

Abstracts

Mycotoxins – General

GONZALEZ, H.H.L., MARTINEZ, E.J., PACIN, M., RESNIK, S.L. and SYDENHAM, E.W. 1999. **Natural co-occurrence of fumonisins, deoxynivalenol, zearalenone and aflatoxins in field trial corn in Argentina.** *Food Additives and Contaminants* **16**: 565–569.

Corn samples collected from the main production area in Argentina in 1995 were surveyed for the natural occurrence of *Fusarium* mycotoxins and aflatoxins. Fumonisin B₁ (FB₁), B₂ and B₃ and zearalenone were found in all samples. A positive relationship was found between FB₁, B₂ and B₃, B₁ and B₃, and B₂ and B₃. Deoxynivalenol and aflatoxins were not detected. The predominant fungus was *Fusarium moniliforme*.

FREIRE, F.C.O., KOZAKIEWICZ, Z. and PATERSON, R.R.M. 1999. **Mycoflora and mycotoxins of Brazilian cashew kernels.** *Mycopathologia* **145**: 95–103.

Kernel samples of common and dwarf Brazilian cashew nuts were examined for fungi and mycotoxins. In general, dwarf cashews were more contaminated than common cashews. A total of 37 fungal species were identified with *Aspergillus niger* being the dominant species. Multimycotoxin analysis by TLC and HPLC were positive for mycotoxins, particularly from the infected samples. Aflatoxins were not detected by HPLC.

FINOLI, C., VECCHIO, A., GALLI, A. and FRANZETTI, L. 1999. **Production of cyclopiazonic acid by molds isolated from Taleggio cheese.** *Journal of Food Protection* **62**: 1198–1202.

Twenty seven strains of *Penicillium* were isolated from the rind of Taleggio, a typical Italian cheese, and their capacity to produce

cyclopiazonic acid (CPA) was determined. All strains produced CPA. Strains incubated at 25°C for 7 days always produced CPA in mannitol broth, with concentrations ranging from 0.02 to 1 mg/L, whereas only 33% of strains grown in yeast extract broth produced CPA, with a maximum value of 0.1 mg/L. The capacity of CPA for migrating into the cheeses, the rind, the cheese near the rind and the cores was determined. CPA was present in five cheeses and in one cheese, the toxin migrated to the core. There was a positive correlation between CPA production and surface mould infection.

JANARDHANA, G.R., RAVEESHA, K.A. and SHETTY, H.S. 1999. **Mycotoxin contamination of maize grains grown in Karnataka (India).** *Food and Chemical Toxicology* **37**: 863–868.

Maize samples representing different cultivars were collected from different agroclimatic regions of Karnataka, India, and analysed for moisture content, mould incidence, ergosterol and mycotoxin contamination. Mycotoxin analyses using ELISA and TLC revealed mycotoxin contamination in 69 of the 197 samples. High levels of mycotoxins were found in many samples.

NIELSEN, K.F., GRAVESEN, S., NIELSEN, P.A., ANDERSEN, B., THRANE, U. and FRISVAD, J.C. 1999. **Production of mycotoxins on artificially and naturally infested building materials.** *Mycopathologia* **145**: 43–56.

The ability to produce mycotoxins during growth on artificially infected building materials was investigated for *Penicillium chrysogenum*, *P. polonicum*, *P. brevicompactum*, *Chaetomium* species, *Aspergillus ustus*, *A. niger*, *Ulocladium* species, *Alternaria* species and *Paecilomyces* species, all isolated from water damaged building materials. Spores were inoculated on gypsum board, with and without wallpaper, and on chipboard, with and without wallpaper. All

six isolates of *C. globosum* produced the toxic chaetoglobosins A and C. The quantities of secondary metabolites produced by *Penicillia* were generally low, and no toxin production was detected from any of the five isolates of *P. chrysogenum*. Both isolates of *P. polonicum* produced 3-methoxyviridicatin, verrucosidin and verrucofortine and 2/5 isolates of *P. brevicompactum* produced mycophenolic acid. Alternariol and alternariol monomethyl ether were detected from 5/6 isolates of *Alternaria*. No mycotoxins were detected from *Ulocladium* species, *Paecilomyces* species and *A. ustus*. Analyses of wallpaper and glass-fibre wallpaper naturally infected with *A. versicolor* revealed sterigmatocystin (STG) and 5-methoxySTG. Analyses of naturally infected wallpaper showed that *C. globosum* produced the chaetoglobosins A and C, and *P. chrysogenum* produced the antibiotic meleagrin.

SUGIURA, Y., BARR, J.R., BARR, D.B., BROCK, J.W., ELIE, C.M., UENO, Y., PATTERSON, D.G., POTTER, M.E. and REISS, E. 1999. **Physiological characteristics and mycotoxins of human clinical isolates of *Fusarium* species.** *Mycological Research* **103**: 1462–1468.

Thirty three strains of *Fusarium* species from clinical sources including *F. solani*, *F. oxysporum*, *F. moniliforme*, *F. proliferatum*, *F. subglutinans* and *F. chlamydosporum*, were examined for their physiological characteristics and mycotoxin production. Two strains of *F. moniliforme* and two strains of *F. proliferatum* produced fumonisins. No strains produced detectable amounts of trichothecenes. All 18 strains of *F. solani* tested produced the immunosuppressive agent cyclosporin A.

BOCKELMANN, W., PORTIUS, S., LICK, S. and HELLER, K.J. 1999. **Sporulation of *Penicillium camemberti* in submerged batch culture.** Systematic and Applied Microbiology **22**: 479–485.

Sporulation of *Penicillium camemberti* was studied in submerged batch fermentation using a defined medium with glucose and ammonium as C and N sources at a temperature of 25°C and at pH 5.6. CPA was produced during fermentation. The levels observed (0.5–4 mg/kg at 96 hr) were strain specific and not related to spore yield.

XU, G.R., LU, C., MU, X.Q., CHEN, J.L., CHEN, Y., GU, Y.M., WU, Y.P., SHENG, F. and WU, M.Y. 1999. **A study on the production of citrinin by *Monascus* spp.** Archiv fur Lebensmittelhygiene **50**: 88–91.

Citrinin was detected by TLC and HPLC in *Monascus* cultured on “red rice” collected from various manufacturers. Thirty two of 35 samples contained citrinin in the range 0.2–140 mg/kg. *Monascus* strains were isolated from these samples of “red rice” and some strains, *M. anka* and *M. ruber*, were identified as potential citrinin producers when cultivated in YES medium. When monosodium glutamate or histidine was used as the sole N source to cultivate *M. ruber* strain JH-2, no citrinin was detected in the fermented broth, while fermentation on rice or wheat resulted in high toxin concentrations.

COMBINA, M., DALCERO, A.M., VARSAVSKY, E. and CHULZE, S. 1999. **Effects of food preservatives on *Alternaria alternata* growth and tenuazonic acid production.** Food Additives and Contaminants **16**: 433–437.

The effects of different organic acids on *Alternaria alternata* growth and tenuazonic acid production were evaluated. Sodium benzoate, potassium sorbate and sodium propionate, all preservatives commonly used by food industry in Argentina, were tested. Levels above 10 mg/kg of sodium benzoate and potassium sorbate resulted in total inhibition of fungal development and toxin biosynthesis. Sodium propionate produced a 59% decrease in *A. alternata* growth and total inhibition of tenuazonic production only at 200 mg/kg, the highest concentration employed.

RITIENI, A., MONTI, S.M., MORETTI, A., LOGRIECO, A., GALLO, M., FERRACANE, R. and FOGLIANO, V. 1999. **Stability of fusaproliferin, a mycotoxin from *Fusarium* spp.** Journal of the Science of Food and Agriculture **79**: 1676–1680.

The decomposition of fusaproliferin, a mycotoxin produced by some phytopathogenic *Fusarium* species, was measured in dry or wet

contaminated wheat samples incubated at various temperatures (80, 120, 180 and 240°C) for various times (15, 20, 45 and 60 min). Water increased fusaproliferin decomposition at 80, 120 and 180°C, but a complete destruction of fusaproliferin occurred at 240°C only under dry conditions. Treatment of samples with a saturated solution of dichloroisocyanuric acid reduced fusaproliferin contamination, while physical treatments such as UV irradiation and sonication did not.

VISCHETTI, C. and ESPOSITO, A. 1999. **Degradation and transformation of a potential natural herbicide in three soils.** Journal of Agricultural and Food Chemistry **47**: 3901–3904.

The methyl ester of fusaric acid is one of four toxins produced by *Fusarium nygamai*, which could be used as a natural herbicide against *Striga hermonthica*, a parasitic weed of sorghum and corn in a vast zone of West and Central Africa. A laboratory study was performed to measure the degradation of the toxin in three soil types and under different temperature and soil moisture conditions. The results show that the persistence in all soils and under all incubation conditions is long enough to protect the crops for the first week of growth, excluding the trial at 30°C in humic soil where the half-life of 6 days would require more than one treatment.

GIORDANO, W. and DOMENECH, C.E. 1999. **Aeration affects acetate destination in *Gibberella fujikuroi*.** FEMS Microbiology Letters **180**: 111–116.

Gibberellins, fatty acids and the polyketides bikaverin and fusarin C are synthesised from a common precursor, acetylCoA. The production of these compounds in *Gibberella fujikuroi* was strongly influenced by aeration. Higher aeration resulted in increased growth and the production of bikaverin and gibberellins. Low aeration stimulated fatty acid and fusarin C production.

ITO, Y., PETERSON, S.W. and GOTO, T. 1998. **Properties of *Aspergillus tamarii*, *A. caelatus* and related species from acidic tea field soils in Japan.** Mycopathologia **144**: 169–175.

Aspergillus caelatus is a recently described non-aflatoxigenic species in *Aspergillus* section Flavi. *A. caelatus* has some common characteristics with *A. tamarii*, such as yellowish brown colour and double walled spores. In contrast to the morphological similarities, all of the *A. caelatus* isolates tested produced no CPA whereas most isolates of *A. tamarii* produce this compound. There are six nucleotide differences that distinguish the DNA sequences of these two species in the regions of ITS1, ITS2, 5.8S rDNA and 28S rDNA and this is a consistent difference.

ABRAMSON, D., USLEBER, E. and MARTLBAUER, E. 1999. **Rapid determination of citrinin in corn by fluorescence liquid chromatography and enzyme immunoassay.** Journal of AOAC International **82**: 1353–1356.

A rapid assay procedure was developed for citrinin in corn using liquid–liquid extraction (LLE) cartridges. Ground corn was extracted with methylene chloride and phosphoric acid and the extract added to an LLE cartridge containing a diatomaceous earth adsorbant, previously impregnated with sodium bicarbonate solution. After aspiration to dryness, the cartridge was eluted with methanol–water (4 + 1) and aliquots were taken for quantification by reversed phase LC with fluorescence detection. Recoveries of citrinin added to ground corn at 200–1600 µg/kg ranged from 71.2 to 86.3%, with coefficients of variation between 4.1 and 10.6%. The LLE cartridge procedure offers the advantages of low solvent consumption and speed, and is amenable to automation.

REINHARD, H. and ZIMMERLI, B. 1999. **Reversed-phase liquid chromatographic behavior of the mycotoxins citrinin and ochratoxin A.** Journal of Chromatography A **862**: 147–159.

The reversed phase chromatographic behaviour of citrinin and ochratoxin A (OA) were studied as a function of hydrophobicity and silanophilic activities of the stationary phase, pH, type of acid in the eluent, its composition as well as of the column temperature. While the affinity of OA to reversed phase materials was not influenced by phase material properties, citrinin showed a high affinity to hydrophobic phase materials, and its elution order, compared to OA, depended strongly on the phase material chosen.

MORESSI, M.B., ZON, A., FERNANDEZ, H., RIVAS, G. and SOLIS, V. 1999. **Amperometric quantification of *Alternaria* mycotoxins with a mushroom tyrosinase modified carbon paste electrode.** Electrochemistry Communications **1**: 472–476.

The affinity of mushroom tyrosinase for alternariol (AOH) and alternariol monomethyl ether (AME) produced by *Alternaria alternata* was verified both spectroscopically and electrochemically. Commercial enzyme and fresh mushroom tissue were employed for electrochemical measurements. The results demonstrate for the first time that both AME and AOH are substrates for mushroom tyrosinase. It is concluded that the enzyme has relatively good affinities for both substrates which is a promising result for developing an enzymatic biosensor.

SEWRAM, V., NIEUWOUDT, T.W., MARASAS, W.F.O., SHEPHARD, G.S. and RITIENI, A. 1999. **Determination of the *Fusarium* mycotoxins, fusaproliferin and beauvericin by high-performance liquid chromatography–electrospray ionization mass spectrometry.** Journal of Chromatography A **858**: 175–185.

A method is described using LC–MS for the detection of fusaproliferin and beauvericin in cultures of *Fusarium subglutinans* and in naturally contaminated maize. Detection of fusaproliferin was best performed in the MS–MS mode while beauvericin displayed a stronger signal in the MS mode. The on-column instrumental detection limits for pure fusaproliferin and beauvericin were 2 ng and 20 pg while those in naturally contaminated maize were 1 and 0.5 µg/kg, respectively. Five South African strains of *F. subglutinans* were analysed following methanol extraction of which four produced fusaproliferin at levels between 330 and 2630 mg/kg while only three produced beauvericin at levels between 140 and 700 mg/kg. Naturally contaminated maize samples from the Transkei region of South Africa showed fusaproliferin at levels of 8.8–39.6 µg/kg and beauvericin at 7.6–238.8 µg/kg.

