

## Abstracts

### Mycotoxins – General

TOURNAS, V.H. and STACK, M.E. 2001. **Production of alternariol and alternariol methyl ether by *Alternaria alternata* grown on fruits at various temperatures.** *Journal of Food Protection* **64**: 528–532.

Two toxigenic strains of *Alternaria alternata* were grown on surface disinfected, fresh, ripe fruits and tested for the production of alternariol (AOH) and alternariol methyl ether (AME). Examined fruits included strawberries, red and green seedless grapes, concord grapes, red delicious and golden delicious apples, gala apples and blueberries. After inoculation, fruits were incubated at 4, 10 or 21°C for up to 3 weeks. *A. alternata* and its metabolites were not a major problem in strawberries due to the presence of fast growing moulds that outgrew and possibly inhibited *Alternaria*. Both *Alternaria* strains showed limited growth on apples and AOH and AME were produced only by the ATCC 56836 strain on the golden delicious and gala varieties, (ranging from 0.1 to 5 mg/kg and 0.1 to 14 mg/kg for AOH and AME, respectively). Restricted growth of both strains without toxin production occurred in blueberries, whereas moderate growth and AOH (up to 3.336 mg/kg) and AME (up to 1,716 mg/kg) production took place in grapes.

THUVANDER, A., MOLLER, T., BARBIERI, H.E., JANSSON, A., SALOMONSSON, A.C. and OLSEN, M. 2001. **Dietary intake of some important mycotoxins by the Swedish population.** *Food Additives and Contaminants* **18**: 696–706.

In Sweden, approximately 600 samples of foods were collected and analysed for aflatoxins, ochratoxin A (OA), patulin and trichothecenes. Intakes were calculated for average and high consumers among adults

and children and compared with the tolerable daily intake (TDI) of the respective toxin. Mycotoxin levels in the food samples were generally below the European/national maximum limits. The intake of OA, patulin and trichothecenes was found to be below the temporary TDI values (tTDI) proposed for these toxins although the intake of trichothecenes (expressed as T-2 toxin equivalents) in children with a high consumption of cereals was close to the tTDI for T-2 toxin. Since there is to date no established numerical tTDI for aflatoxins, such a value was estimated for use within the project. The calculated intake of aflatoxins in high consumers exceeded this tTDI by a factor of two.

ESKOLA, M., PARIKKA, P. and RIZZO, A. 2001. **Trichothecenes, ochratoxin A and zearalenone contamination and *Fusarium* infection in Finnish cereal samples in 1998.** *Food Additives and Contaminants* **18**: 707–718.

A total of 68 Finnish cereal samples, including 43 rye, 4 wheat, 15 barley and 6 oats samples, were collected after a cool and very rainy growing season in 1998. Heavy rainfalls in the summer and autumn of 1998 caused abundant *Fusarium* mould infection, particularly in rye. *Fusarium avenaceum* was the most common *Fusarium* species found in cereals. Mycotoxin concentrations were low and only deoxynivalenol (DON), nivalenol (NIV) and HT-2 toxin were detected. DON was detected in 54 samples in the concentration range 5–111 µg/kg. NIV and HT-2 toxin were detected in three and two samples, respectively, in the concentration range 10–20 µg/kg.

NGOKO, Z., MARASAS, W.F.O., RHEEDER, J.P., SHEPHARD, G.S., WINGFIELD, M.J. and CARDWELL, K.F. 2001. **Fungal infection and mycotoxin contamination of maize in the**

**Humid Forest and the Western Highlands of Cameroon.** *Phytoparasitica* **29**: 352–360.

Maize samples were collected at 2 and 4 months after stocking from 72 farmers' stores in 1996 and 1997 in the Humid Forest (HF) and Western Highlands (WHL) of Cameroon. *Nigrospora* species were the most prevalent fungi in HF (32%) and WHL (30%) in 1996. *Fusarium verticillioides* (22%) and *F. graminearum* (27%) were also isolated from these samples. In 1997 the levels of fungal contamination were lower than in 1996. The incidence of *Aspergillus* species was low in general, ranging from 0 to 5.9% infected kernels. Low levels of aflatoxins were detected in a few samples. Fumonisin B<sub>1</sub> (FB<sub>1</sub>) at levels in the range 300–26,000 µg/kg, DON in the range <100–1,300 µg/kg and zearalenone in the range <50–110 µg/kg, were detected. Storage time (2 vs 4 months after stocking) had a significant positive effect on the level of FB<sub>1</sub>.

MACHINSKI, M., SOARES, L.M.V., SAWAZAKI, E., BOLONHEZI, D., CASTRO, J.L. and BORTOLLETO, N. 2001. **Aflatoxins, ochratoxin A and zearalenone in Brazilian corn cultivars.** *Journal of the Science of Food and Agriculture* **81**: 1001–1007.

Past surveys indicated that the occurrence of aflatoxins, zearalenone (ZEA) and OA was not a problem in corn and corn products in the state of Sao Paulo, Brazil. However, in recent studies, a change in pattern was observed. A total of 110 samples of freshly harvested corn, corresponding to 48 commercial cultivars planted at three different locations in the state, were assayed. Aflatoxin B<sub>1</sub> contamination was found in 60/110 samples at levels ranging from 6 to 1600 µg/kg. OA was found in two samples (206 and 128 µg/kg) and ZEA in one sample (4640 µg/kg). Possible causes of the increase in aflatoxin levels are discussed.

LOPEZ-DIAZ, T.M., SANTOS, J.A., GARCIA-LOPEZ, M.L. and OTERO, A. 2001. **Surface mycoflora of a Spanish fermented meat sausage and toxigenicity of *Penicillium* isolates.** International Journal of Food Microbiology **68**: 69–74.

The surface mycoflora of “chorizo de Cantimpalos”, a Spanish variety of fermented meat sausage characterised by a natural white covering, has been investigated. Among 54 mould strains isolated, 38 belonged to *Penicillium* subgenus *Penicillium*. The major species found was *Penicillium commune*. All *P. commune* isolates produced cyclopiazonic acid.

CURTUI, V.G., GAREIS, M., USLEBER, E. and MARTLBAUER, E. 2001. **Survey of Romanian slaughtered pigs for the occurrence of mycotoxins ochratoxins A and B, and zearalenone.** Food Additives and Contaminants **18**: 730–738.

Blood serum, kidney, liver and muscle samples were collected from slaughtered pigs in Romania. The samples were analysed for OA, OB and ZEA. The incidences of OA in kidney and liver were very similar (79 and 75%) with mean levels of 0.54 and 0.16 µg/kg, respectively. The lowest incidence (17%) and the lowest mean level contamination (0.15 µg/kg) were in muscle samples. The mean distribution in tissues followed the pattern: serum > kidney > liver > muscle (100:26:8.5:2.57%). No sample contained OA above the maximum permitted limit in Romania (5 µg/kg). ZEA was detected in 17.3% of the serum samples with a maximum concentration of 0.96 µg/L.

TANIWAKI, M.H., HOCKING, A.D., PITT, J.I. and FLEET, G.H. 2001. **Growth of fungi and mycotoxin production on cheese under modified atmospheres.** International Journal of Food Microbiology **68**: 125–133.

The use of modified atmospheres to prevent fungal growth and mycotoxin production in cheese was evaluated. Eight fungal species: *Mucor plumbeus*, *Fusarium oxysporum*, *Byssoschlamys fulva*, *B. nivea*, *Penicillium commune*, *P. roqueforti*, *Aspergillus flavus* and *Eurotium chevalieri* were inoculated onto cheese and incubated under conditions of decreasing concentrations of O<sub>2</sub> (5% to less than 0.5%) and increasing concentrations of CO<sub>2</sub> (20–40%). The formation of aflatoxins B<sub>1</sub> and B<sub>2</sub>, roquefortine C and cyclopiazonic acid was greatly decreased but not totally inhibited in these atmospheres.

TRUNG, T.S., BAILLY, J.D., QUERIN, A., LEBARS, P. and GUERRE, P. 2001. **Fungal contamination of rice from south Vietnam, mycotoxinogenesis of**

**selected strains and residues in rice.** Revue de Medecine Veterinaire **152**: 555–560.

Twenty-five samples of Vietnamese rice coming from the Mekong delta were analysed for fungal contamination. *Aspergillus* was the most important genus (43.75% of isolates) followed by *Fusarium* (21.8%) and *Penicillium* (10.9%). Isolated strains of *Aspergillus flavus*, *A. ochraceus* and *Penicillium citrinum* were tested for their toxigenic potential on either YES liquid medium or on autoclaved rice. Eighty percent of *A. flavus* strains were able to produce cyclopiazonic acid and all strains of *A. ochraceus* synthesised OA. The *P. citrinum* isolate produced citrinin. Two rice samples were found to be contaminated with OA at 21.3 and 26.2 µg/kg.

GIRIDHAR, P. and REDDY, S.M. 2001. **Phenolic antioxidants for the control of some mycotoxigenic fungi.** Journal of Food Science and Technology—Mysore **38**: 397–399.

The efficacy of three phenolic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG), in the control of eight mycotoxigenic fungal strains was investigated. BHA was found to be highly effective compared to BHT and PG, as it inhibited the vegetative growth of 7/8 fungi tested. Similarly, it inhibited secalonic acid and satratoxin production by *Penicillium purpurogenum* and *Stachybotrys atra*, respectively, at 100 mg/L, while the other fungi were inhibited at 250 mg/L. BHT completely inhibited the growth of *S. atra* at 250 mg/L. *A. terreus* produced patulin in the presence of BHT even at 1000 mg/L. PG was comparatively a mild inhibitor of mould growth and mycotoxin production.

HOVERMALE, J.T. and CRAIG, A.M. 2001. **Correlation of ergovaline and lolitrem B levels in endophyte-infected perennial ryegrass (*Lolium perenne*).** Journal of Veterinary Diagnostic Investigation **13**: 323–327.

Lolitrem B is generally considered to be the predominant alkaloid in endophyte-infected perennial ryegrass. Ergovaline, a vasoconstrictor normally associated with endophyte-infected tall fescue (*Festuca arundinacea*), is also present in endophyte infected perennial ryegrass. While clinical signs of animals ingesting endophyte-infected perennial ryegrass are consistent with the presence of lolitrem B, signs normally associated with ergovaline poisoning are not usually observed. A survey was conducted to quantitate both lolitrem B and ergovaline in 459 samples of perennial ryegrass straw. A strong positive correlation between the two alkaloids was observed,

especially in the samples containing lolitrem B at less than 3,000 µg/kg. The threshold levels above which clinical signs typically occur for lolitrem B is 2,000 µg/kg and for ergovaline 300–400 µg/kg.

CAZZANIGA, D., BASILICO, J.C., GONZALEZ, R.J., TORRES, R.L. and DEGREEF, D.M. 2001. **Mycotoxins inactivation by extrusion cooking of corn flour.** Letters in Applied Microbiology **33**: 144–147.

Samples of corn flour experimentally contaminated with aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) at 50 µg/kg and DON at 5 mg/kg were extruded and the effects of three extrusion variables, flour moisture, extrusion temperature and sodium metabisulphite addition, were analysed. The process was effective for the reduction of DON content in corn flour with higher than 95% inactivation under all the conditions assessed, but was only partially successful (10–25%) for the decontamination of AFB<sub>1</sub>.

HUWIG, A., FREIMUND, S., KAPPELI, O. and DUTLER, H. 2001. **Mycotoxin detoxication of animal feed by different adsorbents.** Toxicology Letters **122**: 179–188.

The most frequently applied method for protecting animals against mycotoxicosis is the utilisation of adsorbents mixed with the feed which are supposed to bind the mycotoxins efficiently in the gastrointestinal tract. Aluminosilicates are the preferred adsorbents, followed by activated charcoal and special polymers. This review with 73 references describes the most important types of adsorbents and the respective mechanisms of adsorption. Data of the *in vitro* and *in vivo* efficacy of detoxication are given.

BENKERROUM, S. and TANTAOU-ELARAKI, A. 2001. **Study of toxigenic moulds and mycotoxins in poultry feeds.** Revue de Medecine Veterinaire **152**: 335–342.

Seventy samples of poultry feeds including ‘starting’, mixed feed, corn, barley, wheat barn, fish-meal and soja, sunflower and colza cakes, were analysed for fungal contamination. *Penicillium* and *Aspergillus* were the dominant genera and were 35.7 and 20.4% of isolates, respectively. Three out of ten *A. flavus* isolates, were able to produce AFB<sub>1</sub> on rice. One of four *A. ochraceus* isolates and 2/14 *P. verrucosum* isolates produced OA on wheat. The toxigenesis of these strains was lower on the substrates from which they had been isolated from than on reference substrate. When cultured on a yeast extract and sucrose medium, 40 isolates including *Peni-*

*cillium*, *Aspergillus*, *Fusarium* and *Alternaria* species produced toxic metabolites in biological tests.

VAZQUEZ, B.I., FENTE, C., FRANCO, C.M., VAZQUEZ, M.J. and CEPEDA, A. 2001. **Inhibitory effects of eugenol and thymol on *Penicillium citrinum* strains in culture media and cheese.** International Journal of Food Microbiology **67**: 157–163.

The antifungal effects of eugenol and thymol on the growth and production of citrinin from *Penicillium citrinum* in culture media and in different Spanish cheeses (Arzria-Ulloa, Cebreiro and San Simon) were evaluated. Eugenol at 100 mg/L delayed citrinin production until the sixth day, after which a limiting effect persisted. In Arzria-Ulloa cheese, no citrinin was detected at a eugenol concentration of 150 mg/L, but citrinin was detected after 5 days in the case of thymol at the same concentration. In Cebreiro cheese, neither eugenol nor thymol prevented the production of citrinin at the concentrations applied.

## Mycotoxicoeses

KUBENA, L.F., BAILEY, R.H., BYRD, J.A., YOUNG, C.R., CORRIER, D.E., STANKER, L.H. and ROTTINGHAUS, G.E. 2001. **Cecal volatile fatty acids and broiler chick susceptibility to *Salmonella typhimurium* colonization as affected by aflatoxins and T-2 toxin.** Poultry Science **80**: 411–417.

In day-of-hatch broiler chicks the effects of aflatoxins and T-2 toxin on caecal volatile fatty acids (VFA) and the susceptibility to *Salmonella* colonisation were examined. Chicks were challenged orally with *Salmonella typhimurium* on Day 3. In chicks fed diets containing aflatoxins at 2.5 or 7.5 mg/kg of diet and allowed to develop their microflora naturally, there were no treatment effects on caecal propionic acid, total VFA or incidence or severity of *Salmonella* colonisation. In chicks orally gavaged on the day of hatch with a competitive exclusion (CE) culture and fed diets containing T-2 toxin at 2.5 or 7.5 mg/kg there was a significant reduction in total VFA at 5 days in chicks fed the 2.5 mg T-2 toxin. No significant treatment differences were observed for numbers of *Salmonella* caecal culture-positive chicks or for numbers of *Salmonella* in the caecal contents. In chicks orally gavaged with CE and fed diets containing T-2 toxin at 15.0 mg/kg, chicks showed a decrease in propionic acid concentration at 3 and 11 days and an increase in susceptibility to *Salmonella* colonisation.

PELTOLA, J., ANDERSSON, M.A., HAAHTELA, T., MUSSALO-RAUHAMAA, H., RAINEY, F.A., KROPENSTEDT, R.M., SAMSON, R.A. and SALKINOJA-SALONEN, M.S. 2001. **Toxic-metabolite-producing bacteria and fungus in an indoor environment.** Applied and Environmental Microbiology **67**: 3269–3274.

Microbes emitting toxic metabolites were isolated from the indoor environment of a building where the occupant was suffering serious building-related health symptoms. Toxic substances soluble in methanol and inhibitory to spermatozoa at less than 10 mg/L were found from six bacterial isolates and one fungus. The substances from the fungal isolate, *Trichoderma harzianum*, damaged the cell membrane barrier function of sperm cells.

TROUT, D., BERNSTEIN, J., MARTINEZ, K., BIAGINI, R. and WALLINGFORD, K. 2001. **Bioaerosol lung damage in a worker with repeated exposure to fungi in a water-damaged building.** Environmental Health Perspectives **109**: 641–644.

The case is reported of a worker with a respiratory illness related to bioaerosol exposure in a water-damaged building with extensive fungal contamination. Mycotoxin-specific IgG antibody was used in serologic studies in the attempt to evaluate exposure to mycotoxins. The serologic test was not useful in differentiating workers who were probably occupationally exposed to mycotoxins from those who were not; however, the studies did yield evidence that individuals may make specific IgE antibodies to macrocyclic trichothecene mycotoxins.

MEKY, F.A., HARDIE, L.J., EVANS, S.W. and WILD, C.P. 2001. **Deoxyvalenol-induced immunomodulation of human lymphocyte proliferation and cytokine production.** Food and Chemical Toxicology **39**: 827–836.

