

Abstracts

Mycotoxins—General

VANBARNEVELD, R.J. 1999. **Physical and chemical contaminants in grains used in livestock feeds.** Australian Journal of Agricultural Research **50**: 807–823.

This review with 67 references focuses on 3 main sources of contamination of Australian grains used in livestock feeds, namely chemical residues, moulds and mycotoxins, and weed seeds. The amount of research and literature available on the effects of mould and mycotoxin contamination of grain on livestock production, and techniques available for the measurement of moulds and mycotoxins, far exceeds the potential risk these contaminants pose to the livestock industries in Australia. The actual economic impact of moulds and mycotoxin contamination is difficult to assess due to a lack of systematic surveys and varying livestock production responses to the presence of these compounds.

SOHN, H.B., SEO, J.A. and LEE, Y.W. 1999. **Co-occurrence of *Fusarium* mycotoxins in mouldy and healthy corn from Korea.** Food Additives and Contaminants **16**: 153–158.

A total of 71 samples consisting of 36 mouldy and 35 visibly healthy corn were collected from Kangwon province of Korea and analysed for 8-ketotrichothecenes, zearalenone (ZEA) and fumonisins. Deoxynivalenol (DON), 15-acetylDON, 3-acetylDON, nivalenol (NIV) and 4-acetylNIV, ZEA, fumonisin B₁ (FB₁), FB₂ and FB₃ were detected in mouldy corn with mean values of 4.0, 0.9, 0.2, 1.7, 0.4, 0.6, 23.2, 7.5 and 6.3 mg/kg, respectively. Visibly healthy corn samples were contaminated with lower levels of mycotoxins than mouldy corn samples. This is the first report on the simultaneous occurrence of trichothecenes, ZEA and fumonisins in corn from Korea.

CURTUI, V., USLEBER, E., DIETRICH, R., LEPSCHY, J. and MARTLBAUER, E. 1998. **A survey on the occurrence of mycotoxins in wheat and maize from western Romania.** Mycopathologia **143**: 97–103.

Samples of wheat and maize for animal consumption, collected in 1997 after harvest from western Romania, were analysed for mycotoxin contamination. DON and acetylDONs were the major contaminants and were detected in 100% of wheat samples and 46% of maize samples. Median values for DON, 3-acetylDON and 15-acetylDON in wheat were 880, 66 and 150 µg/kg, respectively, and in maize 890, 180 and 620 µg/kg, respectively. Additionally, 3,19-diacetylDON, T-2 toxin, diacetoxyscirpenol, ZEA, FB₁, ochratoxin A and citrinin were found in some samples. No samples contained aflatoxin B₁ or fusarenone X. This is the first reported natural occurrence of a range of mycotoxins in Romanian feeding stuff and shows that DON and acetylDONs may be present at levels which may affect animal production.

BERLETH, M., BACKES, F. and KRAMER, J. 1998. **[Mould spectrum and mycotoxins (deoxynivalenol and ochratoxin A) in grain samples from ecological and integrated cultivated sites].** Agribiologische Zeitschrift für Agrarbiologie Agrikulturchemie Ökologie **51**: 369–376.

In the years 1994–1996, 329 samples of winter wheat and winter rye collected from 65 sites in the area of Northrhine-Westfalia were analysed for DON and ochratoxin A (OA). Freshly harvested crop material was obtained from integrated and ecological managed farms. All samples were dominated by black moulds of the genera *Cladosporium* and *Alternaria*. Nevertheless, DON was detected in each sample. DON content was

higher in 1996 due to colder and moister weather following flowering. No significant difference in DON concentration was observed in samples from integrated and ecological cultivated sites. OA was not detected. (In German).

PRASONGSIDH, B.C., KAILASAPATHY, K., STURGESS, R., SKURRAY, G.R. and BRYDEN, W.L. 1999. **Influence of manufacturing and storing of ice cream on cyclopiazonic acid.** Milchwissenschaft – Milk Science International **54**: 141–144.

Raw whole milk was artificially contaminated with cyclopiazonic acid (CPA) at 1 mg/kg and the stability of CPA in the milk during the preparation of ice cream and its storage over 3 months was studied. Hot and cold treatment during the manufacture of ice cream did not greatly affect levels of CPA in the mix and reduction of CPA levels from mixing until ageing was less than 12% in total. Results show that the potential of CPA to reach dairy consumers will be high if ice cream is made from a high level CPA contaminated milk.

PRASONGSIDH, B.C., STURGESS, R., SKURRAY, G.R. and BRYDEN, W.L. 1999. **Fate of cyclopiazonic acid in Cheddar cheese.** Milchwissenschaft – Milk Science International **54**: 200–203.

Cheddar cheese was prepared from whole milk spiked with CPA at 1 mg/L. The cheeses were analysed for CPA content during ageing for 10 months. The average of CPA increase in the final curd was 1.95 fold over the spiked milk. The percentage of CPA carry-over from milk into cheese-curd and the enrichment factor of CPA in the cheese making varied from 24.7 to 34.1% and 2.3 to 3.4, respectively. Carry-over of CPA into butterfat was also observed following the manufacture of the butter with contaminated milk.

GATENBY, W.A., MUNDAY-FINCH, S.C., WILKINS, A.L. and MILES, C.O. 1999. **Terpendole M, a novel indole-diterpenoid isolated from *Lolium perenne* infected with the endophytic fungus *Neotyphodium lolii***. Journal of Agricultural and Food Chemistry **47**: 1092–1097.

Terpendole M was isolated from perennial ryegrass (*Lolium perenne*) infected with the endophytic fungus *Neotyphodium lolii*. It was identified as 14 alpha-hydroxyterpendole C by NMR and mass spectral techniques. Paspaline and 13-desoxyxipaxilline were also isolated from perennial ryegrass for the first time. Terpendole M was less tremorgenic than terpendole C in a standard mouse bioassay. These findings provide clues to the biogenesis of the lolitrem neurotoxins as well as information on the structure-activity relationships within the indole-diterpenoids.

CHEN, J.W., LIU, B.L. and TZENG, Y.M. 1999. **Purification and quantification of destruxins A and B from *Metarhizium anisopliae***. Journal of Chromatography A **830**: 115–125.

Purification of the insecticidal cyclodepsipeptides, destruxins A (DA) and B (DB), from *Metarhizium anisopliae* fermentation broth was performed. The destruxins were isolated using ion exchange chromatography, silica gel chromatography and semi-preparative HPLC chromatography. Over 90% purity was achieved for both DA and DB. The purified destruxins were further identified by [¹H] NMR and fast atom bombardment MS. HPLC quantification methods for both destruxins in fermentation broth have been established.

MACKINNON, S.L., KEIFER, P. and AYER, W.A. 1999. **Components from the phytotoxic extract of *Alternaria brassicicola*, a black spot pathogen of canola**. Phytochemistry **51**: 215–221.

Six new fusicoccane-like diterpenoids, brassicene A to F, have been isolated from the liquid culture filtrates of the canola pathogen *Alternaria brassicicola* and their structures determined. This is the first report of the isolation of this class of diterpenoids from the genus *Alternaria*.

MORISSEAU, C., WARD, B.L., GILCHRIST, D.G. and HAMMOCK, B.D. 1999. **Multiple epoxide hydrolases in *Alternaria alternata* f. sp. *lycopersici* and their relationship to medium composition and host-specific toxin production**. Applied and Environmental Microbiology **65**: 2388–2395.

The production by *Alternaria alternata* f. sp. *lycopersici* of host-specific toxins

(AAL toxins) and epoxide hydrolase (EH) activity were studied during growth in liquid cultures. Media containing pectin as the primary carbon source displayed peaks of EH activity at days 4 and 12. When pectin was replaced by glucose there was a single peak of EH activity at day 6. Partial characterisation of the EH activities suggests the presence of three biochemically distinguishable EH activities. The EH activities present at day 6 (glucose) or day 12 (pectin) are concomitant with AAL toxin production.

HUEBNER, H.J., LEMKE, S.L., OTTINGER, S.E., MAYURA, K. and PHILLIPS, T.D. 1999. **Molecular characterization of high affinity, high capacity clays for the equilibrium sorption of ergotamine**. Food Additives and Contaminants **16**: 159–171.

Diverse phyllosilicate clays and other adsorbent materials differing in chemical and structural characteristics were tested for their ability to adsorb ergotamine, a prevalent ergot mycotoxin, from acidic solution. Results indicated minimal binding to those sorbents possessing low surface area, cation exchange capacity and inaccessible interlayer regions. Cetyl pyridinium-exchanged montmorillonite (organoclay) exhibited decreased propensity for ergotamine in acidic solution as compared with the unexchanged hydrophilic parent clay. The highest ergotamine sorption was observed with cation exchanged montmorillonite clays, whereas, when collapsed, these same clays adsorbed very little ligand.

DIXON, D.J., LEY, S.V., GRACZA, T. and SZOLCSANYI, P. 1999. **Total synthesis of the polyenoyltetramic acid mycotoxin erythrokyrine**. Journal of the Chemical Society – Perkin Transactions 1 1999(8): 839–841.

The first total synthesis of erythrokyrine, a polyenoyl-tetramic acid mycotoxin and principal pigment of *Penicillium islandicum* Sopp., is described.

Mycotoxins—Methodology

MONTI, S.M., FOGLIANO, V., RANDAZZO, G., PELUSO, G., LOGRIECO, A. and RITIENI, A. 1999. **Polyclonal antibodies against fusaproliferin**. Canadian Journal of Microbiology **45**: 45–50.

The toxic activity of fusaproliferin (FP) on haematopoietic human cell lines and its teratogenic effects on chicken embryos have been recently proved. In this study FP-hemiglutarate conjugated to modified bovine serum albumin was synthesised, characterised and used as an antigen for raising

polyclonal antibodies by immunising rabbits. Indirect and competitive ELISA and immunoblotting analyses were performed to determine antibody specificity towards the mycotoxin. The determination of 10 mg/L was achieved using antibodies purified by means of affinity chromatography on a FP-lysine-Sepharose column. This unsatisfactory detection limit is due to high background values. Thus, this method is not competitive with traditional UV-HPLC methods.

CASTLEBURY, L.A., SUTHERLAND, J.B., TANNER, L.A., HENDERSON, A.L. and CERNIGLIA, C.E. 1999. **Short communication. Use of a bioassay to evaluate the toxicity of beauvericin to bacteria**. World Journal of Microbiology & Biotechnology **15**: 131–133.