TRUCKSESS, M.W. and TANG, Y.F. 1999. **Solid-phase extraction method for patulin in apple juice and unfiltered apple juice.** Journal of AOAC International **82**: 1109–1113.

A solid-phase extraction method is described for the determination of patulin in apple juice and unfiltered apple juice. A portion of the test sample was passed through a macroporous copolymer cartridge and was washed with 1% sodium bicarbonate and then with 1% acetic acid. Patulin was eluted with 2% acetonitrile in anhydrous ethyl ether and was determined by reversed phase LC with UV detection. Recoveries ranged from 93 to 104% in test samples spiked at 20–100 µg/L.

RYCHLIK, M. and SCHIEBERLE, P. 1999. **Quantification of the mycotoxin patulin by a stable isotope dilution assay.** Journal of Agricultural and Food Chemistry **47**: 3749–3755.

Two stable isotope dilution assays for the quantification of patulin in foods were developed using [¹³C]-patulin as the internal standard. One method was performed by means of LC/MS in negative electrospray ionisation mode without derivatisation. The other used high resolution GC/high resolution MS (HRGC/HRMS) after trimethylsilylation of the patulin isotopomers. In comparison with previously reported methods based on HPLC with UV detection, HRGC/HRMS of the derivatised samples showed better repeatability, higher recovery rates and

a 100 times lower detection limit (12 ng/L). In contrast, LC/MS showed a much lower performance as compared to HPLC/UV or HRGC/HRMS.

Mycotoxicoses

PERAICA, M., RADIC, B., LUCIC, A. and PAVLOVIC, M. 1999. **Toxic effects of mycotoxins in humans.** Bulletin of the World Health Organisation **77**: 754–766.

A review with 113 references. Outbreaks of mycotoxicoses where the mycotoxic etiology of the disease is supported by mycotoxin analysis or identification of mycotoxin producing fungi are reviewed. Epidemiological, clinical and histological findings in outbreaks of mycotoxicoses resulting from exposure to aflatoxins, ergot, trichothecenes, ochratoxins, 3-nitropropionic acid, zearalenone and fumonisins are discussed.

STEYN, P.S. and STANDER, M.A. 1999. **Mycotoxins as causal factors of diseases in humans.** Journal of Toxicology – Toxin Reviews **18**: 229–243.

A review with 76 references. The criteria of human mycotoxicoses are discussed and the role of certain mycotoxins in human diseases highlighted, including ergotoxins (ergotism), trichothecenes, T2-toxin (alimentary toxic aleukia), aflatoxins (primary liver cancer), ochratoxins (Balkan Endemic Nephropathy and chronic interstitial nephropathy) and fumonisins (oesophageal cancer). The chemical properties and biochemical mechanism of action of aflatoxin B₁, OA and FB₁ are discussed.

GALTIER, P. 1999. **Biotransformation and fate of mycotoxins.** Journal of Toxicology – Toxin Reviews **18**: 295–312.

A review with 81 references. Biotransformations of mycotoxins generally take place in the liver or gastrointestinal tract and are a consequence of the action of tissue enzymes or microflora. Metabolites can correspond to oxidative derivatives which are produced in liver, such as hydroxy-metabolites of aflatoxin B₁ or OA. In some cases highly reactive epoxides represent the first step in the formation of carcinogenic intermediates like exo epoxides of aflatoxins. Hepatic and intestinal phase II enzymes, including transferases, are involved in the conjugation of the above-mentioned oxidative metabolites. In this respect, they are generally considered as detoxifying enzymes. Microbial flora participate generally in toxicological deactivation pathways such as hydrolysis of OA or deepoxidation of trichothecenes. For other mycotoxins, including patulin, fumonisins and rubratoxin B, there is of lack of information regarding

their fate. The major metabolism of zearalenone consists of reduction leading to estrogenic zearalenols which is characterised by large interspecies differences. Consequences of biotransformations are reviewed in terms of residues of metabolites occurring in animal derived food products such as milk or eggs.

BADRIA, F.A., EL-NASHAR, E.M. and HAWAS, S.A. 1999. **Mycotoxins and disease in Egypt.** Journal of Toxicology – Toxin Reviews **18**: 337–353.

A review with 70 references. The progressive increase in the number of patients with hepatocellular carcinoma in Egypt has focused study on factors such as food habits that could be implicated in the pathogenesis of such malignancy. Several epidemiological studies have associated the exposure status of people to aflatoxins B₁ as being important in the etiology of liver cancer in Egypt. Many types of food samples including corn, broad beans and coffee beans have been found to contain aflatoxins, ochratoxins, fumonisin, zearalenone and deoxynivalenol. Several positive associations have been found between incidence of human liver and kidney diseases and mycotoxicosis in Egypt.

FINK-GREMMELES, J. 1999. **Mycotoxins: Their implications for human and animal health.** Veterinary Quarterly **21**: 115–120.

A review with 45 references. In animals, next to acute intoxication, losses in productivity, reduced weight gain and immunosuppression are the most important features of mycotoxicoses. Genotoxic effects and the involvement of certain mycotoxins such as aflatoxin, ochratoxins and fumonisins in the etiology of human cancers have gained particular attention. This implies that recent research activities must concentrate on mechanistic aspects of mycotoxin induced pathologies rather than compiling analytical measures of mycotoxin concentrations in food and feeds.

FLAPPAN, S.M., PORTNOY, J., JONES, P. and BARNES, C. 1999. **Infant pulmonary hemorrhage in a suburban home with water damage and mold (*Stachybotrys atra*).** Environmental Health Perspectives **107**: 927–930.

A case of an infant with pulmonary haemorrhage whose residential environmental assessment revealed the presence of *Stachybotrys atra* is reported. Air samples and surface samples were collected from multiple locations in the home. Air sampling revealed significantly elevated total spore counts in the patient's bedroom and in the attic. *Aspergillus/Penicillium* species were predominant. *Stachybotrys* spores were

found in the air sampled in the patient's bedroom, as well as from surfaces sampled in the patient's closet and the attic ceiling. A small patch in the closet ceiling contaminated with *Stachybotrys* proved to be highly toxic. These results further support the link between the presence of *Stachybotrys* in the home and pulmonary haemorrhage in infants.

FLIEGE, R. and METZLER, M. 1999. **The mycotoxin patulin induces intra- and intermolecular protein crosslinks *in vitro* involving cysteine, lysine, and histidine side chains, and alpha-amino groups.** *Chemico-Biological Interactions* **123**: 85–103.

The ability of patulin to covalently crosslink proteins *in vitro* was investigated. Using sodium dodecylsulphate polyacrylamide gel electrophoresis, the formation of patulin induced intermolecular protein-protein crosslinks was clearly demonstrated for bovine serum albumin containing one thiol group per molecule, but also for the thiol free hen egg lysozyme. The thiol group of cysteine was preferred for patulin mediated crosslink reactions, but the side chains of lysine and histidine, and α -amino groups also exhibited reactivity. Patulin can act both as a homobifunctional as well as a heterobifunctional crosslinking agent. The initial formation of a monoadduct with a thiol group appears to activate patulin for the subsequent reaction with an amino group, but also leads to rapid loss of further electrophilic properties when no second nucleophile for crosslink completion is available. In addition to the amino acid composition, the tertiary and quaternary superstructures of proteins appear to markedly influence their reactivity towards patulin. Under appropriate conditions, the generation of protein crosslinks could easily be observed at concentrations of patulin equal to or even below the concentration of the protein.

VOSS, K.A., PORTER, J.K., BACON, C.W., MEREDITH, F.I. and NORRED, W.P. 1999. **Fusaric acid and modification of the subchronic toxicity to rats of fumonisins in *F. moniliforme* culture material.** *Food and Chemical Toxicology* **37**: 853–861.

The effect of fusaric acid on the *in vivo* toxicity of *Fusarium moniliforme* culture material (CM) was examined in male rats. Rats were fed diets containing 0.025, 0.10 or 2.5% CM (providing dietary levels of fumonisin of 3.4, 18.4 or 437 mg/kg, respectively) with or without the addition of fusaric acid at 0, 20, 100 or 400 mg/kg. Apoptosis and other effects consistent with those caused by fumonisins were present in the kidneys of animals fed 0.025% or more CM and in the livers of animals fed 2.5% CM. Fusaric acid alone showed no effect and exerted no synergistic, additive or antagonistic effects on the subchronic *in vivo* toxicity of CM.

MULLER, G., KIELSTEIN, P., ROSNER, H., BERNDT, A., HELLER, M. and KOHLER, H. 1999. **Studies on the influence of combined administration of ochratoxin A, fumonisin B₁, deoxynivalenol and T2 toxin on immune and defence reactions in weaner pigs.** *Mycoses* **42**: 485–493.

In weaner pigs, combined administration of fumonisin, deoxynivalenol and T2 together with OA in quantities expected to be present in feeds of central European origin resulted, as a rule, in changes identical to those observed after single administration of OA.

GENTLES, A., SMITH, E.E., KUBENA, L.F., DUFFUS, E., JOHNSON, P., THOMPSON, J., HARVEY, R.B. and EDRINGTON, T.S. 1999. **Toxicological evaluations of cyclopiazonic acid and ochratoxin A in broilers.** *Poultry Science* **78**: 1380–1384.

Petersen × Hubbard broiler chickens from 1 day to 3 weeks of age were fed diets containing OA at 0 or 2.5 mg/kg feed and CPA at 0 or 34 mg/kg feed. Body weight gain was reduced by OA, CPA, and OA/CPA in combination at the end of 3 weeks. OA significantly increased the relative weight of the kidney and serum concentrations of uric acid and triglycerides and decreased total protein, albumin and cholesterol. The toxicity of CPA was expressed primarily through increased relative weights of the proventriculus and increased activity of creatine kinase. The interaction of OA and CPA was primarily additive or less than additive in the parameter in which the interaction occurred.

KUBENA, L.F., HARVEY, R.B., BUCKLEY, S.A., BAILEY, R.H. and ROTTINGHAUS, G.E. 1999. **Effects of long-term feeding of diets containing moniliformin, supplied by *Fusarium fujikuroi* culture material, and fumonisin, supplied by *Fusarium moniliforme* culture material, to laying hens.** *Poultry Science* **78**: 1499–1505.

White Leghorn laying hens were fed diets containing moniliformin (MON) at 50 or 100 mg/kg, FB₁ at 100 or 200 mg/kg or a combination of 50 mg MON and 100 mg FB₁/kg of diet for 420 days. The hens were then fed a control diet for an additional 60 days. Egg production was reduced by approximately 50% by the end of the second 28-day laying period and remained at approximately this level for the 420 days only in hens fed the diet containing MON at 100 mg/kg feed. Production returned to control levels or above within 60 days after hens were fed the control diet. Egg weights were reduced by the 100 mg MON diet during the first three 28-day laying periods before returning to weights comparable with controls.

The hens in this group also had significantly lower body weights than the other treatments. In hens fed MON at 100 mg/kg and FB₁ at 100 mg/kg mortality was approximately 20%. Fertility was not affected by the dietary treatments. Toxic synergy between MON and FB₁ was not observed for any of the parameters measured.

KUZNIAK, E., PATYKOWSKI, J. and URBANEK, H. 1999. **Involvement of the antioxidative system in tomato response to fusaric acid treatment.** *Journal of Phytopathology – Phytopathologische Zeitschrift* **147**: 385–390.

Fusaric acid application to tomato leaves resulted in a lasting activation of O₂⁻ and H₂O₂ production. Within the first day after toxin application the H₂O₂ scavenging enzymes, catalase and ascorbate peroxidase, showed a strong activity decrease followed by a gradual recovery to the control level after 2 and 3 days. By contrast fusaric acid markedly stimulated peroxidase activity.



VAN OLDRUITENBORGH-OOSTERBAAN, M.M.S., SCHIPPER, F.C.M., GOEHRING, L.S. and GREMELS, J.F. 1999. **[Pleasure horses with neurological signs: EHVI-infection or mycotoxin intoxication?]** *Tijdschrift voor Diergeneeskunde* **124**: 679–681.

In the course of several days most of the 40 riding school horses turned out in paddocks developed ataxia of variable severity. Five of these horses showed severe ataxia and tremors, became paralysed and were euthanised. The most likely diagnosis, the neurological form of EHVI, was discounted following serum analyses. A few weeks later a second outbreak occurred among the horses. Toxicological examination of hay, delivered just before the first outbreak and stored for the winter, showed a contamination with lolitrem B at 5–6 mg/kg. The hay appeared to have been made of ryegrass used for lawns and playing fields. Retrospectively it became probable that this hay had been fed to the horses just before the onset of clinical problems. It is concluded that the horses showed the ryegrass stagger syndrome. (In Dutch).

CHEN, J.W., LUO, Y.L., HWANG, M.J., PENG, F.C. and LING, K.H. 1999. **Territrem B, a tremorgenic mycotoxin that inhibits acetylcholinesterase with a noncovalent yet irreversible binding mechanism.** *Journal of Biological Chemistry* **274**: 34916–34923.