The effects of DON, T-2 toxin, AFB<sub>1</sub> and FB<sub>1</sub> on human lymphocyte proliferation was evaluated using the MTT assay. Additionally, the effect of DON on cytokine profiles was measured. A 50% inhibition in cell proliferation was observed with a DON concentration of 216 µg/L. T-2 toxin was more potent with 50% inhibition between 1 and 5 µg/L. Negligible effects were observed with AFB<sub>1</sub> and FB<sub>1</sub> and a mixture of DON with either FB<sub>1</sub> or AFB<sub>1</sub> did not show any synergistic effects in this assay. Short-term treatment of PHA-stimulated lymphocytes with DON at 100, 200 and 400 µg/L modulated the kinetics of IL-2, IL-4 and IL-6 production. IL-2 levels were up to 12-fold higher at toxin concentrations of 200 and 400 µg/L 72 hr after treatment. IL-4 levels were only

slightly elevated and IL-6 levels were slightly inhibited by these DON concentrations. The kinetics of cytokine production was followed for an extended period of 8–9 days at DON concentrations of 200 and 400 µg/L. With DON at 200 µg/L, IL-2 levels were elevated 17- to 25-fold with a concomitant mild elevation in IFN-gamma, while IL-6 levels were slightly suppressed. At 400 µg/L, IL-2 levels were again significantly elevated until 6 days post treatment, while the effects on IL-4 and IL-6 were less marked.

CHASSEUR, C., SUETENS, C., MICHEL, V., MATHIEU, F., BEGAUX, F., NOLARD, N. and HAUBRUGE, E. 2001. **A 4-year study of the mycological aspects of Kashin-Beck disease in Tibet.** International Orthopaedics **25**: 154–158.

In order to clarify the association between mycotoxin-producing fungi in food and Kashin-Beck disease (KBD), the occurrence and contamination levels of fungi in samples of barley grain from KBD affected families and from unaffected families in endemic areas in Tibet was examined. In 1995, *Trichothecium roseum*, *Dreschlera* and *Alternaria* were the three most common fungi significantly associated with KBD. In 1996 there was again a significant difference between affected and non-affected families, especially in relation to *T. roseum* and *Ulocladium* occurrence.

HAUBRUGE, E., CHASSEUR, C., DEBOUCK, C., BEGAUX, F., SUETENS, C., MATHIEU, F., MICHEL, V., GASPAS, C., ROOZE, M., HINSENKAMP, M., GILLET, P., NOLARD, N. and LOGNAY, G. 2001. **The prevalence of mycotoxins in Kashin-Beck disease.** International Orthopaedics **25**: 159–161.

A survey of barley grains of KBD-affected families and non-affected families in Tibet found a hitherto unknown metabolite of an *Alternaria* species which was especially common on the barley grains of KBD-affected families.

SUDRE, P. and MATHIEU, F. 2001. **Kashin-Beck disease: From etiology to prevention or from prevention to etiology?** International Orthopaedics **25**: 175–179.

A review with 30 references. The aetiology of KBD remains elusive. Four factors have been convincingly associated with the disease: selenium deficiency, iodine deficiency, grain contamination with mycotoxin-producing fungi, and water pollution with organic material and fulvic acid. Although none of the competing theories prevails when they are compared using a predefined and standard set of causality cri-

teria none should be discounted. This is an indication that a comprehensive and unifying theory is most likely to be multifactorial.

SUETENS, C., MORENO-REYES, R., CHASSEUR, C., MATHIEU, F., BEGAUX, F., HAUBRUGE, E., DURAND, M.C., NEVE, J. and VANDERPAS, J. 2001. **Epidemiological support for a multifactorial aetiology of Kashin-Beck disease in Tibet.** *International Orthopaedics* **25**: 180–187.

A cross-sectional study in 12 rural villages was carried out in order to identify the risk factors for KBD in Tibet. A total of 575 children aged 5–15 years were examined and their corresponding houses were visited. Samples were collected in order to study fungal contamination of stored grain and the organic matter content of drinking water. The degree of fungal contamination of barley grain was related to the highest percentage of cases (65%) in a sample of the study population. The results support the hypothesis that KBD occurs as a consequence of oxidative damage to cartilage and bone cells when associated with decreased antioxidant defence. Another mechanism that may coexist is bone remodelling stimulated by thyroid hormones whose actions can be blocked by certain mycotoxins.

KIHARA, T., SURJONO, T.W., SAKAMOTO, M., MATSUO, T., YASUDA, Y. and TANIMURA, T. 2001. **Effects of prenatal rubratoxin B exposure on behaviors of mouse offspring.** *Toxicological Sciences* **61**: 368–373.

The effects of prenatal rubratoxin B (RB) exposure on eight behavioural parameters in Jc1:ICR mice were assessed. Pregnant mice were injected ip with RB at 0.1 or 0.2 mg/kg/day on days 7–9 (group A) or 10–12 (group B) of gestation. The preweaning offspring in the 0.2 mg/kg B group showed significantly lower success rates and longer response times than controls in the cliff-avoidance response. In swimming development, the offspring in the 0.2 mg/kg B group had significantly lower scores than controls for swimming angle at 10 and 12 days of age. The avoidance learning of the mice in all RB exposed groups was significantly poorer than that of controls. These results indicate that prenatal exposure to RB produced a delay of early response development and impaired learning ability in the offspring of mice exposed to RB during middle pregnancy.

## Alternaria Toxins

DAMOTTA, S. and SOARES, L.M.V. 2001. **Survey of Brazilian tomato products for alternariol, alternariol monomethyl ether, tenuazonic acid and cyclopiazonic acid.** *Food Additives and Contaminants* **18**: 630–634.

Tomato products processed and sold in Brazil were surveyed for alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TA) and cyclopiazonic acid (CPA). Tomato products included 11 juice, 22 pulp, 22 puree, 24 paste and one whole stewed tomato. TA was found in seven samples of tomato pulp at 39–111 µg/kg and four samples of tomato puree at 29–76 µg/kg. CPA was found in six samples of pulp at 64–178 µg/kg and two samples of puree at 36–117 µg/kg. This is the first time that CPA has been reported in tomato products. Neither AME nor AOH were detected in the samples.

JOHNSON, L.J., JOHNSON, R.D., AKAMATSU, H., SALAMIAH, A., OTANI, H., KOHMOTO, K. and KODAMA, M. 2001. **Spontaneous loss of a conditionally dispensable chromosome from the *Alternaria alternata* apple pathotype leads to loss of toxin production and pathogenicity.** *Current Genetics* **40**: 65–72.

The *Alternaria alternata* apple pathotype causes *Alternaria* blotch of susceptible apple cultivars through the production of a cyclic peptide, host-specific toxin, AM-toxin. A cyclic peptide synthetase gene, AMT, has been cloned whose product catalyses the production of AM-toxin and has been shown to reside on chromosomes of 1.8 Mb or less, depending on the *A. alternata* apple pathotype strain. Contour-clamped homogeneous electric field electrophoresis and Southern hybridisation demonstrated the loss of a 1.1-Mb chromosome in the non-toxin-producing isolate. Since this chromosome can be entirely lost without affecting growth, but is necessary for pathogenicity, it is proposed that this is a conditionally dispensable chromosome.

SCHRADER, T.J., CHERRY, W., SOPER, K., LANGLOIS, I. and VIJAY, H.M. 2001. **Examination of *Alternaria alternata* mutagenicity and effects of nitrosylation using the Ames *Salmonella* Test.** *Teratogenesis Carcinogenesis and Mutagenesis* **21**: 261–274.

The mutagenic effects of nitrosylation were examined with the major *Alternaria* metabolites altenuene (ALT), AOH, AME, alt毒素 1 (ATX 1), tentoxin (TENT), TA, and radicinin (RAD) using the Ames *Salmo-*

*nella* strains TA98 and TA100. In the absence of nitrosylation, ATX I was mutagenic when tested at 1–100 µg/plate in TA98 with rat liver S9 for activation, while AOH and ATX I were weakly mutagenic +/-S9 in TA100. Incubation with nitrite generally increased mutagenic potencies with ATX I strongly mutagenic +/-S9 in both TA98 and TA100, while ALT, AOH, AME and RAD responses were enhanced in TA100 + S9. However, while nitrosylation increases the mutagenicity of ATX I, and to a lesser extent that of several other *Alternaria* toxins, the results demonstrate that *Alternaria* produces a major mutagenic activity with a *S. typhimurium* response different from that found with the purified toxins.

## Patulin

LEGGOTT, N.L., SHEPHARD, G.S., STOCKENSTROM, S., STAAL, E. and VANSCHALKWYK, D.J. 2001. **The reduction of patulin in apple juice by three different types of activated carbon.** *Food Additives and Contaminants* **18**: 825–829.

Three types of activated carbon were investigated for their ability to reduce patulin levels in apple juice at various Brix levels and temperatures. The steam-activated carbons, NORIT SA 4 and NORIT SX 4, exhibited similar adsorption isotherms at a dosage level of 1 g/L. They achieved patulin reductions of 80 and 70% respectively in 12°Brix juice at 55°C. Chemically-activated carbon, NORIT CA 1, was less effective, achieving only a 45% reduction at a dose of 1 g/L. Patulin removal was influenced by juice Brix in that higher carbon doses were required at higher Brix levels for equivalent removal efficiency.

BISSESSUR, J., PERMAUL, K. and ODHAV, B. 2001. **Reduction of patulin during apple juice clarification.** *Journal of Food Protection* **64**: 1216–1219.

The effectiveness of several clarification processes for the reduction of patulin in apple juice were evaluated. Clarification was carried out on a laboratory scale. Apple pulp was spiked with patulin, pressed and clarified using four different processes: fining with bentonite, enzyme (pectinase) treatment, paper filtration and centrifugation. Pressing followed by centrifugation resulted in an average toxin reduction of 89%. Total toxin reduction using filtration, enzyme treatment and fining were 70, 73 and 77%, respectively. Patulin reduction was due to the binding of the toxin to solid substrates which was verified by analysing the clarified juice as well as the filter cake, pellet and sediment.

KRYGER, R.A. 2001. **Volatility of patulin in apple juice.** Journal of Agricultural and Food Chemistry **49**: 4141–4143.

A simple model of the apple juice concentration process was carried out to examine the possible contamination of patulin in apple aroma, a distillate produced commercially in the concentration of apple juice. The results showed no evidence for patulin volatility. A reduction in patulin content by at least a factor of 250 in the apple distillate was observed. A survey of several commercial apple aroma samples found no evidence of patulin content.

AKE, M., EBA, B., MALAN, A.K. and ATINDEHOU, E. 2001. **[Liquid chromatographic determination of patulin in fruit juices sold in Côte d'Ivoire].** Sciences des Aliments **21**: 199–206.

An HPLC method was developed for the determination of patulin in fruit juices sold in Côte d'Ivoire. Following extraction with organic solvent, patulin was resolved with a C<sub>18</sub> reversed phase-phase column followed by UV detection. Application of the method to the analysis of three manufactured types of fruits juices showed that 18% of samples contained patulin. (In French).

## Ochratoxins – General

SKAUG, M.A., EDUARD, W. and STORMER, F.C. 2001. **Ochratoxin A in airborne dust and fungal conidia.** Mycopathologia **151**: 93–98.

The exposure of farm workers to OA through inhalation of organic dust and conidia was investigated. Dust and aerosol samples were collected from three cowsheds in Norway. A method was developed for extraction and determination of OA in small samples of settled dust. OA was extracted with a mixture of methanol, chloroform, HCl and water, purified on immunoaffinity column and analysed by ion-pair HPLC with fluorescence detection. OA was found in 6/14 settled dust samples at levels in the range 0.2–70 µg/kg. Conidia collected from cultures of *Penicillium verrucosum* and *Aspergillus ochraceus* contained OA at 0.4–0.7 and 0.02–0.06 pg/conidium, respectively. Testing of conidial extracts from these fungi in a *Bacillus subtilis* bioassay indicated the presence of toxic compounds in addition to OA.

ABARCA, M.L., ACCENSI, F., BRAGULAT, M.R. and CABANES, F.J. 2001. **Current importance of ochratoxin A-producing *Aspergillus* spp.** Journal of Food Protection **64**: 903–906.

A review with 52 references. OA contamination of commodities such as cereals or pork and poultry meat is well recognised.

OA has now been reported in a range of food commodities including coffee, wine, beer and milk. OA was believed to be produced only by *Aspergillus ochraceus* and closely related species of section *Circumdati* and by *Penicillium verrucosum*. However, OA has now been clearly established as a metabolite of different species of the *Aspergillus* section *Nigri*, such as *A. niger* and *A. carbonarius*.

VARGA, J., RIGO, K., TEREN, J. and MESTERHAZY, A. 2001. **Recent advances in ochratoxin research. I. Production, detection and occurrence of ochratoxins.** Cereal Research Communications **29**: 85–92.

This review with 53 references deals with ochratoxin producing organisms, ochratoxin detection methods, and occurrence and legislation of ochratoxins in foods and feeds.

VARGA, J., RIGO, K., TEREN, J. and MESTERHAZY, A. 2001. **Recent advances in ochratoxin research. II. Biosynthesis, mode of action and control of ochratoxins.** Cereal Research Communications **29**: 93–100.

This review with 84 references deals with the chemistry, biosynthesis, metabolism and mode of action of OA. Possible strategies for controlling ochratoxin levels in feeds and foods, and preventing toxic effects of OA in animals and humans are also discussed.

URBANO, G.R., TANIWAKI, M.H., LEITAO, M.F.D. and VICENTINI, M.C. 2001. **Occurrence of ochratoxin A-producing fungi in raw Brazilian coffee.** Journal of Food Protection **64**: 1226–1230.

OA-producing fungi were identified in coffee at different stages of maturation and OA was quantified in coffee during terrace drying and in coffee stored in barns. A high level of contamination (100%) was found in the coffee beans studied, with the genus *Aspergillus* representing 33.2% of isolates, of which *Aspergillus ochraceus* and *Aspergillus niger* represented 10.3 and 22.9%, respectively. Among 155 strains of *A. ochraceus* and *A. niger*, the capacity to produce ochratoxin was demonstrated in 88.1 and 11.5% of strains, respectively. Analysis for OA in the terrace and barn coffee samples showed that, independent of cultivar, year harvested or production region, all except one of the samples analysed showed OA levels below the limit suggested by the European Common Market.

THIRUMALA-DEVI, K., MAYO, M.A., REDDY, G., EMMANUEL, K.E., LARONDELLE, Y. and REDDY, D.V.R.

2001. **Occurrence of ochratoxin A in black pepper, coriander, ginger and turmeric in India.** Food Additives and Contaminants **18**: 830–835.

OA contamination of black pepper, coriander seeds, powdered ginger and turmeric powder was estimated using indirect competitive ELISA. Samples were extracted with 0.5% KCl in 70% methanol and diluted in phosphate-buffered saline containing 0.05% Tween 20 and 0.2% bovine serum albumin. ELISA results were compared with those made by HPLC. All estimates were within 1–2 standard deviations of the ELISA values. More than 90% of OA added to spice samples was recovered from samples containing between 5 and 100 µg/kg. In 126 samples obtained from retail shops, OA was found to exceed 10 µg/kg in 14/26 black pepper samples, 20/50 coriander samples, 2/25 ginger samples and 9/25 turmeric samples. This is the first record in India of the occurrence of OA in what are some of the most widely used spices in Indian cooking.

KUMAR, S., and ROY, A.K. 2000. **Occurrence of ochratoxin in maize grains. A report.** National Academy Science Letters—India **23(7–8)**: 101–103.

Ochratoxins (A and B) contamination in two commonly cultivated maize varieties, *Zea mays* var. Diara composite and var. Suwan composite, grown in the gangetic plains of Bihar, India, were investigated. Twenty-five percent of samples of var. Diara composite and 36.3% of var. Suwan composite showed OA contamination, which ranged from 0.06–0.74 and 0.09–0.87 mg/kg, respectively. OB was present in both the varieties.

BAXTER, E.D., SLAIDING, I.R. and KELLY, B. 2001. **Behavior of ochratoxin A in brewing.** Journal of the American Society of Brewing Chemists **59**: 98–100.

Malt containing OA at up to 52 µg/kg was prepared on a pilot scale by inoculating malting barley with *Penicillium verrucosum*. This malt was then used for pilot brewing trials to determine the fate of OA during the brewing process. Substantial losses, up to 40% of the OA in the grist, were observed during mashing, most probably by proteolytic degradation. Another 16% was eliminated with the spent grains. Overall, between 13 and 32% of the OA present in the original grist survived into the beer.

CASTELLARI, M., VERSARI, A., FABIANI, A., PARPINELLO, G.P. and GALASSI, S. 2001. **Removal of ochratoxin A in red wines by means of**

**adsorption treatments with commercial fining agents.** *Journal of Agricultural and Food Chemistry* **49**: 3917–3921.

A variety of fining agents, including activated carbon, silica gel, potassium caseinate, egg albumin and gelatine, was evaluated for their abilities to remove OA in fortified wines. Freundlich adsorption isotherms were used to model the adsorption behaviour between OA and the fining agent. Potassium caseinate and activated carbon were the most effective for removing OA from wine. Potassium caseinate removed up to 82% of OA when used at 150 g/hL, whereas activated carbon showed the highest specific adsorption capacity due to a high surface area per mass and low adsorption of total polyphenols.

PIETRI, A., BERTUZZI, T., PALLARONI, L. and PIVA, G. 2001. **Occurrence of ochratoxin A in Italian wines.** *Food Additives and Contaminants* **18**: 647–654.

