

Abstracts

Mycotoxins – General

- SCUDAMORE, K.A. and PATEL, S. 2000. **Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize imported into the United Kingdom.** *Food Additives and Contaminants* **17**: 407–416.
- Raw maize as received at ports or at major maize mills in the UK were examined for aflatoxins B₁, B₂, G₁ and G₂, ochratoxin A (OA), zearalenone (ZEA) and fumonisins B₁, B₂ and B₃ using HPLC methods with detection limits of 0.1 µg/kg for each aflatoxin and OA, 4 mg/kg for ZEA and 10 µg/kg for each fumonisin. Ninety-five percent and 92.1% of samples met the new EC statutory maximum permissible level for total aflatoxins and aflatoxin B₁, respectively. ZEA and fumonisins were detected in almost every sample with 41.7% of maize containing ZEA at more than 100 µg/kg and 48% of samples containing total fumonisins at more than 1000 µg/kg. Initial cleaning of raw maize reduced aflatoxin concentrations by about 40% and total fumonisins by 32%.
- SCHNEWEIS, I., MEYER, K., HORMANSDORFER, S. and BAUER, J. 2000. **Mycophenolic acid in silage.** *Applied and Environmental Microbiology* **66**: 3639–3641.
- Mycophenolic acid, a metabolite of *Penicillium roqueforti*, was detected in 74 of 233 grass and maize silage samples collected in Bavaria, Germany. Levels ranged from 20 to 35,000 (mean 1,400) µg/kg. Mycophenolic acid has well-known immunosuppressive properties and might cause problems if contaminated silage is fed to livestock.
- MEDINA-MARTINEZ, M.S. and MARTINEZ, A.J. 2000. **Mold occurrence and aflatoxin B₁ and fumonisin B₁ determination in corn samples in Venezuela.** *Journal of Agricultural and Food Chemistry* **48**: 2833–2836.
- White and yellow corn samples in Venezuela were examined for mould, aflatoxin B₁ (AFB₁) and fumonisin B₁ (FB₁) contamination. Yellow corn showed higher mould incidence than white corn. *Aspergillus flavus* and *Fusarium moniliforme* were isolated. AFB₁ and FB₁ were detected in 16.6 and 83.8% of samples, respectively.
- VELLUTI, A., MARIN, S., BETTUCCI, L., RAMOS, A.J. and SANCHIS, V. 2000. **The effect of fungal competition on colonization of maize grain by *Fusarium moniliforme*, *F. proliferatum* and *F. graminearum* and on fumonisin B₁ and zearalenone formation.** *International Journal of Food Microbiology* **59**: 59–66.
- The effects of water activity (0.98, 0.95, 0.93) and temperature (15, 25°C) on fungal growth and toxin production were determined when isolates of *Fusarium moniliforme* and *F. proliferatum* producing fumonisin and an isolate of *F. graminearum* producing ZEA were incubated at the same time on irradiated maize grains. Populations (CFUs) of *F. moniliforme* and *F. proliferatum* were reduced to some extent by the presence of *F. graminearum* under all conditions tested, while the presence of *F. moniliforme* or *F. proliferatum* had a minor inhibitory effect on populations of *F. graminearum*. FB₁ production by *F. proliferatum* was inhibited under all conditions tested while production by *F. moniliforme* was inhibited at 15°C and enhanced at 25°C in the presence of *F. graminearum*. ZEA production was not significantly affected in the presence of *F. moniliforme* and *F. proliferatum*.
- LI, F.Q. and YOSHIZAWA, T. 2000. **Alternaria mycotoxins in weathered wheat from China.** *Journal of Agricultural and Food Chemistry* **48**: 2920–2924.
- Twenty-two samples of weathered wheat kernels from the 1998 crop, representing three locations in the suburbs of Beijing, China, were examined for the presence of *Alternaria* toxins. Wheat kernels were significantly invaded by *Alternaria* species, mostly *A. alternata*, with an average infection frequency of 87.3%. Alternariol was detected in 20/22 samples at concentrations ranging between 116 and 731 µg/kg and alternariol methyl ether was detected in 21/22 samples at concentrations ranging between 52 and 1426 µg/kg. Tenuazonic acid was detected in all samples analysed at an average concentration of 2419 µg/kg with a maximum of 6432 µg/kg. All samples were free from altertoxin 1 and altenenuene. This is the first report of the natural occurrence of *Alternaria* toxins in Chinese wheat.
- BEGUM, F. and SAMAJPATI, N. 2000. **Mycotoxin production on rice, pulses and oilseeds.** *Naturwissenschaften* **87**: 275–277.
- Mycotoxin producing fungi were isolated from rice, pulses and oilseeds sold for human consumption in the local markets of Calcutta. Among the isolates, *Aspergillus flavus* and *Aspergillus parasiticus* produced AFB₁, *A. flavus* produced AFG¹, *A. ochraceus* produced ochratoxin, *A. japonicus* produced sterigmatocystin and *Penicillium citrinum* produced citrinin. AFB₁ in the range 333–10416 µg/kg was produced by *Aspergillus* spp. in rice, pulses and oilseeds.
- SWEENEY, M.J., WHITE, S. and DOBSON, A.D.W. 2000. **Mycotoxins in agriculture and food safety.** *Irish Journal of Agricultural and Food Research* **39**: 235–244.

A review with 12 references. The production of two groups of mycotoxins, aflatoxins and OA, is discussed together with the development of molecular based detection techniques to monitor mycotoxin production.

CHELKOWSKI, J., KAPTUR, P., TOMKOWIAK, M., KOSTECKI, M., GOLINSKI, P., PONITKA, A., SLUS-ARKIEWICZ-JARZINA, A. and BOCIANOWSKI, A. 2000. **Moniliformin accumulation in kernels of triticale accessions inoculated with *Fusarium avenaceum* in Poland.** Journal of Phytopathology – Phytopathologische Zeitschrift **148**: 433–439.

Twelve Polish winter triticale cultivars and 14 doubled haploid lines (derived from the cv. Lasko × line SZD 366 hybrids) were inoculated with *Fusarium avenaceum*, a moniliformin (MON) producer, and their susceptibility to *Fusarium* head blight was evaluated. MON accumulated in kernels of all inoculated cultivars. In most of the genotypes examined the reaction to the fungus and MON content changed significantly from season to season. Double haploid lines accumulated on average 2.62 and 0.85 mg/kg of MON in 1997 and 1998, respectively. The correlation coefficient for MON content/*Fusarium* damaged kernels percentage was 0.539 in cultivars and 0.548 in the double haploid lines. This is the first report of *Fusarium* head blight of a segregating population in triticale.

RAFAI, P., BATA, A., JAKAB, L. and VANYI, A. 2000. **Evaluation of mycotoxin-contaminated cereals for their use in animal feeds in Hungary.** Food Additives and Contaminants **17**: 799–808.

In the period 1991 to 1998, 760 maize, 367 wheat, 119 soybean, 222 barley, 85 bran, 32 triticale, 60 oat, 14 rye and 22 sunflower samples collected in Hungary were investigated for the presence and concentration of T-2 toxin, ZEA, deoxynivalenol, nivalenol, diacetoxyscirpenol, HT-2 toxin, fusarenone-X and OA. Results indicate that soybean tends to be good substrate for trichothecene producing fungi and the rate of contamination is regarded as substantial. The commodities were assorted into one of three quality categories. The proportion of objectionable samples was 3.0, 2.2, 2.3 and 1.7% in maize, wheat, barley and soybean samples, respectively. The proportion of objectionable samples was much higher in the case of bran, oat and triticale which were 7.1, 6.7 and 6.3%, respectively. The results of the present investigation indicate a need for regular screening for mycotoxins of

importance and individual appraisal of each commodity from the point of their use in animal feeds.

WILLIAMS, R.M., STOCKING, E.M. and SANZ-CERVERA, J.F. 2000. **Biosynthesis of prenylated alkaloids derived from tryptophan.** Biosynthesis: Aromatic Polyketides, Isoprenoids, Alkaloids **209**: 97–173.