Territrem B has been shown previously to be a potent and irreversible inhibitor of acetylcholinesterase (AChE). A number of

binding and inhibition assays were carried out to further characterise the inhibitory effect of territrein B. It was found that the binding of territrein B is much more selective than a well characterised selective inhibitor of AChE, BW284C51, that it adopts a one-to-one stoichiometry with the enzyme and that it cannot be undone by an AChE-regenerating oxime agent. According to the prediction of a molecular modelling study, the distinct AChE inhibitory characteristics of territrein B may arise from the inhibitor being noncovalently trapped within a unique active-site gorge structure of the enzyme. It was predicted that an optimal territrein B AChE binding would position a narrowing connection of the territrein B structure at a constricted area near the entrance of the gorge, thereby providing a structural basis for the observed irreversible binding.

Ochratoxins – General

PERAICA, M., DOMIJAN, A.M., FUCHS, R., LUCIC, A. and RADIC, B. 1999. **The occurrence of ochratoxin A in blood in general population of Croatia.** *Toxicology Letters* **110**: 105–112.

The exposure of the general population in Croatia to OA was investigated in five cities: Split, Rijeka, Varazdin, Osijek and Zagreb. OA in plasma was determined using HPLC. The mean concentration was 0.39 µg/L of plasma. The highest frequency of OA positive samples (greater than 0.2 µg/L plasma), and the highest number of samples with the concentration exceeding 1.0 µg/L, were found in Osijek. This difference is probably due to the higher consumption of fresh and dried pork by population of Osijek. The calculated daily intake of OA, estimated from the mean OA concentration of all samples in each town, is lower than the tolerable daily intake proposed by Joint FAO/WHO Expert Committee on Food Additives (1995) of 16.0 µg/kg body weight.

GAREIS, M. 1999. **[Contamination of German malting barley and of malt-produced from it with the mycotoxins ochratoxin A and B].** *Archiv für Lebensmittelhygiene* **50**: 83–87.

OA and ochratoxin B (OB) contamination was assessed in malting barley from the harvest in the wet year 1996 and of malt produced from this barley. Samples were obtained from 16 or 17 malting plants from various parts of Germany and included malting barley immediately after harvest and after 5 months of storage, as well as malts obtained from these barley lots. OA was found in 7.1, 10.1 and 11.7 % and OB in 7.8, 4.0 and 5.3 % of the three kinds of samples, respectively. The median values of OA and OB for fresh barley were 0.035 and 0.036

µg/kg, in stored barley 0.1 and 0.14 µg/kg, and in malt 0.26 and 0.04 µg/kg, respectively. Storage and malting did not significantly change the incidence or concentrations of the toxins. (In German).

GILLMAN, I.G., CLARK, T.N. and MANDERVILLE, R.A. 1999. **Oxidation of ochratoxin A by an Fe-porphyrin system: Model for enzymatic activation and DNA cleavage.** *Chemical Research in Toxicology* **12**: 1066–1076.

The enzymatic activation of OA was modelled using the water soluble iron(III) meso-tetrakis(4-sulfonatophenyl)porphyrin (FeTPPS) oxidation system. In its presence, OA has been found to facilitate single strand cleavage of supercoiled plasmid DNA through production of reactive oxygen species. The reaction of OA with the FeTPPS oxidation system also generated three hydroxylated products, which was taken as evidence for production of the known hydroxylated metabolites of OA. This result suggested that the FeTPPS system served as a reasonable model for the enzymatic activation of OA. When the reaction of OA with FeTPPS was carried out in the presence of excess H₂O₂ and sodium ascorbate, a hydroquinone species was detected. Results imply that the hydroquinone and quinone metabolites of OA have the ability to cause alkylation/redox damage and suggest a viable pathway for oxidative damage by OA.

BASILICO, M.Z. and BASILICO, J.C. 1999. **Inhibitory effects of some spice essential oils on *Aspergillus ochraceus* NRRL 3174 growth and ochratoxin A production.** *Letters in Applied Microbiology* **29**: 238–241.

Inhibitory effects of essential oils of oregano (*Origanum vulgare*), mint (*Mentha arvensis*), basil (*Ocimum basilicum*), sage (*Salvia officinalis*) and coriander (*Coriandrum sativum*), on the mycelial growth and OA production by *Aspergillus ochraceus* were studied. At 1000 mg/kg, oregano and mint completely inhibited fungal growth and OA production up to 21 days, while basil was only effective up to 7 days. At 750 mg/kg, oregano was completely effective up to 14 days, whereas mint allowed fungal growth but no OA production up to 14 days. Sage and coriander showed no important effect at any of the concentrations studied.

STEYN, P.S. and PAYNE, B.E. 1999. **The synthesis of bromo- and iodo-ochratoxin B.** *South African Journal of Chemistry – Suid-Afrikaanse Tydskrif vir Chemie* **52**: 69–70.

The effective synthesis of bromo- and iodo-OB by the treatment of OB with pyridinium hydrobromide perbromide, and iodine and mercury(II)oxide, respectively, is reported.

Ochratoxins – Methodology

JORGENSEN, K. and VAHL, M. 1999. **Analysis of ochratoxin A in pig kidney and rye flour using liquid chromatography tandem mass spectrometry (LC/MS/MS).** *Food Additives and Contaminants* **16**: 451–456.

A liquid chromatography electrospray tandem mass spectrometric (LC/MS/MS) method is described for analysis and confirmation of OA in pig kidney and rye flour using derivatisation of OA to the methyl ester. The detection limit is 0.02 µg/kg and the repeatability is between 6 and 16% in the contamination range 0.5–8 µg/kg.

Ochratoxicoses

BACHA, H., MAAROUFI, K., GHEDIRA-CHEKIR, L., ABID, S., CHERIF, A., ACHOUR, A. and CREPPY, E.E. 1999. **Mycotoxins and mycotoxicosis in Tunisia: What do we know and what do we need to know?** *Journal of Toxicology – Toxin Reviews* **18**: 245–262.

A review with 87 references of mycotoxicoses in Tunisia. In Tunisia, a clear-cut correlation has been found between the consumption of food contaminated by toxigenic fungi and specific pathologies. Work has focused on OA and zearalenone (ZEA), which are widely found in Tunisia, contaminating various foods and feedstuffs. Studies on OA in the food chain show that Tunisia appears to be another hot spot for Balkan Endemic Nephropathy. Studies on ZEA show that this toxin adversely affects normal reproductive function of intoxicated animals and produces modifications of haematological and biochemical parameters of hepatic function.

MAAROUFI, K., ABID, S., CHERIF, A., ACHOUR, A., ZAKHAMA, A., CREPPY, E.E. and BACHA, H. 1999. **Molecular aspects of human ochratoxicosis in Tunisia.** *Journal of Toxicology – Toxin Reviews* **18**: 263–276.

A nephropathy similar to Balkan Endemic Nephropathy (BEN) has been found in Tunisian populations especially in nephropathic patients having OA in blood due to chronic exposure by ingestion of contaminated food. Some of these patients have shown several karyomegalic tubular cells in their renal biopsy. In the laboratory, rats exposed to OA showed karyomegalic cells with alteration of renal tubular tissue combined with megacytosis suggesting that the karyomegaly and megacytosis are induced by OA in rats and could be also in humans. In addition, abnormal mitosis is observed at the early stage of the pathology (30 days of

treatment), suggesting that renal tubular cells can regenerate if exposure to OA is limited to a short period. After 90 days of treatment the lesions became irreversible.

CREPPY, E.E. 1999. **Human ochratoxicosis**. *Journal of Toxicology – Toxin Reviews* **18**: 277–293.

An overview with 66 references. OA is nephrotoxic to all animal species studied so far and most likely to humans, who show the longest half-life time for elimination of this toxin among all species examined. For a complete diagnosis of human ochratoxicosis it is necessary to identify the origin of the toxin to relate its presence in human blood with at least a pathology one can cure or prevent. This is still a very difficult task, since humans may be exposed to several toxins simultaneously with synergistic or antagonistic effects. OA is found in human blood everywhere. However, the prevalence is different. Important factors affecting body burdens and pathologies include the quality of the diet in providing antioxidants, vitamins and amino acids, such as phenylalanine in the sweetener Aspartame.

SIMON, P. 1999. **Chronic renal failure: Are there environmental and occupational risk factors?** *Journal of Toxicology – Toxin Reviews* **18**: 313–321.

This review with 45 references discusses studies demonstrating the role of environmental or occupational factors in the appearance of chronic renal failure and the evolution to end stage renal disease, particularly the responsibility of heavy metals, solvents, mycotoxins (OA), phytotoxins and some drugs. Responsible factors are difficult to identify and renal lesions are often unspecific. Rarely is only one risk factor responsible for the appearance of renal disease, and most often several environmental factors are implicated.

KERKADI, A., BARRIAULT, C., MARQUARDT, R.R., FROHLICH, A.A., YOUSEF, I.M., ZHU, X.X. and TUCHWEBER, B. 1999. **Cholestyramine protection against ochratoxin A toxicity: Role of ochratoxin A sorption by the resin and bile enterohepatic circulation**. *Journal of Food Protection* **62**: 1461–1465.

The addition of cholestyramine (CHA, a resin known to bind bile salts in the gastrointestinal tract) to OA contaminated rat diets has been shown to reduce plasma levels of the toxin and prevent OA induced nephrotoxicity. The mechanism of action of CHA was studied in *in vitro* experiments. Results showed that CHA binds both OA and bile salts (taurodeoxycholate [TDC] and taurocholate [TCA]). Also, CHA showed greater affinity for OA and TDC than for TCA. How-

ever, OA and TDC sorption was decreased only at high concentrations of NaCl suggesting a stronger binding to the resin than electrostatic binding. In *in vivo* studies, OA plasma levels at 1 and 3 hr after a single oral dose of OA were significantly decreased in bile salt depleted rats compared to the control. Thus, the alteration of the bile salt biliary pool and OA enterohepatic circulation may be an additional mechanism of action of the resin against mycotoxin toxicity.

OBRECHT-PFLUMIO, S., CHASSAT, T., DIRHEIMER, G. and MARZIN, D. 1999. **Genotoxicity of ochratoxin A by *Salmonella* mutagenicity test after bioactivation by mouse kidney microsomes**. *Mutation Research – Genetic Toxicology and Environmental Mutagenesis* **446**: 95–102.

OA was, up to now, believed to be non-mutagenic in the classical *Salmonella typhimurium* reverse mutation test. Mutagenicity was confirmed using rat liver microsomal fractions with the strains, TA1535, TA1538 and TA98, utilising an Ames microtest. However, using mice kidney microsomal fractions as metabolic activators, reverse mutations were obtained with the three strains used, in the presence of either NADP or arachidonic acid as cofactors. The mutagenicity was higher with arachidonic acid than with NADP using the TA1535 strain indicating that several metabolic pathways of OA can lead to genotoxic compounds. In addition, both base pair substitutions and frameshift mutations can be caused by OA after metabolic activation.

MULLER, G., KIELSTEIN, P., ROSNER, H., BERNDT, A., HELLER, M. and KOHLER, H. 1999. **Studies of the influence of ochratoxin A on immune and defence reactions in weaners**. *Mycoses* **42**: 495–505.

In subtoxic amounts, OA was shown to produce immunomodulation in weaner pigs in a dose dependent manner. Increased counts of total leukocytes and neutrophils in the blood and reduced lymphocyte levels were observed. There was a striking increase in the counts of eosinophils and of apoptotic phagocytes. Functionally, there was a predominance of the production of reactive oxygen radicals in whole blood, reduced phagocytosis performance and reduced expression of a swine specific surface marker (SWC1) on lymphocytes.

MEREDITH, F.I., TORRES, O.R., DETEJADA, S.S., RILEY, R.T. and MERRILL, A.H. 1999. **Fumonisin B₁ and hydrolyzed fumonisin B₁ (AP₁) in tortillas and nixtamalized corn (*Zea mays* L.) from two different geographic locations in Guatemala**. *Journal of Food Protection* **62**: 1218–1222.

In the preparation of tortillas, corn is treated with lime producing nixtamal that when heated hydrolyses at least a portion of the FB₁ to the aminopentol backbone, AP₁, another known toxin. The amounts of FB₁ and AP₁ in tortillas and nixtamal from two communities in the central highlands of Guatemala where corn is a major dietary staple (Santa Maria de Jesus, Sacatepequez, and Patzicia, Chimaltenango) were analysed. The amounts of FB₁ and AP₁ in tortillas from Santa Maria de Jesus were, respectively, 0.85 and 26.1 mg/kg dry weight, and from Patzicia were 2.2 and 5.7 mg/kg dry weight. Less than 6% of the tortillas from both locations contained FB₁ at greater than or equal to 10 mg/kg dry weight, whereas, 66% of the samples from Santa Maria de Jesus and 29% from Patzicia contained AP₁ at greater than or equal to 10 mg/kg dry weight.

MARIN, S., MAGAN, N., SERRA, J., RAMOS, A.J., CANELA, R. and SANCHIS, V. 1999. **Fumonisin B₁ production and growth of *Fusarium moniliforme* and *Fusarium proliferatum* on maize, wheat, and barley grain**. *Journal of Food Science* **64**: 921–924.

Two isolates each of *Fusarium moniliforme* and *Fusarium proliferatum* isolated from maize were compared for growth and FB₁ production on maize, wheat and barley extract agars and on irradiated maize, wheat and barley grain in relation to water availability and temperature. All isolates produced FB₁ only on irradiated maize grain, but not on wheat or barley.

CASTELLA, G., BRAGULAT, M.R. and CABANES, F.J. 1999. **Surveillance of fumonisins in maize-based feeds and cereals from Spain**. *Journal of Agricultural and Food Chemistry* **47**: 4707–4710.