A total of 96 red wines and 15 white dessert wines produced mostly in the years 1995–97 in 19 Italian regions were analysed for OA. The amount of OA ranged from less than 1 to 3856 ng/L, with medians (means) of 90 (419) ng/L for red wines and 8 (736) ng/L for white dessert wines. The geographic region of origin had a strong influence on OA contamination, both for red and for dessert wines with wines produced in southern Italy markedly more contaminated. OA concentrations of more than 1000 ng/L were found in 4/5 samples from southern Italy (1185, 2454, 3477, 3856 ng/L), while central and northern samples showed very low contamination.

FILALI, A., OUAMMI, L., BETBEDER, A.M., BAUDRIMONT, I., SOULAYMANI, R., BENAYADA, A. and CREPPY, E.E. 2001. **Ochratoxin A in beverages from Morocco: A preliminary survey.** *Food Additives and Contaminants* **18**: 565–568.

Samples of Moroccan wines, beers and fruit juices were assayed for OA. All 30 wine samples were contaminated and the overall median concentration was 0.65 µg/L (range 0.028–3.24 µg/L). One of 14 samples of fruit juices was contaminated with a concentration of 1.16 µg/L, and 0/5 beer samples was contaminated.

LARSEN, T.O., SVENDSEN, A. and SMEDSGAARD, J. 2001. **Biochemical characterization of ochratoxin A-producing strains of the genus *Penicillium*.** *Applied and Environmental Microbiology* **67**: 3630–3635.

Forty-eight *Penicillium verrucosum* isolates were screened for the production of secondary metabolites in order to explore

the biochemical scope of OA producing penicillia. By use of average linking clustering based on binary (non-volatile) metabolite data, the 48 isolates could be grouped into two large and clearly separated groups and a small outlying group of four non-ochratoxin producing isolates. The largest group, containing 24 isolates, mainly originating from plant sources, included the type culture of *P. verrucosum*. These isolates produced OA, verrucolones, citrinin and verrucines and had a characteristic dark brown reverse colour on yeast extract-sucrose agar medium. Almost all of a group of 20 isolates originating mainly from cheese and meat products had a pale cream reverse colour on yeast extract-sucrose agar medium and produced OA, verrucolones, anacines and sclerotigenin. This group included the former type culture of *P. nordicum*.

## Ochratoxins – Methodology

CURTUI, V.G. and GAREIS, M. 2001. **A simple HPLC method for the determination of the mycotoxins ochratoxin A and B in blood serum of swine.** *Food Additives and Contaminants* **18**: 635–643.

A simple method for the determination of OA and OB in pig blood serum is described. The method includes serum acidification (pH <1.6) and precipitation of protein with 15% trichloroacetic acid, liquid partitioning with dichloromethane and fluorescence detection. The estimated detection limits for OA and OB were 0.1 and 0.2 µg/L, respectively. The mean recoveries from samples spiked at 0.3, 1 and 3 µg/L, were 86.8% for OA and 90.0% for OB. Forty-nine of 52 Romanian pig blood serum samples were found to be naturally contaminated with OA in the range 0.1–13.4 µg/L. No sample was found positive for OB.

SOLEAS, G.J., YAN, J. and GOLDBERG, D.M. 2001. **Assay of ochratoxin A in wine and beer by high-pressure liquid chromatography photodiode array and gas chromatography mass selective detection.** *Journal of Agricultural and Food Chemistry* **49**: 2733–2740.

Two new methods were developed and evaluated for the routine assay of OA in wines and beers. The first utilised solid-phase extraction on a C<sub>18</sub> cartridge to achieve a 100-fold sample concentration followed by HPLC on a C<sub>18</sub> column with gradient elution and quantitation by means of a photodiode array detection. The limits of detection (LOD) and quantitation (LOQ) are 0.05 and 0.10 µg/L, respectively. With a throughput of 35 assays per working day, this method is ideal for routine OA analysis. A total of 942 wines and 107 beers were

surveyed using this method. OA was detected more frequently in red than white wines, with the highest incidence in red wines from Spain and Argentina. Among the beers analysed there was no association between OA and country of origin or beverage type. The second method utilised gas chromatography with mass selective detection monitoring eight specific ions, preceded by extraction in dichloromethane and derivatisation with bis[trimethylsilyl]trifluoroacetamide. The method is not suitable for routine quantitation but is potentially useful as a confirmatory tool for samples with OA levels of 0.1 µg/L or higher.

JIMENEZ, A.M., DECERAIN, A.L., GONZALEZ-PENAS, E. and BELLO, J. 2001. **Determination of ochratoxin A in pig liver-derived pâtés by high-performance liquid chromatography.** *Food Additives and Contaminants* **18**: 559–563.

A solid phase extraction procedure followed by HPLC analysis with fluorescence detection has been developed for determining OA in pâtés derived from pig liver. After extraction of OA with 60% acetonitrile, samples were purified through C<sub>8</sub> columns. The percentage recovery was 85.7% and the lower limits calculated for accurate detection and quantitation were 0.56 µg/kg and 0.84 µg/kg, respectively. The HPLC procedure showed good linearity for OA in the range 0.84–3 µg/kg.

## Ochratoxicoses

PUNTARIC, D., BOSNIR, J., SMIT, Z., SKES, I. and BAKLAIC, Z. 2001. **Ochratoxin A in corn and wheat: Geographical association with endemic nephropathy.** *Croatian Medical Journal* **42**: 175–180.

Ninety-two wheat and 51 corn samples from near Slavonski Brod, the area of endemic nephropathy were surveyed for OA contamination. OA was present in 76% of wheat samples and 33% of corn samples at concentrations in the range 0.02–160.00 and 0.02–40.00 µg/kg, respectively. For wheat, the highest level of OA (38.8+/-27.2 µg/kg) was in samples from the Slavonski Brod surroundings followed by Osijek (8.7+/-8.3 µg/kg). For corn, OA concentrations were highest (20.0+/-14.8 µg/kg) in samples from the Slavonski Brod surroundings and considerably lower in samples from other regions. These data clearly demonstrate the geographical overlap between endemic nephropathy and OA.

ARLT, V.M., PFOHL-LESZKOWICZ, A., COSYNS, J.P. and SCHMEISER, H.H. 2001. **Analyses of DNA adducts formed by ochratoxin A and aris-**

**tolochic acid in patients with Chinese herbs nephropathy.** Mutation Research—Genetic Toxicology and Environmental Mutagenesis **494**: 143–150.

Chinese herbs nephropathy (CHN) is a unique type of nephropathy has been associated with the intake of weight-reducing pills containing the Chinese herb *Aristolochia fangchi*. Moreover, an association between the use of *A. fangchi* and urothelial cancer in CHN patients has been reported indicating that aristolochic acid, the major alkaloid of *A. fangchi* might be the causal agent. Similarities of CHN to Balkan endemic nephropathy have led to the hypothesis that OA might be a common etiological agent for both diseases. However, results presented here demonstrate that OA-related DNA adducts do not play a key role in CHN or CHN-associated urothelial cancer.

DIETRICH, D.R., O'BRIEN, E., STACK, M.E. and HEUSSNER, A.H. 2001. **Species- and sex-specific renal cytotoxicity of ochratoxin A and B in vitro.** Experimental and Toxicologic Pathology **53**: 215–225.

Four different cell models were chosen for comparison of OA and OB toxicity: primary porcine (PKC), rat (RPTC) and human renal proximal epithelial cells (HKC) from both sexes and a porcine renal cell line, LLC-PK1. Culture conditions were tested and optimised for each respective cell type (species/sex and origin). Due to possible sensitivity differences, three simple endpoints were chosen to provide basic data for interspecies comparison: neutral red uptake, MTT reduction and cell number. Sex differences were observed between male (m) and female (f) HKC cells following 96 hr exposure to OA, with HKC(m) being more sensitive than HKC(f). No sex-difference was observed in PKC cells, however, the PKC were approximately 3- and 10-times more sensitive than HKC(m) and HKC(f), respectively, to OA and OB. Overall, HKC(m) were nearly as sensitive as PKC towards OA, followed by RPTC, LLC-PK1 and HKC(f).

BAUDRIMONT, I., SOSTARIC, B., YENOT, C., BETBEDER, A.M., DANO-DJEDJE, S., SANNI, A., STEYN, P.S. and CREPPY, E.E. 2001. **Aspartame prevents the karyomegaly induced by ochratoxin A in rat kidney.** Archives of Toxicology **75**: 176–183.

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a structural analogue of both OA and phenylalanine. The effects of aspartame on OA-induced nephrotoxic and genotoxic effects, mainly the karyomegaly, in rat kidney *in vivo* was investigated. Aspartame

at 25 mg/kg body weight (bw) prevented most of the nephrotoxic effects induced by OA at 289 µg/kg bw. It also showed some utility in preventing morphological and histological damage, mainly the karyomegaly.

STANDER, M.A., NIEUWOUDT, T.W., STEYN, P.S., SHEPHARD, G.S., CREPPY, E.E. and SEWRAM, V. 2001. **Toxicokinetics of ochratoxin A in vervet monkeys (*Cercopithecus aethiops*).** Archives of Toxicology **75**: 262–269.

Female vervet monkeys were treated iv with OA at 0.8, 1.5 and 2 mg/kg bw. Blood and urine samples were collected over a period of 21 days. The clearance of OA from plasma followed a two-compartment model. The elimination half-life of OA was determined to be 19–21 days and the average total body clearance was 0.22±0.07 ml/h per kg, and the average apparent distribution volume of the central compartment was 59±9 ml/kg and the peripheral compartment was 59±20 ml/kg. No evidence was found for any metabolic conversion of OA.

### Aflatoxins and Fumonisin – Co-occurrence

LI, F.Q., YOSHIKAWA, T., KAWAMURA, O., LUO, X.Y. and LI, Y.W. 2001. **Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China.** Journal of Agricultural and Food Chemistry **49**: 4122–4126.

A comparative study on the natural occurrence of aflatoxins and *Fusarium* toxins was conducted with corn samples from high- and low-incidence areas for human primary hepatocellular carcinoma in Guangxi, People's Republic of China. In samples from the high-risk area, AFB<sub>1</sub> was the predominant toxin detected in terms of quantity and frequency, with its concentration ranging between 9 and 2496 µg/kg and an 85% incidence of contamination. The average daily intake of AFB<sub>1</sub> from corn in the high-risk area was 184.1 µg, and the probable daily intake is estimated to be 3.68 µg/kg bw/day, 3.20-times the TD<sub>50</sub> in rats. Corn samples from both areas were simultaneously contaminated with FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>.

ONO, E.Y.S., ONO, M.A., FUNO, F.Y., MEDINA, A.E., OLIVEIRA, T.C.R.M., KAWAMURA, O., UENO, Y. and HIROOKA, E.Y. 2001. **Evaluation of fumonisin-aflatoxin co-occurrence in Brazilian corn hybrids by ELISA.** Food Additives and Contaminants **18**: 719–729.

The natural co-occurrence of fumonisins and aflatoxins was investigated in 150 freshly harvested corn kernels samples acquired from three regions of the State of Parana, Brazil. Fumonisin were detected in 98% of samples at concentrations in the range 0.096–22.6 mg/kg, while aflatoxins were detected in 11.3% of samples. All the aflatoxin-positive samples (range 38–460 µg/kg) came from the Central-Western region and were co-contaminated with fumonisins. Fumonisin contamination was higher in corn from the Northern and Central-Western regions when compared with the Central-Southern region.

MARIN, S., ALBAREDA, X., RAMOS, A.J. and SANCHIS, V. 2001. **Impact of environment and interactions of *Fusarium verticillioides* and *Fusarium proliferatum* with *Aspergillus parasiticus* on fumonisin B<sub>1</sub> and aflatoxins on maize grain.** Journal of the Science of Food and Agriculture **81**: 1060–1068.

*Fusarium verticillioides* and *F. proliferatum* isolates were inoculated in mixed cultures with *Aspergillus parasiticus* on irradiated maize grain and incubated under conditions of 0.93–0.98 water activity (a<sub>w</sub>) and 15 and 25°C for 28 days. A complex relationship was found between a<sub>w</sub>, temperature, inoculum concentration and the interactions which took place between fumonisin and aflatoxin producers. In general, *A. parasiticus* reduced *F. verticillioides* and *F. proliferatum* populations (by 6–36%) but did not affect FB<sub>1</sub> production by these species. In contrast, while the *Fusarium* species were not able to decrease *A. parasiticus* populations, they significantly reduced AFB<sub>1</sub> accumulation (by 30–93%).

POZZI, C.R., CORREA, B., XAVIER, J.G., DIREITO, G.M., ORSI, R.B. and MATARAZZO, S.V. 2001. **Effects of prolonged oral administration of fumonisin B<sub>1</sub> and aflatoxin B<sub>1</sub> in rats.** Mycopathologia **151**: 21–27.

Male Wistar rats were fed AFB<sub>1</sub> at 0 or 72 mg/kg bw, either singly or in combination with FB<sub>1</sub> at 5 or 15 mg/kg bw for 21 days. Rats fed with the higher doses of AFB<sub>1</sub> and FB<sub>1</sub> gained less weight and blood concentrations of liver enzymes aspartate aminotransferase and alkaline phosphatase were above control levels. Blood cholesterol increased in the groups treated with the highest dose of FB<sub>1</sub> or FB<sub>1</sub> associated with AFB<sub>1</sub>. Histopathology revealed the occurrence of apoptosis in the liver of rats exposed to FB<sub>1</sub>. The association of AFB<sub>1</sub> with FB<sub>1</sub> at higher dose probably potentiated the effects of the higher dose of FB<sub>1</sub> acting singly.

## Fumonisin – General

HUMPHREYS, S.H., CARRINGTON, C. and BOLGER, M. 2001. **A quantitative risk assessment for fumonisins B<sub>1</sub> and B<sub>2</sub> in US corn.** Food Additives and Contaminants **18**: 211–220.

This analysis for exposure to fumonisin describes the magnitude of adverse effects, variability in the population and uncertainty of models as a range of possible outcomes. The most sensitive adverse response in rats, nephrotoxic lesions, was used for the dose-response analysis. Dietary intake of corn products was estimated from a 3-day consumption survey. Levels of corn in each product were estimated by standard methods. Fumonisin levels in corn products were estimated from Food and Drug Administration surveillance data and distributions of fumonisin consumption were modelled for each eater in the survey population. Results of the dose-response and exposure analyses were assimilated in a two dimensional Monte-Carlo simulation. On the basis of this analysis, current dietary levels of fumonisin would not result in renal lesions even at upper levels of exposure.

DUVICK, J. 2001. **Prospects for reducing fumonisin contamination of maize through genetic modification.** Environmental Health Perspectives **109**: 337–342.

Among the strategies for reducing risk of fumonisin contamination in maize supplied to the market, the development and deployment of *Fusarium* ear mould resistant maize germplasm is a high priority. Breeding for increased ear mould tolerance and reduced mycotoxin levels is being practiced today but the amount of resistance achievable may be limited due to complicated genetics and/or linkage to undesirable agronomic traits. Transgenic approaches to ear mould/mycotoxin resistance are now feasible as well. One example is already on the market, namely transgenic maize expressing *Bacillus thuringiensis* Bt toxin targeted to the European corn borer. A second approach, transgene mediated control of the ability of *Fusarium* to infect and colonise the ear, could potentially be achieved through over-expression of specific antifungal proteins and metabolites, or enhancement of the plant's own defence systems in kernel tissues. A third approach is transgene strategies aimed at preventing mycotoxin biosynthesis, or detoxifying mycotoxins in plants. In one example of such a strategy, enzymes that degrade fumonisins have been identified in a filamentous saprophytic fungus isolated from maize, and corresponding genes have been cloned and are currently being tested in transgenic maize.

MARTINS, M.L., MARTINS, H.M. and BERNARDO, F. 2001. **Fumonisin B<sub>1</sub> and B<sub>2</sub> in black tea and medicinal plants.** Journal of Food Protection **64**: 1268–1270.

A total of 18 black tea samples and 69 samples of four different medicinal plants (chamomile, leaves of the orange tree, leaves and flowers of the linden tree, and corn silk), used for infusions preparations were acquired from supermarkets in Lisbon, Portugal. The samples were analysed for the incidence and levels of FB<sub>1</sub> and FB<sub>2</sub>. The highest number of positive samples was found in black tea (88.8%), with levels ranging from 80 to 280 µg/kg. Among the medicinal plants, the leaves of the orange tree had higher concentrations of FB<sub>1</sub> (range 350–700 µg/kg) followed by leaves and flowers of the linden tree (range 20–200 µg/kg). The samples of corn silk and chamomile had FB<sub>1</sub> concentrations ranging from 50 to 150 µg/kg and 20 to 70 µg/kg, respectively. None of the samples tested were contaminated with FB<sub>2</sub>.

TORRES, A.M., REYNOSO, M.M., ROJO, F.G., RAMIREZ, M.L. and CHULZE, S.N. 2001. ***Fusarium* species (section *Liseola*) and its mycotoxins in maize harvested in northern Argentina.** Food Additives and Contaminants **18**: 836–843.

Maize and maize products harvested in small fields and stored by farmers in northern Argentina were assayed for *Fusarium* and fumonisin and beauvericin contamination. Fumonisin were present in 6/18 samples with levels ranging from 603 to 1888 ng/kg. FB<sub>3</sub> and beauvericin were not detected in the samples. Twenty-five strains of *F. subglutinans* isolated from maize kernels and belonging to *Gibberella fujikuroi* mating population E produced beauvericin in culture. Seven of these strains also produced moniliformin.