An agar diffusion bioassay was used to compare the sensitivities of bacteria to the mycotoxin beauvericin. *Bacillus pumilus* LACB101 was inhibited by filter paper disks containing 0.1 µg of beauvericin. *B. cereus*, *B. mycooides*, *B. sphaericus*, *Paenibacillus alvei*, *P. azotofixans*, *P. macquariensis* and *P. pulvifaciens* were inhibited by 1 µg.

CHAROENPORNSOOK, K., FITZPATRICK, J.L. and SMITH, J.E. 1998. **The effects of four mycotoxins on the mitogen stimulated proliferation of bovine peripheral blood mononuclear cells *in vitro***. Mycopathologia **143**: 105–111.

The effects of T-2 toxin, DON, OA and FB₁ on the response of bovine peripheral blood mononuclear cells (PBM) *in vitro* to the mitogens concanavalin A (Con A), phytohaemagglutinin A (PHA) and pokeweed mitogen (PWM) were assayed after 4 days incubation using [³H]-thymidine uptake and the MTT bioassay. The concentrations of mycotoxin required to reduce the proliferative response of PBM by 50% for Con A, PHA and PWM as measured by [³H]-thymidine incorporation were for T-2 toxin 0.30, 0.40 and 0.18 µg/L; for DON 0.07, 0.09 and 0.04 µg/L; for OA 0.10, 0.20 and 0.15 µg/L, and for FB₁ 35, 18 and 11 µg/L, respectively. The same responses as measured by the MTT bioassay were for T-2 toxin 2.0, 2.0 and 1.0 µg/L; for DON 0.70, 0.50 and 0.50 µg/L; for OA 1.5, 1.5 and 1.5 µg/L and for FB₁ 50, 50 and 20 µg/L, respectively.

Mycotoxicoses

HOLLINGER, K. and EKPERIGIN, H.E. 1999. **Mycotoxicosis in food producing animals**. Veterinary Clinics of North America – Food Animal Practice **15**: 133.

An overview with 73 references. Signs and symptoms of mycotoxicoses in food pro-

ducing animals can range from acute death, immunosuppression to skin lesions or to signs of hepatotoxicity, nephrotoxicity, neurotoxicity or genotoxicity. In addition there is also a public health concern over the potential for humans to consume animal derived food products such as meat, milk or eggs, containing residues of those mycotoxins or their metabolites.

MCLEAY, L.M., SMITH, B.L. and MUNDAY-FINCH, S.C. 1999. **Tremorgenic mycotoxins paxilline, penitrem and lolitrem B, the non-tremorgenic 31-epilolitre B and electromyographic activity of the reticulum and rumen of sheep.** Research in Veterinary Science **66**: 119–127.

The tremorgens penitrem, paxilline and lolitrem B had profound effects on electromyographic activity of smooth muscle of the reticulorumen in conscious sheep, with a similar time course of action to their respective characteristic effects on the induction (1 to 2, 15 to 20 and 20 to 30 min) and the duration (1 to 2, 1 to 2 and 8 to 12 hr) of trembling. Smooth muscle showed greater sensitivity to penitrem than skeletal muscle. Effects included an inhibition of the vagally-dependent cyclical A and B sequences of contraction of the reticulorumen, an increase in their amplitude and an excitation of local intrinsic activity contributing to elevated baselines and the occurrence of chaotic activity of the reticulum. Results indicate that stimulation of muscarinic cholinceptors was involved.

SMITH, B.L., BRIGGS, L.R., EMBLING, P.P., HAWKES, A.D. and TOWERS, N.R. 1999. **Urinary excretion of immunoreactive sporidesmin metabolites in sheep in relation to factors influencing susceptibility to sporidesmin intoxication.** New Zealand Veterinary Journal **47**: 13–19.

The urinary disposition of orally administered sporidesmins A and D in sheep and factors influencing their kinetics, particularly the influence of breeding for resistance and susceptibility to sporidesmin, were studied. Female Romney sheep showed maximum urinary excretion rates of immunoreactive metabolites 2–8 hr after dosing with sporidesmin D and 15–30 hr after dosing with sporidesmin A. Sporidesmin D caused no liver injury, while the liver injury caused by sporidesmin A was greatest for the sheep with the highest cumulative output of metabolite. When sporidesmin D was administered in two separate doses to sheep bred for either resistance or susceptibility to facial eczema caused by sporidesmin, the variability of metabolic output between sheep within groups was much less after the second dose. The mean urinary metabolite excretion was greater for the susceptible than

the resistant sheep but the difference was not significant. Potentiation (caused by pre-administration of small doses of sporidesmin A) resulted in a more severe reaction to the dosed sporidesmin A.

ZONNO, M.C. and VURRO, M. 1999. **Effect of fungal toxins on germination of *Striga hermonthica* seeds.** Weed Research **39**: 15–20.

Fourteen fungal toxins were assayed *in vitro* to evaluate their effect on seed germination of the parasitic weed *Striga hermonthica*. Among them, T-2 toxin was the most active, being able to inhibit seed germination by 100% at 10^{-5} M. DON caused 100% and 69% reduction in germination when assayed at 10^{-4} and 10^{-5} M, respectively. Cytochalasin E, tenuazonic acid, FB₁, enniatin and NIV were shown to have an inhibitory effect of around 50% at 10^{-4} M. The potential for use of fungal toxins as herbicides is discussed.

MOORE, T., MARTINEAU, B., BOSTOCK, R.M., LINCOLN, J.E. and GILCHRIST, D.G. 1999. **Molecular and genetic characterization of ethylene involvement in mycotoxin-induced plant cell death.** Physiological and Molecular Plant Pathology **54**: 73–85.

AAL-toxin and FB₁ cause interveinal cell death in susceptible lines of tomato with morphological characteristics of apoptosis. Concomitant with cell death, an increase in 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene occurs. The primary ACC synthase gene family member involved, LE ACS2, has now been identified. FB₁ caused the accumulation of LE ACS2 mRNA with a similar time course but to a lower level than did AAL-toxin. Nevertheless, ACC levels were similar for AAL and FB₁ treated tissue. ACC oxidase mRNA was also induced by both mycotoxins and again AAL-toxin induction was greater than that with FB₁. The induction of ACC synthase and ACC oxidase mRNA observed here represents the earliest changes in gene expression noted in this cell death system to date.

Ochratoxins—General

OZPINAR, H., AUGONYTE, G. and DROCHNER, W. 1999. **Inactivation of ochratoxin in ruminal fluid with variation of pH-value and fermentation parameters in an *in vitro* system.** Environmental Toxicology and Pharmacology **7**: 1–9.

The effects of different ruminal parameters such as pH and fermentation on the degradation of OA to ochratoxin alpha was investigated under *in vitro* conditions using the

'Hohenheimer gas test'. A mixture of ruminal fluid and a culture medium was incubated with OA for 48 hr. The ruminal degradation rate of the toxins was affected by the relation of roughage and concentrate in the complete diet. In diets comprising of 40% roughage and 60% of concentrate the degradation rate was higher than in diets consisting of 100% concentrate. A shorter half life for OA was estimated when starch was added to the medium. The rate of degradation of OA was not significantly effected by pH.

TSUDA, M., SEKINE, T., TAKEDA, M., CHA, S.H., KANAI, Y., KIMURA, M. and ENDOU, H. 1999. **Transport of ochratoxin A by renal multispecific organic anion transporter 1.** Journal of Pharmacology and Experimental Therapeutics **289**: 1301–1305.

The transport of OA by kidney-specific organic anion transporter 1 (OAT1) was studied. When expressed in *Xenopus laevis* oocytes, OAT1 mediated the sodium-independent uptake of OA. Piroxicam which prevents the nephrotoxicity of OA, inhibited OAT1-mediated uptake of OA. By contrast, another protective compound, aspartame, did not. Using a cell line derived from the mouse kidney terminal proximal tubule (S3) transfected with OAT1 cDNA, the transport of OA and also its effect on cell proliferation and cell viability was investigated. S3 cells expressing OAT1 mediated the saturable transport of OA. Cell proliferation was suppressed in S3 cells expressing OAT1 when exposed to 2 and 10 μ M OA. Results indicate that OA is transported by OAT1 and that the accumulation of OA via OAT1 in proximal tubular cells is the primary event in the development of OA nephrotoxicity.

MCMASTERS, D.R. and VEDANI, A. 1998. **[Ochratoxins. Molecular strategies for developing an antidote].** Altex – Alternativen zu Tierexperimenten **15**: 218–221.

The toxicity of OA is thought to be primarily due to its inhibition of phenylalanine-t-RNA synthetase, a phenylalanine-metabolising enzyme. Based on the three-dimensional structure of phenylalanine-t-RNA synthetase, its interactions with OA were analysed by means of molecular-dynamical simulations and three quite different binding modes were identified, all of which suggest an affinity only in the millimolar range. This would seem to be in conflict with toxicological findings frequently cited in textbooks but is in agreement with recent *in vitro* studies on purified phenylalanine-t-RNA synthetase, which all exclude this enzyme as the main target for OA action. *In vivo*, OA binds preferentially to serum albumin. Based on the three dimensional structure of serum albumin, its interaction with OA was simu-

lated with the long-term goal of identifying a synthetic antagonist which could lead to an enhanced elimination rate of OA from the body. (In German).

Ochratoxins—Methodology

MACDONALD, S., WILSON, P., BARNES, K., DAMANT, A., MASSEY, R., MORTBY, E. and SHEPHERD, M.J. 1999. **Ochratoxin A in dried vine fruit: method development and survey.** *Food Additives and Contaminants* **16**: 253–260.

A method is described for the determination of OA in dried vine fruits (currants, raisins and sultanas) using acidic methanolic extraction, immunoaffinity chromatography cleanup and HPLC determination. The limit of detection was estimated as 0.2 µg/kg and recoveries of 63–77% were achieved at 5 µg/kg. OA and aflatoxins were determined in 60 samples of retail dried vine fruits purchased in the United Kingdom. OA was found in excess of 0.2 µg/kg in 19/20 currant, 17/20 sultana and 17/20 raisin samples examined giving an overall incidence of 88%. The maximum level of OA found was 53.6 µg/kg. No aflatoxin was found in any sample analysed.

DEGELMANN, P., BECKER, M., HERDERICH, M. and HUMPF, H.U. 1999. **Determination of ochratoxin A in beer by high-performance liquid chromatography.** *Chromatographia* **49**: 543–546.