In Spain, the levels of fumonisins in 171 samples of maize based feeds and cereals were surveyed. The highest levels of fumonisins were detected in maize. Overall, FB₁ and FB₂ were detected in 79.5 and 14.6% of samples, respectively, with average FB₁ levels of 3.3 mg/kg and average FB₂ levels of 1.7 mg/kg. Low levels of fumonisins in wheat, barley and soybean were detected. This appears to be the first report of concentrations of fumonisins in these commodities.

TSENG, T.C. and LIU, C.Y. 1999. **Natural occurrence of fumonisins B₁ and B₂ in domestic maize of Taiwan.** Journal of Agricultural and Food Chemistry **47**: 4799–4801.

Samples of maize grown in various districts of Taiwan were collected and analysed for the presence of FB₁ and FB₂ using HPLC. Of 110 samples, 49 were found to contain FB₁ (109–1148 µg/kg) and 2 were found to contain FB₂ (222–255 µg/kg). More than 79% of tested samples had FB₁ concentrations less than 100 µg/kg, whereas 2.7% (or 3 samples) contained FB₁ at greater than 300 µg/kg. These results clearly illustrated that domestically produced maize for human consumption is frequently contaminated with FB₁.

MARIN, S., MAGAN, N., BELLI, A., RAMOS, A.J., CANELA, R. and SANCHIS, V. 1999. **Two-dimensional profiles of fumonisin B₁ production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize grain.** International Journal of Food Microbiology **51**: 159–167.

The effects of temperature and water activity (a_w) on FB₁ production by *Fusarium moniliforme* and *F. proliferatum* on irradiated maize grain was examined after incubation for 28 days. The optimum conditions for *F. moniliforme* and *F. proliferatum* were 30°C at 0.97 a_w and 15°C at 0.97 a_w , respectively. The maximum concentrations were 2861 mg/kg and 17,628 mg/kg dry weight maize grain, respectively. These data were used to construct two dimensional diagrams of all the $a_w \times$ temperature conditions favourable for FB₁ production. The data were also subjected to a polynomial regression, which demonstrated that there was a very good fit for the 15–30°C range of temperature and at 0.97 a_w . This suggests that it may be possible to predict within a limited environmental range the potential for significant FB₁ production.

MAGNOLI, C.E., SAENZ, M.A., CHIACCHIERA, S.M. and DALCERO, A.M. 1999. **Natural occurrence of *Fusarium* species and fumonisin production by toxigenic strains isolated from poultry feeds in Argentina.** Mycopathologia **145**: 35–41.

During 1996–1998, 158 samples of poultry feeds were collected from a factory located in Rio Cuarto Cordoba province, Argentina, and assayed for *Fusarium* species and fumonisin production by these isolates. Among the 43 isolates of *F. moniliforme*, *F. nygamai* and *F. proliferatum* assayed, there was a high degree of variability in the quantities of FB₁, FB₂ and FB₃ produced. The range of concentrations of FB₁, FB₂ and FB₃ produced in corn grain varied from 5.4 to

3,991, 1.01 to 189 and 0.4 to 765 µg/kg of corn, respectively. Two strains of *F. moniliforme* produced exceptionally high concentrations of FB₃ and minor concentrations of FB₂ and FB₁.

MASOERO, F., MOSCHINI, M., ROSSI, F., PRANDINI, A. and PIETRI, A. 1999. **Nutritive value, mycotoxin contamination and *in vitro* rumen fermentation of normal and genetically modified corn (*cryIA(b)*) grown in northern Italy.** Maydica **44**: 205–209.

An assessment was made on the effect of inserting the *cryIA(b)* (Bt) gene of *Bacillus thuringiensis* into the genome of two corn hybrids (the newly-developed hybrid from Cargill Semences identified as CR and the traditional B73xMo17) on the analytical composition, the *in vitro* rumen degradability and the mycotoxin contamination of the plant. Transgenicity changed the plant chemical composition as a function of the recipient genotype: starch was increased in the CR-Bt⁺ plant whereas higher lignin content, lower protein and soluble nitrogen contents were observed for the B73xMo17-Bt⁺ plants. Transgenic plants had less ergosterol and fumonisin content than standard corn, suggesting a reduced susceptibility to mould attack.

REID, L.M., NICOL, R.W., OUELLET, T., SAVARD, M., MILLER, J.D., YOUNG, J.C., STEWART, D.W. and SCHAAFSMA, A.W. 1999. **Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: Disease progress, fungal biomass, and mycotoxin accumulation.** Phytopathology **89**: 1028–1037.

Maize ears were inoculated with either *Fusarium graminearum*, *F. moniliforme* or an equal mixture of the two. Silk and kernel tissues were periodically harvested throughout the growing season and a time course of the experimental variables including disease severity, ergosterol content, fungal DNA content, and mycotoxin concentration, were recorded. Over the 3 years tested the highest levels of disease and ergosterol were found in the *F. graminearum* treatment, followed by the mixture treatment and, finally, the *F. moniliforme* treatment. FB₁ levels did not differ between the *F. moniliforme* treatment and the mixed inoculum treatment.

YATES, I.E., MEREDITH, F., SMART, W., BACON, C.W. and JAWORSKI, A.J. 1999. ***Trichoderma viride* suppresses fumonisin B₁ production by *Fusarium moniliforme*.** Journal of Food Protection **62**: 1326–1332.

Biocontrol activity against *Fusarium moniliforme* was analysed for a *Trichoderma viride* strain isolated from root segments of

corn plants grown in Piedmont, Georgia. The isolate suppressed radial extension of *F. moniliforme* colonies during co-cultivation on potato dextrose agar and FB₁ production during incubation of both fungi on corn kernels. FB₁ production by *F. moniliforme* on corn kernels decreased by 85% when both organisms were inoculated the same day onto corn kernels and by 72% when inoculation of *T. viride* was delayed by 7 days after *F. moniliforme* inoculation. These results are the first to demonstrate that *T. viride* can suppress FB₁ production by *F. moniliforme*, thereby functioning to control mycotoxin production.

RYU, D., MUNIMBAZI, C. and BULLERMAN, L.B. 1999. **Fumonisin B₁ production by *Fusarium moniliforme* and *Fusarium proliferatum* as affected by cycling temperatures.** Journal of Food Protection **62**: 1456–1460.

The effects of temperatures cycling between 5 and 20°C, 10 and 25°C, and 15 and 30°C on the production of FB₁ and ergosterol by *Fusarium moniliforme* and *F. proliferatum* on rice was studied. Temperatures were cycled at 12 hr intervals. The maximum yields of FB₁ were found to be 247 mg/kg by *F. moniliforme* at temperatures that cycled between 10 and 25°C after 2 weeks and 284 mg/kg by *F. proliferatum* when the temperatures cycled between 5 and 20°C after 6 weeks. Overall, the two *Fusarium* species showed differences in production of FB₁ and ergosterol under the various temperature treatments.

MARIN, S., SANCHIS, V., SANZ, D., CASTEL, I., RAMOS, A.J., CANELA, R. and MAGAN, N. 1999. **Control of growth and fumonisin B₁ production by *Fusarium verticillioides* and *Fusarium proliferatum* isolates in moist maize with propionate preservatives.** Food Additives and Contaminants **16**: 555–563.

The effect of propionic acid, its sodium salt or a commercial formulation of propionates (0.03, 0.05 and 0.07%), on growth and FB₁ production by *Fusarium verticillioides* and *F. proliferatum* isolates was evaluated on irradiated maize at different water activities and temperatures. None of the assayed treatments had any effect on FB₁ production by *F. verticillioides* isolates. For *F. proliferatum*, higher FB₁ production occurred in the absence of propionates, and in general concentration decreased with increasing doses of preservatives. Single factors (a_w , propionate concentrations and temperature) and temperature \times a_w , and propionate concentration \times temperature interactions had a significant effect on fumonisin production. Moreover, propionate concentration was the single most important factor, besides temperature, which affected FB₁ production.

ZHI-GANG, W., LI-MING, C., XIU-MEI, L., ZHE, T., SU-YUN, C. and SU-JUN, G. 1998. **Dimorphic fungus characteristic of fumonisin-producing strains of *Fusarium moniliforme* from Zhejiang.** *Mycopathologia* **144**: 165–167.

Twenty fumonisin producing strains of *Fusarium moniliforme*, isolated from food-stuffs in Zhejiang, China, were shown to form yeast-like colonies with mostly budding reproduction on Sabouraud's agar containing 9% NaCl at 37°C. In blood agar plates these strains of *F. moniliforme* appear grass-green and show haemolytic reactions. This is the first report that yeast-like growth, a dimorphic pathogenic fungus feature, is found in *F. moniliforme*.

Fumonins – Methodology

KARUNA, R. and SASHIDHAR, R.B. 1999. **Use of ion-exchange chromatography coupled with TLC-laser scanning densitometry for the quantitation of fumonisin B₁.** *Talanta* **50**: 381–389.

A simple TLC–Laser scanning densitometric (TLC–LSD) method is described for the quantification of FB₁. FB₁ was isolated from cultures of *Fusarium moniliforme* by solvent extraction (methanol–water, 3:1) and purified in a single step by ion-exchange chromatography using Dowex-1. FB₁ in the purified extracts was detected by TLC analysis using *p*-anisaldehyde as a post-chromatographic derivatising agent. The sensitivity of the TLC–LSD method was found to be 500 µg/kg with a correlation coefficient (*r*) value of 0.9 and recovery of standard FB₁ in the range of 87–96%.

YU, F.Y. and CHU, F.S. 1999. **Production and characterization of a monoclonal anti-anti-idiotypic antibody against fumonisin B₁.** *Journal of Agricultural and Food Chemistry* **47**: 4815–4820.

A monoclonal anti-anti-idiotypic antibody (mAb3) against FB₁ was produced from the hybridoma cell line 7C7F4, which was generated by the fusion of P3/NS-1/I-AG4-1 myeloma cells with spleen cells isolated from a Balb/c mouse that had been immunised with the Fab fragments of affinity-purified anti-idiotypic antibodies. The mAb3 belongs to the immunoglobulin M, kappa light chain. A direct competitive ELISA and an indirect competitive ELISA were established for antibody characterisation and toxin analysis. In an indirect competitive ELISA using FB₁-ovalbumin as the coating antigen, the concentrations causing 50% inhibition of binding (IC₅₀) of mAb3 to the solid-phase FB₁-ovalbumin by free FB₁, FB₂ and FB₃ were found to be 75, 95 and 450 µg/L, respectively. In the direct competitive ELISA, the IC₅₀ of FB₁-horseradish peroxidase to the solid-phase mAb3 by free FB₁ was 233 µg/L.

Fumonins – Toxicoses

KEYSER, Z., VISMER, H.F., KLAASEN, J.A., SNIJMAN, P.W. and MARASAS, W.F.O. 1999. **The antifungal effect of fumonisin B₁ on *Fusarium* and other fungal species.** *South African Journal of Science* **95**: 455–458.

The antifungal effect of FB₁ on *Fusarium moniliforme*, *F. proliferatum*, *F. globosum*, *F. subglutinans*, *F. graminearum*, *Penicillium expansum*, *Aspergillus flavus*, *Alternaria alternata* and *Botrytis cinerea* was tested by an agar diffusion method on PDA plates at FB₁ concentrations of 40–0.05 mM at pH 5.45. FB₁ inhibited mycelial growth of 5/9 fungi tested. The minimum inhibitory concentration ranged from 0.25–0.5 mM for *A. alternata*, 1–5 mM for *P. expansum* and *B. cinerea*, and 5–10 mM for *F. graminearum*, whereas the other fungi tested showed no sensitivity to the mycotoxin. A small inhibition zone was visible with *F. proliferatum* at 40 mM. This is the first report on the antifungal activity of FB₁.

GELDERBLOM, W.C.A., ABEL, S., SMUTS, C.M., SWANEVELDER, S. and SNYMAN, S.D. 1999. **Regulation of fatty acid biosynthesis as a possible mechanism for the mitoinhibitory effect of fumonisin B₁ in primary rat hepatocytes.** *Prostaglandins Leukotrienes And Essential Fatty Acids* **61**: 225–234.

The mitoinhibitory effect of FB₁ on the mitogenic response of epidermal growth factor (EGF) was investigated in primary hepatocyte cultures with respect to the alterations in the omega 6 fatty acid metabolic pathway. FB₁ inhibited the EGF induced mitogenic response in a dose dependent manner. The inhibitory effect was counteracted by the addition of prostaglandin E-2. FB₁ also disrupts the omega 6 fatty acid metabolic pathway in primary hepatocytes, resulting in the accumulation of C18:2 omega 6 in phosphatidylcholine and triacylglycerol. The disruption of the omega 6 fatty acid metabolic pathway and/or prostaglandin synthesis is likely to be an important event in the mitoinhibitory effect of FB₁ on growth factor responses.

ARTINEZ-LARRANAGA, M.R., ANADON, A., DIAZ, M.J., FERNANDEZ-CRUZ, M.L., MARTINEZ, M.A., FREJO, M.T., MARTINEZ, M., FERNANDEZ, R., ANTON, R.M., MORALES, M.E. and TAFUR, M. 1999. **Toxicokinetics and oral bioavailability of fumonisin B₁.** *Veterinary and Human Toxicology* **41**: 357–362.