CASTELO, M.M., JACKSON, L.S., HANNA, M.A., REYNOLDS, B.H. and BULLERMAN, L.B. 2001. **Loss of fumonisin B<sub>1</sub> in extruded and baked corn-based foods with sugars.** Journal of Food Science **66**: 416–421.

The effect of added sugars on FB<sub>1</sub> levels in baked corn muffins and extruded corn grits was studied. Muffins containing added glucose had significantly lower FB<sub>1</sub> levels than muffins with sucrose, fructose or no added sugar. Extrusion cooking of the grits resulted in significant reductions of FB<sub>1</sub> in all treatments relative to unextruded controls, but use of glucose resulted in greater reductions of FB<sub>1</sub> (45.3–71%) than did the use of fructose (29.5–53%) or sucrose (19.2–39%). When extrusion conditions

were optimised, 92.1% loss of FB<sub>1</sub> was found when grits were extruded with glucose.

VOSS, K.A., POLING, S.M., MEREDITH, F.I., BACON, C.W. and SAUNDERS, D.S. 2001. **Fate of fumonisins during the production of fried tortilla chips.** Journal of Agricultural and Food Chemistry **49**: 3120–3126.

The fate of FB<sub>1</sub> during the commercial manufacture of fried tortilla chips was studied. FB<sub>1</sub> and hydrolysed FB<sub>1</sub> (HFB<sub>1</sub>) concentrations in four lots of corn and in the masa, other intermediates, liquid and waste by-products, and fried chips were determined by HPLC. FB<sub>1</sub> concentrations in the masa and chips were reduced significantly, up to 80%, in the fried chips, compared to that in the raw corn. HFB<sub>1</sub> was also found in the masa and chips, but at low concentrations compared to FB<sub>1</sub>. Partially hydrolysed FB<sub>1</sub> (PHFB<sub>1</sub>), which, like HFB<sub>1</sub>, is formed during the nixtamalisation step, was found predominantly in the cooking/steeping liquid and solid waste. No significant amounts of N-(carboxymethyl)-FB<sub>1</sub> or N-(1-deoxy-D-fructos-1-yl)-FB<sub>1</sub>, indicative of fumonisin-sugar adduct formation, were found.

## Fumonisin – Methodology

WHITAKER, T.B., HAGLER, W.M., JOHANSSON, A.S., GIESBRECHT, F.G. and TRUCKSESS, M.W. 2001. **Distribution among sample test results when testing shelled corn lots for fumonisin.** Journal of AOAC International **84**: 770–776.

The statistical distribution known as the compound gamma function was studied for suitability in describing the distribution of sample test results associated with testing lots of shelled corn for fumonisin. Thirty-two 1.1 kg test samples were taken from each of 16 contaminated lots of shelled corn. An observed distribution consisted of 32 sample fumonisin test results for each lot. The mean fumonisin concentration and the variance among the 32 sample fumonisin test results along with the parameters for the compound gamma function were determined for each of the 16 observed distributions. The 16 observed distributions of sample fumonisin test results were compared with the compound gamma function using the Power Divergence test. Parameters of the compound gamma distribution were calculated from the 32 fumonisin sample test results using the method of moments. Using regression analysis, equations were developed that related the parameters of the compound gamma distribution to fumonisin concentration and the variance associated with a fumonisin test procedure.

SEWRAM, V., NAIR, J.J., NIEUWOUDT, T.W., GELDERBLOM, W.C.A., MARASAS, W.F.O. and SHEPHARD, G.S. 2001. **Assessing chronic exposure to fumonisin mycotoxins: The use of hair as a suitable non-invasive matrix.** Journal of Analytical Toxicology **25**: 450–455.

This study describes for the first time the accumulation of measurable levels of fumonisins in the hair of nonhuman primates (vervet monkeys, *Cercopithecus aethiops*) and rats exposed to contaminated feed. Hair was subjected to reflux with methanol and the resulting extract was cleaned up on strong anion exchange (SAX) and C<sub>18</sub> solid-phase sorbents. FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> as well as their hydrolysis products were detected in monkey hair using HPLC coupled to electrospray ionisation mass spectrometry. In vervet monkeys, the levels of exposure related well to the levels of toxin detected in hair. Analysis of rat hair indicated that mean levels up to 34.50 mg/kg were detectable by the 4th week in rats treated by gavage with FB<sub>1</sub> at 10 mg/kg bw. This relationship indicates that hair can provide an easily applicable non-invasive matrix for assessing chronic exposure to fumonisins.

DILKIN, P., MALLMANN, C.A., DEALMEIDA, C.A.A. and CORREA, B. 2001. **Robotic automated clean-up for detection of fumonisins B<sub>1</sub> and B<sub>2</sub> in corn and corn-based feed by high-performance liquid chromatography.** Journal of Chromatography A **925**: 151–157.

An HPLC system with fluorescence detection and an automated on-line solid-phase extraction procedure for FB<sub>1</sub> and FB<sub>2</sub> in corn and corn-based products is described. The best methodology was found to be extraction with acetonitrile-water and cleanup on C<sub>18</sub> end capped disposable extraction cartridges. The system has the advantage of running in an unattended mode of operation and allows processing of 40 samples without system refuel, performing cleanup, *o*-phthalaldehyde derivatisation, injection and fumonisin detection by fluorescence detection linked to a computer integrator for automated data processing. Average recoveries for corn and corn-based feed were 92.6 and 88.3%, respectively, with relative standard deviations (RSDs) of 5.04 and 6.22% for FB<sub>1</sub>, and 91.2 and 89.0% with RSDs of 5.84 and 7.88% for FB<sub>2</sub>. Detection limits for corn and corn-based feed were approximately 0.03 and 0.05 mg/kg for FB<sub>1</sub> and FB<sub>2</sub>, respectively.

OMURTAG, G.Z. 2001. **Determination of fumonisin B<sub>1</sub> and B<sub>2</sub> in corn and corn-based products in Turkey by**

**high-performance liquid chromatography.** Journal of Food Protection **64**: 1072–1075.

Corn and corn-based products consumed by the Turkish population were assayed for FB<sub>1</sub> and FB<sub>2</sub> using HPLC with fluorescence detection. Recoveries from corn meal spiked at 1 mg/kg for both FB<sub>1</sub> and FB<sub>2</sub> were 94.4 and 86.5%, respectively. The minimum detectable amount for the *o*-phthalaldehyde derivatives of FB<sub>1</sub> and FB<sub>2</sub> were 1 and 5 ng, respectively. Of a total of 82 products tested, FB<sub>1</sub> was detected in 25.6% of samples at levels in the range 0.25–2.66 mg/kg. FB<sub>2</sub> was detected in only one sample at 0.55 mg/kg.

KATAY, G., SZECSI, A. and TYIHAK, E. 2001. **Separation of fumonisins by OPLC.** JPC—Journal of Planar Chromatography—Modern TLC **14**: 53–56.

A quick and efficient two-step reversed phase overpressured layer chromatography (OPLC) method was developed for the determination of FB<sub>1</sub>, FB<sub>2</sub>, FB<sub>3</sub> and FB<sub>4</sub>. Of six strains of *Fusarium moniliforme* and *F. proliferatum* grown in rice culture, only one isolate of each species produced all the fumonisins investigated. The other isolates produced no fumonisins. This work is the first report of the separation of all the fumonisins of the B series by use of a planar layer liquid chromatographic technique.

## Fumonisin – Toxicology

GELDERBLOM, W.C.A., SEIER, J.V., SNIJMAN, P.W., VANSCHALKWYK, D.J., SHEPHARD, G.S. and MARASAS, W.F.O. 2001. **Toxicity of culture material of *Fusarium verticillioides* strain MRC 826 to nonhuman primates.** Environmental Health Perspectives **109**: 267–276.

In a chronic feeding study in vervet monkeys (*Cercopithecus aethiops*) over 13.5 years, two dietary treatment groups were fed varying levels of culture material of *Fusarium verticillioides* (Sacc.) Nirenberg (= *F. moniliforme* Sheldon) strain MRC 826 mixed into their daily food ration. The apparent fumonisin consumption patterns were related to changes observed in the biochemical parameters in the blood and urine, including the liver function enzymes and creatinine clearance as well as differential blood counts and sphingolipid levels in the serum and urine. An apparent no-effect threshold for kidney and liver damage is estimated to be fumonisin levels between 0.11 and 0.18 mg/kg body weight/day. Apart from the effects on the liver and kidney, a wide variety of parameters, including cholesterol and creatine kinase, were also

adversely affected. Several blood parameters, including white and red blood cells, also significantly decreased in the treated animals. The serum Sa level and the So/Sa ratio, monitored toward the end of the experiment, significantly increased in both the low dose and high dose animals.

GELDERBLOM, W.C.A., GALENDO, D., ABEL, S., SWANEVELDER, S., MARASAS, W.F.O. and WILD, C.P. 2001. **Cancer initiation by fumonisin B<sub>1</sub> in rat liver.** Role of cell proliferation. Cancer Letters **169**: 127–137.

FB<sub>1</sub> causes cancer initiation in rat liver in a similar manner to genotoxic carcinogens although apparently with different kinetics. The role of regenerative cell proliferation, effected by partial hepatectomy and CCl<sub>4</sub> and direct mitogen-induced hyperplasia, induced by PbNO<sub>3</sub>, on FB<sub>1</sub>-induced cancer initiation was investigated. Initiation was effected over a period of 14 days by gavage with FB<sub>1</sub> at 1.4–35 mg/kg bw/day while the stimuli for cell proliferation were introduced 7 days after the start of the FB<sub>1</sub> treatment. Cancer initiation by FB<sub>1</sub> was associated with a hepatotoxic effect and an increase in lipid peroxidation. In contrast to compensatory liver cell proliferation induced by partial hepatectomy and CCl<sub>4</sub>, mitogen-induced hyperplasia failed to enhance the cancer initiating potential of FB<sub>1</sub> suggesting that cancer induction by a non-genotoxic carcinogen is supported by regenerative cell proliferation.

LIU, H.J., LU, Y., HAYNES, J.S., CUNNICK, J.E., MURPHY, P. and HENDRICH, S. 2001. **Reaction of fumonisin with glucose prevents promotion of hepatocarcinogenesis in female F344/N rats while maintaining normal hepatic sphinganine/sphingosine ratios.** Journal of Agricultural and Food Chemistry **49**: 4113–4121.

The reaction of the primary amine of FB<sub>1</sub> with glucose was hypothesised to detoxify this mycotoxin. Ten-day-old female F344/N rats were injected ip with diethylnitrosamine then from 4 weeks of age were fed diets containing FB<sub>1</sub>-glucose (containing FB<sub>1</sub> at 25 mg/kg) or FB<sub>1</sub> at 8 or 25 mg/kg. Rats fed pure FB<sub>1</sub> had greater alanine aminotransferase activity at 9 and 20 weeks of age, greater endogenous hepatic prostaglandin E-2 production at 20 weeks of age and significantly lower plasma cholesterol at 20 weeks of age compared to FB<sub>1</sub>-glucose fed rats. Placental glutathione S-transferase-positive and gamma -glutamyltransferase-positive altered hepatic foci occurred only in rats fed 25 mg/kg at 20 weeks of age. Modifying FB<sub>1</sub> with glucose seems to prevent FB<sub>1</sub>-induced hepatotoxicity and promotion of hepatocarcinogenesis.

GARREN, L., GALENDO, D., WILD, C.P. and CASTEGNARO, M. 2001. **The induction and persistence of altered sphingolipid biosynthesis in rats treated with fumonisin B<sub>1</sub>**. Food Additives and Contaminants **18**: 850–856.

The effect of increasing daily gavage doses of FB<sub>1</sub> at 0.01–5 mg/kg bw on the sphinganine (Sa) to sphingosine (So) ratio in various tissues and urine was examined in BDIV rats. The lowest dose of FB<sub>1</sub> which induced an alteration of Sa/So in any tissue was 1 mg/kg. The tissue most affected was the kidney, with the liver affected to a much lesser extent and the lung and oesophagus not at all. Only a modest indication of an alteration in Sa/So ratio was obtained in the urine. The large interindividual variations in urinary Sa and So in this strain of rat, even in the absence of FB<sub>1</sub> treatment, made interpretation of these data difficult. After cessation of treatment, the Sa/So ratio returned to normal in kidney after 1–3 weeks.

HE, Q.R., RILEY, R.T. and SHARMA, R.P. 2001. **Fumonisin-induced tumor necrosis factor-alpha expression in a porcine kidney cell line is independent of sphingoid base accumulation induced by ceramide synthase inhibition**. Toxicology and Applied Pharmacology **174**: 69–77.

FB<sub>1</sub> inhibits ceramide synthase, resulting in accumulation of free Sa and So. As well, tumour necrosis factor-alpha (TNF alpha) plays an important role in FB<sub>1</sub> toxicity and the expression of TNF alpha mRNA in liver and kidney is increased following FB<sub>1</sub> exposure in mice. In this study using LLC-PK1 cells, inhibition of sphingoid base biosynthesis by ISP-1, a specific inhibitor of serine palmitoyltransferase, the first enzyme in *de novo* sphingolipid biosynthesis, efficiently blocked the accumulation of free sphingoid bases in response to FB<sub>1</sub>, but it did not prevent the induction of TNF alpha expression. Results indicate that FB<sub>1</sub>-induced increase in TNF alpha expression is independent of sphingoid base accumulation induced by ceramide synthase inhibition in LLC-PK1 cells.

SPOTTI, M., POMPA, G. and CALONI, F. 2001. **Fumonisin B<sub>1</sub> metabolism by bovine liver microsomes**. Veterinary Research Communications **25**: 511–516.

The hepatic metabolism of FB<sub>1</sub> by bovine liver microsomes was studied. No biodegradation or metabolisation of the mycotoxin by liver microsomes was detectable after incubating FB<sub>1</sub> with bovine microsomes in the presence of a regenerating system for 1 hr. No aminopolyol 1, aminopolyol 2 or aminopentol, metabolites of FB<sub>1</sub>, were detected in any of the incubated

samples. The tolerance of ruminants to FB<sub>1</sub> is apparently not dependent on its detoxification in the rumen.

RAYNAL, M., BAILLY, J.D., BENARD, G. and GUERRE, P. 2001. **Effects of fumonisin B<sub>1</sub> present in *Fusarium moniliforme* culture material on drug metabolising enzyme activities in ducks**. Toxicology Letters **121**: 179–190.

The effects of FB<sub>1</sub> at 5, 15 and 45 mg/kg/day on drug metabolising enzyme activities were investigated in growing ducks by daily oral administration over 12 days. Although the total microsomal P450 content was unaffected, benzphetamine, ethylmorphine erythromycin N-demethylases and ethoxyresorufin O-deethylase activities were together increased. By contrast, aminopyrine and nitrosodimethylamine N-demethylases, methoxyresorufin and pentoxyresorufin O-dealkylases and UDP-glucuronyltransferase activities were only increased at 45 mg/kg/day. Glutathione S-transferases activities remained unaffected.

BAILLY, J.D., BENARD, G., JOUGLAR, J.Y., DURAND, S. and GUERRE, P. 2001. **Toxicity of *Fusarium moniliforme* culture material containing known levels of fumonisin B<sub>1</sub> in ducks**. Toxicology **163**: 11–22.

The effects of FB<sub>1</sub> on growing ducks receiving FB<sub>1</sub> at 5, 15 or 45 mg/kg by daily oral administration over 12 days was investigated. FB<sub>1</sub> caused a dose-dependant increase in liver weight. In the plasma, protein, cholesterol, alanine aminotransferase, lactate dehydrogenase, gamma-glutamyltransferase and Sa/So ratio were increased. No sign of apoptosis was present neither in the liver nor in peripheral blood lymphocytes and only moderate oxidative damages were obtained. Although ducks appeared resistant to FB<sub>1</sub> toxicity in terms of mortality, liver alterations were obtained with only 5 mg/kg/day for 12 days.

### Trichothecenes – General

MIEDANER, T., REINBRECHT, C., LAUBER, U., SCHOLLENBERGER, M. and GEIGER, H.H. 2001. **Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to *Fusarium* head blight in rye, triticale, and wheat**. Plant Breeding **120**: 97–105.

The effects of winter cereal species, host genotype and environment on DON accumulation and FHB were analysed by inoculating 12 rye, eight wheat and six triticale genotypes of different resistance levels with

a DON-producing isolate of *Fusarium culmorum* at three locations in 2 years. Although rye genotypes were, on average, affected by *Fusarium* infections much the same as wheat genotypes, wheat accumulated twice as much DON as rye. Triticale was least affected and the grain contained slightly more DON than rye. The differences between and within cereal species in the field experiments were highly influenced by environment for resistance traits and mycotoxin contents. Nevertheless, mean mycotoxin content of the grain could not be associated with general weather conditions in the individual environments. Correlations between resistance traits and DON content were high in wheat with the most resistant varieties also accumulating less DON, but with variability in rye.

ABRAMSON, D., CLEAR, R.M., GABA, D., SMITH, D.M., PATRICK, S.K. and SAYDAK, D. 2001. **Trichothecene and moniliformin production by *Fusarium* species from western Canadian wheat**. Journal of Food Protection **64**: 1220–1225.