This review with 134 references considers the biosynthesis of prenylated indole alkaloids and related natural substances derived from tryptophan. The families of compounds covered include the brevi-anamides, austamides, paraherquamides, marcfortine, roquefortine, aszonalenin, echinulin, verruculogen, the fumitremorgins, alpha-cyclopiazonic acid and the ergot alkaloids. The biosyntheses of the families selected for this chapter have not, to the best of the authors' knowledge, been reviewed previously.

Mycotoxins – Methodology

MONTI, S.M., FOGLIANO, V., LOGRIECO, A., FERRACANE, R. and RITIENI, A. 2000. **Simultaneous determination of beauvericin, enniatins, and fusaproliferin by high performance liquid chromatography.** Journal of Agricultural and Food Chemistry **48**: 3317–3320.

A rapid, sensitive and inexpensive HPLC method for routine screening of beauvericin, fusaproliferin and enniatin B₁, A1 and B has been optimised. Detection limits for the different compounds ranged between 0.5 and 3.6 ng and recoveries averaged from 56 to 74%. LC–MS conditions for enniatin analyses by API electrospray technique were set up which allowed for unique identification of three different enniatins.

DAMOTTA, S. and SOARES, L.M.V. 2000. **Simultaneous determination of tenuazonic and cyclopiazonic acids in tomato products.** Food Chemistry **71**: 111–116.

A procedure for the simultaneous determination of tenuazonic acid (TA) and cyclopiazonic acid (CPA) is described for the first time. It has been applied to tomato products and involves simple extraction, defatting and partitioning steps followed by metal complexation chromatography on a reverse phase C18 column. The quantification limits in tomato products for TA was 11.0 µg/kg and for CPA 8.0 µg/kg. Average recoveries for TA and CPA were 88 and 78%, respectively.

PITT, J.I. 2000. **Toxigenic fungi and mycotoxins.** British Medical Bulletin **56**: 184–192.

Growth of commonly occurring filamentous fungi in foods may result in production of mycotoxins, which can cause a variety of ill effects in humans, from allergic responses to immunosuppression and cancer. The most important mycotoxins are aflatoxins, OA, fumonisins, trichothecenes and ZEA. Currently available records and statistics do not reflect the major role played by mycotoxins in mortality attributable to foodborne microorganisms.

KRISTENSEN, P., ANDERSEN, A. and IRGENS, L.N. 2000. **Hormone-dependent cancer and adverse reproductive outcomes in farmers' families – effects of climatic conditions favoring fungal growth in grain.** Scandinavian Journal of Work Environment & Health **26**: 331–337.

The impact of grain farming and climate on late-term abortion among female farmers, male genital birth defects among their sons, and hormone dependent cancer among male and female farmers and their adult children was investigated. National registers were cross-matched in Norway and 246,043 male and female farmers born in 1925–1971 were identified, as were their 264,262 children, born in 1952–1980. Categories of high exposure were associated with reproductive outcomes and cancer among female farmers, the strongest occurring for late-term abortion. Exposure associations for ovarian and breast cancer, and male genital defects, were more moderate. Endometrial cancer was associated with grain farming across all levels of fungal warnings. Exposure associations for cancer were strongest for premenopausal, parous women. In conclusion, climatic conditions favouring fungal growth in grain were associated with hormone dependent adverse outcomes among female farmers. The results are consistent with hormonal effects of inhaled mycotoxins during pregnancy.

WOOLF, A. 2000. **Witchcraft or mycotoxin? The Salem witch trials.** Journal of Toxicology – Clinical Toxicology **38**: 457–460.

The Salem witchcraft trials of 1692 have been studied extensively for the complex social, political and psychological determinants behind the community-wide hysteria that led to the deaths of 20 Puritans. Recently, ergot poisoning has been put forward as a previously unsuspected cause of the bizarre behaviours. In this essay, the circumstances behind the ergot poisoning the-

ory for this historical event are described. When the evidence is weighed carefully it seems unlikely that ergotism explains much of what went on in colonial Salem.

PEDRAS, M.S.C., BIESENTHAL, C.J. and ZAHARIA, I.L. 2000. **Comparison of the phytotoxic activity of the phytotoxin destruxin B and four natural analogs.** *Plant Science* **156**: 185–192.

A quantitative bioassay utilising staining of plant cell suspension cultures of *Sinapis alba* was employed to establish a structure-phytotoxic activity correlation among destruxin B, homodestruxin B and desmethyldestruxin B, toxins produced by *Alternaria brassicae*, the causative agent of *Alternaria* blackspot of brassicas. In addition, the phytotoxicity of destruxin B, homodestruxin B and their respective metabolites hydroxydestruxin B and hydroxyhomodestruxin B were tested on resistant and susceptible plant species. Overall, the results obtained indicated that homodestruxin B was the most toxic of the five compounds, followed by destruxin B and desmethyldestruxin B. The hydroxylated destruxins were significantly less phytotoxic than the parent toxins.

HOHENBOKEN, W.D., ROBERTSON, J.L., BLODGETT, D.J., MORRIS, C.A. and TOWERS, N.R. 2000. **Sporidesmin-induced mortality and histological lesions in mouse lines divergently selected for response to toxins in endophyte-infected fescue.** *Journal of Animal Science* **78**: 2157–2163.

For eight generations, mouse lines were selected for smaller or larger reduction in postweaning gain from endophyte infected fescue seed in the diet. After five generations in which there was no further selection for divergence in response to fescue toxicosis, an experiment was conducted to determine whether resistant (R) and susceptible (S) lines differed in response to the mycotoxin sporidesmin. At approximately 8 weeks of age, R and S mice that had never consumed endophyte infected fescue seed were given sporidesmin at 0–40 mg/kg by oral gavage. At death or euthanasia 14 days after treatment, livers and kidneys were collected for histological examination. The R mice were more resistant to sporidesmin than S mice; LD50 values were 23.6 and 31.8 mg/kg for the S and R lines, respectively. Sporidesmin caused dose related liver and kidney lesions in both lines. However, at 30 and 40 mg/kg doses, severity of this lesion was higher in affected S than in affected R mice. At the higher sporidesmin doses, there also was a greater incidence of hepatic subacute cholangitis in S mice than in R mice.

Ochratoxins – General

VRABCHEVA, T., USLEBER, E., DIETRICH, R. and MARTLBAUER, E. 2000. **Co-occurrence of ochratoxin A and citrinin in cereals from Bulgarian villages with a history of Balkan endemic nephropathy.** *Journal of Agricultural and Food Chemistry* **48**: 2483–2488.

Cereal samples were collected from three Bulgarian villages with a history of Balkan endemic nephropathy (BEN) and one village without a history of BEN. Samples including foods (wheat, corn) and feeds (barley, oats, wheat bran) were analysed for OA and citrinin. Highest toxin levels were found in wheat, wheat bran and oats. For OA, the percentages of positives for the one non-endemic and three endemic villages were 35, 29, 30 and 47%, respectively, with mean values of positives of 1.5, 11, 18 and 3.5 µg/kg, respectively. For citrinin, 5, 14, 3.3 and 13% were positive, and the mean values of positives were 6.1, 180, 10 and 84 µg/kg, respectively. Highest concentrations of OA and citrinin were found in samples from endemic villages.

BRESCH, H., URBANEK, M. and NUSSER, M. 2000. **Ochratoxin A in food containing liquorice.** *Nahrung* **44**: 276–278.

Samples of liquorice, the roots of *Glycyrrhiza* spp., and sweets containing a liquorice extract were purchased in shops in or around Karlsruhe, Germany, and analysed for OA. About 50% of the root samples contained detectable OA concentrations in the range 0.3–216 µg/kg. Among 19 samples of liquorice containing sweets, 18 had OA concentrations in the range 0.4–3 µg/kg.

OTTENEDER, H. and MAJERUS, P. 2000. **Occurrence of ochratoxin A (OTA) in wines: Influence of the type of wine and its geographical origin.** *Food Additives and Contaminants* **17**: 793–798.