The kinetics of FB₁ after single doses of 10 mg/kg (po) or 2 mg/kg (iv) were studied in male Wistar rats. The FB₁ plasma profile could be adequately described by a 2-com-

partment open model. The elimination half-life from plasma was 1.03 hr after iv and 3.15 hr after po administration. The total plasma clearance of FB₁ was the same for both the po and iv routes. After the single po dose, FB₁ was rapidly absorbed with a T-max of 1.02 hr. The maximum plasma concentration of FB₁ was 0.18 mg/L. The po bioavailability of FB₁ was 3.5%. The tissue concentration time data for FB₁ fit a 1-compartment open model. Considerable concentrations of FB₁ were found in the liver and kidney tissues. The elimination half-life for liver was 4.07 hr and for kidney was 7.07 hr. Tissue accumulation of FB₁ was evidenced by the tissue/plasma area under the concentration-time curve ratios and was 2.03 in liver and 29.89 in kidney.

ATROSHI, F., RIZZO, A., BIESE, I., VEIJALAINEN, P., SALONIEMI, H., SANKARI, S. and ANDERSSON, K. 1999. **Fumonisin B₁-induced DNA damage in rat liver and spleen: Effects of pretreatment with coenzyme Q(10), L-carnitine, alpha-tocopherol and selenium.** *Pharmacological Research* **40**: 459–467.

The contribution of oxidative stress to the initiation or progression of hepatic and splenic cell DNA damage induced by FB₁ in rats was investigated. Rats were injected iv with a single dose of FB₁ at 1.55 mg/kg body weight. Treatment with FB₁ led to splenic and hepatic DNA fragmentation in 85% of the test animals. DNA fragmentation was investigated as a critical event in toxic cell death by testing total Ca²⁺ in liver. FB₁ administration caused total Ca²⁺ in liver to increase within 4 hr (204% of control). Measurement of liver enzyme activities showed an increase in aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT). FB₁ also markedly decreased splenic and hepatic glutathione (GSH) levels. Pretreatment with CoQ(10) together with L-carnitine, alpha-tocopherol and selenium, decreased DNA damage and the activities of Ca²⁺, ASAT and ALAT in the liver. However, the level of GSH was slightly increased.

DANTZER, W.R., HOPPER, J., MULLIN, K., HENDRICH, S. and MURPHY, P.A. 1999. **Excretion of C¹⁴-fumonisin B₁, C¹⁴-hydrolyzed fumonisin B₁, and C¹⁴-fructose in rats.** *Journal of Agricultural and Food Chemistry* **47**: 4291–4296.

Male and female F344/N rats were each dosed by gavage with [¹⁴C]-FB₁, [¹⁴C]-hydrolysed FB₁, or [¹⁴C]-FB₁-fructose at 0.69 µmol/kg body weight. Urinary excretion of [¹⁴C]-FB₁ and [¹⁴C]-FB₁-fructose was 0.5% and 4.4% of the total dose, respectively, and was similar between male and female rats. Urinary excretion of [¹⁴C]-hydrolysed FB₁ was signifi-

cantly greater in female rats as compared with male rats. There were no significant differences in biliary excretion of the three fumonisin compounds with a mean of 1.4% of the dose excreted at 4 hr after dosing. Increased urinary excretion of the hydrolysed FB₁ as compared to FB₁ and FB₁-fructose indicated a greater absorption of the hydrolysed form.

LEMMER, E.R., GELDERBLUM, W.C.A., SHEPHARD, E.G., ABEL, S., SEYMOUR, B.L., CRUSE, J.P., KIRSCH, R.E., MARASAS, W.F.O. and HALL, P.D.M. 1999. **The effects of dietary iron overload on fumonisin B₁-induced cancer promotion in the rat liver.** *Cancer Letters* **146**: 207–215.

The effects of excess hepatic iron on the cancer initiating and promoting properties of FB₁ were examined in male F344 rats fed diets containing FB₁ at 250 mg/kg, with and without carbonyl iron (CI) at 1–2% diet. All the FB₁ fed rats, in the presence or absence of CI, developed a toxic hepatitis with a 4 fold rise in serum alanine transaminase levels. FB₁ appeared to augment iron induced hepatic lipid peroxidation, as measured by the generation of thiobarbituric acid reacting substances. Morphometric analysis showed that FB₁ caused a significantly greater mean number of enzyme-altered foci and nodules per cm², as well as a greater area of liver occupied by foci and nodules, compared with FB₁ plus CI.

SHIER, W.T. and ABBAS, H.K. 1999. **Current issues in research on fumonisins, mycotoxins which may cause nephropathy?** *Journal of Toxicology – Toxin Reviews* **18**: 323–335.

An overview with 71 references. Structure-activity relationship studies on natural and synthetic fumonisins indicate that extensive alterations in structure are possible without loss of biological activity. This observation raises the concern that the FB₁ eliminated during food processing may actually be converted to other biologically active forms. The full extent of the threat to food safety posed by fumonisins will not be known until it is determined what substances the toxin is converted to during food processing, the bioavailability of those substances, the extent they retain biological activity and the additivity of their toxic effects with other nephrotoxic mycotoxins such as OA and OB.

PELAGALLI, A., BELISARIO, M.A., SQUILLACIOTI, C., DELLAMORTE, R., DANGELO, D., TAFURI, S., LUCISANO, A. and STAIANO, N. 1999. **The mycotoxin fumonisin B₁ inhibits integrin-mediated cell-matrix adhesion.** *Biochimie* **81**: 1003–1008.

The effects of FB₁ on cell growth and integrin mediated cell matrix adhesion in

B16–BL6 mouse melanoma cells was investigated. Treatment with the highest tested dose (75 µM) of FB₁ for 72 hr induced about 20% inhibition of cell growth. FB₁ strongly affected B16–BL6 cell adhesion to immobilised fibronectin by causing a dose dependent inhibition of cell attachment to this substrate. FB₁ also inhibited in a dose dependent manner the adhesion of B16–BL6 cells to the immobilised anti-fibronectin receptor antibody, whereas it affected only to a low extent cell attachment to concanavalin A. Results demonstrate that FB₁ treatment alters integrin adhesive activity, thus affecting all cellular integrin dependent functions.

SMITH, G.W., CONSTABLE, P.D., TUMBLESON, M.E., ROTTINGHAUS, G.E. and HASCHEK, W.M. 1999. **Sequence of cardiovascular changes leading to pulmonary edema in swine fed culture material containing fumonisin.** *American Journal of Veterinary Research* **60**: 1292–1300.

The sequence of cardiovascular and blood gas changes induced by ingestion of fumonisin containing culture material in swine and the temporal relationship of these changes to plasma sphinganine and sphingosine concentrations were investigated. Pigs were fed a standard grower diet with culture material added to provide FB₁ at 20 mg/kg/body weight per day. Sphinganine and sphingosine concentrations were increased in plasma of treated pigs within 24 hr of initial fumonisin exposure and continued to increase dramatically until euthanasia. Fumonisin treated pigs had increased respiratory rate, mean pulmonary artery pressure and pulmonary artery wedge pressure, along with decreased heart rate and cardiac output in the 12 hr period before euthanasia. Pigs also had systemic arterial hypotension, arterial and mixed venous hypoxemia, metabolic acidosis, decreased oxygen delivery and increased oxygen consumption immediately before euthanasia.

LI, Y.C., LEDOUX, D.R., BERMUDEZ, A.J., FRITSCHKE, K.L. and ROTTINGHAUS, G.E. 1999. **Effects of fumonisin B₁ on selected immune responses in broiler chicks.** *Poultry Science* **78**: 1275–1282.

Immune responses in chicks fed FB₁ at 0, 50, 100 or 200 mg/kg diet were investigated. One group of chicks was injected iv with *Escherichia coli* on day 21. Chicks fed the 200 mg/kg diet had significantly higher numbers of bacterial colonies in blood, spleen and liver than controls. A second group of chicks was injected with inactivated Newcastle Disease virus vaccine on weeks 2 and 3 of the experiment and primary and secondary antibody titres were measured 7

days after each injection. The secondary antibody response in chicks fed the 200 mg/kg diet was significantly lower than that of control chicks. In a third experiment, lymphocyte proliferation in chicks exposed to FB₁ *in vivo* or *in vitro* was shown to be reduced in chicks fed 200 mg/kg diet compared to controls.

Trichothecenes – General

LAUREN, D.R. and DIMENNA, M.E. 1999. **Fusaria and Fusarium mycotoxins in leaves and ears of maize plants 2. A time course study made in the Waikato region, New Zealand, in 1997.** *New Zealand Journal of Crop and Horticultural Science* **27**: 215–223.

The patterns of fungal infection and mycotoxin contamination in leaf and ear sections of plants of two maize hybrids, one resistant to mycotoxin accumulation under New Zealand conditions (Pioneer 3902 (P3902)) and one less so (Pioneer 3751 (P3751)), have been measured. *Fusarium* infection was evident in ears of P3902 from March to April, although heavy infection by the toxigenic species tended to occur later towards May–June, especially for the basal ear fractions. For P3751 ear infection commenced in May, and then was predominantly by toxigenic species. Mycotoxins were found in most plant fractions measured, especially as the plants aged. The highest mycotoxin concentration in a leaf fraction was 16.6 mg/kg of ZEA in an upper leaf axil sample. Nivalenol (NIV) was also found at up to 7.4 mg/kg in leaf axils. The most contaminated ear fraction was the rachis, with over 40–95 mg/kg of ZEA, NIV or deoxynivalenol (DON) at various times. The highest concentration found in kernels was 3.8 mg/kg of DON found in apical kernels of P3751 two weeks before harvest. The results suggest that the mechanisms of maize infection by *Fusarium* in New Zealand may not be controlled by factors at silk emergence but rather by later season events such as high rainfall and warmer temperatures.

ARSENIUK, E., FOREMSKA, E., GORAL, T. and CHELKOWSKI, J. 1999. **Fusarium head blight reactions and accumulation of deoxynivalenol (DON) and some of its derivatives in kernels of wheat, triticale and rye.** *Journal of Phytopathology – Phytopathologische Zeitschrift* **147**: 577–590.

Fifty three commercially grown cultivars and germplasm lines of winter triticale, wheat and rye and spring triticale, wheat and rye were inoculated at mid anthesis with a spore suspension consisting of a mixture of *Fusarium culmorum*, *F. avenaceum* and *F. graminearum* isolates of known toxigenic activity. Wheat suffered from the largest ker-

nel weight reductions and accumulated the largest amounts of DON and 3-acetylDON in kernels. DON was not detected in grain samples of winter rye cv. Dankowskie Zlote and spring rye cv. Ludowe. 15-acetylDON was only detected in genotypes of triticale, and 3-acetylDON only in a few genotypes of winter wheat and rye. MON was detected at low concentrations in kernels of some genotypes. A moderately strong Pearson correlation was found between head blight severity parameters and the accumulation of DON and its derivatives in grain of the cereal genotypes studied. Both *Fusarium* head blight resistant and susceptible genotypes of the three cereal species accumulated DON in kernels. This suggests that the system regulating DON accumulation may be independent of *Fusarium* head blight reaction.

GRABARKIEWICZ-SZCZESNA, J., FOREMSKA, E., KOSTECKI, N., GOLINSKI, P. and CHELKOWSKI, J. 1999. **Trichothecenes accumulation in kernels of corn inoculated with *Fusarium poae* (Peck) Wollenw.** *Nahrung* **43**: 330–332.

NIV and fusarenone X (FX) accumulation in kernels of 12 corn genotypes inoculated with *Fusarium poae* was analysed. All kernels with visible symptoms of scab were contaminated with NIV and FX at the average concentration level of 1.34 and 1.17 mg/kg, respectively. Healthily looking kernels were free of detectable amounts of the trichothecenes. The highest concentration of NIV with high levels of FX was observed in Smolimag genotype kernels with visible symptoms of disease.

RYU, D. and BULLERMAN, L.B. 1999. **Effect of cycling temperatures on the production of deoxynivalenol and zearalenone by *Fusarium graminearum* NRRL 5883.** *Journal of Food Protection* **62**: 1451–1455.

The effects of three regimens of cycling incubation temperatures and incubation at constant 25°C on the growth of *Fusarium graminearum* and production of DON and ZEA on rice were compared. The greatest fungal growth was found in cultures incubated at temperatures cycling between 15 and 30°C during a 6 week period. The highest amounts of DON and ZEA were produced in cultures incubated at a constant 25°C for 2 weeks prior to incubation at a constant 15°C for an additional 4 weeks. Under cycling incubation temperatures, maximum amounts of DON and ZEA were produced in cultures incubated at temperatures cycling between 15 and 30°C for 6 weeks. Overall, there was no correlation between mould growth and production of either DON or ZEA. However, DON production and ZEA production were correlated.

RYU, D., HANNA, M.A. and BULLERMAN, L.B. 1999. **Stability of zearalenone during extrusion of corn grits.** *Journal of Food Protection* **62**: 1482–1484.

The effects of extrusion cooking on the stability of ZEA in spiked food-grade corn grits were investigated using a twin screw extruder. Extrusion cooking of the corn grits resulted in significant reductions of ZEA in grits extruded with either mixing screws or nonmixing screws, but use of mixing screws was more effective (66 to 83%) overall than nonmixing screws (65 to 77%). Greater reduction of ZEA was observed at either 120 or 140°C than at 160°C. The moisture content of corn grits was not a significant factor affecting reduction of ZEA during extrusion with either mixing or nonmixing screws.

ACCERBI, M., RINALDI, V.E.A. and NG, P.K.W. 1999. **Utilization of highly deoxynivalenol-contaminated wheat via extrusion processing.** *Journal of Food Protection* **62**: 1485–1487.