*Fusarium graminearum*, *F. culmorum* and *F. avenaceum*, isolated from *Fusarium*-damaged wheat harvested in western Canada, were cultured and evaluated for mycotoxin production. DON was found in 28/42 isolates of *F. graminearum* and all 42 isolates of *F. culmorum* at levels ranging from 0.5 to 25.0 mg/kg. 15-AcetylDON was found in 28/42 isolates of *F. graminearum* at levels ranging from 1.0 to 7.1 mg/kg. 3-AcetylDON was found in 41/42 isolates of *F. culmorum* at levels ranging from 0.8 to 13.0 mg/kg. Moniliformin was present in 40/42 isolates of *F. avenaceum* at levels ranging from 1.3 to 138.1 mg/kg, but was not present in any of the isolates of *F. graminearum* or *F. culmorum*.

MULLER, H.M., REIMANN, J., SCHUMACHER, U. and SCHWADORF, K. 2001. **Further survey of the occurrence of *Fusarium* toxins in wheat grown in southwest Germany**. Archives of Animal Nutrition—Archiv für Tierernährung **54**: 173–182.

A total of 276 wheat samples for feed use were collected randomly after harvests from 1989 to 1993 from farms in an area of southwest Germany. DON was the major toxin, with incidences of 77–93 % and mean contents of 167–735 µg/kg. In contrast, incidences of ZEA, 3-acetylDON, NIV, HT-2 toxin and T-2 toxin were at 13–37%, 10–44%, 15–67%, 0–11%, and 0–12%, respectively, with mean contents in positive samples between 2 and 73 µg/kg. For the years 1991 to 1993, 15-acetylDON was found in 0–11% of samples at mean levels

up to 17 µg/kg. Diacetoxyscirpenol, alpha and beta-zearalenol and fusarenon-X were not detected in any sample.

OMURTAG, G.Z. and YAZICIOGLU, D. 2001. **Occurrence of T-2 toxin in processed cereals and pulses in Turkey determined by HPLC and TLC.** Food Additives and Contaminants **18**: 844–849.

A total of 50 commercially available cereal and pulse product samples collected from markets and street bazaars in Turkey were analysed for T-2 toxin by HPLC and TLC. T-2 toxin was detected in cereal and pulse products in 23.5 and 31.2% of samples, respectively, and the maximum detected amount was 1.9 mg/kg in a sample of dried beans.

YI, C., KAUL, H.P., KUBLER, E., SCHWADORF, K. and AUFHAMMER, W. 2001. **Head blight (*Fusarium graminearum*) and deoxynivalenol concentration in winter wheat as affected by pre-crop, soil tillage and nitrogen fertilization.** Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz—Journal of Plant Diseases and Protection **108**: 217–230.

The influence of different pre-crops and crop husbandry on *Fusarium* head blight (FHB) incidence in winter wheat test crops was studied in a 2-year factorial field experiment. Infection by FHB and grain contamination with DON were similar following maize and spring wheat crops, either for silage or for grain, but the method of pre-crop inoculation by infected grains might have masked differences between pre-crops. The reductions of FHB incidence due to ploughing or nitrolime application were 27–32% or 31–59% compared with residues remaining on the surface or calcium ammonium nitrate fertilisation, respectively. Contemporary reductions in DON were less consistent. Results show that crop health can be supported by crop husbandry to some degree, but FHB cannot be reliably controlled in susceptible rotations with abundant sources of inoculum.

SIRANIDOU, E. and BUCHENAUER, H. 2001. **Chemical control of *Fusarium* head blight on wheat.** Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz—Journal of Plant Diseases and Protection **108**: 231–243.

The effects of different chemical substances on infection and mycotoxin production by *Fusarium culmorum* in winter wheat cultivars were investigated in field trials during two years. Spray treatments of the cvs. Kontrast and Agent with the fungicide tebuconazole either two days before or two days post-inoculation reduced of the disease index of the spikes by 61–89% and the DON in the grain by 50–70%. Application of met-

conazole in the cvs. Kontrast, Agent and Piko two days before inoculation led to decreases of the disease index by 68–71% and the DON content in the grain by 61–69%. Application of the fungicidal substances chlorothalonil, prochloraz and benomyl two days before inoculation did not satisfactorily control the pathogen. Treatment of the plants with azoxystrobin reduced the disease index of the spikes, but the DON content of the grain was higher than in the untreated control. Application of piperonylbutoxide, a possible inhibitor of trichothecene biosynthesis, increased the efficacy of the fungicides in the cv. Agent.

MIEDANER, T. and REINBRECHT, C. 2001. **Trichothecene content of rye and wheat genotypes inoculated with a deoxynivalenol- and a nivalenol-producing isolate of *Fusarium culmorum*.** Journal of Phytopathology—Phytopathologische Zeitschrift **149**: 245–251.

The covariation between DON and NIV accumulation of 12 rye and eight wheat genotypes that differed in resistance to FBH were analysed by inoculating them with a DON- and a NIV-producing chemotypes of *Fusarium culmorum*. Wheat and rye genotypes significantly differed in their DON and NIV accumulation. All genotypes reacted in a similar manner to both chemotypes of *F. culmorum* for the resistance traits and the respective mycotoxin contents with the exception of one wheat variety. Results showed that resistance to head blight and tolerance to mycotoxin accumulation seems to be most likely the same for DON- and NIV-producing isolates of *F. culmorum*.

SIMPSON, D.R., WESTON, G.E., TURNER, J.A., JENNINGS, P. and NICHOLSON, P. 2001. **Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain.** European Journal of Plant Pathology **107**: 421–431.

*Fusarium* head blight of wheat is caused by a disease complex comprised of toxigenic pathogens, predominantly *Fusarium* species, and a non-toxicigenic pathogen *Microdochium nivale*, which causes symptoms visually indistinguishable from *Fusarium* and is often included as a causal agent. In this study, the effects of fungicide treatments on colonisation by *Fusarium* and *Microdochium*, and on the production of DON were investigated. Tebuconazole selectively controlled *F. culmorum* and *F. avenaceum* and reduced levels of DON, but showed little control of *M. nivale*. Application of azoxystrobin, however, selectively controlled *M. nivale* and allowed greater

colonisation by toxigenic *Fusarium* species and this treatment also resulted in increased levels of DON. Azoxystrobin application two days post-inoculation increased the production of DON per unit of pathogen in an artificially inoculated field trial.

LEE, T., OH, D.W., KIM, H.S., LEE, J., KIM, Y.H., YUN, S.H. and LEE, Y.W. 2001. **Identification of deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae* by using PCR.** Applied and Environmental Microbiology **67**: 2966–2972.

The gene clusters for trichothecene biosynthesis from two chemotypes of *Gibberella zeae*, H-11, a DON producer isolated from corn, and 88-1, a NIV producer from barley, were cloned and sequenced. Each gene cluster contained 10 *Tri* gene homologues in the same order and transcriptional directions as those of *Fusarium sporotrichioides*. Between H-11 and 88-1 all of the *Tri* homologues except *Tri7* were conserved. The *Tri7* genes were aligned and it was found that the *Tri7* open reading frame of H-11 carried several mutations and an insertion containing 10 copies of an 11-bp tandem repeat. One-hundred *G. zeae* isolates of both chemotypes were assayed by PCR amplification with a primer pair derived from the *Tri7* gene and the chemotypes could be differentiated by polyacrylamide gel electrophoresis. The PCR-based method developed in this study should provide a simple and reliable diagnostic tool for differentiating the two chemotypes of *G. zeae*.

HARRIS, L.J. and GLEDDIE, S.C. 2001. **A modified *Rpl3* gene from rice confers tolerance of the *Fusarium graminearum* mycotoxin deoxynivalenol to transgenic tobacco.** Physiological and Molecular Plant Pathology **58**: 173–181.

DON is a potent inhibitor of eukaryotic protein synthesis and is believed to play a role in fungal pathogenesis on cereal crops worldwide. The putative site of action by DON has been theorised to be the 60S ribosomal protein L3 (RPL3). A rice (*Oryza saliva*) cDNA encoding the ribosomal protein RPL3 was modified so that amino acid residue 258 is changed from tryptophan to cysteine, a change which is believed to confer resistance to similar mycotoxins in yeast. Both versions of the rice *Rpl3* genes were introduced into two species of tobacco by *Agrobacterium tumefaciens* co-cultivation and expressed under the control of a constitutive promoter. Results indicate a possible mechanism of host plant resistance to the fungal pathogen *Fusarium graminearum* among the susceptible cereal species based on the expression of modified *Rpl3* genes.

BRACHER, F. and KRAUSS, J. 2001. **Total synthesis of (+/-)-zearalanone.** Monatshefte für Chemie **132**: 805–811.

A new approach to the macrocyclic lactone ZEA is described utilising an alkenol and an arene trifluoromethanesulfonate as starting materials. The key step is a Pd(0)-catalysed cross-coupling of the arene trifluoromethanesulfonate with a 9-alkyl-9-borabicyclo[3.3.1]nonane derived from the alkenol.

## Tricothecenes – Toxicoses

SHIER, W.T., SHIER, A.C., XIE, W. and MIROCHA, C.J. 2001. **Structure-activity relationships for human estrogenic activity in zearalenone mycotoxins.** Toxicon **39**: 1435–1438.

Using an improved MCF7 human breast cell proliferation assay, the oestrogenicity of 17 chromatographically homogeneous zearalenones have been compared. Both similarities and substantial differences from published results in intact animal systems were observed. Substantial human oestrogenicity was retained even in analogues lacking hydroxylation on the aromatic and macrocyclic rings.

LEGUEVEL, R. and PAKDEL, F. 2001. **Assessment of oestrogenic potency of chemicals used as growth promoter by *in vitro* methods.** APMIS **109**: S473–S479.

Three *in vitro* bioassays were used to compare the oestrogenic potency of chemicals used as growth promoters in beef cattle or found as feed contaminants such as the mycotoxin ZEA and some of its metabolites. The assessment of oestrogenic potency of these chemicals clearly demonstrated the strong oestrogenicity of ZEA and its metabolites and particularly alpha-zearalenol which was as potent as ethinyl oestradiol and diethylstilboestrol in the human endometrial Ishikawa cell line.

FU, Y.T., LIN, W.G., ZHAO, B.C. and QUAN, G. 2001. **The effect of T-2 toxin on IL-1 beta and IL-6 secretion in human fetal chondrocytes.** International Orthopaedics **25**: 199–201.

The effects of T-2 toxin on IL-1 beta and IL-6 secretion in human foetal chondrocytes *in vitro* were investigated. The evaluation was realised on primary monolayer culture of human foetal epiphyseal chondrocytes with or without PMA stimulation. There was a significant increase of IL-1 beta and IL-6 in supernatants of chondrocytes cultivated for 24 hr with T-2 toxin at 8 µg/L after PMA stimulation. In the absence of PMA, IL-1 beta was increased alone after 48 hr.

These data suggested that superinduction of cytokines might be one of the key mechanisms of chondrocyte injuries by T-2 toxin.

SROBAROVA, A. and PAVLOVA, A. 2001. **Toxicity of secondary metabolites of the fungus *F. culmorum* in relation to resistance of winter wheat cultivars.** Cereal Research Communications **29**: 101–108.

The toxicity of secondary metabolites of *Fusarium culmorum* were studied in wheat seedlings. Seedlings were exposed to different concentrations of DON, diacetoxyscirpenol and culture filtrates of *F. culmorum*. Phytotoxicity was evaluated according to biomass production and level of free proline accumulation. Tricothecenes are specific inhibitors of protein synthesis which cause free amino acids to accumulate in the tissue. Free proline was determined as indicator of this accumulation. The highest level of accumulation of free proline was found in the susceptible cultivar Barbara. Highly resistant to the toxins was the medium resistant cv. Samanta, but not cv. Hana which is most resistant to the fungus.

DAHLEEN, L.S. and MCCORMICK, S.P. 2001. **Tricothecene toxin effects on barley callus and seedling growth.** Cereal Research Communications **29**: 115–120.

The effects of DON and the related toxin diacetoxyscirpenol (DAS) on barley callus growth and seedling germination, root and shoot growth were investigated. The effects of the toxins on callus growth were small. Although statistically significant differences were seen at the higher toxin levels after eight weeks, the differences were not large enough for selection between toxin resistant and toxin susceptible calli. Neither toxin had an effect on seed germination. DON at 5 and 10 mg/L significantly reduced root growth by day 7, and 10 mg/L significantly reduced shoot growth by day 4. The effects of DAS were greater, with all toxin levels significantly reducing root growth by day 2 and shoot growth by day 4. DAS may be usable for screening progeny of transgenic plants for expression and segregation of anti-toxin genes.

DEBOUCK, C., HAUBRUGE, E., BOLLAERTS, P., VANBIGNOOT, D., BROSTAU, Y., WERRY, A. and ROOZE, M. 2001. **Skeletal deformities induced by the intraperitoneal administration of deoxynivalenol (vomitoxin) in mice.** International Orthopaedics **25**: 194–198.

The pattern of skeletal malformations induced by the ip administration of DON to pregnant mice is reported. Costo-vertebral

segmentation abnormalities were the main deformities observed. The chondrodysplasia previously described was not seen. The possible implications for Kashin Beck disease are discussed.

CONKOVA, E., LACIAKOVA, A., PASTOROVA, B., SEIDEL, H. and KOVAC, G. 2001. **The effect of zearalenone on some enzymatic parameters in rabbits.** Toxicology Letters **121**: 145–149.

The effects of ZEA at 10 and 100 µg/kg bw orally administered to rabbits on selected blood serum enzyme activities was studied. In rabbits receiving 100 µg/kg bw, significant increases in activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase and lactate dehydrogenase were observed at 168 and 336 hr, indicating possible liver toxicity.

NITAO, J.K., MEYER, S.L.F., SCHMIDT, W.F., FETTINGER, J.C. and CHITWOOD, D.J. 2001. **Nematode-antagonistic tricothecenes from *Fusarium equiseti*.** Journal of Chemical Ecology **27**: 859–869.

A strain of *Fusarium equiseti* isolated from soybean cyst nematode secretes nematode-antagonistic compounds. Bioassay-guided fractionation of an extract of the culture broth was undertaken to identify the compounds. Fractions were assayed for activity against a root-knot nematode (*Meloidogyne incognita*), a plant pathogen that attacks the roots of numerous plant species. Two tricothecene compounds were isolated that inhibited egg hatch and immobilised second-stage juveniles of this nematode: 4,15-diacetylNIV and DAS. This is the first published report of these compounds affecting plant-parasitic nematodes.

## Aflatoxins – General

PEREZ, M., HENKE, S.E. and FEDYNICH, A.M. 2001. **Detection of aflatoxin-contaminated grain by three granivorous bird species.** Journal of Wildlife Diseases **37**: 358–361.

Wild birds including northern bobwhites (*Colinus virginianus*), white-winged doves (*Zenaida asiatica*) and green jays (*Cyanocorax yncas*) were presented with feeders that had four compartments. The compartments contained feed that was contaminated with aflatoxin at levels of 0, 100, 500 and 1,000 µg/kg, respectively. White-winged doves and northern bobwhites did not avoid contaminated feed. However, green jays selected against aflatoxin-containing grain.

GALVANO, F., GALOFARO, V., RITIENI, A., BOGNANNO, M., DEANGELIS, A. and GALVANO, G. 2001. **Survey of the occurrence of aflatoxin M<sub>1</sub> in dairy products marketed in Italy: Second year of observation.** Food Additives and Contaminants **18**: 644–646.

During 1996, 161 samples of milk, 92 samples of dry milk for infant formula and 120 samples of yoghurt, were randomly collected in supermarkets and drug stores in four big Italian cities and assayed for AFM<sub>1</sub>. AFM<sub>1</sub> was detected in 78% of milk samples ranging from <1 to 23.5 ng/L, in 53% of dry milk samples ranging from <1 to 79.6 ng/kg and in 61% of yoghurt samples ranging from <1 to 32.1 ng/kg.

HIRANO, S., SHIMA, T. and SHIMADA, T. 2001. **[Proportion of aflatoxin B<sub>1</sub> contaminated kernels and its concentration in imported peanut samples.]** Journal of the Food Hygienic Society of Japan **42**: 237–242.

Mouldy and split peanut kernels were separated by visual inspection from peanuts exported from Brazil, Sudan, India and Taiwan. The remaining peanuts from Brazil, Sudan and India were roasted lightly and the skins were removed. Stained peanuts were separated from the others. Aflatoxin was detected in mouldy and stained peanuts. Aflatoxin concentrations in mouldy peanuts was higher than that in stained peanut kernels. (In Japanese).

HORN, B.W., GREENE, R.L., SORENSEN, R.B., BLANKENSHIP, P.D. and DORNER, J.W. 2001. **Conidial movement of nontoxigenic *Aspergillus flavus* and *A. parasiticus* in peanut fields following application to soil.** Mycopathologia **151**: 81–92.

The use of non-toxicogenic strains of *Aspergillus flavus* and *A. parasiticus* in biological control effectively reduces aflatoxin in peanuts when conidium-producing inoculum is applied to the soil surface. The movement of conidia of these fungi in soil was examined following natural rainfall and controlled precipitation from a sprinkler irrigation system. Conidia remained near the soil surface despite repeated rainfall and varying amounts of applied water from irrigation. In addition, rainfall washed the conidia along the peanut furrows for up to 100 m downstream from the experimental plot boundary.