The results of more than 450 samples taken from the literature together with 400 samples tested by the authors have been taken into account to quite extensively describe the situation of OA contamination of wine. OA has been detected in 25% of white wine samples, 40% of rose and 54% of red wine samples. Red wine samples from cooler regions showed a contamination of 12% in contrast to those from warmer regions which showed a contamination of about 95%.

ROMANI, S., SACCHETTI, G., LOPEZ, C.C., PINNAVAIA, G.G. and DALLAROSA, M. 2000. **Screening on the**

occurrence of ochratoxin A in green coffee beans of different origins and types. *Journal of Agricultural and Food Chemistry* **48**: 3616–3619.

The influence of green coffee type and origin on OA content was determined by analysis of 162 samples of green coffee beans from various countries (84 from Africa, 60 from America and 18 from Asia). The results showed that 106 of the overall samples were positive for OA with concentrations ranging up to 48 µg/kg. African samples were more contaminated compared to samples from other origins in terms of frequency and level of OA. The highest concentrations observed were 18 and 48 µg/kg in two samples from The Congo.

VARGA, J., RIGO, K. and TEREN, J. 2000. **Degradation of ochratoxin A by *Aspergillus* species.** *International Journal of Food Microbiology* **59**: 1–7.

Several *Aspergillus* species were examined for their ability to degrade OA. *A. fumigatus* and black *Aspergillus* strains were found to detoxify OA in culture media. The kinetics of OA detoxification by an atoxigenic *A. niger* strain were examined by TLC, HPLC and an immunochemical technique. *A. niger* CBS 120.49 was found to effectively eliminate OA from both liquid and solid media, and the degradation product, ochratoxin alpha, was also decomposed.

ELMHOLT, S. and HESTBJERG, H. 1999. **Field ecology of the ochratoxin A-producing *Penicillium verrucosum*: Survival and resource colonisation in soil.** *Mycopathologia* **147**: 67–81.

The survival of *Penicillium verrucosum* and indigenous soil fungi in soil and in soil containing waste grain was evaluated in steel cylinders which were buried for the period October 1994 to March 1996. *P. verrucosum* seemed well adapted to survival in arable soil and little affected by indigenous fungi. During the first autumn and winter the grain caused a proliferation of *P. verrucosum* while its abundance in bulk soil was more constant. In the soil plus waste grain sample, *P. verrucosum* initially infected more than 50% of the kernels but during the first few months it was ousted by other fungi. A hypothesis regarding waste grain as the natural niche for the fungus in the field was therefore partly rejected. A gradual decrease in the abundance of *P. verrucosum* in soil during spring, a die-off in the dry summer and a proliferation during the second winter were found in both samples.

FRISVAD, J.C. and SAMSON, R.A. 2000. ***Neopetromyces* gen. nov and an overview of teleomorphs of *Aspergillus* subgenus *Circumdati*.** *Studies in Mycology* **45**: 201–207.

Species in the anamorph genus *Aspergillus* are associated with several teleomorphic genera in the Eurotiales and the most important mycotoxin producers are concentrated in *Aspergillus* subgenus *Circumdati*. A new genus, *Neopetromyces*, is proposed for the recently described *Petromyces muricatus*, because this species differs distinctly from the two other species, *P. alliaceus* and *P. albertensis*. This new classification is in accord with DNA sequence data. Strains identified as *A. melleus* producing OA proved to be the anamorph of *N. muricatus*, while no strains of *A. melleus sensu stricto* produced OA. This is the first report of OA production by *N. muricatus*. OA is thus produced by some species in both sections *Flavi* and *Circumdati*.

VARGA, J., KEVEI, E., TOTH, B., KOZAKIEWICZ, Z. and HOEKSTRA, R. 2000. **Molecular analysis of variability within the toxigenic *Aspergillus ochraceus* species.** Canadian Journal of Microbiology **46**: 593–599.

Genetic variability of *Aspergillus ochraceus* was examined at the DNA level. Based on the HaeIII–BglII generated mitochondrial DNA restriction profiles, most isolates could be classified into two distinct groups. These two groups could also be distinguished by the random amplified polymorphic DNA technique and with telomeric PCR amplifications. Phylogenetic analysis of sequences of the intergenic transcribed spacer region of some of the strains resulted in a dendrogram with the same topology as that based on mitochondrial DNA and amplified DNA data. None of the isolates with type 2 mtDNA profiles produce ochratoxins.

Ochratoxins – Methodology

VISCONTI, A., PASCALE, M. and CENTONZE, G. 2000. **Determination of ochratoxin A in domestic and imported beers in Italy by immunoaffinity clean-up and liquid chromatography.** Journal of Chromatography A **888**: 321–326.

A method first developed to quantify OA in wine has been applied to the analysis of domestic and imported beers in Italy. Beer was degassed then diluted with a polyethylene glycol–sodium hydrogencarbonate solution and applied to an OchraTest immunoaffinity column. OA was eluted from the immunoaffinity column with methanol and quantified by reversed-phase HPLC with fluorometric detector. Average recoveries of OA from beer spiked at levels from 0.04–1.0 $\mu\text{g/L}$ ranged from 93.8 to 100.4%. The detection limit was 0.01 $\mu\text{g/L}$. Analysis of 61 samples of domestic and

imported beers showed OA levels ranging from <0.01 to 0.135 $\mu\text{g/L}$ with an incidence of contamination of 50% and no substantial difference between strong and pale beers.

CASTELLARI, M., FABBRI, S., FABIANI, A., AMATI, A. and GALASSI, S. 2000. **Comparison of different immunoaffinity clean-up procedures for high-performance liquid chromatographic analysis of ochratoxin A in wines.** Journal of Chromatography A **888**: 129–136.

Three immunoaffinity cleanup procedures for the analysis of OA in wines were compared. The direct cleanup with Ochraprep and OchraTest columns gave equivalent results in terms of recovery and precision if compared with the reference procedure involving a preliminary extraction of OA with chloroform. OA quantification limit ranged from 0.020 to 0.045 $\mu\text{g/L}$. The 'on-flow' OA emission spectrum (excitation 333 nm) showed a maximum at 460 nm and could be used to confirm the quantitative results. There were no significant quantitative differences between the three cleanup techniques.

JORNET, D., BUSTO, O. and GUASCH, J. 2000. **Solid-phase extraction applied to the determination of ochratoxin A in wines by reversed-phase high-performance liquid chromatography.** Journal of Chromatography A **882**: 29–35.

A reversed-phase HPLC method is described for the analysis of OA at low $\mu\text{g/L}$ levels in samples of artificially contaminated wines. The method involves solid phase extraction of samples using octadecylsilane cartridges and an additional preconcentration step prior to chromatography with isocratic elution and fluorimetric detection. Recoveries of OA added to wines over the range 0.1–3.0 $\mu\text{g/L}$ were higher than 80%. The method compared very favourably with results of other published studies of OA which use immunoaffinity columns or solvent extraction techniques.

FESTAS, I., HERBERT, P., SANTOS, L., CABRAL, M., BARROS, P. and ALVES, A. 2000. **Ochratoxin A in some Portuguese wines: Method validation and screening in Port Wine and Vinho Verde.** American Journal of Enology and Viticulture **51**: 150–154.

A methodology for the determination of OA in wines by HPLC with fluorescence detection was validated and used to assay some quality wines from the northern region of Portugal – Port Wine and Vinho Verde. The detection limit of the method was 0.02 $\mu\text{g/L}$ and recoveries ranged between 87 and 107%. Sixty-four samples of adulterated

Port Wines and Vinho Verde wines were analysed. OA was not detected in any of the Port Wine or Vinho Verde samples. OA was detected in three of the adulterated samples, but at levels not exceeding 0.08 $\mu\text{g/L}$.

Ochratoxins – Toxicology

PETZINGER, E. and ZIEGLER, K. 2000. **Ochratoxin A from a toxicological perspective.** Journal of Veterinary Pharmacology and Therapeutics **23**: 91–98.