The effects of sodium bisulfite and extrusion cooking under high temperature and pressure on DON levels in wheat grain and mill fractions was investigated. Samples of highly naturally DON contaminated soft winter wheat were soaked for 1 hr in water or aqueous sodium bisulfite solutions and extruded. The soaking treatment with sodium bisulfite solution (5% SO₂ equivalent) lowered DON from 7.3 to 0.8 mg/kg without extrusion and to 0.3 mg/kg with an extrusion process. When the contaminated kernels were tempered with water or sodium bisulfite solutions (5 or 10% SO₂ equivalent) and milled, the flour samples showed lower levels of DON. Extrusion of milled flour and whole meal samples, both obtained from sodium bisulfite tempered wheat, did not change DON levels significantly as compared to the nonextruded milled flour and whole meal samples.

HUANG, W.L., LEE, K.R. and SHIAO, M.S. 1999. **Inhibition of trichothecin and ergosterol biosynthesis in *Trichothecium roseum* by lovastatin.** *Journal of the Chinese Chemical Society* **46**: 687–692.

The effect of lovastatin, an HMG-CoA reductase inhibitor, on the biosynthesis of trichothecin, ergosterol and fatty acids in *Trichothecium roseum* was investigated. Treatment of lovastatin at 50 µM reduced the incorporation of [¹⁴C]-acetate into trichothecin by 79%, whereas the conversion of [³H]-mevalonate was reduced by only 28%. These results indicate that HMG-CoA reductase is a major, but not strict, regulatory site in mevalonic acid pathway leading to the formation of trichothecin and ergosterol.

HARRIS, L.J., DESJARDINS, A.E., PLATTNER, R.D., NICHOLSON, P., BUTLER, G., YOUNG, J.C., WESTON, G., PROCTOR, R.H. and HOHN, T.M. 1999. **Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize.** *Plant Disease* **83**: 954–960.

Trichothecene producing and nonproducing *Fusarium graminearum* strains were tested for their ability to cause *Gibberella* ear rot in field trials at two locations, Ottawa, Ontario, and Peoria, Illinois. Maize ears were inoculated with wild-type or transgenic *F. graminearum* strains in which the trichothecene biosynthetic pathway had been disabled by the specific disruption of the trichodiene synthase gene and with a derivative revertant strain in which trichothecene production had been restored through recombination. The trichothecene nonproducing strains were still pathogenic but appeared less virulent on maize than the trichothecene producing progenitor and revertant strains, as assayed by most parameters. This suggests that the trichothecenes may act as virulence factors to enhance the spread of *F. graminearum* on maize.

MCCORMICK, S.P., ALEXANDER, N.J., TRAPP, S.E. and HOHN, T.M. 1999. **Disruption of *TRI101*, the gene encoding trichothecene 3-O-acetyltransferase, from *Fusarium sporotrichioides*.** *Applied and Environmental Microbiology* **65**: 5252–5256.

A *Fusarium sporotrichioides* NRRL 3299 cDNA expression library was screened in a toxin-sensitive *Saccharomyces cerevisiae* strain lacking a functional *PDR5* gene. Fourteen yeast transformants were identified as resistant to 4,15-diacetoxyscirpenol (DAS) and each carried a cDNA encoding the trichothecene 3-O-acetyltransferase that is the *F. sporotrichioides* homologue of the *F. graminearum TRI101* gene. Mutants of *F. sporotrichioides* NRRL 3299 produced by disruption of *TRI101* were altered in their abilities to synthesise T-2 toxin and accumulated isotrichodermol and small amounts of 3,15-didecalonecitrin and 3-decalonecitrin. Results indicate that *TRI101* converts isotrichodermol to isotrichodermin and is required for the biosynthesis of T-2 toxin.

MATSUMOTO, G., WUCHIYAMA, J., SHINGU, Y., KIMURA, M., YONEYAMA, K. and YAMAGUCHI, I. 1999. **The trichothecene biosynthesis regulatory gene from the type B producer *Fusarium* strains: Sequence of *Tri6* and its expression in *Escherichia coli*.** *Bioscience Biotechnology and Biochemistry* **63**: 2001–2004.

A genomic DNA fragment containing *Tri6*, a transcription activator gene of

trichothecene biosynthesis, was cloned by vectorette PCR from *Fusarium graminearum* F15. The nucleotide sequence of the gene showed 84% of identity to that of the type A trichothecene producer *F. sporotrichioides* but the sequence around the initiation codon was not highly conserved between these producers. Based on the upstream and downstream sequences of the coding region of *F. graminearum*, *Tri6* could be amplified by PCR from other type B trichothecene producers. *Tri6* appeared to be expressed for only a limited period prior to the toxin production.

JARVIS, B.B. and WANG, S.J. 1999. **Stereochemistry of the roridins. Diastereomers of roridin E.** Journal of Natural Products **62**: 1284–1289.

Myrothecium verrucaria produces all four roridin E isomers, diastereomeric at the C-6' and C-13' centers. The stereochemistries at these centres were established by a combination of X-ray crystallographic analysis, NMR spectroscopy and chemical transformations. NMR data from these and other macrocyclic trichothecenes allows for the assignment of configurations at the C-6' and C-13' centres for most of these compounds.

Trichothecenes—Methodology

KRUGER, S.C., KOHN, B., RAMSEY, C.S. and PRIOLI, R. 1999. **Rapid immunoaffinity-based method for determination of zearalenone in corn by fluorometry and liquid chromatography.** Journal of AOAC International **82**: 1364–1368.

An immunoaffinity based method was developed to determine ZEA in corn. Corn samples were extracted in acetonitrile–water (90 + 10, v/v), applied to an immunoaffinity column and eluted with methanol. The isolated toxin was quantified either by reaction with aluminium chloride hexahydrate prior to measurement with a fluorometer or injection into an LC system with a fluorescence detector. With the immunoaffinity column cleanup procedure, only ZEA and its metabolites were recognised by the antibody. Limits of detection were 0.10 mg/kg for the fluorometer and 0.10 or 0.0025 mg/kg (sensitive method) for the LC method. Percentage recovery averaged 105% (fluorometer) and 93% (LC method), with average relative standard deviations of 15.7 and 9.3%. Comparative analysis of 17 naturally contaminated corn samples using ZearalaTest LC and the official AOAC LC method for detection of ZEA showed that ZearalaTest is statistically comparable to the AOAC Official Method 985.18.

ZOLLNER, P., JODLBAUER, J. and LINDNER, W. 1999. **Determination of zearalenone in grains by high-performance liquid chromatography-tandem mass spectrometry after solid-phase extraction with RP-18 columns or immunoaffinity columns.** Journal of Chromatography A **858**: 167–174.

A robust, sensitive and selective LC–MS–MS method for the determination of ZEA in several cereals is described. Samples were extracted with a mixture of acetonitrile and water followed by solid phase extraction with RP-18 columns or immunoaffinity columns. The selective determination of ZEA was achieved with an atmospheric pressure chemical ionisation interface. Using the negative ion mode, a detection limit of 0.5 µg/kg and a determination limit of 1 µg/kg grain was achieved, which is by a factor of 100 more sensitive than the positive ion mode. A linear working range from 1.0 to 1000 µg/kg could be achieved in grains with a standard deviation of 4% and recovery rates around 100%. All these results were independent from the grain matrices (maize, barley, oats, wheat) when zearalanone was used as internal standard.

CAHILL, L.M., KRUGER, S.C., MCALICE, B.T., RAMSEY, C.S., PRIOLI, R. and KOHN, B. 1999. **Quantification of deoxynivalenol in wheat using an immunoaffinity column and liquid chromatography.** Journal of Chromatography A **859**: 23–28.

A simple and accurate method to quantify DON in wheat which uses immunoaffinity chromatography for DON isolation and LC for toxin detection and quantification is described. Wheat samples are extracted in water, filtered and applied to an immunoaffinity column. Following a water wash, DON is eluted from the column with methanol and injected onto a LC system with a UV detector for quantification. Specificity of the immunoaffinity column cleanup procedure was confirmed with only DON and its 15-C derivatives being recognised by the antibody while 3-C DON derivatives, NIV, T-2 and fusarenon X did not bind. The limit of detection is at least 0.10 mg/kg. Percentage recovery for the entire assay range averages 90% with an average relative standard deviation of 8.3%. Naturally contaminated samples showed comparable precision.

KANG, Z. and BUCHENAUER, H. 1999. **Immunocytochemical localization of *Fusarium* toxins in infected wheat spikes by *Fusarium culmorum*.** Physiological and Molecular Plant Pathology **55**: 275–288.

Two antisera raised against DON and 3-acetylDON were used to investigate the sub-

cellular localisation of DON, 3-acetylDON and 15-acetylDON in *Fusarium culmorum* infected wheat spikes and kernels by means of the immunogold labelling technique. In hyphal cells, the toxins were localised in the cytoplasm, mitochondria, vacuoles and the cell wall. Results suggest that the toxins can be translocated upwards through the xylem vessels and phloem sieve tubes, and downwards through the phloem sieve tubes. In the infected wheat kernels, the toxins were shown not only in the hyphae, but also in the pericarp tissues, pigment strand, aleurone cells and starchy endosperm in different concentrations.

BERGER, U., OEHME, M. and KUHN, F. 1999. **Quantitative determination and structure elucidation of type A- and B-trichothecenes by HPLC/ion trap multiple mass spectrometry.** Journal of Agricultural and Food Chemistry **47**: 4240–4245.

A method is presented for the quantification and structure confirmation of trichothecenes in wheat by HPLC combined with multiple MS. Nine type A and B trichothecenes were determined, including NIV, DON, FX, 3-acetylDON, 15-acetylDON, neosolaniol, DAS, HT-2 toxin and T-2 toxin. Extraction was carried out with acetonitrile–water. The extract was purified on a MycoSep column. Quantification was based on an internal standard and atmospheric pressure chemical ionisation in the positive ion mode. Recoveries from spiked wheat were in the range of 80–106% at levels of 500 µg/kg. The limits of quantification were between 10 and 100 µg/kg. A scheme has been established for the partial structure elucidation of type A and B trichothecenes in fungal cultures.

HSUEH, C.C., LIU, Y. and FREUND, M.S. 1999. **Indirect electrochemical detection of type-B trichothecene mycotoxins.** Analytical Chemistry **71**: 4075–4080.

A simple and inexpensive method for the detection of type B trichothecenes is described. By hydrolysing the toxin under basic conditions at 80°C for 1 hr it is possible to detect the toxin with simple electrochemical techniques. The detection limit for DON using this method was 9.1 µM in solution, corresponding to 0.24 mg/kg in a 25 g grain sample if the final extraction volume is 2.2 mL. The linear dynamic detection range was from 0.32 mg/kg to greater than 32 mg/kg. In rice samples spiked with DON there is no electrochemical interference from rice extract and 1 mg/kg of DON in rice samples can be quantified. Since the active moiety in DON is common to virtually all type B trichothecenes, this approach may be ideal for type-specific screening.

D'MELLO, J.P.F., PLACINTA, C.M. and MACDONALD, A.M.C. 1999. **Fusarium mycotoxins: A review of global implications for animal health, welfare and productivity.** *Animal Feed Science and Technology* **80**: 183–205.

A review with 93 references. The metabolism and adverse effects of the *Fusarium* mycotoxins are considered in this review with particular reference to recent data on specific and proposed syndromes and to interactions among co-occurring mycotoxins. Within the trichothecene group, DON is associated with emesis, feed refusal and depressed feed intake in pigs, while T-2 toxin and DAS are now clearly linked with oral lesions in poultry. ZEA and its metabolites possess estrogenic activity in pigs, cattle and sheep, but T-2 toxin has also been implicated in reproductive disorders in farm livestock. Fumonisin is positively linked with pulmonary oedema in pigs, leukoencephalo-malacia in equines and with deranged sphingolipid metabolism in these animals. *Fusarium* mycotoxins have also been provisionally implicated in ovine ill-thrift, acute mortality of poultry and in duodenitis/proximal jejunitis of horses. In general, combinations of *Fusarium* mycotoxins result in additive effects, but synergistic and/or potentiating interactions have been observed and are of greater concern in livestock health and productivity.

SPRANDO, R.L., PESTKA, J., COLLINS, T.F.X., RORIE, J., O'DONNELL, M., HINTON, D. and CHIRTEL, S. 1999. **The effect of vomitoxin (deoxynivalenol) on testicular morphology, testicular spermatid counts and epididymal sperm counts in IL-6KO [B6129-IL6 (TmlKopf) (IL-6 gene deficient)] and WT [B6129F2 (wild type to B6129-IL6 with an intact IL-6 gene)] mice.** *Food and Chemical Toxicology* **37**: 1073–1079.

The potential of DON to affect testicular morphology and testicular and epididymal sperm counts was assessed in three strains of mice: IL-6KO (IL-6 gene deficient), WT (wild type with an intact IL-6 gene) and B6C3F(1) mice in a 90 day feeding study. Treated mice received DON at 10 mg/kg diet. Slight changes, not statistically significant, were observed in relative testis weight and testicular spermatid counts. Histological changes were not apparent in the testes of DON treated animals. The IL-6KO and B6C3F(1) DON treated mice had significantly reduced cauda epididymal weights compared with their respective controls. These changes were not attributed to decreased sperm counts and this finding suggests that DON may exert an adverse effect on the epididymis.