ABDEL-SATER, M.A., ZOHRI, AA. and ISMAIL, M.A. 2001. **Natural contamination of some Egyptian fruit juices and beverages by mycoflora and mycotoxins.** Journal of Food Science and Technology—Mysore **38**: 407–411.

The mycoflora analysis of some Egyptian canned fruit juices and beverages showed a wide range of species. The most prevalent fungi in all types of juices and beverages were members of *Aspergillus* and *Penicillium*. Analysis of five different fruit juice and beverages (five samples each) revealed that all the five apple beverages tested were contaminated with AFB<sub>1</sub> and G<sub>1</sub> with concentrations ranging from 20 to 30 µg/L, while 2/5 guava juice samples tested were naturally contaminated with AFB<sub>1</sub> at 12 µg/L. All samples of mango juice, grape and peach beverages were free from naturally occurring mycotoxins.

CHOURASIA, H.K. 2001. **Response of some Indian maize samples for aflatoxin production by *Aspergillus flavus* strains.** Journal of Food Science and Technology—Mysore **38**: 387–389.

The laboratory response of five maize varieties commonly marketed in Bihar State, India, to aflatoxin-producing and non-producing strains of *Aspergillus flavus* was studied. In the small number of samples studied, it was possible to separate the maize genotypes into three groups according to their ability to support aflatoxin production.

WILSON, R.A., GARDNER, H.W. and KELLER, N.P. 2001. **Cultivar-dependent expression of a maize lipoxygenase responsive to seed infesting fungi.** Molecular Plant-Microbe Interactions **14**: 980–987.

Fatty acid derivatives of the plant lipoxygenase (LOX) pathway are implicated in the *Aspergillus*-seed interaction in maize. The 9(S)-hydroperoxide derivative of linoleic acid promotes transcription of aflatoxin genes, whereas the 13(S)-hydroperoxide derivative decreases aflatoxin gene expression and production; both are sporulation factors. In this study, screening maize LOX expressed sequence tags identified one clone, cssap 92, which is highly expressed in *Aspergillus*-infected seed susceptible to aflatoxin contamination and repressed in lines with resistance to aflatoxin contamination. The accumulation of cssap 92 transcript was similar during *Fusarium* infection. Product-specificity analysis of the CSSAP 92 protein shows that it preferentially adds oxygen to carbon 9 of linoleic acid. Because 9(S)-hydroperoxy linoleic acid has been implicated as an aflatoxin-signalling molecule, it is possible that cssap 92 could be used as a biomarker that is indicative of aflatoxin resistance in maize lines.

MINERVINI, F., VISCONTI, A., BOTTALICO, A. and MONTAGNA, M.T. 2001. **[On the occurrence of aflatoxin**

**M<sub>1</sub> in cheeses in some southern Italian areas].** Industrie Alimentari **40(403)**: 513–516.

A survey of the occurrence of AFM<sub>1</sub> was carried out on 317 samples of cheeses made from the milks of cows (98 samples), buffalo (85), sheep (94), goat (23) and sheep-goat (17) collected in southern Italy in 1997–99. AFM<sub>1</sub> was found in 19.2% of all samples, with a higher positive incidence for cheeses from cows' milk (29.6%). Low levels of contamination, ranging from 50 to 250 ng/kg, were found in contaminated cheeses. (In Italian).

RAO, S.B.N. and CHOPRA, R.C. 2001. **Influence of sodium bentonite and activated charcoal on aflatoxin M<sub>1</sub> excretion in milk of goats.** Small Ruminant Research **41**: 203–213.

Crossbred (Alpine x Beetal and Saanen x Beetal) lactating goats in early lactation were fed AFB<sub>1</sub> at 100 µg/kg in the diet with or without the addition of sodium bentonite or activated charcoal at 1% of diet. There was no effect of aflatoxin and/or adsorbent on dry matter intake or daily milk yield during the experimental period of 14 days. Bentonite and activated charcoal reduced the excretion of AFM<sub>1</sub> 66.57±4.85 and 75.99±7.13%, respectively, and reduced carry over into milk by 65.26±3.24 and 76.09±6.36%, respectively.

MATSUSHIMA, K., YASHIRO, K., HANYA, Y., ABE, K., YABE, K. and HAMASAKI, T. 2001. **Absence of aflatoxin biosynthesis in koji mold (*Aspergillus sojae*).** Applied Microbiology and Biotechnology **55**: 771–776.

Ten strains isolated from industrial soy sauce producing koji mould were identified as *Aspergillus sojae*. There was no detectable aflatoxin in any culture extracts of *A. sojae* strains. Strain 477 was chosen as a representative strain of industrial *A. sojae* for further molecular analysis. All enzymatic activities associated with the aflatoxin biosynthesis were not detected or negligible in strain 477. Southern analysis suggested that the genomic DNA of strain 477 contained aflatoxin biosynthetic pathway genes. All industrial strains lacked detectable transcripts of *aflR*, the main regulatory gene for aflatoxin biosynthesis suggesting that defects in *aflR* expression cause the lack of expression of aflatoxin-related genes which results in the absence of aflatoxin biosynthesis in *A. sojae* strains.

HASKARD, C.A., EL-NEZAMI, H.S., KANKAANPAA, P.E., SALMINEN, S. and AHOKAS, J.T. 2001. **Surface binding of aflatoxin B<sub>1</sub> by lactic acid bacteria.** Applied and Environmental Microbiology **67**: 3086–3091.

The stability of the AFB<sub>1</sub> complexes formed with 12 bacterial strains in both viable and nonviable (heat- or acid-treated) forms was assessed by repetitive aqueous extraction. By the fifth extraction, up to 71% of the total AFB<sub>1</sub> remained bound. Nonviable bacteria retained the highest amount of AFB<sub>1</sub>. *Lactobacillus rhamnosus* strain GG (ATCC 53103) and *L. rhamnosus* strain LC-705 (DSM 7061) removed AFB<sub>1</sub> from solution most efficiently and were selected for further study. The accessibility of bound AFB<sub>1</sub> to an antibody in an indirect competitive inhibition ELISA suggests that surface components of these bacteria are involved in binding.

REDDY, S.V., MAYI, D.K., REDDY, M.U., THIRUMALA-DEVI, K. and REDDY, D.V.R. 2001. **Aflatoxin B<sub>1</sub> in different grades of chillies (*Capsicum annum* L.) in India as determined by indirect competitive-ELISA.** Food Additives and Contaminants **18**: 553–558.

Samples of the three grades of chilli pod (grades 1 to 3) were collected during surveys in 1998 and 1999 from the principal market yards and cold storage facilities of the major chilli-growing areas of Andhra Pradesh (AP), India. Chilli powders were collected from different supermarkets in Hyderabad, AP. Samples were analysed for AFB<sub>1</sub> by an indirect competitive ELISA. Of the 182 chilli samples tested, 59% were contaminated with AFB<sub>1</sub> and 18% contained the toxin at non-permissible levels. Overall the maximum percentage of chilli pods showing AFB<sub>1</sub> levels higher than 30 µg/kg (non-permissible levels) was in grade 3. Nearly 9% of the chilli powders sold in supermarkets contained non-permissible aflatoxin levels.

TORRES, P., GUZMAN-ORTIZ, M. and RAMIREZ-WONG, B. 2001. **Revising the role of pH and thermal treatments in aflatoxin content reduction during the tortilla and deep frying processes.** Journal of Agricultural and Food Chemistry **49**: 2825–2829.

Corn (*Zea mays*) naturally contaminated with aflatoxin was made into tortillas, tortilla chips and corn chips by the traditional and commercial alkaline cooking processes. The traditional nixtamalisation (alkaline-cooking) process involved cooking and steeping the corn, whereas the commercial nixtamalisation process only steeps the corn in a hot alkaline solution. The traditional process eliminated 51.7, 84.5 and 78.8% of the aflatoxins content in tortilla, tortilla chips and corn chips, respectively. The commercial process was less effective and removed 29.5, 71.2 and 71.2% of the aflatoxin in the same products. Intermediate

and final products did not reach a high enough pH to allow permanent aflatoxin reduction during thermal processing. The cooking or steeping liquor is the only component of the system with a sufficiently high pH (10.2–10.7) to allow modification and detoxification of aflatoxins present in the corn grain.

CHAVAN, J.K., DESHMUKH, R.A. and DALVI, U.S. 2001. **Pearling treatment of discoloured sorghum grains.** Journal of Food Science and Technology—Mysore **38**: 263–265.

A simple process was standardised to obtain about 80% clear, white edible-grade product from mould-infested and discoloured sorghum grains. The discoloured grains were suspended in 40% NaCl (sp.gr. 1.25) to separate heavily infested and light weight grains. The sinkers were dried and pearled on an abrasive polisher at 650 rpm for 5 minutes. The pearling treatment removed completely the surface discoloration and aflatoxins.

DUTTA, T.K. and DAS, P. 2001. **Isolation of aflatoxigenic strains of *Aspergillus* and detection of aflatoxin B<sub>1</sub> from feeds in India.** Mycopathologia **151**: 29–33.

A total of 256 feed samples collected from different parts of Northern India were examined for the presence of aflatoxigenic strains of *Aspergillus flavus* and *A. parasiticus* and for AFB<sub>1</sub>. Of 198 *A. flavus* and 15 *A. parasiticus* strains isolated, 76 and 86%, respectively, were found to be toxigenic. The AFB<sub>1</sub> contents of the feeds averaged 0.412±0.154 mg/kg in comparison to the permissible Indian regulation level of 0.03 mg/kg. The incidence and level of toxin in feed was high during the monsoon/post monsoon period.

OBERHEU, D.G. and DABBERT, C.B. 2001. **Aflatoxin production in supplemental feeders provided for northern bobwhite in Texas and Oklahoma.** Journal of Wildlife Diseases **37**: 475–480.

A common practice in which birds, such as northern bobwhite (*Colinus virginianus*), contact stored agricultural grain is through supplemental feeding. Aflatoxin production and the relationship between weather and aflatoxin production in supplemental feeders was studied. Mean monthly aflatoxin concentration of samples from feeders ranged from 0.57±2.86 to 15.47±14.69 µg/kg. Aflatoxin production was related to the highly variable relative humidity of the study area which influences moisture content of grain.

SCHWEITZER, S.H., QUIST, C.F., GRIMES, G.L. and FORSTER, D.L. 2001. **Aflatoxin levels in corn available as wild turkey feed in Georgia.** Journal of Wildlife Diseases **37**: 657–659.

Samples of corn available as wild turkey (*Meleagris gallopavo*) feed from retailers throughout Georgia, USA, were collected during April 1997 and analysed for aflatoxin. Three of 31 samples collected from a 40-country area were positive. Two samples contained up to 20 µg/kg of aflatoxin. A third sample contained aflatoxin at 380 µg/kg. Because aflatoxin levels ranging from 100 to 400 µg/kg may cause liver dysfunction and immunosuppression in turkey poult and other wildlife, grains known to be contaminated with aflatoxin at levels unacceptable for domestic animal feeds (greater than or equal to 100 µg/kg) should not be sold as wildlife feed.

BOYD, M.L. and COTTY, P.J. 2001. ***Aspergillus flavus* and aflatoxin contamination of leguminous trees of the Sonoran Desert in Arizona.** Phytopathology **91**: 913–919.

*Aspergillus* species in section *Flavi* were frequently associated with desert tree legumes in uncultivated areas of the Sonoran Desert in Arizona. Of 270 samples of debris and fruits of mesquite (*Prosopis* species), ironwood (*Olneya tesota*), acacia (*Acacia* species) and palo verde (*Cercidium* and *Parkinsonia* species), 87% were positive for *A. flavus* (S and L strains) and *A. tamarii*. Mesquite pods were both the substrate from which *A. flavus* was recovered most frequently and the substrate from native habitats with the greatest aflatoxin content. Twenty percent of legume pods collected in the desert contained measurable quantities of aflatoxin, ranging from 1 to more than 2,500 µg/kg.

ZERINGUE, H.J., SHIH, B.Y. and BHATNAGAR, D. 2001. **Effects of clarified neem oil on growth and aflatoxin B<sub>1</sub> formation in submerged and plate cultures of aflatoxigenic *Aspergillus* spp.** Phytoparasitica **29**: 361–366.

In 5-day-old submerged cultures of aflatoxigenic *Aspergillus* species containing either 0.5 or 1.0 ml clarified neem oil, an increase of 11–31% of dry mycelial mass was observed along with a slight decrease of 5–10% in AFB<sub>1</sub> production. In potato dextrose agar plate cultures inoculated with aflatoxigenic *Aspergillus* species and exposed to an atmosphere of volatiles emitted from clarified neem oil, fungal radial growth was reduced by 7–29% and AFB<sub>1</sub> production was reduced by 0–67%.

KONDO, T., SAKURADA, M., OKAMOTO, S., ONO, M., TSUKIGI, H., SUZUKI, A., NAGASAWA, H. and SAKUDA, S. 2001. **Effects of aflastatin A, an inhibitor of aflatoxin production, on aflatoxin biosynthetic pathway and glucose metabolism in *Aspergillus parasiticus***. *Journal of Antibiotics* **54**: 650–657.

Aflastatin A, which inhibits aflatoxin production by *Aspergillus parasiticus*, has been shown to inhibit production of norsolorinic acid, an early biosynthetic intermediate of aflatoxin, at a concentration of 0.25 mg/L. Reverse-transcriptase PCR and real-time quantitative PCR experiments indicated that the transcription of genes encoding aflatoxin biosynthetic enzymes (*pksA*, *ver-1* and *omtA*) and a gene encoding a regulatory protein for expression of the biosynthetic enzymes (*ajlR*) were significantly reduced by the addition of aflastatin A. Aflastatin A also elevated glucose consumption and ethanol accumulation by *A. parasiticus* and repressed transcription of genes involved in ethanol utilisation.

VERGOPOULOU, S., GALANOPOULOU, D. and MARKAKI, P. 2001. **Methyl jasmonate stimulates aflatoxin B<sub>1</sub> biosynthesis by *Aspergillus parasiticus***. *Journal of Agricultural and Food Chemistry* **49**: 3494–3498.

One factor promoting the production of aflatoxin is the presence of high levels of fatty acid hydroperoxides often found in plant material under stress. Jasmonic acid (JA) and its methyl ester (MeJA) are derived from linolenic acid, and their biosyntheses involve the production of lipid hydroperoxides. In this study the effect of MeJA on the growth of *Aspergillus parasiticus* and AFB<sub>1</sub> biosynthesis is reported. MeJA at 10<sup>-4</sup>M in yeast extract sucrose medium, had no apparent effect on mycelial growth during the 16 days of observation but did increase significantly the levels of AFB<sub>1</sub> after day 7 of growth. After day 9, AFB<sub>1</sub> production was decreased in contrast to the control cultures.

PETERSON, S.W., ITO, Y., HORN, B.W. and GOTO, T. 2001. ***Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius***. *Mycologia* **93**: 689–703.

A new aflatoxigenic species of *Aspergillus*, *A. bombycis*, was discovered during isolation of fungi from insect frass collected in silkworm rearing houses in Japan. The new species produces B and G aflatoxins. It is distinguished from *A. flavus* and *A. nomius* by differences in growth rates at 37 and 42°C, from *A. nomius* by roughness of the stipe, and from both of these species by dif-

ferences in the nucleotide sequences in the beta-tubulin, calmodulin, norsolorinic acid reductase, ITS, and 18S-rDNA genes. Phylogenetic analysis of the DNA sequences shows that *A. bombycis* is a phylogenetically distinct species which is most closely related to *A. nomius* and which belongs in *Aspergillus* section *Flavi*.

## Aflatoxins – Methodology

STROKA, J., ANKLAM, E., JOERISEN, U. and GILBERT, J. 2001. **Determination of aflatoxin B<sub>1</sub> in baby food (infant formula) by immunoaffinity column cleanup liquid chromatography with postcolumn bromination: Collaborative study**. *Journal of AOAC International* **84**: 1116–1123.

A collaborative study was conducted to evaluate the effectiveness of an immunoaffinity column cleanup LC method for determination of AFB<sub>1</sub> in a milk powder based infant formula at a possible future European regulatory limit of 0.1 µg/kg. The test portion was extracted with methanol-water (8 + 2, v + v), filtered, diluted with water, and applied to an immunoaffinity column. The column was washed with water and the purified AFB<sub>1</sub> was eluted with methanol. The separation and determination of AFB<sub>1</sub> was performed by reversed phase LC and detected by fluorescence after postcolumn derivatisation involving bromination with either pyridinium hydrobromide perbromide or an electrochemical (Kobra) cell by addition of bromide to the mobile phase. Test portions were spiked at AFB<sub>1</sub> levels of 0.1 and 0.2 µg/kg. Recoveries ranged from 101 to 92%. The relative standard deviation for repeatability (RSDr) ranged from 3.5 to 14%. The relative standard deviation for reproducibility (RSDR) ranged from 9 to 23%.

WALTON, M., EGNER, P., SCHOLL, P.F., WALKER, J., KENSLER, T.W. and GROOPMAN, J.D. 2001. **Liquid chromatography electrospray-mass spectrometry of urinary aflatoxin biomarkers: Characterization and application to dosimetry and chemoprevention in rats**. *Chemical Research in Toxicology* **14**: 919–926.

A liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS) method for the measurement of aflatoxin biomarkers in urine has been developed and validated. The two major aflatoxin-DNA adducts formed in rat tissues, aflatoxin N-7-guanine and 8,9-dihydro-8-(2,6-diamino-4-oxo-3,4-dihydropyrimid-5-yl formamido)-9-hydroxy-AFB<sub>1</sub>, were detected and quantified in rat urine. Other metabolites derived from

the conjugation and/or oxidation of AFB<sub>1</sub> measured in the urine of dosed rats were also assayed. In the final method the coefficients of variation of *in vivo* rat urine samples for aflatoxin N-7-guanine, AFB<sub>1</sub>-mercapturic acid and AFM<sub>1</sub> were 12.5, 12.8 and 5.8%, respectively. Further, the LC-ESI-MS/MS method to detect aflatoxin N-7-guanine in *in vivo* rat urine samples was at least 20-fold more sensitive than prior techniques. The method was used to determine the modulation of levels of urinary aflatoxin metabolites by treatment with oltipraz. Importantly, even under circumstances where the oltipraz intervention was most efficient in reducing aflatoxin metabolite levels, the LC-ESI-MS/MS method was still sensitive enough to detect the reduced biomarker content.

STROKA, J., PESCHEL, T., TITTELBACH, G., WEIDNER, G., VANOTERDIJK, R. and ANKLAM, E. 2001. **Modification of an office scanner for the determination of aflatoxins after TLC separation**. *JPC—Journal of Planar Chromatography—Modern TLC* **14**: 109–112.

A computer-driven office scanner has been modified to enable measurement of the fluorescence of aflatoxins on TLC plates. The main modifications were substitution of the light tube with a black light tube and inclusion of a filter. The modified scanner can be used to determine aflatoxins at low nanogram levels.

MOGHADDAM, A., LOBERSLI, I., GEBHARDT, K., BRAUNAGEL, M. and MARVIK, O.J. 2001. **Selection and characterisation of recombinant single-chain antibodies to the hapten aflatoxin B<sub>1</sub> from naive recombinant antibody libraries**. *Journal of Immunological Methods* **254**: 169–181.

Selection of antibodies from large repertoire phage display libraries has become a common technique for isolation of specific antibodies to antigens. Many of these libraries are shown to contain antibodies specific to haptens, but only when these haptens are derivatised or conjugated to an immobilising molecule, such as bovine serum albumin (BSA). Two-phage antibody libraries were panned against AFB<sub>1</sub>-BSA and single-chain antibody fragments were screened for binding to AFB<sub>1</sub>-BSA and AFB<sub>1</sub>. Many of the antibodies isolated specifically bound AFB<sub>1</sub>-BSA, but not soluble AFB<sub>1</sub> or BSA. Modification of the protocol led to isolation of single-chain fragment variable antibody domain (scFv) antibodies that specifically bound soluble AFB<sub>1</sub> with an affinity of 6 × 10<sup>-9</sup>M.

STERN, M.C., UMBACH, D.M., YU, M.M.C., LONDON, S.J., ZHANG, Z.Q. and TAYLOR, J.A. 2001. **Hepatitis B, aflatoxin B<sub>1</sub>, and p53 codon 249 mutation in hepatocellular carcinomas from Guangxi, People's Republic of China, and a meta-analysis of existing studies.** *Cancer Epidemiology Biomarkers & Prevention* **10**: 617–625.

In a study using DNA sequencing of hepatocellular carcinomas (HCCs) from patients in southern Guangxi, People's Republic of China, a high aflatoxin exposure area, it was found that 18/50 tumours had a 249(ser) mutation. Also, 30/60 were positive for p53 protein accumulation and 28/36 were positive for hepatitis B virus (HBV) surface antigen. Using these results along with those from 48 published studies examining the interrelationships among aflatoxin exposure, HBV infection and p53 mutations in HCCs, a method was used that takes into account both within-study and study-to-study variability and found that the mean proportion of HCCs with the 249(ser) mutation was positively correlated with aflatoxin exposure. There was little evidence for an HBV-aflatoxin interaction modulating the presence of the p53 249(ser) mutation or any type of p53 mutation.

DEVEREUX, T.R., STERN, M.C., FLAKE, G.P., YU, M.C., ZHANG, Z.Q., LONDON, S.J. and TAYLOR, J.A. 2001. **CTNNB1 mutations and beta-catenin protein accumulation in human hepatocellular carcinomas associated with high exposure to aflatoxin B<sub>1</sub>.** *Molecular Carcinogenesis* **31**: 68–73.

beta-Catenin plays a key role in the Wnt signalling pathway, and mutations of CTNNB1, the gene that encodes beta-catenin, have been identified in about one-fourth of human HCCs from regions of low AFB<sub>1</sub> exposure. In this study 62 HCCs from people highly exposed to AFB<sub>1</sub> in Guangxi, People's Republic of China, were laser-capture micro-dissected and examined for CTNNB1 mutations. In addition, 41 of the HCCs were evaluated for the presence of the beta-catenin protein by immunohistochemical methods. Twenty of the HCCs showed positive results for beta-catenin, with strong membrane staining, while adjacent non-neoplastic liver tissue lacked or showed only weak membrane staining. Mutations of CTNNB1 were identified in five HCCs. These mutations were similar to those previously reported for human HCC, although at a lower frequency. A signature mutation profile associated with AFB<sub>1</sub> exposure could not be identified. The immunohistochemical findings indicate a

role for accumulation of beta-catenin and possibly increased Wnt signalling in AFB<sub>1</sub>-associated HCC. The low frequency of CTNNB1 mutations, however, suggests that mutation of another Wnt signalling component also may be involved in aflatoxin-associated HCC.

SOFOWORA, G.G., CHOO, E.F., MAYO, G., SHYR, Y. and WILKINSON, G.R. 2001. **In vivo inhibition of human CYP1A2 activity by oltipraz.** *Cancer Chemotherapy and Pharmacology* **47**: 505–510.

The ability of oltipraz to induce phase II xenobiotic enzymes that detoxify the ultimate carcinogen formed *in vivo* is thought to be an important mechanism by which aflatoxin-associated HCC risk may be attenuated. However, an additional mechanism could be a reduction in the activation of environmental procarcinogens by certain cytochrome P450 (CYP) isoforms. This hypothesis was tested with respect to CYP1A2, by using the clearance of caffeine by N-demethylation as a phenotypic trait measurement of the isoform's catalytic activity. Subjects received a single oral dose of caffeine (200 mg) on five separate occasions: on the day prior to oltipraz administration (day 0), 2hr after the first (day 1) of eight daily oral doses of oltipraz (125 mg) and 2 hr after the last dose (day 8). Results demonstrate that oltipraz is a potent *in vivo* inhibitor of CYP1A2 in humans and, because this isoform is importantly involved in procarcinogen activation, they also indicate that such inhibition probably contributes to oltipraz's cancer-chemopreventive effect. In addition, the findings also suggest the likelihood of significant drug interactions between oltipraz and drugs whose metabolism is mediated by CYP1A2.

VANVLEET, T.R., KLEIN, P.J. and COULOMBE, R.A. 2001. **Metabolism of aflatoxin B<sub>1</sub> by normal human bronchial epithelial cells.** *Journal of Toxicology and Environmental Health—Part A* **63**: 525–540.

The potential for AFB<sub>1</sub> activation in human lung was studied by examining cytochrome P450 (CYP)-mediated activation and glutathione S-transferase (GST)-mediated detoxification of AFB<sub>1</sub> in cultured normal human bronchial epithelial (NHBE) cells. At 0.15 μM, AFB<sub>1</sub> was metabolised only to the detoxified metabolite AFQ<sub>1</sub>. At 1.5 μM AFB<sub>1</sub>, both AFM<sub>1</sub> and AFQ<sub>1</sub> were produced. Cells pretreated with 50 μM 3-methylcholanthrene (3MC), a CYP1A inducer, for 72 hr prior to 0.15 μM AFB<sub>1</sub>, produced the activated AFB<sub>1</sub> 8,9-epoxide (AFBO). Similarly, microsomes prepared from 3MC-pretreated cells formed AFBO, but microsomes from non-induced cells did

not. While AFB<sub>1</sub>-DNA adducts were not detected at low AFB<sub>1</sub> concentrations in untreated NHBE, 3MC induction caused the production of AFB<sub>1</sub>-DNA adducts at 0.015 and 0.15 μM AFB<sub>1</sub>. The data support earlier findings showing modest CYP-mediated AFB<sub>1</sub> activation in human airways, but indicate that exposure to polycyclic aromatic hydrocarbons, such as 3MC, which induce CYP(s) that specifically activate AFB<sub>1</sub> may increase the harmful effects of AFB<sub>1</sub> exposures in human airways.

STETTLER, P.M. and SENGSTAG, C. 2001. **Liver carcinogen aflatoxin B<sub>1</sub> as an inducer of mitotic recombination in a human cell line.** *Molecular Carcinogenesis* **31**: 125–138.

In the lower eukaryote yeast, the recombinagenic activity of AFB<sub>1</sub> surpasses its mutagenic activity. To investigate whether the recombinagenic activity of AFB<sub>1</sub> also would be identified in human cells, the fate of a heterozygous thymidine kinase (tk) allele in the human lymphoblastoid cell line TK6 was followed after exposure to AFB<sub>1</sub>. In parallel, a similar analysis was performed on TK6 cells exposed to the alkylating agent N-nitrosomethylurea, a well-known classic point mutagen. There was a difference in the molecular mechanism leading to inactivation of the tk allele upon exposure to these two mutagens. In AFB<sub>1</sub>-exposed cells the fraction of recombination-derived mutants predominated, whereas in N-nitrosomethylurea-exposed cells the fraction of point mutants was higher. Thus, the recombinagenic activity of AFB<sub>1</sub> previously identified in a lower eukaryote also was found in the human cell line TK6. The data support the hypothesis that mitotic recombination represents a central mechanism of action in AFB<sub>1</sub>-induced liver carcinogenesis.

YAKICIER, M.C., LEGOIX, P., VAURY, C., GRESSIN, L., TUBACHER, E., CAPRON, F., BAYER, J., DEGOTT, C., BALABAUD, C. and ZUCMAN-ROSSI, J. 2001. **Identification of homozygous deletions at chromosome 16q23 in aflatoxin B<sub>1</sub> exposed hepatocellular carcinoma.** *Oncogene* **20**: 5232–5238.

Loss of heterozygosity (LOH) represents the most frequent genetic alteration observed in HCC. Chromosome 16q is of particular interest as it exhibits LOH in 29% of HCC tumours and is frequently lost in breast, prostate, ovarian and gastric carcinomas. A total of 157 HCC tumours were genotyped for 17 microsatellite markers distributed on chromosome 16q and a common region of LOH, localised between the markers D16S518 and D16S504, was determined. The data revealed a relationship between chromosome 16q homozygous

deletions and R249S *p53* mutations in tumours where the patient had been exposed to AFB<sub>1</sub>. These results are consistent with a role of AFB<sub>1</sub> in the instability of chromosome 16q at the fragile site FRA16D. However, the nature of the specific gene that is altered during hepatocarcinogenesis remains to be elucidated.

SUN, C.A., WANG, L.Y., CHEN, C.J., LU, S.N., YOU, S.L., WANG, L.W., WANG, Q., WU, D.M. and SANTELLA, R.M. 2001. **Genetic polymorphisms of glutathione S-transferases M1 and T1 associated with susceptibility to aflatoxin-related hepatocarcinogenesis among chronic hepatitis B carriers: A nested case-control study in Taiwan.** *Carcinogenesis* **22**: 1289–1294.

The modifying effect of glutathione S-transferase (GST) M1 and T1 polymorphisms on aflatoxin-induced hepatocarcinogenesis among chronic HBV surface antigen (HBsAg) carriers was investigated. A total of 79 HBsAg-positive cases of HCC were studied. Blood samples were tested for hepatitis B and C viral markers and for AFB<sub>1</sub>-albumin adducts. GSTM1 and GSTT1 genotypes were determined by PCR. There was a statistically significant relationship between detectable levels of AFB<sub>1</sub>-albumin adducts in serum and risk of HCC among chronic HBsAg carriers. In addition, the effect of aflatoxin exposure on HCC risk was more pronounced among chronic HBsAg carriers with the GSTT1 null genotype than those who were non-null. The interaction between serum AFB<sub>1</sub>-albumin adduct level and GSTT1 genotype was statistically significant. For GSTM1 the effect of aflatoxin exposure on HCC risk in those with the null genotype was also greater than in those with the gene present, but the difference was not significant. Notably, when the interaction between aflatoxin exposure and GSTT1 genotype was considered, aflatoxin exposure by itself was not a significant determinant of HCC risk among chronic HBsAg carriers.

CHEN, S.Y., CHEN, C.J., TSAI, W.Y., AHSAN, H., LIU, T.Y., LIN, J.T. and SANTELLA, R.M. 2000. **Associations of plasma aflatoxin B<sub>1</sub>-albumin adduct level with plasma selenium level and genetic polymorphisms of glutathione S-transferase M1 and T1.** *Nutrition and Cancer—An International Journal* **38**: 179–185.

Mortality from HCC is extraordinarily high in Matzu, an island off the coast of Southeastern China. In 304 healthy adult residents from Matzu, HBsAg status, genotypes of GSTM1 and T1, and plasma selenium were determined. Men had higher AFB<sub>1</sub>-albumin adduct levels than women. GSTM1-non-null and GSTT1-null geno-

types and low plasma selenium level were significantly associated with an increased level of AFB<sub>1</sub>-albumin adducts among men, whereas age was significantly correlated with adduct level among women. High intake of fermented beans was associated with an increased adduct level among men and women. The inverse associations between plasma selenium level and AFB<sub>1</sub>-albumin adducts were statistically significant among those with null genotypes of GSTM1 and GSTT1, but not among the non-null genotypes.

TIEMERSMA, E.W., OMER, R.E., BUNSCHOTEN, A., VANTVEER, P., KOK, F.J., IDRIS, M.O., KADARU, A.M.Y., FEDAIL, S.S. and KAMPMAN, E. 2001. **Role of genetic polymorphism of glutathione-S-transferase T1 and microsomal epoxide hydrolase in aflatoxin-associated hepatocellular carcinoma.** *Cancer Epidemiology Biomarkers & Prevention* **10**: 785–791.

Aflatoxins can occur in peanut butter and are metabolised by genetically polymorphic enzymes such as GSTM1 and T1, and microsomal epoxide hydrolase encoded by epoxide hydrolase gene (EPHX). GSTM1 has been found to modify the association between peanut butter consumption and HCC. The possible roles of GSTT1 and EPHX polymorphisms in this relationship were examined in a Sudanese case control study on HCC. Univariate analysis showed that GSTT1 polymorphism was not associated with HCC, whereas EPHX 113HH and 139HH genotypes increased the risk of HCC. Results do not suggest significant roles of GSTT1 and EPHX in aflatoxin metabolism.

METHENITOU, G., MARAVELIAS, C., ATHANASELIS, S., DONA, A. and KOUTSELINIS, A. 2001. **Immunomodulative effects of aflatoxins and selenium on human natural killer cells.** *Veterinary and Human Toxicology* **43**: 232–234.

The possible immunomodulative effects of AFB<sub>1</sub> and selenium on natural killer (NK) cells from healthy volunteers were evaluated by a Cr-51 release natural killer assay. AFB<sub>1</sub> and selenium separately had an immunosuppressive effect on NK cell activity. When they were studied in combination, they caused a statistically significant increase of the cytotoxic activity of NK cells in the presence of the lowest concentrations of AFB<sub>1</sub> (0.005 µg/kg) and of sodium selenite (10<sup>-8</sup>M).

KWAK, M.K., EGNER, P.A., DOLAN, P.M., RAMOS-GOMEZ, M., GROOPMAN, J.D., ITOH, K., YAMAMOTO, M. AND KENSLER, T.W. 2001. **Role of**

**phase 2 enzyme induction in chemoprotection by dithiolethiones.** *Mutation Research—Fundamental and Molecular Mechanisms of Mutagenesis* **480**: 305–315.

A placebo-controlled, double blind clinical trial of oltipraz was conducted in residents of Qidong, People's Republic of China, who are exposed to dietary aflatoxins and who are at high risk for the development of liver cancer. Oltipraz significantly enhanced excretion of a phase 2 product, aflatoxin-mercapturic acid, a derivative of the aflatoxin-glutathione conjugate, in the urine of study participants administered oltipraz at 125 mg by mouth daily. Administration of oltipraz at 500 mg once a week led to a significant reduction in the excretion of the primary oxidative metabolite of AFB<sub>1</sub>, AFM<sub>1</sub>, when measured shortly after drug administration. While this study highlighted the general feasibility of inducing phase 2 enzymes in humans, a longer term intervention is addressing whether protective alterations in aflatoxin metabolism can be sustained for extended periods of time in this high-risk population.

BURGOS-HERNANDEZ, A., LOPEZ-GARCIA, R., NJAPAU, H. and PARK, D.L. 2001. **Anti-mutagenic compounds from corn.** *Food Additives and Contaminants* **18**: 797–809.