When ingested as a food contaminant, OA is very persistent in human beings with a blood half-life of 35 days after a single oral dosage due to unfavourable elimination toxicokinetics. This renders the toxin among the most frequent mycotoxin contaminants in human blood in the EU, the USA, Canada and elsewhere. As the toxicological profile includes teratogenesis, nephrotoxicity and immunotoxicity, legislation authorities are currently discussing maximal residue levels (MRL) for OA in various foodstuffs. In the present article arguments are presented which suggest an acceptable daily intake of 1.5 ng/kg body weight and a much lower MRL than 5 $\mu\text{g/kg}$ cereals and cereal products as has been postulated by the EU commission.

EDER, S., BENESIC, A., FREUDINGER, R., ENBERT, J., SCHWERDT, G., DRUMM, K. and GEKLE, M. 2000. **Nephritogenic ochratoxin A interferes with mitochondrial function and pH homeostasis in immortalized human kidney epithelial cells.** Pflugers Archiv – European Journal of Physiology **440**: 521–529.

The effect of nanomolar concentrations of OA on cellular pH (pH(c)) homeostasis and the possible involvement of mitochondria were assessed using immortalised human kidney epithelial (IHKE1) cells. Within seconds, OA evoked a decrease of pH(c) with a threshold concentration of 0.1 nmol/L, followed by a sustained alkalinisation. When Ca^{2+} entry across the plasma membrane was prevented, virtually no OA induced pH changes could be observed. Determination of Na^+/H^+ -exchange (NHE) activity as a function of pH revealed that OA stimulated NHE in a Ca^{2+} dependent manner. OA induced a hyperpolarisation of the mitochondrial membrane potential in a Ca^{2+} dependent manner. Furthermore, OA exposure resulted in a mitochondria dependent increase of the cellular ATP content. Results indicate that OA activates mitochondria and NHE by interfering with cellular Ca^{2+} homeostasis.

OBRECHT-PFLUMIO, S. and DIRHEIMER, G. 2000. **In vitro DNA and dGMP adducts formation caused by ochratoxin A.** *Chemico-Biological Interactions* **127**: 29–44.

Microsomes prepared from mice or rabbit kidney and liver used as metabolic activators, were incubated in the presence of commercial salmon testes DNA and OA. Up to 126 DNA adducts for 10^9 nucleotides were detected using the [32 P] postlabelling method after incubation with the mouse kidney system. Similar results were obtained with rabbit kidney microsomes. Using liver microsomes, the number of DNA adducts detected was much lower. These results lead support to the hypothesis of the preferential activation of OA by the peroxidase activity of prostaglandin synthases and/or lipoxygenases to direct genotoxic metabolites. To identify the nucleotides of DNA modified by the OA metabolites, dAMP, dGMP, dTMP and dCMP were used as substrates under the same conditions as with DNA. The adducts were found only on dGMP. Results showed that OA is metabolised to genotoxic metabolite(s) which interact with the guanine residues of DNA.

STOEV, S.D., GOUNDASHEVA, D., MIRTICHEVA, T. and MANTLE, P.G. 2000. **Susceptibility to secondary bacterial infections in growing pigs as an early response in ochratoxicosis.** *Experimental and Toxicologic Pathology* **52**: 287–296.

Mycotoxic nephropathy was induced in 12 pigs fed a diet containing OA at 1 or 3 mg/kg for up to 3 weeks. Concurrently, salmonellosis arose spontaneously in all animals treated at 3 mg/kg and all died between days 15 and 17. Two of the 6 pigs in the 1 mg/kg group died similarly but the rest, and all control animals, were unaffected. Clinical biochemistry and histology revealed changes typical of renal ochratoxicosis in all OA treated pigs. Clinical and pathomorphological changes typical of salmonellosis were evident in all those that died and *Salmonella choleraesuis* was consistently isolated from their faeces and liver. In a further experiment at 1 mg/kg in animals immunised against *S. choleraesuis* haemorrhagic diarrhoea resulted instead, associated with *Serpulina hyodysenteriae* and *Campylobacter coli*. There was concomitant evidence of immunosuppression and delayed response to immunisation. For the first time, susceptibility to natural infectious disease has been demonstrated in pigs exposed to the immunotoxicity of OA.

Patulin

BERETTA, B., GAIASCHI, A., GALLI, C.L. and RESTANI, P. 2000. **Patulin in apple-based foods: Occurrence and safety evaluation.** *Food Additives and Contaminants* **17**: 399–406.

Patulin levels in samples of apples and apple derivatives were determined by HPLC. In apple juices and in homogenised baby foods, the mycotoxin concentration was always below the established limits of 50 μ g/kg, while in some samples of juice with pulp the mycotoxin content exceeded the safe limits. In rotten apples, not only was the amount of patulin extraordinarily high in the rotten area, but the mycotoxin had also spread to the part unaffected by mould. The data presented indicate that the intake of patulin with apple derivatives is usually below the tolerable level of 0.4 μ g/kg body weight per day, however, the quality of fruits used in the production of apple derivatives should be strictly controlled in order not to exceed the safe limits.

PATERSON, R.R.M., ARCHER, S., KOZAKIEWICZ, Z., LEA, A., LOCKE, T. and O'GRADY, E. 2000. **A gene probe for the patulin metabolic pathway with potential for use in patulin and novel disease control.** *Biocontrol Science and Technology* **10**: 509–512.

The iso-epoxy dehydrogenase gene of the patulin metabolic pathway was detected in environmental samples, *Penicillium expansum* and *P. brevicompactum* isolated from an organic orchard. Patulin was not detected from *P. brevicompactum*. Both traits were negative for other penicillia.

LEGGOTT, N.L., VISMER, H.F., SYDENHAM, E.W., SHEPHARD, G.S., RHEEDER, J.P. and MARASAS, W.F.O. 2000. **Occurrence of patulin in the commercial processing of apple juice.** *South African Journal of Science* **96**: 241–243.

The importance of removing contaminated apples from the initial processing line during apple juice production was studied. Mean patulin concentration in non-processed apples over the three seasons was 2010 \pm 949 μ g/kg. The decrease in patulin concentration to 440 \pm 253 μ g/kg after an initial water wash step was followed by further reduction to 200 \pm 183 μ g/kg after the removal of rotten and damaged apples. A further study was undertaken to determine the effects of processing on patulin concentrations in apple juice. During the preconcentration stage (pasteurisation), a statistically significant decrease in the mean *P. expansum* count accompanied a significant increase in patulin concentration from

105 \pm 44 to 165 \pm 49 mg/L. These results were probably due to the higher temperature and total solids concentration (Brix), respectively, during pasteurisation. The combined depectinisation/charcoal/ultra-filtration stage yielded a significant decrease in mean patulin concentration from 110 \pm 35 to 75 \pm 18 μ g/L, which was probably due to the adsorption of patulin on the activated charcoal. No further removal of patulin occurred during the remainder of the juicing process.

SHEPHARD, G.S. and LEGGOTT, N.L. 2000. **Chromatographic determination of the mycotoxin patulin in fruit and fruit juices.** *Journal of Chromatography A* **882**: 17–22.

This paper with 42 references reviews currently available analytical methods for patulin determination in fruit and fruit juices. Of these, HPLC with UV or, preferably, photodiode array detection is most widely used, although GC and TLC methods have also been described.

Fumonisin – General

ONO, E.Y.S., SUGIURA, Y., HOMECHIN, M., KAMOGAE, M., VIZZONI, E., UENO, Y. and HIROOKA, E.Y. 1999. **Effect of climatic conditions on natural mycoflora and fumonisins in freshly harvested corn of the State of Paraná, Brazil.** *Mycopathologia* **147**: 139–148.