OA was determined in 35 samples of German beers. OA was extracted with toluene and purified by solid phase extraction using silica cartridges followed by HPLC with fluorescence detection. Since fluorescence detection is not specific in this instance, samples were also analysed by HPLC electrospray ionisation-tandem MS. OA levels in the range 0.1–0.2 µg/L were found in 9 beer samples, 21 samples contained trace amounts of OA (0.1 µg/L) and in 5 samples OA was not detected.

BASSEN, B. and BRUNN, W. 1999. **[Ochratoxin A in paprika powder].** *Deutsche Lebensmittel-Rundschau* **95**: 142–144.

A method of the determination of OA in paprika powder is described employing purification by immunoaffinity columns and high-pressure liquid chromatography with fluorescence detection. Two different solvent mixtures were compared for extracting OA. (In German).

Ochratoxins—Toxicoses

PINELLI, E., ELADLOUNI, C., PIPY, B., QUARTULLI, F. and PFOHLESZKOWICZ, A. 1999. **Roles of cyclooxygenase and lipoxygenases in**

ochratoxin A genotoxicity in human epithelial lung cells. *Environmental Toxicology and Pharmacology* **7**: 95–107.

The roles of constitutive prostaglandin-H-synthetase (PGHS) and lipoxygenases in OA genotoxicity were investigated *in vitro* in human epithelial cell culture and in the presence of pig seminal vesicle microsomes. Indomethacin (0.1 µM), which inhibits PGHS and significantly increases leukotriene C-4 production by enhancement of lipoxygenases, enhanced formation of OA-DNA adducts tenfold. Nordihydroguaiaretic acid, which inhibits lipoxygenases, suppressed OA-DNA adduct formation. These data demonstrate that OA is biotransformed into genotoxic metabolites via a lipoxygenase, whereas PGHS decreases OA genotoxicity.

GEKLE, M., SAUVANT, C., SCHWERDT, G. and SILBERNAGL, S. 1998. **Tubulotoxic mechanisms of ochratoxin A.** *Kidney & Blood Pressure Research* **21**: 277–279.

The majority of studies set up to determine the mechanisms of action of OA use high dose concentrations of OA which are not observed during naturally occurring exposure. This paper presents a working hypothesis for the 'specific' actions of OA in the low dose range that OA interacts with certain, so far unknown, cellular key targets (e.g. enzyme regulating messengers) and thereby, leads to the disturbance of cellular signalling and regulatory events (e.g. carrier inhibition, MAPK activation). Those changes in cellular signalling and/or regulation result in specific changes in cell function and/or phenotype, leading to changes in renal function and finally to the possible disturbance of the whole organism homeostasis.

BELMADANI, A., STEYN, P.S., TRAMU, G., BETBEDER, A.M., BAUDRIMONT, I. and CREPPY, E.E. 1999. **Selective toxicity of ochratoxin A in primary cultures from different brain regions.** *Archives of Toxicology* **73**: 108–114.

The mechanism of the cytotoxicity of OA in primary cultures of ventral mesencephalon and cerebellum from rat brain and the relative sensitivities of these regions to OA were investigated. Protein and DNA syntheses, lactate dehydrogenase (LDH) release and production of malondialdehyde (MDA) were assayed in astrocytes and neurones of the selected structures in the presence of OA. After 48 hr incubation, OA at 10–150 µM caused inhibition of protein and DNA syntheses in a concentration dependent manner with a selective higher toxicity in the cells of the ventral mesencephalon compared to the cerebellum values. In parallel, significant increases in levels of MDA and LDH release

were noted. These results indicate that OA is a neurotoxic substance in addition to its well-documented nephrotoxicity.

Fumonisin—General

GROVES, F.D., ZHANG, L.A., CHANG, Y.S., ROSS, P.F., CASPER, H., NORRED, W.P., YOU, W.C. and FRAUMENI, J.F. 1999. **Fusarium mycotoxins in corn and corn products in a high-risk area for gastric cancer in Shandong Province, China.** *Journal of AOAC International* **82**: 657–662.

Consumption of fermented, but not unfermented, corn pancakes has been linked with elevated stomach cancer mortality rates in rural Linqu County in Shandong Province, China. Specimens of corn, cornmeal, unfermented and fermented pancake batter, and cooked fermented pancakes from each of 16 households were assayed for fumonisins and trichothecenes. FB₁, B₂ and B₃ were detected in 19, 25 and 6% of the corn specimens, respectively, as well as in various corn products. DON and 15-acetylDON were detected in 58 and 17% of the corn specimens, respectively, and ZEA was detected in 15% of the cornmeal specimens. The mycotoxins were detected only at low levels (<10 mg/kg), which did not increase with fermentation. These findings do not support the hypothesis that mycotoxin contamination increases the risk of gastric cancer among those who consume fermented Chinese pancakes.

EDWARDS, O.E., BLACKWELL, B.A., DRIEGA, A.B., BENSIMON, C. and APSIMON, J.W. 1999. **The absolute stereochemistry of the ester functions of fumouisin B₁.** *Tetrahedron Letters* **40**: 4515–4518.

Synthesis of an optically active gamma-lactone related to tricarballic acid (TCA) and correlation of this to the same lactone derived from the two side chain TCA esters at C-14 and C-15 of FB₁ has established that these esters have the R configuration.

SHETTY, P.H. and BHAT, R.V. 1999. **A physical method for segregation of fumonisin-contaminated maize.** *Food Chemistry* **66**: 371–374.

A batch of maize naturally contaminated with FB₁ was treated with either water or different concentrations of NaCl in water by immersing for 5 min and removing the upper buoyant fraction. Both the buoyant and non-buoyant fractions were analysed for FB₁. With water alone, up to 74% of the toxin was removed in the buoyant layer. A solution of 30% NaCl and above was effectively used for physical separation of fumonisin

contaminated maize. Maximum effective removal was 86% with a saturated NaCl solution.

Fumonisin–Toxicology

TOLLESON, W.H., COUCH, L.H., MELCHIOR, W.B., JENKINS, G.R., MUSKHELISHVILI, M., MUSKHELISHVILI, L., MCGARRITY, L.J., DOMON, O., MORRIS, S.M. and HOWARD, P.C. 1999. **Fumonisin B₁ induces apoptosis in cultured human keratinocytes through sphinganine accumulation and ceramide depletion.** *International Journal of Oncology* **14**: 833–843.

The role of sphingolipid changes in FB₁ stimulated apoptosis in cultured human keratinocytes was examined. During FB₁ exposure sphinganine accumulated rapidly, sphingosine levels remained unchanged and ceramides decreased. Increased DNA fragmentation, decreased viability and apoptotic morphology were observed in cells exposed to FB₁, sphinganine or N-acetylsphingosine. Coexposure to N-acetylsphingosine or beta-chloroalanine, which blocks sphinganine accumulation, partially protected cells from FB₁ induced apoptosis.

SHEPHARD, G.S. and SNIJMAN, P.W. 1999. **Elimination and excretion of a single dose of the mycotoxin fumonisin B₂ in a nonhuman primate.** *Food and Chemical Toxicology* **37**: 111–116.

FB₂ was dosed both iv and by gavage to vervet monkeys. In monkeys dosed iv with 2 mg/kg body mass, the concentration of FB₂ in plasma was characterised by an initial distributional phase and a subsequent elimination phase with a mean half-life of 18 min. When two monkeys were dosed by gavage, only one showed detectable trace levels of FB₂ in plasma. This indicates that, like FB₁, FB₂ has a limited bioavailability. In total, a mean of 4.1% of the iv dose and 0.2% of the gavage dose was recovered in urine over a 7 day period. The predominant route of excretion was via the faeces, mainly as the unmetabolised toxin or as a partially hydrolysed analogue. A maximum of 1.1% of the fully hydrolysed aminopolyol backbone of FB₂ was recovered in faeces.

ZHANG, Y.G., DICKMAN, M.B. and JONES, C. 1999. **The mycotoxin fumonisin B₁ transcriptionally activates the p21 promoter through a cis-acting element containing two Sp1 binding sites.** *Journal of Biological Chemistry* **274**: 12367–12371.

Previous studies have concluded that FB₁ represses cyclin-dependent kinase 2 (CDK2)

activity but induces CDK inhibitors p21(Waf1/Cip1), P27(Kip1), and p57(Kip2) in monkey kidney cells (CV-1). In contrast, CV-1 cells transformed by simian virus 40 are resistant to the antiproliferative or apoptotic effects of FB₁. Consequently, FB₁ treatment of CV-1 cells leads to cell cycle arrest and apoptosis. In this study, it is shown that FB₁ transcriptionally activates the p21 promoter. Functional analysis of the p21 promoter by reporter gene assays mapped the FB₁ responsive region to –124 to –47. DNase I footprinting analysis revealed two protected motifs that span the FB₁ responsive region. Further studies demonstrated that DNA sequences from –124 to –101 were sufficient for FB₁ stimulation. DNA sequences from –124 to –101 contain two Sp1 binding sites and disruption of these sites abrogated the binding of nuclear proteins and prevented activation by FB₁. Taken together, these results suggest that Sp1 or Sp1-related proteins mediate FB₁ induced activation of the p21 promoter.

BAKER, D.C. and ROTTINGHAUS, G.E. 1999. **Chronic experimental fumonisin intoxication of calves.** *Journal of Veterinary Diagnostic Investigation* **11**: 289–292.

To determine whether cattle develop pulmonary hypertension in response to consumption of fumonisins for prolonged periods of time at altitudes that are not usually associated with pulmonary hypertension, calves were fed FB₁ at 2.36 mg/kg body weight per day for 23 weeks. Prolonged feeding and a higher dose of FB₁ did not result in pulmonary oedema or pulmonary hypertension.

LEMMER, E.R., HALL, P.D., OMORI, N., OMORI, M., SHEPHARD, E.G., GELDERBLOM, W.C.A., CRUSE, J.P., BARNARD, R.A., MARASAS, W.F.O., KIRSCH, R.E. and THORGEIRSSON, S.S. 1999. **Histopathology and gene expression changes in rat liver during feeding of fumonisin B₁, a carcinogenic mycotoxin produced by *Fusarium moniliforme*.** *Carcinogenesis* **20**: 817–824.