GHEDIRA-CHEKIR, L., MAAROUFI, K., CREPPY, E.E. and BACHA, H. 1999. **Cytotoxic and genotoxic effects of zearalenone: Prevention by vitamin E.** *Journal of Toxicology – Toxin Reviews* **18**: 355–368.

ZEA induces sterility in female rats treated before or after fertilisation and also causes hepatic and renal lesions and haematological and biochemical effects as evidenced by modification of several parameters. Its cytotoxicity has been confirmed *in vitro* in Vero cells ($IC_{50} = 20 \mu M$), in which it causes inhibition of protein and DNA synthesis and production of malondialdehyde. ZEA induces the formation of DNA adducts primarily in the liver in mice. ZEA at 1.5 mM induces the expression of genes of the SOS repair system in lysogenic *E. coli* which have integrated the 1 phage, indicating the induction of lesions in the DNA. Vitamin E, which has some structural similarities with ZEA, prevented all the observed effects, when preincubated in the medium 1hr prior to the toxin. Thus vitamin E appears efficient in preventing cytotoxic, genotoxic and mutagenic effects of ZEA and possibly the carcinogenic ones.

LEAL, M., SHIMADA, A., RUIZ, F. and DEMEJIA, E.G. 1999. **Effect of lycopene on lipid peroxidation and glutathione-dependent enzymes induced by T-2 toxin *in vivo*.** *Toxicology Letters* **109**: 1–10.

Male broiler chicks were fed diets containing T-2 toxin at 1.5 mg/kg body weight per day with or without the addition of lycopene at 25 mg/kg body weight. After 7 days of treatment, T-2 toxin increased hepatic malondialdehyde concentration (128%). A significant consumption of endogenous antioxidant glutathione (GSH) (45%) was induced as well as a marked increase in hepatic enzymatic activities of glutathione S transferase, gamma glutamyltransferase and glutathione peroxidase (312, 187 and 324%, respectively). The addition of T-2 toxin plus lycopene, at an approximate ratio of 1:17 in the diet, diminished some parameters measured. Apparently lycopene participated as an antioxidant agent as well as protecting the cellular level of GSH.

BOEIRA, L.S., BRYCE, J.H., STEWART, G.G. and FLANNIGAN, B. 1999. **Inhibitory effect of *Fusarium* mycotoxins on growth of brewing yeasts. 1. Zearalenone and fumonisin B₁.** *Journal of the Institute of Brewing* **105**: 366–374.

ZEA and FB₁ added to the growth medium in low and high concentrations were investigated as a possible cause of inhibition of growth of *Saccharomyces cerevisiae* lager and ale strains. Low concentrations (0.1–2 mg/L) had no significant effect on growth

compared to controls. Although high concentrations of both mycotoxins strongly affected growth, the inhibitory effect depended on toxin concentration and type, yeast strain, length of incubation and method used to assess growth. The lowest concentrations of mycotoxin causing significant inhibition of growth were for ZEA, 50 mg/L for both yeast strains, and for FB₁, 10 mg/L for the lager strain and 50 mg/L for the ale strain.

BOEIRA, L.S., BRYCE, J.H., STEWART, G.G. and FLANNIGAN, B. 1999. **Inhibitory effect of *Fusarium* mycotoxins on growth of brewing yeasts. 2. Deoxynivalenol and nivalenol.** *Journal of the Institute of Brewing* **105**: 376–381.

The effects of DON and NIV on the growth of *Saccharomyces cerevisiae* lager and ale strains was investigated. The inhibitory effect of both toxins on yeast growth was dependent on toxin concentration and was subject to yeast strain, length of incubation and method used to assess yeast growth. The lowest concentrations of mycotoxin causing significant inhibition of growth were for DON, 100 mg/L for the lager strain and 50 mg/L for the ale strain, and for NIV, 50 mg/L for the ale strain.

ARUKWE, A., GROTMOL, T., HAUGEN, T.B., KNUDSEN, F.R. and GOKSOYR, A. 1999. **Fish model for assessing the *in vivo* estrogenic potency of the mycotoxin zearalenone and its metabolites.** *Science of the Total Environment* **236**: 153–161.

The *in vivo* estrogenic potency of ZEA and its metabolites, alpha and beta zearalenol, have been studied in fish. Estrogenicity was evaluated using an *in vitro* competitive receptor binding assay and *in vivo* induction of vitellogenesis and zonagenesis, two estrogen receptor-mediated responses that are integral aspects of fish oogenesis. Generally, alpha-zearalenol and ZEA possess estrogenic potencies that are approximately 50% compared to that of estradiol-17 beta. Their order of estrogenic potency in both *in vitro* receptor competitive binding and *in vivo* induction of vitellogenin (Vtg) and zona radiata proteins (Zr-proteins) is: alpha-zearalenol > ZEA > beta-zearalenol. Results show that blood plasma analysis of Vtg and Zr-proteins levels provides a suitable *in vivo* fish model for assessing the estrogenic potencies of ZEA and its metabolites.

SABINO, M., MILANEZ, T.V., LAMARDO, L.C.A., INOMATA, E.I., ZORZETTO, M.A.P., NAVAS, S.A. and STOFER, M. 1999. **Occurrence of aflatoxins in peanuts and peanut products consumed in the State of Sao Paulo, Brazil from 1995 to 1997.** *Revista de Microbiologia* **30**: 85-88.

Samples of peanuts and peanut containing foods were collected in markets in the State of Sao Paulo, Brazil, from 1995 to 1997. The foods included raw peanuts, peanut candies, peanut butter, fried or roasted salted peanuts, "torrone", chocolate coated peanuts and salt coated peanuts. About 45% of the 137 samples were positive for aflatoxins and 27% exceeded the limits of the Brazilian legislation (30.0 µg/kg for AFB₁+G₁). The aflatoxins concentration in the raw peanut samples ranged from 5 to 382 µg/kg and 27.1% were above the legal limits. Contamination in peanut candies was above the limit in 32.8% of the samples and the aflatoxins levels ranged from 6 to 494 µg/kg. Contamination of salty peanuts was less frequent, around 10% of the samples, and the toxin levels were usually below 10 µg/kg.

WICKLOW, D.T. 1999. **Influence of *Aspergillus flavus* strains on aflatoxin and bright greenish yellow fluorescence of corn kernels.** *Plant Disease* **83**: 1146–1148.

The diversity of a naturally occurring population of *Aspergillus flavus* isolated from a corn field in Illinois, and their ability to contaminate grain with aflatoxin and produce bright greenish yellow fluorescent (BGYF) kernels was investigated in 1996 and 1998. The 19 strains included 16 genotypes representing both aflatoxin producers and non-producers. Ears of corn hybrid Pioneer 3394 in the late-milk to early-dough stage of maturity were inoculated with each *A. flavus* strain using a toothpick wound procedure. At harvest, 20 to 24 of the kernels nearest to each wounded site were separated into three categories: wound inoculated kernels, intact BGYF kernels and all other intact kernels. Removal of the individual wound inoculated kernels and the intact BGYF kernels from corn ears inoculated with 13 aflatoxin producing strains of *A. flavus* lowered mean aflatoxin values from 115 to 2 µg/kg in 1996 and from 744 to 33 µg/kg in 1998. Results indicated substantial variation among *A. flavus* genotypes in their ability to produce aflatoxin in the germ and endosperm of infected BGYF kernels.

REN, P., AHEARN, D.G. and CROW, S.A. 1999. **Comparative study of *Aspergillus* mycotoxin production on enriched media and construction material.** *Journal of Industrial Microbiology Biotechnology* **23**: 209–213.

Isolates of *Aspergillus flavus* and *A. fumigatus* from indoor air were grown on enrichment media and construction materials. In enrichment media, 4/7 isolates of *A. flavus* produced at least one aflatoxin and both isolates of *A. fumigatus* produced mycotoxins. When the mycotoxin positive strains were grown to a dense concentration on indoor construction and finishing materials such as ceiling tile and wall boards, mycotoxins were not detected in extracts of the materials.

BEUCHAT, L.R., CHMIELEWSKI, R., KESWANI, J., LAW, S.E. and FRANK, J.F. 1999. **Inactivation of aflatoxigenic *Aspergilli* by treatment with ozone.** *Letters in Applied Microbiology* **29**: 202–205.

Conidia of aflatoxigenic *Aspergillus flavus* and *A. parasiticus* were exposed to ozone at 1.74 mg/kg in 1 mM potassium phosphate buffer (pH 7.0 and 5.5) at 25°C and the D-values were determined. D-values of *A. flavus* conidia were 1.72 and 1.54 min at pH 5.5 and pH 7.0, respectively. D-values of *A. parasiticus* were 2.08 and 1.71 min, respectively. None of these D-values was significantly different from each other.

PASTER, N., ZHOU, L.C., MENASHROV, M. and SHAPIRA, R. 1999. **Possible synergistic effect of nisin and propionic acid on the growth of the mycotoxigenic fungi *Aspergillus parasiticus*, *Aspergillus ochraceus*, and *Fusarium moniliforme*.** *Journal of Food Protection* **62**: 1223–1227.

The effects of nisin and propionic acid (PA) on aflatoxin production and on mycelial growth and spore germination of *Aspergillus parasiticus*, *A. ochraceus* and *Fusarium moniliforme* were investigated. For all three fungi tested, the inhibitory effect on mycelial growth was found to be fungistatic rather than fungicidal. The combined treatment of PA with nisin produced better fungistatic activity than treatment involving either material alone. Nisin, applied alone, did not stimulate aflatoxin production but the combined treatment at certain concentrations was inhibitory to AFB₁ or G₁. The production of AFG₁, but not of B₁, was stimulated in 0.05% PA with 1,000 mg/kg nisin and on media containing 0.1% PA with 100 mg/kg nisin. These results indicate that a combined treatment of nisin in small concentrations of PA might be useful in preventing mould damage in certain foods and stored grain.

TRAN-DINH, N., PITT, J.I. and CARTER, D.A. 1999. **Molecular genotype analysis of natural toxigenic and nontoxigenic isolates of *Aspergillus flavus* and *A. parasiticus*.** *Mycological Research* **103**: 1485–1490.

The genetic relationship between toxigenic and non-toxigenic isolates of *Aspergillus flavus* and *A. parasiticus* was examined using RAPD and Neighbour Joining analysis. Twenty four RAPD amplifications using a combination of 17 primers discriminated between 20 isolates of *A. flavus* and 15 isolates of *A. parasiticus*. *A. flavus* isolates divided into two distinct groups with both toxigenic and non-toxigenic isolates occurring in each group. No association was seen between RAPD genotype and the ability to produce toxin. Five non-toxigenic isolates of *A. parasiticus* separated into two groups and these groups occurred on branches in which toxigenic isolates also occurred. This study suggests that either multiple losses of toxigenicity in *A. flavus* and *A. parasiticus* have occurred, or that recombination has reassorted this phenotype into a variety of different genetic backgrounds.

ZHOU, R. and LINZ, J.E. 1999. **Enzymatic function of the nor-1 protein in aflatoxin biosynthesis in *Aspergillus parasiticus*.** *Applied and Environmental Microbiology* **65**: 5639–5641.

The *nor-1* gene is involved in aflatoxin biosynthesis in *Aspergillus parasiticus* and was predicted to encode a norsolorinic acid ketoreductase. Recombinant Nor-1 expressed in *Escherichia coli* was shown to convert the 1' keto group of norsolorinic acid to the 1' hydroxyl group of averantin in crude *E. coli* cell extracts confirming that Nor-1 functions as a ketoreductase *in vitro*.

MOTOMURA, M., CHIHAYA, N., SHINOZAWA, T., HAMASAKI, T. and YABE, K. 1999. **Cloning and characterization of the *O*-methyltransferase I gene (*dmT*) from *Aspergillus parasiticus* associated with the conversions of demethylsterigmatocystin to sterigmatocystin and dihydrodemethylsterigmatocystin to dihydrosterigmatocystin in aflatoxin biosynthesis.** *Applied and Environmental Microbiology* **65**: 4987–4994.

O-Methyltransferase I catalyzes both the conversion of demethylSTG to STG and the conversion of dihydrodemethylSTG to dihydroSTG during aflatoxin biosynthesis. In this study, both genomic cloning and cDNA cloning of the gene encoding *O*-methyltransferase I, *dmT*, were accomplished by using PCR strategies, such as conventional PCR based on the N-terminal amino acid sequence of the purified enzyme, 5' and 3' rapid amplification of cDNA ends

PCR, and thermal asymmetric interlaced PCR (TAIL-PCR), and genes were sequenced by using *Aspergillus parasiticus* NIAH-26. The *dmtA* gene is located in the aflatoxin biosynthesis cluster between the *omtA* and *ord-2* genes. Northern blotting revealed that expression of the *dmtA* gene is influenced by both medium composition and culture temperature and that the pattern correlates with the patterns observed for other genes in the aflatoxin gene cluster. Furthermore, Southern blotting and PCR analyses of the *dmtA* gene showed that a *dmtA* homologue is present in *A. oryzae* SYS-2.

KRAUS, G.A. and WANG, X.M. 1999. A direct synthesis of aflatoxin M₂. Tetrahedron Letters **40**: 8513–8514.

A six step synthesis of AFM₂ starting from 1,3,5-trimethoxybenzene is described. The key step is the addition of dichloromethylithium.

BUTCHKO, R.A.E., ADAMS, T.H. and KELLER, N.P. 1999. *Aspergillus nidulans* mutants defective in *stc* gene cluster regulation. Genetics **153**: 715–720.