Natural mycoflora associated with fumonisins were analysed in 150 samples of freshly harvested corn from Central-Southern, Central-Western and Northern regions of the State of Paraná, Brazil, and correlated to climatic conditions. The highest contamination with potentially mycotoxigenic fungi occurred in corn harvested in the Central-Western region where *F. moniliforme* was the predominant *Fusarium* species, and was isolated in 85.9% of the samples. FB₁ was detected in 100% of the samples (mean of 2.39 mg/kg) and FB₂ in 97.7% (mean of 1.09 mg/kg). Fumonisin were also detected in all samples from Northern region, with mean concentrations of FB₁ and FB₂ of 4.56 and 2.20 mg/kg, respectively. Seventy-two percent of the corn samples from the Central-West and 92% from the North were contaminated with concentrations above 1 mg/kg, in contrast to a 18.5% contamination rate from Central-Southern samples. The higher fumonisin contamination of corn from Northern region when compared to the Central-South is attributed to the differences in rainfall levels (92.8 mm in Central-Southern, 202 mm in Northern) during the month preceding harvest.

CALONI, F., SPOTTI, M., AUERBACH, H., OPDENCAMP, H., GREMMELS, J.F. and POMPA, G. 2000. **In vitro metabolism of fumonisin B₁ by ruminal microflora.** Veterinary Research Communications **24**: 379–387.

Ruminants are reported to be tolerant towards FB₁. The fate of FB₁ was evaluated in a model rumen where FB₁ at 1 mg/L was incubated in ruminal fluid for up to 72 hr in the presence or absence of alfalfa as a substrate for microbial growth. After 72 hr incubation, FB₁ depletion was 12 and 18% in samples with and without alfalfa, respectively. No hydrolysed metabolites (aminopolyols or aminopentol) were detected. These results indicate that FB₁ is poorly metabolised in the rumen and suggest that such metabolism is not the cause of the tolerance to this toxin displayed by ruminants.

CAMILO, S.B., ONO, C.J., UENO, Y. and HIROOKA, E.Y. 2000. **Anti-Fusarium moniliforme activity and fumonisin biodegradation by corn and silage microflora.** Brazilian Archives of Biology and Technology **43**: 159–164.

Microorganisms isolated from corn and silage were screened for their ability to inhibit *Fusarium moniliforme* growth in association with fumonisin detoxification. Four Gram positive bacilli and one yeast with inhibitory activity were selected. The inhibition zone ranged from 50 to 72.5 mm using cultures and from 25 to 52.5 mm for crude alcoholic extract. Three sporulated bacilli and one yeast degraded the initial FB₁ by 43, 48, 83 and 57%, respectively. The pH increased gradually in the medium during incubation for biodegradation process.

Fumonisin – Methodology

BARNA-VETRO, I., SZABO, E., FAZEKAS, B. and SOLTI, L. 2000. **Development of a sensitive ELISA for the determination of fumonisin B₁ in cereals.** Journal of Agricultural and Food Chemistry **48**: 2821–2825.

Monoclonal FB₁ antibodies with high titre were raised by using FB₁-glutaraldehydekeyhole limpet hemocyanin immunogen prepared by a short cross-linker reagent. Mean cross reactivities of the selected monoclonal antibody for FB₁, FB₂ and FB₃ were 100, 91.8 and 209%, respectively. No reactivity was found with hydrolysed fumonisin. A direct, competitive ELISA for the quantitative determination of FB₁ in cereals was developed with this antibody. The mean within assay and interassay coefficients of variation for the standard curve were 10%. The measuring range of this test is 10–500 µg/kg, with a detection limit of 7.6 µg/kg. Recovery from cereals varied between 61

and 84%. This test proved to be suitable for the rapid screening of food and feed samples for the presence of fumonisins.

PREIS, R.A. and VARGAS, E.A. 2000. **A method for determining fumonisin B₁ in corn using immunoaffinity column clean-up and thin layer chromatography/densitometry.** Food Additives and Contaminants **17**: 463–468.

A method for determining FB₁ in corn was developed and the cleanup optimised in order to give an extract suitable for one-dimensional TLC analysis. FB₁ was extracted with a solution of methanol–water (80:20) purified through an immunoaffinity column and separated on a C₁₈ reversed phase TLC plate. The FB₁ was visualised with 0.1 mol/L sodium tetraborate, 0.40 g/L fluorescamine in acetonitrile and 0.01 mol/L boric acid–acetonitrile (2:3) for fluorescence detection and quantified by densitometric analysis. The mean recovery for FB₁ in spiked samples was 85% and the linear equation of standard calibration curve by densitometric analysis gave an r(2) value higher than 0.99. The maximum coefficient of variation for replicate analysis of spiked samples was 19%. The absolute amount of FB₁ standard detectable was 2 ng, giving a detection limit for the method of 0.1 mg/kg. In 214 samples of corn collected in different regions of Brazil, FB₁ was detected in 99% of samples in the range of 0.2 to 6 mg/kg.

Fumonisin – Toxicology

SHARMA, R.P., BHANDARI, N., TSUNODA, M., RILEY, R.T., VOSS, K.A. and MEREDITH, F.I. 2000. **Fumonisin toxicity in a transgenic mouse model lacking the *mdr1a/1b* P-glycoprotein genes.** Environmental Toxicology and Pharmacology **8**: 173–182.

The toxicity of FB₁ was investigated in male *mdr1a/1b* double knockout (MDRK) mice, lacking the drug transporting P-glycoproteins. These transgenic animals are deficient in their blood:brain barrier and accumulate different drugs in brain and other tissues. The MDRK and their wild-type counterparts, FVB mice, were injected sc with FB₁ at 2.25 mg/kg/day for 5 days. FB₁ caused liver enlargement in both FVB and MDRK and plasma levels of alanine aminotransferase and aspartate aminotransferase were increased in both strains. Kidney lesions were induced by FB₁ in both strains. Concentrations of free sphingosine and sphinganine increased in liver and kidney of both strains, although the increase in liver sphingoid bases was half as much in MDRK. Results showed that mice deficient in P-glycoprotein do not exhibit greater sen-

sitivity to FB₁ and the cellular or brain transport of FB₁ appears to be independent of this multi-drug transporting system.

SHARMA, R.P., BHANDARI, N., TSUNODA, M., RILEY, R.T. and VOSS, K.A. 2000. **Fumonisin hepatotoxicity is reduced in mice carrying the human tumour necrosis factor alpha transgene.** Archives of Toxicology **74**: 238–248.

Male transgenic mice expressing human tumour necrosis factor alpha (TNF alpha) gene (TG) and their wild-type equivalent C57BL/6 (WT) were used to investigate the role of TNF alpha in FB₁ toxicity. The mRNA for TNF alpha in liver increased in both TG and WT after FB₁ treatment at 2.25 mg/kg/day for 5 days, providing evidence that FB₁ induces hepatic TNF alpha expression. Liver lesions seen in FB₁ treated TG were considerably less than those observed in WT and correlated with plasma concentrations of alanine aminotransferase and aspartate aminotransferase. The increase of free sphinganine in the liver from TG mice was 40% less than in WT mice and paralleled the changes in serum liver enzymes. Regional brain neurotransmitters and their metabolites were increased to a similar extent by FB₁ in both WT and TG mice. Cytosolic NF kappa B was significantly higher in TG compared with WT. Induction of NF kappa B, caused by increased endogenous production of TNF alpha, is a possible explanation of decreased FB₁ hepatotoxicity in TG. The results suggest a protective role for NF kappa B in FB₁ induced liver damage.

ENONGENE, E.N., SHARMA, R.P., BHANDARI, N., VOSS, K.A. and RILEY, R.T. 2000. **Disruption of sphingolipid metabolism in small intestines, liver and kidney of mice dosed subcutaneously with fumonisin B₁.** Food and Chemical Toxicology **38**: 793–799.

Mice were administered a single sc injection of FB₁ and the level of free sphingoid bases in the intestinal epithelial cells was followed for 24 hr. A significant time dependent increase in sphingoid bases occurred in the intestine and liver peaking at 4–8 hr and declining to control levels by 24 hr. In the kidney the increase in free sphinganine was persistent. The parallel time course of the change in sphinganine in the intestine and liver suggested FB₁ was rapidly excreted into the small intestine. Rapid cell turnover in the intestine could account for the reversal of the sphinganine increase. The rapid return to the control level in liver was unexpected since ceramide synthase inhibition in cultured cells is persistent suggesting that liver handles FB₁ or sphingoid bases quite differently than kidney.

