yield (>99%). This reaction is characterised by intercalation between base pairs and rapid reaction with the guanyln-7 atom. A proton field on the periphery of DNA is postulated to catalyse hydrolysis and also conjugation. Rat and especially human epoxide hydrolase show very little rate acceleration of hydrolysis of AFB$_1$ exo- or endo-8,9-epoxide. However, glutathione S-transferases (GSTs) can catalyse AFB$_1$ exo-8,9-epoxide conjugation. Polymorphic GST M1-1 has the highest activity of the human GSTs and studies with human hepatocytes indicate a major role for GST M1-1 in AFB$_1$.


The diterpene cafestol and kaurenoic acid (CK) have been identified in animal models as two potentially chemoprotective agents present in green and roasted coffee beans. Male Sprague-Dawley rats were treated with increasing amounts of a mixture of CK in the diet (0–6.2 µg/kg) for 28 and 90 days. A dose dependent inhibition of AFB$_1$-DNA binding was observed using S9 and microsomal subcellular fractions from CK treated rat liver in an in vitro binding assay. Significant inhibition was detected at 2.3 µg/kg and maximal reduction of DNA adduct formation to nearly 50% of the control was achieved with 6.2 µg/kg of dietary CK. Two complementary mechanisms may account for the chemopreventive action of CK against AFB$_1$ in rats—a decrease in the expression of the rat activating cytochrome P450 enzymes in a dose-dependent manner and a decrease in the activation of CK to AFB$_1$. The interactions between S9 activating systems and CK require further investigation.


Three PCR based subtractive techniques were used to identify AFB$_1$ responsive genes in cultured primary rat hepatocyte RNA—
The effects of four vegetables commonly consumed in Thailand, namely, flowers of the neem tree (Azadirachta indica var. siamensis), fruits of Thai and the Chinese bitter gourd (Momordica charantia Linn.) and leaves of sweet basil (Ocimum basilicum), on the levels of phase I enzymes, as well as the capacity to activate the mutagenicity of AFB₁, and to induce phase II enzymes in rat liver, were studied. Neem flowers and Thai bitter gourd fruits contain mono- and multifunctional phase II enzyme inducers and compounds capable of repressing some monooxygenases, especially those involved in the metabolic activation of chemical carcinogens, while sweet basil leaves contain compounds, probably bifunctional inducers, capable of inducing both phase I and phase II enzymes and Chinese bitter gourd fruits contain only compounds capable of repressing some monooxygenases. These results therefore suggest that neem flowers and Thai bitter gourd fruits may possess chemopreventive potential.


The effect of extracts of Thonningia sanguinea, a plant used prophylactically against bronchial asthma in Ghana, on certain biochemical indices in serum and liver of Fischer 344 rats given a single ip dose of AFB₁ at 1 mg/kg was investigated. AFB₁ caused significant increases in serum alanine aminotransferase (ALT) and GST levels and a significant decrease in aniline hydroxylase activity in liver microsomes. When T. sanguinea at 5 ml/kg was administered ip to rats 12 hr and 1 hr before AFB₁, liver injury was significantly reduced as seen in the decreased levels of serum ALT and serum GST. The decrease in aniline hydroxylase activity was not recovered but enhanced by T. sanguinea pre-treatment. The data indicate a hepatoprotective action of T. sanguinea against AFB₁ induced liver injury.


The rat can be protected against AFB₁ hepatocarcinogenesis by being fed a diet containing the synthetic antioxidant, ethoxyquin. The GST I-isoenzymes in rat liver that contribute to ethoxyquin-induced chemoprotection against AFB₁ have been identified by protein purification. This has resulted in the isolation of several heterodimeric class alpha GST all of which contained the A5 subunit and possessed at least 50 fold greater activity towards AFB₁-8,9-epoxide than previously studied transferases.