A novel screen is described for detecting *Aspergillus nidulans* mutants defective in *stc* gene cluster activity using a genetic block early in the sterigmatocystin biosynthetic pathway that results in the accumulation of the first stable intermediate, norsolorinic acid (NOR), an orange-coloured compound visible with the unaided eye. This NOR accumulating strain was mutagenised and 176 Nor⁻ mutants, 83 of which appear to be wild type in growth and development, were isolated. Sixty of these 83 mutations are linked to the *stc* gene cluster and are likely defects in *afIR* or known *stc* biosynthetic genes. Of the 23 mutations not linked to the *stc* gene cluster, 3 prevent accumulation of NOR due to the loss of *afIR* expression.

FAKHOURY, A.M. and WOLOSHUK, C.P. 1999. *Amy1*, the alpha-amylase gene of *Aspergillus flavus*: Involvement in aflatoxin biosynthesis in maize kernels. Phytopathology **89**: 908–914.

The alpha-amylase gene *Amy1* was isolated from *Aspergillus flavus* and its DNA sequence was determined to be nearly identical to *Amy3* of *A. oryzae*. When *Amy1* was disrupted in an aflatoxigenic strain of *A. flavus*, the mutant failed to produce extracellular alpha-amylase and grew at 45% the rate of the wild-type strain on starch medium. The mutant produced aflatoxin in medium containing glucose but not in a medium containing starch. The alpha-amylase-deficient mutant produced aflatoxin in maize kernels with wounded embryos and occasionally produced aflatoxin only in embryos of kernels with wounded endosperm. The mutant strain

failed to produce aflatoxin when inoculated onto degermed kernels. Results suggest that alpha-amylase facilitates aflatoxin production and growth of *A. flavus* from a wound in the endosperm to the embryo.

Aflatoxins – Methodology

TSAI, G.J. and YU, S.C. 1999. Detecting *Aspergillus parasiticus* in cereals by an enzyme-linked immunosorbent assay. International Journal of Food Microbiology **50**: 181–189.

An ELISA was used for the specific detection of *Aspergillus parasiticus* in artificially contaminated corn, rice, wheat and peanut. The growth of *A. parasiticus* was monitored by plate count and ELISA, and the aflatoxin content was measured. Higher a_w levels (a_w 0.98 vs. a_w 0.92) resulted in higher fungal growth rates and the plate count and ELISA measurements were better correlated, with correlation coefficients of 0.94, 0.93, 0.96 and 0.86 for corn, rice, wheat and peanuts, respectively. Aflatoxin was produced sooner and degraded more rapidly at a_w 0.98. Standard plate counting techniques detected *A. parasiticus*/*A. flavus* in 5/40 cereal samples bought from retail stores, however ELISA did not give a positive result in any of them. After moisturisation and incubation of these commercial samples at 28°C for 29 days, the incident rates of aflatoxigenic moulds increased to 65% and 52% by plate count and ELISA, respectively.

BHATTACHARYA, D., BHATTACHARYA, R. and DHAR, T.K. 1999. A novel signal amplification technology for ELISA based on catalyzed reporter deposition. Demonstration of its applicability for measuring aflatoxin B₁. Journal of Immunological Methods **230**: 71–86.

In an earlier communication a novel signal amplification technology termed Super-CARD, which is able to significantly improve antigen detection sensitivity in conventional Dot-ELISA was described. In this paper, the utilisation of this Super-CARD amplification technique in ELISA and its applicability for the rapid determination of AFB₁ in infected seeds is described. The limit of detection of AFB₁ by this method was 0.1 pg/well, the sensitivity enhancement being 5 fold over the optimised CARD ELISA. Furthermore, the total incubation time was reduced to 16 min compared to 50 min for the CARD method. Assay specificity was not adversely affected and the amount of AFB₁ measured in seed extracts correlated well with the values obtained by conventional ELISA.

ABD-ALLAH, G.A., EL-FAYOUMI, R.I., SMITH, M.J., HECKMANN, R.A. and O'NEILL, K.L. 1999. A comparative evaluation of aflatoxin B₁ genotoxicity in fish models using the Comet assay. Mutation Research – Genetic Toxicology and Environmental Mutagenesis **446**: 181–188.

The alkaline Comet assay is a simple and rapid method by which DNA damage can be demonstrated as a function of tail moment. The present work is the first to evaluate the genotoxicity of AFB₁ in fish using the Comet assay. Rainbow trout (a species sensitive to AFB₁) and channel catfish (resistant to AFB₁) were injected ip with AFB₁ at 0.5 mg/kg body weight. Trout blood and kidney tissue displayed significant and extensive DNA damage (shown by increased tail moment) after 4 hr which then decreased by 24 hr. In liver cells, damage progressively increased over time. Conversely, similarly treated catfish showed no elevation in DNA damage over controls. These results suggest that the Comet assay is a useful tool for monitoring the genotoxicity of mycotoxins such as AFB₁.

Aflatoxicoses

CHAO, H.K., TSAI, T.F., LIN, C.S. and SU, T.S. 1999. Evidence that mutational activation of the *ras* genes may not be involved in aflatoxin B₁-induced human hepatocarcinogenesis, based on sequence analysis of the *ras* and *p53* genes. Molecular Carcinogenesis **26**: 69–73.

In rats, activation of the *ras* gene is a prevalent event in AFB₁ induced hepatocarcinogenesis. By analysis of codon 249 of the *p53* gene, six of 36 human hepatoma samples were found to show a G to T transversion suggesting that AFB₁ may be a risk factor for hepatocarcinogenesis. However, analysis at codons 12, 13, and 61 in the *ras* family genes revealed a A to T transversion at codon 61 of the N-*ras* gene in a single tumour. Apparently, *ras* activation is rare in human hepatoma and the mutation detected might not be induced by AFB₁. This suggests that activation of the *ras* gene may not be a major event in AFB₁ related human hepatocarcinogenesis.

WANG, S.S., O'NEILL, O.J.P., QIAN, G.S., ZHU, Y.R., WANG, J.B., ARMENIAN, H., ZARBA, A., WANG, J.S., KENSLER, T.W., CARIELLO, N.F., GROOPMAN, J.D. and SWENBERG, J.A. 1999. Elevated *HPRT* mutation frequencies in aflatoxin-exposed residents of Daxin, Qidong County, People's Republic of China. Carcinogenesis **20**: 2181–2184.

The effects of aflatoxin exposure, as measured by serum aflatoxin-albumin adduct levels, on somatic mutation frequency in the human hypoxanthine guanine phosphoribosyl transferase gene (*HPRT*) were examined. *HPRT* mutant frequency was determined in individuals by a T cell clonal assay and the samples were categorised as low or high according to mean values. An odds ratio of 19.3 was demonstrated for a high *HPRT* mutation frequency in individuals with high aflatoxin exposure compared with those with low aflatoxin exposure. This association indicates that aflatoxin induced DNA damage in T lymphocytes, assessed using the validated surrogate albumin adduct markers, leads to increased mutations reflected as elevated *HPRT* gene mutations. This cross-sectional study suggests the potential use of mutation frequency of the *HPRT* gene as a long term biomarker of aflatoxin exposure in high risk populations.

HOQUE, A., PATT, Y.Z., YOFFE, B., GROOPMAN, J.D., GREENBLATT, M.S., ZHANG, Y.J. and SANTELLA, R.M. 1999. **Does aflatoxin B₁ play a role in the etiology of hepatocellular carcinoma in the United States?** *Nutrition and Cancer – An International Journal* **35**: 27–33.

A search for AFB₁ adducts and *p53* alterations, potentially induced by AFB₁, was conducted in the United States in 23 hepatocellular carcinoma (HCC) patients with available tissue samples. Hepatitis B virus (HBV) and hepatitis C virus (HCV) serology, and serum HBV-DNA were also determined. Thirteen patients were positive for HBV. Nine patients were free of HBV and HCV markers. Five of 22 sera tested were anti-HCV positive. *p53* protein expression was present in 5/23 tumour tissues, whereas *p53* codon 249 mutations were not observed in the 5 cases in which tissue was available for study. AFB₁ tumour-DNA adducts were present in 3/19 tumour tissues, and in 1 of these 3 samples *p53* protein was also detected. Sera from only 5 of the patients were tested for AFB₁-lysine adducts, and all were positive. In these 5 patients, neither *p53* protein nor a mutation on codon 249 was detected. The demonstration that AFB₁-DNA and -lysine adducts are present in HCC patients in the United States is intriguing but requires further substantiation because of the small number of subjects in this pilot study.

STEWART, R.K., SMITH, G.B.J., DONNELLY, P.J., REID, K.R., PETSİKAS, D., CONLAN, A.A. and MASSEY, T.E. 1999. **Glutathione S-transferase-catalyzed conjugation of bioactivated aflatoxin B₁ in human lung: Differential cellular distribution and lack of significance of the GSTM1 genetic polymorphism.** *Carcinogenesis* **20**: 1971–1977.

Among the purified human GST enzymes studied, the polymorphic hGSTM1-1 has the highest activity towards AFB₁ exo-epoxide. The influence of the GSTM1 polymorphism on AFB₁-GSH formation, as well as the abilities of cytosols from preparations enriched in different isolated lung cell types to conjugate AFB₁-epoxides, were examined. The results demonstrate that human lung GSTs exhibit very low conjugation activity for both AFB₁-8,9-epoxide stereoisomers and that this activity is heterogeneously distributed among cell types, with alveolar type II cells exhibiting relatively high activity. Of the GSTs present in human peripheral lung which contribute to AFB₁ exo- and endo-epoxide detoxification, hGSTM1-1 appears to play, at most, only a minor role.

PREMALATHA, B. and SACHDANANDAM, P. 1999. **Alterations in lipid metabolism during the development of aflatoxin B₁ induced experimental hepatocellular carcinoma.** *Medical Science Research* **27**: 779–782.

HCC was induced in rats with a single dose of AFB₁. The amounts of total cholesterol, free cholesterol and triglycerides were significantly higher and those of ester cholesterol, free fatty acids and phospholipids significantly lower, in carcinoma bearing animals. The activities of total lipase, lipoprotein lipase, lecithin cholesterol acyl transferase and cholesterol ester synthetase were significantly lower and cholesterol ester hydrolase activity significantly raised. Due to these alterations in lipid metabolism, rats with HCC developed hyperlipidaemia and this may be a useful indicator for the early detection of AFB₁ mediated HCC.

LIU, J., YANG, C.F., LEE, B.L., SHEN, H.M., ANG, S.G. and ONG, C.N. 1999. **Effect of *Salvia miltiorrhiza* on aflatoxin B₁-induced oxidative stress in cultured rat hepatocytes.** *Free Radical Research* **31**: 559–568.

The protective effect of *Salvia miltiorrhiza* (Sm; a herbal plant that has been used extensively in traditional Chinese medicine for treating cardiovascular and liver diseases) against AFB₁ induced cytotoxicity was investigated in cultured primary rat hepatocytes. Sm was able to suppress the lactate dehydrogenase leakage induced by AFB₁ in a dose dependent manner. A dose

dependent inhibitory effect on AFB₁ induced lipid peroxidation was also observed and Sm produced an inhibitory effect on ROS formation caused by AFB₁. Concomitantly, the GSH content in Sm treated groups increased substantially compared to those without Sm treatment. The major component of the aqueous extract of Sm was identified as D(+)-beta 3,4-dihydroxyphenol lactic acid.

WATZL, B., NEUDECKER, C., HANSCH, G.M., RECHKEMMER, G. and POOL-ZOBEL, B.L. 1999. **Short-term moderate aflatoxin B₁ exposure has only minor effects on the gut-associated lymphoid tissue of Brown Norway rats.** *Toxicology* **138**: 93–102.

The toxic effect of short term moderate AFB₁ exposure on the intestinal epithelium and on the immune cells associated with the intestinal tract was investigated in rats. The toxicological potential of AFB₁ and its metabolites to the intestinal epithelium was determined by measuring viability and genotoxic damage in isolated jejunal epithelial cells (comet assay) after 30 min incubation *in vitro*. *In vivo* toxicology studies were carried out with Brown Norway rats, which were dosed orally once a week with AFB₁ at 100 µg/kg body weight for 5 consecutive weeks. Viability and genotoxicity were measured in explanted jejunal epithelial cells. Results showed that exposure to moderate doses of AFB₁ does not damage the intestinal epithelium and has only minor effects on the gut associated lymphoid tissue.

PARLAT, S.S., YILDIZ, AO. and OGUZ, H. 1999. **Effect of clinoptilolite on performance of Japanese quail (*Coturnix coturnix japonica*) during experimental aflatoxicosis.** *British Poultry Science* **40**: 495–500.

Clinoptilolite (CLI) a natural zeolite, was incorporated into the diet of growing Japanese quail chicks and its ability to reduce the deleterious effects of aflatoxins on the chicks was evaluated. CLI at 50 g/kg diet significantly reduced the effects of aflatoxins at 2.0 mg/kg diet on food consumption, body weight gain and food conversion ratio. CLI alone significantly decreased food consumption and body weight gain during week 4, but these parameters were similar to the controls in week 5.

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Compiled and edited by J.C. Eyles, A. Hocking, and J.I. Pitt, Food Science Australia, Sydney. Produced by Arawang Communication Group, Canberra.

Please direct correspondence to ACIAR Postharvest Technology Program, GPO Box 1571, Canberra, ACT 2601, Australia. Fax: +61 2 6217 0501. Email: johnson@aciargov.au



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