Male Fischer rats were fed either FB₁ at 250 mg/kg or control diet for up to 5 weeks. FB₁ caused a predominantly zone 3 'toxic' liver injury with hepatocyte death due to necrosis and apoptosis. Hepatocyte injury and death were mirrored by hepatic stellate cell proliferation and marked fibrosis, with progressive disturbance of architecture and formation of regenerative nodules. Despite ongoing hepatocyte mitotic activity, oval cell proliferation was noted from week 2, glutathione S-transferase pi-positive hepatic foci and nodules developed and, at later time points, oval cells were noted inside some of

the 'atypical' nodules. Northern blot (mRNA) analysis of liver specimens from weeks 3 to 5 showed a progressive increase in gene expression for alpha-fetoprotein, hepatocyte growth factor, transforming growth factor alpha (TGF-alpha) and especially TGF-beta 1 and c-myc. Immunostaining with LC(1–30) antibody demonstrated a progressive increase in expression of mature TGF-beta 1 protein by hepatocytes over the 5 week feeding period.

ABEYWICKRAMA, K., BEAN, G.A. and KENNEDY, K.A. 1998. **Effects of *Fusarium moniliforme* culture extracts and fumonisin B₁ on DNA, RNA and protein synthesis by baby hamster kidney cells.** *Mycopathologia* **143**: 59–63.

Baby hamster kidney cells (BHK-21) were exposed to culture filtrates of 4 *Fusarium moniliforme* isolates containing varying levels of FB₁ and the effects upon RNA, DNA and protein synthesis were monitored. FB₁ at 1 mg/L reduced protein synthesis by 4% after 24 hr and by 18% after 48 hr. Culture filtrates containing the highest levels of FB₁ (3.6 mg/L) also caused the greatest inhibition in protein synthesis after 24 hr but after 48 hr protein synthesis levels were the same as controls. The culture filtrates containing the highest levels of FB₁ reduced DNA synthesis more than 50% after 24 hr and 48 hr. Culture filtrates containing lesser amounts of FB₁ in some instances stimulated DNA synthesis and inhibited it in others. There was also no correlation in the level of FB₁ with the inhibition of RNA synthesis. It appears that metabolites other than fumonisin produced by *F. moniliforme* in culture can affect and both stimulate and inhibit RNA, DNA and protein synthesis by BHK cells.

Trichothecenes–General

PLACINTA, C.M., D'MELLO, J.P.F. and MACDONALD, A.M.C. 1999. **A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins.** *Animal Feed Science and Technology* **78**: 21–37.

A review with 57 references. From a global perspective, three classes of *Fusarium* mycotoxins may be considered to be of particular importance in animal health and productivity. Within the trichothecene group, DON is widely associated with feed rejection in pigs, while T-2 toxin can precipitate reproductive disturbances in sows. Another group comprising ZEA and its derivatives is endowed with oestrogenic properties. The third category includes the fumonisins which have been linked with specific toxicity syndromes such as equine leukoencephalomalacia and porcine pulmonary

oedema. Although sample size has been small in a number of surveys, there is nevertheless unequivocal evidence of global contamination of cereal grains and animal feed with several trichothecenes, ZEA and fumonisins. Furthermore, it is clear that legislation for the control of these mycotoxins in animal feed is now overdue and that further work is required to exploit cereal genotypes that are resistant to diseases caused by toxigenic *Fusarium* phytopathogens.

PROM, L.K., HORSLEY, R.D., STEFFENSON, B.J. and SCHWARZ, P.B. 1999. **Development of *Fusarium* head blight and accumulation of deoxynivalenol in barley sampled at different growth stages.** Journal of the American Society of Brewing Chemists **57**: 60–63.

In 1995 and 1996, a study was conducted to assess the development of *Fusarium* head blight (FHB) and the accumulation of DON in cv. Stander barley at the heading, early milk, late milk, soft dough, hard dough and mature developmental stages. In 1995, FHB severity increased markedly from the heading (0.7%) to soft dough (9.6%) developmental stages, and DON concentration increased from the heading (4.9 mg/kg) to late milk (36.8 mg/kg) stages. The increase in FHB and DON coincided with frequent and significant precipitation. In 1996, the level of FHB was less than 1% up to the soft dough stage, after which it increased from 1.7% at the hard dough stage to 8.2% at maturity. DON concentrations were low and ranged from 0.2 to 0.7 mg/kg after the heading stage. These data indicate that FHB severity and possibly DON concentration can increase in barley at any time between the heading stage and maturity.

JONES, R.K. and MIROCHA, C.J. 1999. **Quality parameters in small grains from Minnesota affected by *Fusarium* head blight.** Plant Disease **83**: 506–511.

Recent epidemics of *Fusarium* head blight (FHB) severely damaged the hard red spring wheat and barley crops in Minnesota. Samples of commercial grain were analysed in 1993 and 1994 to determine the effects of FHB on several quality parameters. DON was detected in 493/500 samples with concentrations in the range 0.0 to 44.7 mg/kg. Scab in wheat could rapidly be estimated using easy-to-prepare visual comparison standards and scores of percent 'visual scabby kernels' were correlated with DON concentration at $r = 0.897$ and 0.908 in 1993 and 1994, respectively. 'Wheat test weight' and 'thousand kernel weight' were less effective estimators of DON.

NEWTON, B. 1999. **Managing vomitoxin in the cereal processing industry.** Cereal Foods World **44**: 338–341.

There are well recognised, potentially serious consequences of DON contamination, both commercially and clinically. Also, the incidence of DON is increasing, as seen from the hitherto unobserved contamination of wheat and corn in the south eastern US in 1997. These factors, coupled with the advent of new onsite DON test kits, recommend the approach "test every load".

MESTERHAZY, A., BARTOK, T., MIROCHA, C.G. and KOMOROCZY, R. 1999. **Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding.** Plant Breeding **118**: 97–110.

Wheat genotypes with different degrees of resistance and origins were tested with seven and eight isolates, respectively, of *Fusarium graminearum* and four *Fusarium culmorum* isolates of diverse origin in Europe. *Fusarium* head blight (FHB) values, yield response and kernel infection values revealed close but varying relationships with DON content. This variability is explained by the presence of tolerance mechanisms which affect the relationship between FHB severity and yield response. Kernel infection resistance accounted for decreasing FHB values. Genotypes were found with lower infection severity and higher DON contamination and *vice versa*. However, in the most resistant genotypes showing no infection to any of the isolates or only sporadic symptom development, nil or very low accumulation of DON was detected. Resistant genotypes gave a stable reaction with b-values close to zero for all trials tested. Susceptible genotypes were unstable under different epidemic conditions and their stability was different for the traits investigated. Therefore, the mean of b-values is suggested to better describe the stability of the wheat genotypes.

MATTHIES, A., WALKER, F. and BUCHENAUER, H. 1999. **Interference of selected fungicides, plant growth retardants as well as piperonyl butoxide and 1-aminobenzotriazole in trichothecene production of *Fusarium graminearum* (strain 4528) in vitro.** Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz – Journal of Plant Diseases and Protection **106**: 198–212.

The effects of selected fungicides, plant growth retardants, piperonyl butoxide and aminobenzotriazole on the inhibition of DON and 3-acetylDON production relative to mycelium growth in *Fusarium graminearum* were examined. Various fungicides (e.g., prochloraz, carbendazim) and plant growth retardants (e.g., ancymidol, flurprimidol and BAS 111) reduced 3-acetylDON production to a similar extent as mycelium growth. On the other hand, in the presence of some compounds (e.g., fluquinconazole, thiabendazole,

tebuconazole), 3-acetylDON production was increased compared to mycelium growth. However, at higher dosages, these compounds reduced 3-acetylDON production and mycelium growth either to similar levels (e.g., tebuconazole) or mycotoxin production was more effectively inhibited than mycelium growth (e.g., thiabendazole). The guanidine-derivatives guazatine and iminoctadine induced a marked increase of 3-acetylDON production in relation to the mycelium growth. Pyrimethanil retarded 3-acetylDON production more effectively than mycelium growth. Piperonyl butoxide and 1-aminobenzotriazole impaired 3-acetylDON production more effectively than mycelium growth.

ZAMIR, L.O., NIKOLAKAKIS, A., SAURIOL, F. and MAMER, O. 1999. **Biosynthesis of trichothecenes and apotrichothecenes.** Journal of Agricultural and Food Chemistry **47**: 1823–1835.

Fusarium culmorum produces two major trichothecenes, 3-acetylDON and sambucinol, and some minor apotrichothecenes. The biosynthesis of these trichothecenes was investigated using labelled trichodiene. The result established for the first time that 2 alpha-hydroxy-11 alpha-apotrichothecene is a precursor to sambucinol. A biosynthetic scheme for the production of trichothecenes and apotrichothecenes is described.

ZAMIR, L.O., NIKOLAKAKIS, A., HUANG, L.R., ST-PIERRE, P., SAURIOL, F., SPARACE, S. and MAMER, O. 1999. **Biosynthesis of 3-acetyldeoxynivalenol and sambucinol.** Identification of the two oxygenation steps after trichodiene. Journal of Biological Chemistry **274**: 12269–12277.

The first two oxygenation steps post-trichodiene in the biosyntheses of 3-acetylDON and sambucinol were investigated. It was shown that 2 alpha-hydroxytrichodiene is the first oxygenated step in the biosynthesis of both 3-acetylDON and sambucinol. It was also demonstrated that the dioxygenated 12,13-epoxy-9,10-trichoene-2 alpha-ol is a biosynthetic precursor to trichothecenes.

Trichothecenes—Methodology

KOTAL, F., HOLADOVA, K., HAJŠLOVA, J., POUŠTKA, J. and RADOVA, Z. 1999. **Determination of trichothecenes in cereals.** Journal of Chromatography A **830**: 219–225.

An effective method for the determination of DON, NIV, T-2 tetraol, fusarenon X, diacetoxyscirpenol, T-2 toxin and HT-2 toxin

in cereals is presented. Gel permeation chromatography on Bio-Beads S-X3 was used for cleanup of acetonitrile-methanol extract. GC-ECD was used for identification and quantification of trifluoroacetylated trichothecenes. The limit of quantitation for the method was in the range 40–200 µg/kg. Recoveries at a spiking level of 2 mg/kg ranged from 76 to 100%.

DAWLATANA, M., COKER, R.D., NAGLER, M.J., GIBBS, J. and BLUNDEN, G. 1999. **An HPTLC method for the quantitative determination of T-2 toxin and deoxynivalenol in rice.** *Chromatographia* **49**: 547–551.