RAMLJAK, D., CALVERT, R.J., WIESENFELD, P.W., DIWAN, B.A., CATIPOVIC, B., MARASAS, W.F.O., VICTOR, T.C., ANDERSON, L.M. and GELDERBLOM, W.C.A. 2000. **A potential mechanism for fumonisin B₁-mediated hepatocarcinogenesis: Cyclin D1 stabilization associated with activation of Akt and inhibition of GSK-3 beta activity.** *Carcinogenesis* **21**: 1537–1546.

In a long-term feeding study of FB₁ in rats, overexpression of cyclin D1 protein was observed in both preneoplastic and neoplastic liver samples. In rats fed FB₁ short-term, cyclin D1 protein levels in liver were increased up to five-fold in a dose responsive manner. Results suggest that overexpression of cyclin D1 results from stabilisation due to a lack of phosphorylation mediated by glycogen synthase kinase-3 beta (GSK-3 beta). In summary, the activation of protein kinase B leads to increased survival, inhibition of GSK-3 beta activity and posttranslational stabilisation of cyclin D1, all events responsible for disruption of the cell cycle G(1)/S restriction point in hepatocytes. This is the first report suggesting the mechanism by which FB₁ acts as a carcinogen.

LI, W., RILEY, R.T., VOSS, K.A. and NORRED, W.P. 2000. **Role of proliferation in the toxicity of fumonisin B₁: Enhanced hepatotoxic response in the partially hepatectomized rat.** *Journal of Toxicology and Environmental Health – Part A* **60**: 441–457.

Partially hepatectomised (PH) rats and sham operated rats were dosed ip with FB₁ 24 hr after the operation and were killed 24 hr later. The dose related increase in free sphingoid bases was enhanced in the PH treated rats. Serum cholesterol and enzymes were higher in PH treated rats dosed with FB₁ than in those given PH without FB₁ or in sham operated, FB₁ dosed rats. Multiple daily doses of FB₁ after surgery elevated the number of apoptotic hepatocytes in both sham operated and PH treated rats to about the same degree suggesting that apoptosis is not associated with the enhanced cytotoxicity of FB₁ in regenerating liver.

KWON, O.S., SLIKKER, W. and DAVIES, D.L. 2000. **Biochemical and morphological effects of fumonisin B₁ on primary cultures of rat cerebrum.** *Neurotoxicology and Teratology* **22**: 565–572.

The short-term consequences of direct FB₁ exposure on astrocytes and oligodendrocytes were assessed in primary cultures of rat cerebrum. Beginning at 5 days, cultures were exposed to FB₁ at 0.5–75 μM and

then evaluated at 10 and 15 days. FB₁ treated cultures showed significantly increased sphinganine levels and sphinganine/sphingosine ratios. FB₁ treated cultures exhibited a two-fold increase in the number of process-bearing cells by 15 days. Also, the activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase, an enzyme associated with myelin and oligodendrocytes, was increased in treated cultures. This study suggests that short-term exposure to FB₁ may modify the proliferation or differentiation of glial cells.

SPOTTI, M., MAAS, R.F.M., DENIJS, C.M. and FINK-GREMMELS, J. 2000. **Effect of fumonisin B₁ on rat hepatic P450 system.** *Environmental Toxicology and Pharmacology* **8**: 197–204.

The effects of FB₁ on the hepatic cytochrome P450 system were investigated in male rats fed FB₁ at 3 mg/kg body weight per day for 9 days. CYP2E activity was increased, which is considered to mark the metabolic changes inherent to growth retardation in young rats. Treatment with FB₁ also resulted in a selective inhibition of CYP2C11 and to a lesser extent, CYP1A2 in liver microsomes, whereas it did not affect significantly the activity of CYP2A1/2A2, CYP2B1/2B2, CYP3A1/3A2 and CYP4A. The significant inhibition of CYP2C11 is considered to reflect a suppressed activity of protein kinase activity resulting from the inhibition of sphingolipid biosynthesis caused by FB₁.

SMITH, G.W., CONSTABLE, P.D., EPPLEY, R.M., TUMBLESON, M.E., GUMPRECHT, L.A. and HASCHEK-HOCK, W.M. 2000. **Purified fumonisin B₁ decreases cardiovascular function but does not alter pulmonary capillary permeability in swine.** *Toxicological Sciences* **56**: 240–249.

Pigs were injected iv with FB₁ at 1 mg/kg for 4 days. On day 5, pigs were anaesthetised and instrumented for haemodynamic studies. FB₁ treated pigs had marked decreases in the maximal rate of change of left ventricular pressure, mean aortic pressure, cardiac output and arterial pO₂, accompanied by increases in mean pulmonary artery pressure, oxygen extraction ratio and blood haemoglobin concentration. Plasma and left ventricular sphingosine and sphinganine concentrations were markedly increased in treated pigs at day 5, however, there was no difference in the relative permeability index between groups. Results indicate that cardiovascular function is altered by FB₁ and that fumonisin-induced pulmonary oedema is caused by left-sided heart failure and not by altered endothelial permeability.

REID, L.M., ZHU, X., SAVARD, M.E., SINHA, R.C. and VIGIER, B. 2000. **Pre-harvest accumulation of deoxynivalenol in sweet corn ears inoculated with *Fusarium graminearum*.** *Food Additives and Contaminants* **17**: 689–701.

Three types of commercial sweet corn hybrids (sugary (su1), shrunken or 'super-sweet' (sh2) and sugary enhancer (se1)) were silk channel inoculated with a macroconidial suspension of *Fusarium graminearum* and the accumulation of DON in kernels was monitored. On all hybrids, disease symptoms were apparent 4 days after inoculation. Toxin levels were greater than 1 mg/kg in kernels as early as 2 weeks after silk emergence and rapidly increased to extremely high levels. Susceptibility in all hybrids decreased as the silk dried out. DON concentrations were correlated to disease severity. There was some indication that the sh2 genotype was more susceptible than the su1 or se1 genotypes.

ABBAS, H.K., CARTWRIGHT, R.D., XIE, W., MIROCHA, C.J., RICHARD, J.L., DVORAK, T.J., SCIUMBATO, G.L. and SHIER, W.T. 1999. **Mycotoxin production by *Fusarium proliferatum* isolates from rice with *Fusarium sheath rot* disease.** *Mycopathologia* **147**: 97–104.

Fifteen cultures of *Fusarium proliferatum* were established from 20 rough rice samples collected in Arkansas and Texas from fields exhibiting *Fusarium sheath rot* disease or panicle blight. Single spore isolates of each culture were grown on rice and tested for the production of fumonisins, moniliformin (MON) and beauvericin (BEA). All 15 isolates produced FB₁, FB₂, MON and BEA in culture on rice. No deoxynivalenol, its derivatives or ZEA was detected. Seven cultures produced FB₁ in the range 80–230 mg/kg, with the rest producing FB₁ in the range 14–43 mg/kg. FB₂ was produced in the range 5–47 mg/kg. Of the 15 cultures producing MON, 11 produced it in the range 188–6018 mg/kg, with the rest producing in the range 7–64 mg/kg. BEA was produced in the range 109–1350 mg/kg. Other derivatives of fumonisins as well as several unknown metabolites were identified.

MOLTO, G., SAMAR, M.M., RESNIK, S., MARTINEZ, E.J. and PACIN, A. 2000. **Occurrence of trichothecenes in Argentinean beer: A preliminary exposure assessment.** *Food Additives and Contaminants* **17**: 809–813.

Fifty samples of Argentinean beer (nine different brands) were surveyed for the presence of trichothecenes. The only mycotoxin detected was deoxynivalenol (DON) which was present in 44% of the samples. Eighteen percent were contaminated with more than 20 µg/L. Toxin levels ranged from 4–221 µg/L in positive samples. This is the first report on DON contamination of Argentinean beer.