In previous studies with aflatoxin-contaminated corn an uncharacteristic response for AFB<sub>1</sub> in the *Salmonella*/microsomal mutagenicity assay (Ames test) was observed and the presence of anti-aflatoxin factors in the corn was suggested. In the current study, corn was extracted and fractionated using TLC, and the Ames test was used to monitor for anti-mutagenic activity in the corn fractions. Several corn fractions, at different stages in the isolation and purification process, showed anti-mutagenic dose responses when exposed to pure AFB<sub>1</sub>.

GUENGERICH, F.P., CAI, H.L., MCMAHON, M., HAYES, J.D., SUTTER, T.R., GROOPMAN, J.D., DENG, Z.W. and HARRIS, T.M. 2001. **Reduction of aflatoxin B<sub>1</sub> dialdehyde by rat and human aldo-keto reductases.** *Chemical Research in Toxicology* **14**: 727–737.

AFB<sub>1</sub> 8,9-epoxide nonenzymatically hydrolyses rapidly to a dihydrodiol that in turn undergoes slow, base-catalysed ring opening to a dialdehyde. One enzyme induced by cancer chemopreventive agents is AFB<sub>1</sub> aldehyde reductase (AFAR), which catalyses the NADPH-dependent reduction of the dialdehyde to a dialcohol. The enzymic reaction of AFB<sub>1</sub> dialdehyde with rat and human AFAR isozymes was investigated. Two monoalcohols were identified as products. The monoalcohol corresponding

to reduction at C-8 formed first in reactions catalysed by either the rat or the human AFAR. This C-8 monoalcohol was further reduced to AFB<sub>1</sub> dialcohol by AFAR. The other monoalcohol (C-6a) was formed but not reduced to the dialcohol rapidly. A survey of 12 human liver cytosol samples showed a variation of 2.3-fold in AFAR activity. Rats treated with AFB<sub>1</sub> excreted the dialcohol and a monoalcohol in urine. The results of these studies are consistent with a role of rat and human AFAR in protection against AFB<sub>1</sub> toxicity.

QIN, G.Z., NING, Y.Y. and LOTLIKAR, P.D. 2000. **Chemoprevention of aflatoxin B<sub>1</sub>-initiated and carbon tetrachloride-promoted hepatocarcinogenesis in the rat by green tea.** Nutrition and Cancer—An International Journal **38**: 215–222.

Chemoprevention of hepatocarcinogenesis by green tea (GT) has been examined in male Fischer rats fed AFB<sub>1</sub> and CCl<sub>4</sub> as the initiator and promoter, respectively. Animals were fed AFB<sub>1</sub> at 0.25 mg/kg bw twice a week for 2 weeks, and 2 weeks later, CCl<sub>4</sub> was injected at 0.8 ml/kg bw once per week for 11 weeks. Rats were given 0.5% GT in their drinking water before and during initiation (0–4 wk) or during promotion (6–16 wk) or throughout the experimental period. Feeding of GT during initiation or promotion inhibited the number of glutathione S-transferase placental form- and gamma-glutamyl transpeptidase positive hepatic foci by 30–40% and the area and volume by 50%. GT treatment throughout the period inhibited the number of both types of hepatic foci by 60% and the area and volume by 75–80%. Cell proliferation was inhibited 35% by GT given during promotion, whereas inhibition was 65% when GT was given during initiation or throughout the period.

MISTRY, K.J., KRISHNA, M. and BHATTACHARYA, R.K. 2001. **Effect of aflatoxin B<sub>1</sub> on phosphoinositide signal transduction pathway during regeneration of liver cells following partial hepatectomy.** Indian Journal of Biochemistry & Biophysics **38**: 270–273.

AFB<sub>1</sub> administered to partially hepatectomised rats 4 hr prior to sacrifice, activated the signalling pathway in regenerating rat liver. Activation of protein kinase C by AFB<sub>1</sub> during regeneration of liver cells when protein kinase C is normally inhibited, may create conditions conducive to carcinogenesis.

HIRUMA, S., GOPALAN-KRICZKY, P., QIN, G.Z., GAUGHAN, J.P. and LOTLIKAR, P.D. 2001. **Differential effects of acetaminophen pretreatment**

**on hepatic aflatoxin B<sub>1</sub>-DNA binding, cellular proliferation, and aflatoxin B<sub>1</sub>-induced hepatic foci in rats and hamsters.** Cancer Letters **170**: 117–124.

Effects of acetaminophen (AAP) pretreatment on hepatic AFB<sub>1</sub>-DNA binding, cellular proliferation and AFB<sub>1</sub>-induced glutathione S-transferase placental form (GST-P) positive hepatocytes and foci have been examined in young male rats and hamsters. Intraperitoneal dosing with AAP 3 hr before AFB<sub>1</sub> ip injection showed 3-fold more AFB<sub>1</sub>-DNA binding in hamsters and 40% less binding in rats. Cell proliferation was not significant 24–96 hr after AAP treatment of rats; however, proliferation was stimulated and was maximum (11%) in hamsters at 72 hr after AAP treatment. Dosing of rats with AFB<sub>1</sub> alone gave an appreciable number of GST-P positive minifoci; pretreatment with AAP 48 hr before AFB<sub>1</sub> had no effect on the number and focal area of foci. In hamsters, AFB<sub>1</sub> alone yielded GST-P positive hepatocytes without any minifoci. Pretreatment with AAP 48 or 72 hr before AFB<sub>1</sub> produced increases in both GST-P positive hepatocytes and minifoci.

KIM, B.R., KIM, D.H., PARK, R., KWON, K.B., RYU, D.G., KIM, Y.C., KIM, N.Y., JEONG, S., KANG, B.K. and KIM, K.S. 2001. **Effect of an extract of the root of *Scutellaria baicalensis* and its flavonoids on aflatoxin B<sub>1</sub> oxidizing cytochrome P450 enzymes.** Planta Medica **67**: 396–399.

The inhibition of AFB<sub>1</sub> metabolism by a water extract of the root of *Scutellaria baicalensis* and its flavonoids was examined in liver microsomes. The water extract potently inhibited the production of AFM<sub>1</sub> by cytochrome P450 (CYP)1A1/2 and slightly reduced AFBO formation by CYP1A1/2, CYP2B1, CYP2C11 and CYP3A1/2 in TCDD treated rat liver microsomes. IC<sub>50</sub> values for AFM<sub>1</sub> and AFBO formation were 6.8 and 122.4 mg/L, respectively. Among the flavonoids isolated from the extract, wogonin showed the highest inhibitory activity towards AFM<sub>1</sub> formation. The extract had no effects on the formation of AFBO and AFQ<sub>1</sub> in human liver microsomes, or on the activities of CYP2B1, CYP2C11 and CYP3A1/2.

RAJ, H.G., KOHLI, E., ROHIL, V., DWARAKANATH, B.S., PARMAR, V.S., MALIK, S., ADHIKARI, J.S., TYAGI, Y.K., GOEL, S., GUPTA, K., BOSE, M. and OLSEN, C.E. 2001. **Acetoxy-4-methylcoumarins confer differential protection from aflatoxin B<sub>1</sub>-induced micronuclei and apoptosis in lung and bone marrow cells.** Mutation Research—Genetic Toxicology and Environmental Mutagenesis **494**: 31–40.

The ability of various acetoxy derivatives of 4-methylcoumarins to inhibit the genotoxic changes due to AFB<sub>1</sub> is reported. Several 4-methylcoumarins were separately administered ip to male Wistar rats followed by AFB<sub>1</sub> administration ip or intratracheally at 2–8 mg/kg bw and another dose of the test compound. The animals were sacrificed 26 hr after AFB<sub>1</sub> administration. AFB<sub>1</sub> caused significant dose dependent induction of micronuclei in bone marrow as well as lung cells. Apoptotic bodies were observed in lung cells and were found to correlate with micronuclei induction. 7,8-Diacetoxy-4-methylcoumarin (DAMC) injection caused a significant decrease in apoptosis due to AFB<sub>1</sub> in lung cells and micronuclei in both bone marrow and lung cells. Results suggest that DAMC-mediated acetylation of microsomal P-450 through the action of microsomal transacetylase is responsible for the protective action of DAMC. The relative number and position of acetoxy groups on the coumarin nucleus determine the specificity to the transacetylase necessary for the chemopreventive action.

YATIM, A.M. and SACHAN, D.S. 2001. **Carnitine alters binding of aflatoxin to DNA and proteins in rat hepatocytes and cell-free systems.** Journal of Nutrition **131**: 1903–1908.

The effects of L-carnitine on AFB<sub>1</sub>-DNA adduct formation in isolated rat hepatocytes was investigated. There was negative linear correlation between the dosage of carnitine and formation of [<sup>3</sup>H]AFB<sub>1</sub>-DNA adducts in the hepatocytes; however, the partitioning of AFB<sub>1</sub> into cellular compartments was not affected by carnitine. The attenuating effect of carnitine on AFB<sub>1</sub>-DNA adduct formation was also present in a cell-free system. Carnitine appears to interfere with bioactivation of AFB<sub>1</sub> and binding of AFB<sub>1</sub>-epoxide to DNA. On the contrary, carnitine enhanced the binding of AFB<sub>1</sub> and its epoxide to microsomal proteins, plasma proteins and bovine serum albumin. These results indicate that carnitine diverts AFB<sub>1</sub>-epoxide away from DNA by promoting binding to proteins. It is concluded that modulation of AFB<sub>1</sub> binding to proteins and DNA by carnitine alters the carcinogenic and hepatotoxic potential of AFB<sub>1</sub> and poses concerns about the human AFB<sub>1</sub>-exposure data based on the AFB<sub>1</sub>-albumin adduct concentrations as a biomarker.

RASTOGI, R., SRIVASTAVA, A.K. and RASTOGI, A.K. 2001. **Long term effect of aflatoxin B<sub>1</sub> on lipid peroxidation in rat liver and kidney: Effect of picroliv and silymarin.** Phytotherapy Research **15**: 307–310.

The comparative antioxidant effects of picroliv, a standardised iridoid glycoside fraction of *Picrorhiza kurroa*, and silymarin, a well known standard hepatoprotective, on AFB<sub>1</sub>-induced lipid peroxidation in rat liver and kidney were investigated. Marked increases in lipid peroxide levels and a concomitant decrease in enzymic antioxidant levels were observed in rats injected ip with AFB<sub>1</sub> at 2 mg/kg, while both picroliv and silymarin treatment reversed the condition to near normal levels. The effects of picroliv and silymarin were comparable.

SHEEN, L.Y., WU, C.C., LII, C.K. and TSAI, S.J. 2001. **Effect of diallyl sulfide and diallyl disulfide, the active principles of garlic, on the aflatoxin B<sub>1</sub>-induced DNA damage in primary rat hepatocytes.** *Toxicology Letters* **122**: 45–52.

The effect of the active principles in garlic, diallyl sulfide (DAS) and diallyl disulfide (DADS), on AFB<sub>1</sub>-induced DNA damage in primary rat hepatocytes treated with AFB<sub>1</sub> at 10 µM was investigated. DAS at 0.5 and 2 mM or DADS at 0.5 and 1 mM significantly increased the viability of hepatocytes compared with the AFB<sub>1</sub> controls and significantly decreased DNA damage induced by AFB<sub>1</sub>. DAS and DADS increased glutathione S-transferase (GST) and glutathione peroxidase activities indicating their protection of rat hepatocytes from AFB<sub>1</sub>-induced DNA damage is via increased activities of GST and glutathione peroxidase.

LIU, J., YANG, C.F., WASSER, S., SHEN, H.M., TAN, C.E.L. and ONG, C.N. 2001. **Protection of *Salvia miltiorrhiza* against aflatoxin-B<sub>1</sub>-induced hepatocarcinogenesis in Fischer 344 rats. Dual mechanisms involved.** *Life Sciences* **69**: 309–326.

Extract of *Salvia miltiorrhiza* (SM) has been widely used in traditional Chinese medicine for treating liver diseases. The effect of SM on AFB<sub>1</sub>-induced hepatocarcinogenesis was investigated in male Fischer 344 rats given AFB<sub>1</sub> at 400 µg/kg bw by gavage once a week for 24 weeks with or without SM at 2.5g/kg bw 5 days/week by gavage for a total of 28 weeks, including 4 weeks before and 24 weeks during AFB<sub>1</sub> exposure. The elevation of serum alanine aminotransferase and aspartate aminotransferase activities due to AFB<sub>1</sub> dosing was almost completely abolished by the treatment of SM. SM substantially reduced

GST-P positive foci formation and GST-P mRNA expression caused by AFB<sub>1</sub>. Furthermore, the inhibition on AFB<sub>1</sub> hepatocarcinogenesis was associated with a corresponding decrease in AFB<sub>1</sub>-DNA adducts formation as well as AFB<sub>1</sub>-induced oxidative DNA damage.

ABBAS, T.A. and ALI, B.H. 2001. **Retinol values in the plasma of the Arabian camel (*Camelus dromedarius*) and the influence of aflatoxicosis.** *Veterinary Research Communications* **25**: 517–522.

In male and female adult camels (*Camelus dromedarius*) in the United Arab Emirates the mean concentration of retinol in healthy camels was 460.1±49.3 ng/L. Camels with aflatoxicosis had a mean concentration of the vitamin in the plasma of 243.4±32.3 ng/L. The concentrations of aflatoxins in the liver and rumin contents of these animals were 18.2 and 243.4 µg/kg, respectively.

SOLIMAN, K.M., EL-FARAMAWY, A.A., ZAKARIA, S.M. and MEKKAWY, S.H. 2001. **Monitoring the preventive effect of hydrogen peroxide and gamma-radiation of aflatoxicosis in growing rabbits and the effect of cooking on aflatoxin residues.** *Agricultural and Food Chemistry* **49**: 3291–3295.

Growing rabbits were fed a diet contaminated with aflatoxins at 833 µg/kg or a diet decontaminated by treatment using 5% H<sub>2</sub>O<sub>2</sub> and gamma-radiation at a dose level of 5 kGy. There were no differences in feed consumption between the groups, but feed efficiency was reduced significantly in both the contaminated and decontaminated groups. Mortality percentage was 25% for both groups. These findings indicate that the use of H<sub>2</sub>O<sub>2</sub> and gamma-radiation for the destruction of aflatoxins in contaminated diet induces adverse effects in the animals. Results of cooking rabbit livers showed that the percentage of aflatoxin reduction ranged between 67 and 80% in boiled liver and between 79 and 90.5% in fried liver, whereas complete reduction in aflatoxin was found after boiling followed by frying.

SALEM, M.H., KAMEL, K.I., YOUSEF, M.I., HASSAN, G.A. and EL-NOUTY, F.D. 2001. **Protective role of ascorbic acid to enhance semen quality of rabbits treated with sublethal doses of aflatoxin B<sub>1</sub>.** *Toxicology* **162**: 209–218.

The effects of ascorbic acid on productive and reproductive characteristics of mature male rabbits given AFB<sub>1</sub> at 15 or 30 µg/kg bw on alternating days was investigated. Live body weight, dry matter intake, relative testes weight and serum testosterone were significantly reduced by treatment with AFB<sub>1</sub> in a dose dependent manner. Aflatoxin treatment also decreased ejaculate volume, sperm concentration, total sperm output, sperm motility index and semen initial fructose concentration in a dose dependent manner. Treatment with ascorbic acid alleviated the negative effects of AFB<sub>1</sub>.

SAHOO, P.K. and MUKHERJEE, S.C. 2001. **Immunosuppressive effects of aflatoxin B<sub>1</sub> in Indian major carp (*Labeo rohita*).** *Comparative Immunology Microbiology and Infectious Diseases* **24**: 143–149.

AFB<sub>1</sub> at 1.25 and 5 mg/kg bw were injected ip into rohu (*Labeo rohita*) fingerlings weighing 30–50 g, and the fish were observed for a period of 90 days. Treated fish showed a reduction of total protein, globulin levels, bacterial agglutination titre, NET and serum bactericidal activities, as well as an enhanced A:G ratio without change in albumin concentration, irrespective of dose levels of toxin treatment.

VALDIVIA, A.G., MARTINEZ, A., DAMIAN, F.J., QUEZADA, T., ORTIZ, R., MARTINEZ, C., LLAMAS, J., RODRIGUEZ, M.L., YAMAMOTO, L., JARAMILLO, F., LOARCA-PINA, M.G. and REYES, J.L. 2001. **Efficacy of N-acetylcysteine to reduce the effects of aflatoxin B<sub>1</sub> intoxication in broiler chickens.** *Poultry Science* **80**: 727–734.

N-acetylcysteine (NAC) has been used safely in humans and in other mammals as an antidote against several toxic and carcinogenic agents, including AFB<sub>1</sub>. The capability of dietary supplementation with NAC to ameliorate the effects of subacute intoxication with AFB<sub>1</sub> in broiler chickens was studied. One-day-old chickens were fed AFB<sub>1</sub> at 3 mg/kg feed with or without the addition of NAC at 800 mg/kg bw for 21 days. Broilers treated with AFB<sub>1</sub> plus NAC were shown to be partially protected against the deleterious effects of AFB<sub>1</sub> on body weight, daily weight gain, feed conversion index, plasma and hepatic total protein concentration, plasma alanine aminotransferase, hepatic GST and reduced GSH liver concentration.

## **AUSTRALIAN MYCOTOXIN NEWSLETTER**

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