A GC method for the quantitative estimation of T-2 toxin which utilises a florisil SPE cleanup procedure has been adapted for the quantitative analysis of both T-2 toxin and DON using a high performance TLC quantification step. The method was validated by spiking rice extracts with T-2 toxin and DON over the range 100 to 1000 µg/kg. The mean recoveries for T-2 toxin and DON were 84 and 91%, respectively, with mean coefficients of variation of 5.2 and 6.0 %, respectively.

ENGLER, K.H., COKER, R. and EVANS, I.H. 1999. **A novel colorimetric yeast bioassay for detecting trichothecene mycotoxins.** *Journal of Microbiological Methods* **35**: 207–218.

A novel colorimetric microbial bioassay showing particular sensitivity to trichothecenes is described. The assay uses inhibition of expression of beta-galactosidase activity within the yeast *Kluyveromyces marxianus* as a sensitive toxicity indicator, cultures remaining yellow, rather than turning deep green-blue, in the presence of X-gal, a chromogenic substrate. The assay is conducted in standard microtitre plates and can be scored either automatically by a plate-reader, or by eye. Polymyxin B nonapeptide was the most effective toxicity-enhancing membrane modulating agents (MMA) tested, enabling verrucaric acid to be detected at a concentration of about 1 µg/L. Using polymyxin B sulfate as an alternative MMA, and T2 toxin, reproducibility and sensitivity were better for the beta-galactosidase X-gal endpoint than for an alternative chromogenic toxicity indicator, the respiratory substrate MTT.

ENGLER, K.H., COKER, R.D. and EVANS, I.H. 1999. **A colorimetric technique for detecting trichothecenes and assessing relative potencies.** *Applied and Environmental Microbiology* **65**: 1854–1857.

A novel colorimetric toxicity test, based on the inhibition of beta-galactosidase activity in *Kluyveromyces marxianus*, was tested

for a range of mycotoxins. The order of toxicity established with this bioassay was verrucaric acid > roridin A > T-2 toxin > diacetoxyscirpenol > HT-2 toxin > acetyl T-2 toxin > neosolaniol > fusarenon X > T-2 triol > scirpentriol > NIV > DON > T-2 tetraol. Verrucaric acid had a 50% effective concentration of 2 µg/L. Other mycotoxins including cyclopiazonic acid, FB₁, OA, patulin, sterigmatocystin, tenuazonic acid and ZEA, could not be detected at up to 10 mg/L.

Trichothecenes-Toxicoses

ZHOU, H.R., HARKEMA, J.R., YAN, D. and PESTKA, J.J. 1999. **Amplified proinflammatory cytokine expression and toxicity in mice coexposed to lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol).** *Journal of Toxicology and Environmental Health - Part A* **57**: 115–136.

The effects of oral DON exposure on tumour necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6) and IL-1 beta expression were measured in mice that were injected ip with lipopolysaccharide (LPS), a prototypic inflammatory agent. DON alone at 1, 5 and 25 mg/kg body weight increased splenic mRNA expression of all three cytokines after 3 hr in a dose response fashion. LPS injection at 1 and 5 mg/kg body weight also induced proinflammatory cytokine mRNA expression. There was a synergistic increase in TNF-alpha splenic mRNA levels in mice treated with both DON and LPS, whereas the effects were additive for IL-6 and IL-1 beta mRNA expression. Mice exposed to a single oral dose of DON at 25 mg/kg body weight and an ip dose of LPS at 1 or 5 mg/kg body weight became moribund in less than 40 hr. The principal histologic lesions in the moribund mice were marked cell death and loss in thymus, Peyer's patches, spleen and bone marrow. In all of these lymphoid tissues, treatment induced cell death had characteristic histologic features of apoptosis causing lymphoid atrophy.

SHIFRIN, V.I. and ANDERSON, P. 1999. **Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis.** *Journal of Biological Chemistry* **274**: 13985–13992.

The trichothecene family of mycotoxins inhibit protein synthesis by binding to the ribosomal peptidyltransferase site. Inhibitors of the peptidyltransferase reaction can trigger a ribotoxic stress response that activates c-Jun N-terminal kinase (JNK)/p38 mitogen-activated protein kinases, components of a signalling cascade that regulates cell survival

in response to stress. This study shows that some trichothecenes strongly activate JNK/p38 kinases and induce rapid apoptosis in Jurkat T cells. Although the ability of individual trichothecenes to inhibit protein synthesis and activate JNK/p38 kinases are dissociable, both effects contribute to the induction of apoptosis. Among trichothecenes that strongly activate JNK/p38 kinases, induction of apoptosis increases linearly with inhibition of protein synthesis. Among trichothecenes that strongly inhibit protein synthesis, induction of apoptosis increases linearly with activation of JNK/p38 kinases.

ALBARENQUE, S.M., SHINOZUKA, J., IWAMOTO, S., NAKAYAMA, H. and DOI, K. 1999. **T-2 toxin-induced acute skin lesions in Wistar-derived hypotrichotic WBN/ILA-Ht rats.** *Histology and Histopathology* **14**: 337–342.

Acute lesions in dorsal skin topically applied with T-2 toxin were examined in Wistar-derived hypotrichotic WBN/ILA-Ht rats up to 24 hr after treatment. In the epidermis, depression of basal cell proliferating activity was detected at 3 hr after treatment by immunostaining for proliferating cell nuclear antigen (PCNA), and the percentage of PCNA-positive basal cells decreased thereafter. At 12 hr after treatment, in addition to intracytoplasmic edema of spinous cells, acidophilic degeneration of basal cells characterised by shrinkage of cell body with acidophilic cytoplasm and pyknotic or karyorrhectic nuclei became prominent. Most of these nuclei were positive for TUNEL, an immunostaining used for the *in situ* detection of fragmented DNA.

Aflatoxins-General

COTTY, P.J. and CARDWELL, K.F. 1999. **Divergence of West African and North American communities of *Aspergillus* section *Flavi*.** *Applied and Environmental Microbiology* **65**: 2264–2266.

West African isolates of *Aspergillus flavus* S differ from North American isolates. Both groups produced AFB₁. However, 40 and 100% of West African isolates also produced AFG₁ in NH₄ medium and urea medium, respectively. No North American S strain isolate produced AFG₁.

ABDULLAH, N., NAWAWI, A. and OTHMAN, I. 1998. **Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch-based foods.** *Mycopathologia* **143**: 53–58.

Starch based foods sampled from retail outlets in Malaysia were surveyed for moulds and aflatoxins. Screening for aflatoxins was

carried out on 84 samples of ordinary rice grains and 83 samples of wheat flour. Of the rice grain samples 2.4% were positive for AFG₁ and 3.6% were positive for AFG₂ at concentrations ranging from 3.69–77.50 µg/kg. All the positive samples were collected from private homes. Of the wheat flour samples 1.2% were positive for AFB₁ (25.62 µg/kg), 4.8% were positive for AFB₂ (11.25–252.50 µg/kg), 3.6% were positive for AFG₁ (25.00–289.38 µg/kg) and 13.25% were positive for AFG₂ (16.25–436.25 µg/kg). Similarly, positive wheat flour samples were mostly collected from private homes.

HORN, B.W. and DORNER, J.W. 1999. **Regional differences in production of aflatoxin B₁ and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States.** Applied and Environmental Microbiology **65**: 1444–1449.

Soil isolates of *Aspergillus flavus* from a transect extending from eastern New Mexico through Georgia to eastern Virginia were examined for the production of AFB₁ and CPA in a liquid medium. Peanut fields from major peanut-growing regions were sampled, and fields with other crops were sampled in regions where peanuts are not commonly grown. The *A. flavus* isolates were identified as members of either the L strain which produces sclerotia that are >400 µm in diameter, or the S strain which produces numerous small sclerotia that are <400 µm in diameter. The S strain isolates generally produced high levels of AFB₁, whereas the L strain isolates were more variable in aflatoxin production; variation in CPA production also was greater in the L strain than in the S strain. Significant differences in production of AFB₁ and CPA by the L strain isolates were detected among regions. In the western half of Texas and the peanut-growing region of Georgia and Alabama, 62–94% of the isolates produced AFB₁ at >10 mg/L. The percentages of isolates producing >10 mg/L ranged from 0 to 52% in the remaining regions of the transect; other isolates were often nonaflatoxigenic.

RAMOS, A.J., MUNOZ, J., MARIN, S., SANCHIS, V. and MAGAN, N. 1999. **Calorific losses in maize in relation to colonisation by isolates of *Aspergillus ochraceus* under different environmental conditions.** Journal of Cereal Science **29**: 177–183.

The effect of water activity (a_w), temperature and incubation period on growth and calorific losses in relation to colonisation by three mycotoxigenic isolates of *Aspergillus ochraceus* was studied on maize based substrates. The calorific losses (kJ) of maize flour due to fungal growth in relation to temperature and a_w were quantified and found

to be maximal (10.5–16.2%) at 20–30°C after 4 weeks at 0.95 a_w, with only slight losses (0–7.1%) at 0.85 a_w at both 15 and 20°C.

SCHINDLER, B., ROTH, S., MOSENTHIN, R. and RAIBLE, H.P. 1999. **[Short communication. Nutmegs – contamination with aflatoxins. Study for the evaluation of raw material].** Deutsche Lebensmittel-Rundschau **95**: 104–106.

Aflatoxin contamination in nutmegs was studied to obtain information for a spot sampling plan for nutmegs. Aflatoxins were not detected in whole nutmegs, but were detected in 7% of 'shrunked' nutmegs, especially Siauw. These samples exceeded the maximum allowed amount of AFB₁ of 2 µg/kg nutmegs. (In German).

MORENO, O.J. and KANG, M.S. 1999. **Aflatoxins in maize.** The problem and genetic solutions. Plant Breeding **118**: 1–16.

A review with 230 references of progress in research and current knowledge of the genetics of resistance and breeding strategies to develop resistance to aflatoxin contamination, the genetic manipulation of the fungus, and new strategies for controlling aflatoxin contamination of maize in the field. Genetic manipulation of both the host plant and the fungus provides the most promising route for achieving a satisfactory control of aflatoxin in maize grain.

AHMED, I.A. and ROBINSON, R.K. 1999. **The ability of date extracts to support the production of aflatoxins.** Food Chemistry **66**: 307–312.