LANGSETH, W. and RUNDBERGET, T. 1999. **The occurrence of HT-2 toxin and other trichothecenes in Norwegian cereals.** *Mycopathologia* **147**: 157–165.

A total of 449 grain samples, 102 barley, 169 wheat and 178 oat samples were collected from different regions of Norway from 1996–1998 crops, mainly from grain loads and silos. The samples were analysed for type A and B trichothecenes. DON and HT-2 toxin were the trichothecenes most frequently detected, followed by T-2 toxin, nivalenol (NIV) and scirpentriol. Oats were most heavily contaminated grain with an incidence HT-2 toxin, T-2 toxin, DON and NIV of 70, 30, 57 and 10%, respectively. The corresponding values for barley were 22, 5, 17 and 6%, and for wheat 1.2, 0.6, 14 and 0%, respectively. Norwegian oats were found to contain HT-2 toxin and T-2 toxin in concentrations that might be at threat to human health for high consumers of oats.

TORP, M. and LANGSETH, W. 1999. **Production of T-2 toxin by a *Fusarium* resembling *Fusarium poae*.** *Mycopathologia* **147**: 89–96.

A *Fusarium* species with a micro morphology similar to *F. poae* and a metabolite profile resembling that of *F. sporotrichioides* has been identified. Like typical *F. poae*, the microconidia have a globose to pyriform shape, but the powdery appearance, especially on Czapek-Dox Iprodione Dichloran agar, less aerial mycelium and the lack of fruity odour on Potato Sucrose Agar (PSA) make it different from *F. poae*. The lack of macroconidia, polyphialides and chlamydo-spores differentiates it from *F. sporotrichioides*. All 18 isolates investigated, 15 Norwegian, two Austrian and one Dutch, produced T-2 toxin on PSA or Yeast Extract Sucrose agar. In addition, neosolanol, iso-neosolanol, HT-2 toxin, 4- and 15-acetyl T-2 tetraol, T-2 triol and T-2 tetraol and 4,15-diacetoxyscirpenol were formed.

MUHITCH, M.J., MCCORMICK, S.P., ALEXANDER, N.J. and HOHN, T.M. 2000. **Transgenic expression of the TRI101 or PDR5 gene increases resistance of tobacco to the phytotoxic effects of the trichothecene 4,15-diacetoxyscirpenol.** *Plant Science* **157**: 201–207.

The effectiveness of two strategies, metabolic alteration and extracellular transport, to protect plant cells from the deleterious effects of 4,15-diacetoxyscirpenol (DAS) were tested. Tobacco plants were transformed with either the *Saccharomyces cerevisiae* gene *PDR5*, which encodes a multi-drug transporter, or with the *Fusarium sporotrichioides* gene *TRI101*, which encodes a trichothecene 3-O-acetyltransferase. Both genes conferred significant increased tolerance to DAS as measured by a sensitive seed germination assay. Expression of *PDR5* or *TRI101* in a seed specific manner in crop plants such as wheat could lower the incidence of head blight as well as reduce mycotoxin levels within the seed.

DESJARDINS, A.E., BAI, G.H., PLATTNER, R.D. and PROCTOR, R.H. 2000. **Analysis of aberrant virulence of *Gibberella zeae* following transformation-mediated complementation of a trichothecene-deficient (*Tri5*) mutant.** *Microbiology* – UK **146**: 2059–2068.

In previous field tests of wheat ear blight, trichothecene non-producing mutants were less virulent than the wild-type progenitor strain from which they were derived. Trichothecene producing revertants also were restored to wild-type levels of virulence. In contrast, in the field test of wheat ear blight reported here, trichothecene producing strains obtained by *Tri5* mutant complementation were not restored to wild-type levels of virulence. The complemented mutants showed a slightly reduced radial growth compared to the wild-type strain, but otherwise appeared normal in morphology, pigmentation and sexual fertility. Genetic analysis indicated that the aberrant virulence of a complemented mutant was likely due to non-target effects that occurred during the process of transforming the trichothecene non-producing mutant with *Tri5*.

YUAN, Q.P., HU, W.Q., PESTKA, J.J., HE, S.Y. and HART, L.P. 2000. **Expression of a functional antizearalenone single-chain Fv antibody in transgenic *Arabidopsis* plants.** *Applied and Environmental Microbiology* **66**: 3499–3505.

The efficacy of cloning a recombinant mycotoxin antibody in plants was tested using *Arabidopsis* as a model. An anti ZEA single chain Fv (scFv) DNA fragment was first cloned in the newly constructed phage display vector (pEY.5) and then re-cloned in the plant transformation vector pKYLX71::35S². After transformation, constructs of anti ZEA scFv were introduced into immature *Arabidopsis* seeds via *Agrobacterium tumefaciens* mediation by vacuum infiltration. Only plants transformed with the construct containing a PR-Ib signal

peptide sequence produced transgenic offspring. The anti ZEA scFv “plantibody” from these transgenic plants bound ZEA with a high affinity that was comparable to that of bacterially produced scFv antibody and the parent monoclonal antibody. Expression of specific plantibodies in crops might be useful for neutralising mycotoxins in animal feeds and for reducing mycotoxin associated plant diseases.

SOKOLOVA, G.D. 2000. **[Clonal variability of *Fusarium graminearum* toxicogenicity and vegetative compatibility].** *Mikologiya i Fitopatologiya* **34**: 63–66.

Monoconidial isolates obtained from a culture of *Fusarium graminearum* were shown to vary considerably in toxigenicity. Vegetative compatibility type between toxigenic and nontoxigenic strains indicates the possibility for heterokaryosis. (In Russian).

MILLER, J.D. and MACKENZIE, S. 2000. **Secondary metabolites of *Fusarium venenatum* strains with deletions in the *Tri5* gene encoding trichodiene synthetase.** *Mycologia* **92**: 764–771.

The secondary metabolites of a number of strains of *Fusarium venenatum*, some of which had the gene for trichodiene synthetase deleted, were studied. Under optimum fermentation conditions, wild-type isolates produced DAS and related metabolites, isotrichodermin and isotrichodermol, the modified trichothecenes sambucinol and apotrichothecene, culmorin, culmorone and enniatin B. These results are compared to metabolite production by other members of the section *Discolor*. Strains without trichodiene synthetase produced no trichothecenes or modified trichothecenes.

Trichothecenes – Methodology

FAZEKAS, B., HAJDU, E.T., TAR, A.K. and TANYI, J. 2000. **Natural deoxynivalenol (DON) contamination of wheat samples grown in 1998 as determined by high-performance liquid chromatography.** *Acta Veterinaria Hungarica* **48**: 151–160.

An HPLC–diode array detection (DAD) method was developed for determining the DON content of wheat and other cereals. Samples were extracted with a mixture of acetonitrile and water (84 + 16). Part of the extract was evaporated and purified on Florisil and activated charcoal columns. HPLC separation was performed on a C₁₈ column, using acetonitrile–water (8 + 92) as eluent. DAD was performed at 218 and 236 nm by determination of the UV spectrum. The recovery rate of DON was 75% and the detection limit was 0.05 mg/kg. Using this

method, the DON content of feeding wheat samples grown in Hungary was determined. DON contamination of wheat was of higher prevalence (100%) and severity (0.27–4.3 mg/kg) in the southeastern county of Bekes than in Szabolcs county located in the north-eastern part of Hungary (82% and 0.05–1.3 mg/kg).

WIDESTRAND, J., LUNDH, T., PETERSSON, H. and LINDBERG, J.E. 1999. **Cytotoxicity of four trichothecenes evaluated by three colorimetric bioassays.** *Mycopathologia* **147**: 149–155.