Aqueous extracts of the fruit of the date palm (*Phoenix dactylifera* L.) are used widely in the Middle East, and they are often stored at high ambient temperatures and in vessels of dubious hygienic standards. The levels of aflatoxin generated in experimental extracts depended upon the sugar content (glucose and fructose) of the solution, but extracts from all the varieties of dates tested were able to support mycelial growth and aflatoxin production.

WINDHAM, G.L., WILLIAMS, W.P. and DAVIS, F.M. 1999. **Effects of the southwestern corn borer on *Aspergillus flavus* kernel infection and aflatoxin accumulation in maize hybrids.** Plant Disease **83**: 535–540.

Field studies conducted in 1995 to 1997 to determine the effects of the southwestern corn borer (SWCB) on *Aspergillus flavus* kernel infection and aflatoxin accumulation in maize hybrids showed that SWCB can substantially increase aflatoxin levels when combined with *A. flavus*. However, inoculation and infestation techniques, placement of

the fungus and the insect, and timing of inoculation and infestation are all critical in demonstrating a synergistic relationship between *A. flavus* and SWCB on aflatoxin contamination of maize.

DORNER, J.W., COLE, R.J. and WICKLOW, D.T. 1999. **Aflatoxin reduction in corn through field application of competitive fungi.** Journal of Food Protection **62**: 650–656.

Soil in corn plots was inoculated with nonaflatoxigenic strains of *Aspergillus flavus* and *A. parasiticus* during crop years 1994 to 1997 to and the effect of application on preharvest aflatoxin contamination of corn was determined. Inoculation resulted in significant increases in the total *A. flavus/parasiticus* soil population in treated plots, and that population was dominated by the applied strain of *A. parasiticus*. In the years when weather conditions favoured aflatoxin contamination (1996 and 1997), corn was predominately colonised by *A. flavus* as opposed to *A. parasiticus*. In 1996 and 1997, the aflatoxin concentrations in corn from treated plots averaged 24.0 and 29.8 µg/kg, respectively, reductions of 87 and 66%, respectively, compared with control plots. Together, the data indicated that although the applied strain of *A. parasiticus* dominated in the soil, the nonaflatoxigenic strains of *A. flavus* were more responsible for the observed reductions in aflatoxin contamination.

WARKE, R.G., KAMAT, A.S. and KAMAT, M.Y. 1999. **Irradiation of chewable tobacco mixes for improvement in microbiological quality.** Journal of Food Protection **62**: 678–681.

The microbiological quality of chewable tobacco mixes from India, traditionally known as "gutkha", was studied. Analysis of 15 samples revealed high bacterial and fungal counts. AFB₁, B₂ and G₂ were found to be present in all the samples. Gamma radiation at 3 kGy resulted in the sterilisation for gutkha.

GREENE-MCDOWELLE, D.M., INGBER, B., WRIGHT, M.S., ZERINGUE, H.J., BHATNAGAR, D. and CLEVELAND, T.E. 1999. **The effects of selected cotton-leaf volatiles on growth, development and aflatoxin production of *Aspergillus parasiticus*.** Toxicon **37**: 883–893.

Previous studies have shown that aflatoxigenic strains of *A. flavus* and *A. parasiticus* grown in the presence of specific cotton leaf volatiles exhibit alterations in aflatoxin production accompanied by variations in growth of the fungi. In this study, two alcohols (3-methyl-1-butanol (3-MB) and nonanol) and two terpenes (camphene and limonene) were chosen as representative

cotton leaf volatiles and their effects on fungal growth and/or aflatoxin production were investigated. Aflatoxin production increased in cultures exposed to 3-MB but decreased when exposed to the other three volatiles studied.

HASAN, H.A.H. 1999. **Role of caffeine and tannin in anti-toxicogenic properties of coffee and tea.** *Cryptogamie Mycologie* **20**: 17–21.

AFB₁, B₂, G₁ and G₂ produced by *Aspergillus parasiticus* var *globosus* in detannin-caffeinated coffee and black tea were five times more concentrated than in normal tea and coffee. Extracts of normal coffee and tea powders significantly reduced aflatoxin production in liquid broth at 1 and 3% concentrations, with tea extract having a more pronounced effect than coffee extract. Anti-aflatoxicogenic properties appear to be due to tannin and caffeine which induced 95% inhibition of aflatoxin production at 0.3 and 0.6%, respectively.

FAN, J.J. and CHEN, J.H. 1999. **Inhibition of aflatoxin-producing fungi by Welsh onion extracts.** *Journal of Food Protection* **62**: 414–417.

Welsh onion ethanol extracts were tested for their inhibitory activity against the growth and aflatoxin production of *Aspergillus flavus* and *A. parasiticus*. Mycelial growth of both fungi cultured on yeast extract sucrose broth was completely inhibited in the presence of the extract at a concentration of 10 g/L during 30 days of incubation at 25°C. The extracts inhibited aflatoxin production at a concentration of 10 g/L and permitted only a small amount of aflatoxin production at 5 g/L after 2 weeks incubation.

JAYASHREE, T. and SUBRAMANYAM, C. 1999. **Antiaflatoxicogenic activity of eugenol is due to inhibition of lipid peroxidation.** *Letters in Applied Microbiology* **28**: 179–183.

Eugenol inhibited aflatoxin production by *Aspergillus parasiticus* in a dose dependent manner up to a concentration of 0.75 mmol/L without inhibiting growth. When grown for 3 days in the presence of eugenol at 0.45 mmol/L (the concentration inhibiting aflatoxin production by 50%) for 3 days, *in vivo* activities of components of polysubstrate monooxygenase were decreased at idiophase, concomitant with decreased activities of enzymes involved in free radical scavenging, lipid peroxidation and maintenance of redox potential.

NORTON, R.A. 1999. **Inhibition of aflatoxin B₁ biosynthesis in *Aspergillus flavus* by anthocyanidins and related flavonoids.** *Journal of Agricultural and Food Chemistry* **47**: 1230–1235.

Anthocyanidins and precursors or related flavonoids were tested at concentrations from 0.3 to 9.7 mM (similar to 0.1–3.0 g/L) for activity against growth and AFB₁ biosynthesis by *Aspergillus flavus*. AFB₁ production was inhibited by all anthocyanidins tested, and 3-hydroxy compounds were more active than 3-deoxy forms. Monoglycosides of cyanidin were 40% less inhibitory than the aglycon, whereas a monoglucoside and a diglucoside of pelargonidin were 80 and 5%, respectively, as active as the aglycon. Of eight flavonoids tested, only kaempferol was moderately active, whereas luteolin and catechin were weakly inhibitory. Results with an aflatoxin pathway mutant indicated that anthocyanidin inhibition occurred before norsolorinic acid synthesis.

HASAN, H.A.H. 1999. **Mode of action of pesticides on aflatoxin biosynthesis and oxidase system activity.** *Microbiological Research* **154**: 95–102.

The effects of nine pesticides on the biosynthesis of aflatoxin and oxidase activity in wild-type *Aspergillus flavus* and mutant strains of *A. parasiticus* *avr1* and *A. parasiticus* *ver1* were investigated. In *A. parasiticus*, phosphonic acid derivative reduced the formation of AFB₂, but B₁, G₁ and G₂ and anthraquinones (versicolorin A, versiconal hemiacetal acetate and averufin) accumulated. Phosphorothioic acid derivatives reduced the formation of AFB₂ and G₂ but B₁ and G₁ and anthraquinones accumulated. Phosphorodithioic acid derivatives blocked AFB₂, reduced B₁ and G₂ but G₁ and anthraquinones accumulated. Phosphoric acid derivative inhibited the formation of all aflatoxins, versicolorin A and versiconal hemiacetal acetate but averufin accumulated. The phenylurea derivatives inhibited all aflatoxin but anthraquinones accumulated. On the other hand, the dicarboximide derivative inhibited the whole pathway in the mutant strains of *A. parasiticus*.

HUA, S.S.T., BAKER, J.L. and FLORES-ESPIRITU, M. 1999. **Interactions of saprophytic yeasts with a *nor* mutant of *Aspergillus flavus*.** *Applied and Environmental Microbiology* **65**: 2738–2740.

The *nor* mutant of *Aspergillus flavus* has a defective norsolorinic acid reductase and thus the aflatoxin biosynthetic pathway is blocked, resulting in the accumulation of norsolorinic acid, a bright red-orange pigment. A visual agar plate assay has been developed to monitor yeast strains for their ability to inhibit aflatoxin production by visually scoring the accumulation of this pigment of the *nor* mutant. Yeast strains were identified that reduced the red-orange pigment accumulation in the *nor* mutant. These yeasts also reduced aflatoxin accumulation by a toxigenic strain of *A. flavus*.

CASILLAS, L.K. and TOWNSEND, C.A. 1999. **Total synthesis of O-methylsterigmatocystin using N-alkylnitrilium salts and carbonyl-alkene interconversion in a new xanthone synthesis.** *Journal of Organic Chemistry* **64**: 4050–4059.

A general strategy is described for the preparation of substituted xanthenes and embodied in the preparation of (+/-)-O-methylsterigmatocystin (OMST). The essential features of this approach are the reaction of N-alkylnitrilium salts with activated aromatic rings, and protection of the derived xanthenes as their corresponding alkenyl xanthenes.

EHRlich, K.C., CARY, J.W. and MONTALBANO, B.G. 1999. **Characterization of the promoter for the gene encoding the aflatoxin biosynthetic pathway regulatory protein AFLR.** *Biochimica et Biophysica Acta – Gene Structure and Expression* **1444**: 412–417.

Most genes in the aflatoxin biosynthetic pathway in *Aspergillus parasiticus* are regulated by the binuclear zinc cluster DNA-binding protein AFLR. The aflR promoter was analysed in beta-glucuronidase reporter assays to elucidate some of the elements involved in the gene's transcription control. Truncation at 118 bp upstream of the translational start site increased promoter activity 5 fold, while truncation at -100 reduced activity about 20 fold. These findings indicate the presence of an important positive regulatory element between -100 and -118 and a negative regulatory region further upstream.

EHRlich, K.C., MONTALBANO, B.G. and CARY, J.W. 1999. **Binding of the C6-zinc cluster protein, AFLR, to the promoters of aflatoxin pathway biosynthesis genes in *Aspergillus parasiticus*.** *Gene* **230**: 249–257.

AFLR is a Zn(2)Cys(6)-type sequence-specific DNA-binding protein that is thought to be necessary for expression of most of the genes in the aflatoxin pathway gene cluster in *Aspergillus parasiticus* and *A. flavus*, and the sterigmatocystin gene cluster in *A. nidulans*. Recently, *A. nidulans* AFLR was shown to bind to the motif 5'-TCGN(5)CGA-3'. In the present study, the binding of AFLR to promoter regions of 11 genes in the *A. parasiticus* cluster was examined. Based on electrophoretic mobility shift assays, the genes *nor1*, *pksA*, *adhA*, *norA*, *ver1*, *omtA*, *ordA* and *vbs*, had at least one 5'-TCGN(5)CGA-3' binding site within 200 bp of the translation start site, and *pksA* and *ver1* had an additional binding site further upstream. Based on a comparison of 16 possible sites, the preferred binding sequence was 5'-TCGSWNNSCGR-3'.

BROWN, M.P., BROWN-JENCO, C.S. and PAYNE, G.A. 1999. **Genetic and molecular analysis of aflatoxin biosynthesis.** *Fungal Genetics and Biology* **26**: 81–98.

This review with 124 references covers recent data on the genetics and molecular biology of aflatoxin/sterigmatocystin biosynthesis. The review also covers some of the early studies on the genetics of *Aspergillus flavus* and *A. parasiticus*. Although *A. nidulans* provides a powerful genetic system to study the aflatoxin/sterigmatocystin pathway, there is some evidence that the two pathways may be regulated differently.

Aflatoxins—Methodology

NESHEIM, S., TRUCKSESS, M.W. and PAGE, S.W. 1999. **Molar absorptivities of aflatoxins B₁, B₂, G₁, and G₂ in acetonitrile, methanol, and toluene-acetonitrile (9+1) (modification of AOAC official method 971.22).** Collaborative study. *Journal of AOAC International* **82**: 251–258.

Four laboratories participated in a mini-collaborative study of AOAC Official Method 971.22, Standards for Aflatoxins, Thin-Layer Chromatographic Method, to extend the method to 3 replacement solvents for benzene for calibration of standard aflatoxin solutions. AOAC Official Method 971.22 has been modified to extend its applicability to 3 replacement solvents for benzene, namely methanol, acetonitrile and toluene-acetonitrile (9+1), for calibration of standard aflatoxin solutions.

VONGBUDDHAPITAK, A., TRUCKSESS, M.W., ATISOOK, K., SUPRASERT, D. and HORWITZ, W. 1999. **Laboratory proficiency testing of aflatoxins in corn and peanuts.** A cooperative project between Thailand and the United States. *Journal of AOAC International* **82**: 259–263.

An aflatoxin proficiency test program in government, academia and industry laboratories was conducted in Thailand. The test was conducted according to the ISO/IUPAC/AOAC International Harmonized Protocol with z scores indicating laboratory performance. The participants used 3 methods: ELISA, TLC and the minicolumn. Among laboratories that reported results for aflatoxins in naturally contaminated corn and naturally contaminated peanuts, 68 and 48%, respectively, performed satisfactorily on the basis of the mean obtained by an expert laboratory, a calculated target value for standard deviation, and the z score. Subsequently, a workshop of lectures and laboratory sessions was conducted to improve performance.

WHITAKER, T.B., HAGLER, W.M. and GIESBRECHT, F.G. 1999. **Performance of sampling plans to determine aflatoxin in farmers' stock peanut lots by measuring aflatoxin in high-risk-grade components.** *Journal of AOAC International* **82**: 264–270.

Five 2 kg test samples were taken from each of 120 farmers' stock peanut lots contaminated with aflatoxin. Kernels from each 2 kg sample were divided into the following USDA grade components: sound mature kernels plus sound splits, other kernels, loose shelled kernels and damaged kernels. The kernel mass, aflatoxin mass and aflatoxin concentration were measured for each of the 2400 component samples. The variance associated with measuring aflatoxin in each of the 4 combinations of components increased with aflatoxin, and functional relationships were developed from regression analysis. The variability associated with estimating the lot concentration from each of the 4 combinations of components was also determined.

PEARSON, S.M., CANDLISH, A.A.G., AIDOO, K.E. and SMITH, J.E. 1999. **Determination of aflatoxin levels in pistachio and cashew nuts using immunoaffinity column clean-up with HPLC and fluorescence detection.** *Bio-technology Techniques* **13**: 97–99.

A technique for the detection of aflatoxins in pistachio and cashew nuts using immunoaffinity column cleanup with HPLC and fluorescent detection is presented. Recoveries were in the range of 79 to 99% for pistachio samples artificially contaminated with aflatoxins at 10 µg/kg of food sample. For cashew samples recoveries ranged from 80 to 106%.

OTTA, K.H., PAPP, E., MINCSOVICS, E. and ZARAY, G. 1998. **Determination of aflatoxins in corn by use of the personal OPLC basic system.** *JPC – Journal of Planar Chromatography – Modern TLC* **11**: 370–373.

AFB₁, B₂, G₁ and G₂ were analysed after isolation from corn by extraction with different solvents. The extracts were purified by overpressured-layer chromatography (OPLC) rather than by liquid-liquid extraction and solid-phase extraction. After preconcentration of the extracts the aflatoxins were separated using the Personal OPLC System and quantitative measurements were performed by fluorodensitometry. The OPLC technique combines the advantages of the HPLC and HPTLC and enables high sample throughput and low operating costs.

LEE, R.C. and CHU, F.S. 1999. **Production and characterization of monoclonal**

antibodies against norsolorinic acid reductase involved in aflatoxin biosynthesis. *Food and Agricultural Immunology* **11**: 29–42.

Norsolorinic acid reductase (NSR) is responsible for the conversion of norsolorinic acid to averantin in the early stage of aflatoxin biosynthesis. Using a partially purified NSR preparation as the immunogen, monoclonal antibodies (mAbs) against NSR were produced. An ELISA, using partially purified NSR as coating antigen and a second antibody-peroxidase conjugate as indicator, was established for measurement of the antibody titre and enzyme level. HPLC-postcolumn ELISA, immunoblot analysis and enzyme inhibition study revealed that a mAb elicited in cell line 10D2 specifically bound with the 43 kDa NSR. The mAb produced by 10D2 cell line was capable of partially neutralising the NSR activity and has also been used for detection of expression of the gene encoding the enzyme.

PHILLIPS, J.C., DAVIES, S. and LAKE, B.G. 1999. **Dose-response relationships for hepatic aflatoxin B₁-DNA adduct formation in the rat *in vivo* and *in vitro*: The use of immunoslot blotting for adduct quantitation.** *Teratogenesis Carcinogenesis and Mutagenesis* **19**: 157–170.

An immunoslot blotting method for quantitating AFB₁-DNA adduct levels has been developed and used to examine the relationship between dose and hepatic AFB₁-DNA adduct levels in rats fed AFB₁ in the diet at 0.5 or 10 µg/kg/day. The time course for the accumulation of AFB₁-DNA adducts in rat liver slices incubated with AFB₁ at 0.5 µM was also investigated and the relationship between adduct formation and AFB₁ concentration over a wide concentration range in liver slices was determined.

MARRAZZA, G., CHIANELLA, I. and MASCINI, M. 1999. **Disposable DNA electrochemical biosensors for environmental monitoring.** *Analytica Chimica Acta* **387**: 297–307.

Two disposable electrochemical biosensors are described. The first one is useful for the detection of the hybridisation of DNA and the second one is able to detect low molecular weight compounds with affinity for nucleic acids. The DNA biosensor is able to detect known intercalating and groove binding compounds. A detection limit of 10 mg/L was obtained for AFB₁.

BRACKLEY, M.E., DEBOER, J.G. and GLICKMAN, B.W. 1999. **Use of log-linear analysis to construct explanatory models for TDBP- and AFB₁-induced mutation spectra in *lacI* transgenic animals.** *Mutation Research – Fundamental*

and Molecular Mechanisms of Mutagenesis **425**: 55–69.

Mutation spectra recovered from *lacI* transgenic animals exposed to AFB₁ were examined using log-linear analysis. Log-linear analysis is a categorical procedure that analyses contingency table data. Expected contingency table cell counts are estimated by maximum likelihood as effects of main variables and variable interactions. Evaluation of hierarchical models of decreasing complexity indicates when significant explanatory power is lost by the sequential omission of interactions between variables. Use of this technique allows construction of the most parsimonious models to account for the mutation spectra obtained. The resulting statistical models are consistent with previous analyses of these data and with biological explanations for causes of the observed spectra.

Aflatoxicoses

ANDERSON, D., YU, T.W., HAMBLY, R.J., MENDY, M. and WILD, C.P. 1999. **Aflatoxin exposure and DNA damage in the Comet assay in individuals from the Gambia, West Africa.** *Teratogenesis Carcinogenesis and Mutagenesis* **19**: 147–155.

The single cell gel electrophoresis assay (Comet assay) was used to measure DNA damage in peripheral lymphocytes from a group of individuals from The Gambia in order to determine whether such damage could be associated with increased exposure to aflatoxin in this population. Responses obtained were correlated to responses previously obtained in a cross-sectional study in the same individuals of various cytogenetic alterations and aflatoxin-albumin adducts. A comparison was also made between The Gambian individuals and a group of volunteers in the United Kingdom where aflatoxin exposure would be expected to be low. DNA damage was not significantly higher than in the healthy United Kingdom volunteers. In addition, there were no associations between cytogenetic damage, GSTM1 genotype, age, sex, smoking and aflatoxin exposure, and Comet response at the individual level. It would appear that DNA damage as measured in the Comet assay in peripheral blood lymphocytes is not a sensitive genotoxic marker of aflatoxin exposure in this population.

ROSSANO, F., DELUNA, L.O., BUOMMINO, E., CUSUMANO, V., LOSI, E. and CATANIA, M.R. 1999. **Secondary metabolites of *Aspergillus* exert immunobiological effects on human monocytes.** *Research in Microbiology* **150**: 13–19.

The effects of AFB₁ on the release and genetic expression of some important cytokines, interleukin-1 alpha (IL-1 alpha), IL-6 and tumour necrosis factor-alpha (TNF alpha), by human monocytes was investigated. Monocytes, preincubated for different time periods with concentrations of AFB₁ ranging from 0.01 to 1.0 µg/L, were activated with bacterial LPS. Cytokine levels were measured by immunoassay and mRNA by cDNA amplification. Pretreatment of monocytes with AFB₁ resulted in a decrease in IL-1, IL-6 and TNF alpha release already at a concentration of 0.05 µg/L. AFB₁ completely blocked the transcription of IL-1 alpha, IL-6 and TNF alpha mRNAs, while it did not affect beta-actin mRNA at the concentrations used. It therefore appears that AFB₁ exerts its effect on cytokine release through selective inhibition of specific mRNA, without affecting general protein synthesis.

RASHID, A., WANG, J.S., QIAN, G.S., LU, B.X., HAMILTON, S.R. and GROOPMAN, J.D. 1999. **Genetic alterations in hepatocellular carcinomas: association between loss of chromosome 4q and p53 gene mutations.** *British Journal of Cancer* **80**: 59–66.

Genetic alterations in liver resection specimens from Shanghai and Qidong were studied. Hepatitis B virus was integrated in all patient samples, and a null phenotype for the GSTM1 enzyme was present in 63% of patients. Alteration of p53 was present in 23/24 of cases, mutations of the p53 gene in 12 HCC, p53 overexpression in 13 and loss of heterozygosity (LOH) of chromosome 17p in 17. All seven hepatocellular carcinomas (HCCs) with a p53 mutation from Qidong and 3/5 from Shanghai had the aflatoxin-associated point mutation with a G to T transversion at codon 249, position 3. No HCC had microsatellite instability. LOH of chromosome 4q, 1p, 16q and 13q was present in 50, 46, 42 and 38%, respectively, and 4q was preferentially lost in HCCs containing a p53 mutation: LOH of 4q was present in 9/12 of HCC with, but only 3/12 of HCC without, a p53 gene mutation.

DENISSENKO, M.F., CAHILL, J., KOUDRIAKOVA, T.B., GERBER, N. and PFEIFER, G.P. 1999. **Quantitation and mapping of aflatoxin B₁-induced DNA damage in genomic DNA using aflatoxin B₁-8,9-epoxide and microsomal activation systems.** *Mutation Research – Fundamental and Molecular Mechanisms of Mutagenesis* **425**: 205–211.

Quantitative PCR (QPCR) and ligation-mediated PCR (LMPCR) were used to map total AFB₁ adducts in genomic DNA treated with AFB₁-8,9-epoxide and in hepatocytes exposed to AFB₁ activated by rat liver microsomes or human liver and enterocyte

microsomal preparations. The p53 gene-specific adduct frequencies in DNA, modified in cells with 40–400 µM AFB₁, were 0.07–0.74 adducts per kb. *In vitro* modification with 0.1–4 ng AFB₁-8,9-epoxide per microgram DNA produced 0.03–0.58 lesions per kb. The adduct patterns obtained with the epoxide and the different microsomal systems were virtually identical indicating that adducts form with a similar sequence specificity *in vitro* and *in vivo*. The lesions were detected exclusively at guanines with a preference towards GpG and methylated CpG sequences. The methods utilising QPCR and LMPCR thus provide means to assess gene specific and sequence specific AFB₁ damage.

STANLEY, L.A., MANDEL, H.G., RILEY, J., SINHA, S., HIGGINSON, F.M., JUDAH, D.J. and NEAL, G.E. 1999. **Mutations associated with *in vivo* aflatoxin B₁-induced carcinogenesis need not be present in the *in vitro* transformations by this toxin.** *Cancer Letters* **137**: 173–181.

An immortalised, non-transformed liver epithelial cell line derived from a male F344 rat was transformed *in vitro* by AFB₁ as demonstrated by tumour formation in nude mice. Transfection of DNA extracted from these tumours into NM 3T3 fibroblasts conferred a stable, malignant transforming capacity. However, no mutations in codon 12 of the K-ras or codon 13 of the N-ras genes were detected in any of these tumours. These results indicate that *in vitro* transformation does not necessarily involve the same mutations as those observed *in vivo*. Also, no mutations in codon 243 or adjacent codons of the p53 gene, paralleling those observed in the human cell line treated with AFB₁, were detected. The results serve to emphasise the *in vivo* and *in vitro* variation in the oncogene activation in the same target organ or cell lines derived from that organ, even when using a single carcinogen activated by a known metabolic pathway.

ABDEL-WAHHAB, M.A., NADA, S.A. and AMRA, H.A. 1999. **Effect of aluminosilicates and bentonite on aflatoxin-induced developmental toxicity in rat.** *Journal of Applied Toxicology* **19**: 199–204.

The pregnant rat was used as an *in vivo* model to compare the potential of hydrated sodium calcium aluminosilicate (HSCAS) and bentonite to prevent the developmental toxicity of aflatoxin. HSCAS and bentonite were added to the diet at a level of 0.5% (w/w) and fed to the pregnant rat throughout pregnancy. Test animals were fed a diet containing aflatoxin at 2.5 mg/kg with or without sorbents during gestation days 6–15. Sorbents alone were not toxic and aflatoxin alone resulted in significant maternal and de-

velopmental toxicity. Animals treated with phyllosilicate plus aflatoxin were comparable to controls following evaluations for resorptions, live fetuses and foetal body weights, as well as biochemical parameters. While bentonite plus aflatoxin resulted in significant reduction in foetal body weight, none of the fetuses from HSCAS or bentonite plus aflatoxin treated groups had any gross, internal soft tissue or major skeletal malformations.

KIM, N.D. and KIM, S.G. 1999. **Chemopreventive effects of 2-(allylthio)pyrazine.** Archives of Pharmacological Research **22**: 99–107.

A series of organosulfur compounds were synthesised with the aim of developing chemopreventive compounds active against hepatotoxicity and chemical carcinogenesis. 2-(Allylthio) pyrazine (2-AP) was effective in inhibiting cytochrome P450 2E1 mediated catalytic activities and protein expression, and in inducing microsomal epoxide hydrolase and major glutathione S-transferases. Anticarcinogenic effects of 2-AP at the stage of initiation of tumours were observed in the AFB₁ induced three-step medium-term hepatocarcinogenesis model. Reduction of AFB₁-DNA adduct by 2-AP appeared to result from the decreased formation of AFB₁-8,9-epoxide via suppression of cytochrome P450, while induction of GST by 2-AP increases the excretion of glutathione-conjugated AFB₁.

SOUZA, M.F., TOME, A.R. and RAO, V.S.N. 1999. **Inhibition by the bioflavonoid ternatin of aflatoxin B₁-induced lipid peroxidation in rat liver.** Journal of Pharmacy and Pharmacology **51**: 125–129.

Ternatin, a tetramethoxyflavone isolated from *Egletes viscosa*, was investigated for possible protection against liver injury induced by AFB₁ in rats. Seventy two hours after a single ip dose of AFB₁ at 1 mg/kg, the concentration of malondialdehyde, the product of lipid peroxidation in liver homogenates, and serum levels of alanine aminotransferase and aspartate aminotransferase, were significantly elevated. Subcutaneous ternatin at 25 mg/kg pretreatment greatly reduced AFB₁ induced increases in the levels of serum enzymes and elevated malondialdehyde levels in a manner similar to oral vitamin E at 300 mg/kg, a standard antioxidant. Histological changes induced by AFB₁ such as hepatocellular necrosis and bile duct proliferation were markedly inhibited in animals pretreated with ternatin or vitamin E.

PREMALATHA, B., MUTHULAKSHMI, V. and SACHDANANDAM, P. 1999. **Anticancer potency of the milk extract of**

***Semecarpus anacardium* Linn. nuts against aflatoxin B₁ mediated hepatocellular carcinoma bearing Wistar rats with reference to tumour marker enzymes.** Phytotherapy Research **13**: 183–187.

The influence of *Semecarpus anacardium* Linn. nut milk extract on hepatocarcinogenicity of AFB₁ was evaluated in adult albino male Wistar rats. AFB₁ was administered ip to induce HCC then animals were treated with the extract at 200 mg/kg body weight/day orally for 14 days. Plasma and the liver tumour tissue were assayed for lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase. The levels of these enzymes were altered in cancer bearing animals but levels were reversed to near normal control values in extract treated animals. Results indicate the antitumour efficacy of *Semecarpus anacardium* nut milk extract on AFB₁ induced HCC.

PREMALATHA, B. and SACHDANANDAM, P. 1998. **Regulation of mineral status by *Semecarpus anacardium* Linn. nut milk extract in aflatoxin B₁-induced hepatocellular carcinoma.** Journal of Clinical Biochemistry and Nutrition **25**: 63–70.

Semecarpus anacardium Linn. nut milk extract was investigated for its modulating effect on mineral levels in experimental HCC. HCC was induced in rats by a single ip injection of AFB₁. A decreased level of sodium and increased levels of potassium, calcium and magnesium were observed in carcinoma bearing rats. Oral administration of the nut milk extract at 200 mg/kg body weight/day significantly reversed the mineral contents to near normal levels.

MOON, E.Y., RHEE, D.K. and PYO, S. 1999. **Inhibition of various functions in murine peritoneal macrophages by aflatoxin B₁ exposure *in vivo*.** International Journal of Immunopharmacology **21**: 47–58.

Various functions of murine peritoneal macrophages following treatment with AFB₁ were investigated. AFB₁ decreased phagocytosis and the production of superoxide anion and hydrogen peroxide compared to controls. In addition, the production of NO and TNF-alpha was decreased in macrophages of AFB₁ treated mice. *In vitro* antitumour activity of *in vivo* AFB₁ treated macrophages was reduced against target cell L929. These results suggested that AFB₁ might have the immunosuppressive effect on macrophages after *in vivo* exposure, which was related to the antitumour activity reduction.

BARRAUD, L., GUERRET, S., CHEVALLIER, M., BOREL, C., JAMARD, C., TREPO, C., WILD, C.P. and COVA, L. 1999. **Enhanced duck hepatitis B virus gene expression following aflatoxin B₁ exposure.** Hepatology **29**: 1317–1323.

Six weeks old chronic DHBV-carrier or uninfected ducks were exposed to AFB₁ for 5 weeks. Histological analysis showed more marked changes in the livers of AFB₁ treated ducks and these were enhanced by DHBV infection. A significant increase in serum and liver DHBV DNA level was observed in AFB₁ treated ducks compared with controls. In addition, viral RNAs, in particular the pregenomic RNA that is the template of viral replication, and intrahepatic DHBV DNA replicative intermediates, were significantly increased by AFB₁ treatment. Moreover, an overexpression and accumulation of DHBV large envelope protein was observed in the hepatocytes of AFB₁ exposed animals.

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