Three colorimetric bioassays were used to determine the cytotoxicity of T-2 toxin, HT-2 toxin, DON and NIV to 3T3 mouse fibroblasts. The bioassays assess DNA synthesis (incorporation of 5-bromo-2-deoxyuridine; BrdU), metabolic activity (cleavage of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTT) and cell membrane damage (release of lactate dehydrogenase; LDH). The BrdU bioassay was the most sensitive with IC₅₀ values of T-2 toxin, HT-2 toxin, DON and NIV of 4.6, 13, 263 and 365 µg/L, respectively. At the same toxin concentrations used in the BrdU bioassay, only T-2 toxin and HT-2 toxin were toxic enough to obtain IC₅₀ values using the MTT bioassay and were 12 and 68 µg/L, respectively. In the LDH bioassay, the IC₅₀ values of T-2 toxin and HT-2 toxin were 18 and 42 µg/L, respectively.

TANAKA, T., YONEDA, A., INOUE, S., SUGIURA, Y. and UENO, Y. 2000. **Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography–mass spectrometry.** *Journal of Chromatography A* **882**: 23–28.

This paper describes GC–MS analysis for the simultaneous determination of trichothecenes and ZEA contaminants in cereals.

JODLBAUER, J., ZOLLNER, P. and LINDNER, W. 2000. **Determination of zeranol, taleranol, zearalenone, alpha- and beta-zearalenol in urine and tissue by high-performance liquid chromatography–tandem mass spectrometry.** *Chromatographia* **51**: 681–687.

An LC–MS/MS method for the sensitive determination of zeranol, its epimer taleranol and the mycotoxins ZEA, alpha-zearalenol and beta-zearalenol in animal urine and meat samples is described. Sample preparation included extraction of meat samples and enzymatic digest of urine samples followed by solid phase extraction with RP₁₈ columns for sample cleanup. MS determination was carried out with an atmospheric

pressure chemical ionisation interface in the multi reaction monitoring mode. Using the negative ion mode, a limit of detection between 0.1 and 0.5 µg/kg and a limit of determination between 0.5 and 1 µg/kg was achieved. Depending on the biological matrix and analyte, standard deviations were below 8.5% with average recovery rates around 86 to 102%.

Trichothecenes – Toxicoses

RAO, C.Y., BRAIN, J.D. and BURGE, H.A. 2000. **Reduction of pulmonary toxicity of *Stachybotrys chartarum* spores by methanol extraction of mycotoxins.** *Applied and Environmental Microbiology* **66**: 2817–2821.

The role of mycotoxins in the pulmonary effects caused by *Stachybotrys chartarum* spores and the dose dependency of these effects were investigated. *S. chartarum* spores were extracted in methanol to reduce the mycotoxin content of the spores. Then either untreated (toxin containing) or methanol extracted spores were intratracheally instilled into male rats. Methanol extraction dramatically reduced the toxicity of *S. chartarum* spores. No statistically significant effects were observed in the bronchoalveolar lavage fluids of the animals that were treated with methanol extracted spores at any dose. Conversely, dose dependent effects of the toxin containing spores were observed when lactic dehydrogenase, albumin and haemoglobin concentrations and the polymorphonuclear leukocyte counts in bronchoalveolar lavage fluids, and weight loss were examined.

CHOI, C.Y., NAKAJIMA-ADACHI, H., KAMINOGAWA, S. and SUGITAKONISHI, Y. 2000. **Nivalenol inhibits total and antigen-specific IgE production in mice.** *Toxicology and Applied Pharmacology* **165**: 94–98.

The effect of NIV on antigen specific IgE production was examined using ovalbumin specific T cell receptor alpha beta-transgenic mice. The mice produced significant amounts of total and antigen specific IgE, IgG1 and IgA in serum when given ovalbumin orally. Administration of NIV with ovalbumin suppressed total IgE and ovalbumin specific IgE, IgG1 and IgA production significantly. Cytokine assay using splenocytes obtained from mice given the ovalbumin plus NIV diet revealed that interleukin 4 (IL-4) production was suppressed and IL-2 production was enhanced.

PESTKA, J.J. and ZHOU, H.R. 2000. **Interleukin-6-deficient mice refractory to IgA dysregulation but not anorexia**

induction by vomitoxin (deoxynivalenol) ingestion. *Food and Chemical Toxicology* **38**: 565–575.

Dietary exposure to DON causes feed refusal and elevates IgA production in the mouse. Since IL-6 can cause anorexia and promote IgA production and gene expression of this cytokine is increased with DON exposure, the relationship between IL-6 and DON induced feed refusal and IgA dysregulation was investigated. The effects of dietary DON on feed intake, weight gain, serum IgA levels and kidney mesangial IgA deposition in an IL-6 “knockout” mouse (B6129-IL6 (tmi Kopf)) were compared to those in both a corresponding “wild type” (B6129F2) and a previously characterised “sentinel” strain (B6C3F1) that possess the intact gene for this cytokine. IL-6 deficiency did not alter the capacity of DON to cause feed refusal or impair weight gain. DON fed B6129F2 and B6C3F1 mice had significantly higher serum IgA concentrations than did their corresponding controls, whereas significant differences were not observed between IL-6 knockout mice fed DON or control diets. The results suggested that IL-6 deficient mice were refractory to DON induced dysregulation of IgA production and development of IgA nephropathy, whereas chronic DON mediated nutritional effects related to feed intake and weight gain were unaffected.

ALBARENQUE, S.M., SHINOZUKA, J., SUZUKI, K., NAKAYAMA, H. and DOI, K. 2000. **Kinetics and distribution of transforming growth factor (TGF)-beta 1 mRNA in the dorsal skin of hypotrichotic WBN/ILA-Ht rats following topical application of T-2 toxin.** *Experimental and Toxicologic Pathology* **52**: 297–301.

The kinetics of transforming growth factor (TGF)-beta 1 mRNA was investigated in the dorsal skin of hypotrichotic WBN/ILA-Ht rats following topical application of T-2 toxin. The level of TGF-beta 1 mRNA of the whole skin tissue showed a slight elevation from 6 to 12 hr after treatment and reached a significantly higher level at 24 hr after treatment compared with the control skin. The increase in signals of TGF-beta 1 mRNA detected by *in situ* hybridisation method started at 3 hr after treatment in the epidermis and progressed thereafter both in the epidermis and in the dermis. These results suggest that the elevated level of TGF-beta 1 mRNA may have a close relation to the induction of epidermal basal cell apoptosis as well as to the intradermal infiltration of mast cells and fibroblasts following the topical application of T-2 toxin.

HUSZENICZA, G., FEKETE, S., SZIGETI, G., KULCSAR, M., FEBEL, H., KELLEMS, R.O., NAGY, P., CSEH, S., VERESEGYHAZY, T. and HULLAR, I. 2000. **Ovarian consequences of low dose peroral *Fusarium* (T-2) toxin in a ewe and heifer model.** *Theriogenology* **53**: 1631–1639.

The effect of low dose T-2 toxin intake on ovarian function was evaluated in ewes and heifers. Half of the ewes and all of the heifers were fed rich, acidosis inducing concentrate. Ewes were fed T-2 toxin at 0.5 or 15 µg/kg for 21 days. Heifers were fed T-2 toxin at 25 µg/kg for 20 days. The oestrus cycles in all animals were synchronised prior to the experiment and the T-2 toxin exposure was started in the mid luteal phase. In ewes fed concentrate and T-2 toxin, ovarian malfunction manifested as lower P4 peak concentration in the mid luteal phase, shortening of the CL lifespan and prolonged follicular phases. None of the control and acidotic groups (0 µg T-2 toxin) or ewes fed regular diet with T-2 toxin at 5 µg/kg showed any ovarian malfunction. In T-2 toxin treated heifers, ovulation occurred later and the plasma progesterone level remained low for a longer period compared to controls.

FAZEKAS, B., HAJDU, E.T. and TANYI, J. 2000. [Effect of MYCO-AD on experimental T-2 toxicosis in broiler chickens]. *Magyar Allatorvosok Lapja* **122**: 412–416.

The effect of MYCO-AD containing hydrated sodium calcium aluminium silicate on T-2 toxicosis in broiler chickens was studied. Chickens were given feed containing T-2 toxin at 1 mg/kg feed, with or without the addition of MYCO-AD at 2.5 g/kg feed. The effect of T-2 toxin was almost completely prevented by feeding MYCO-AD. Feed intake, weight gain and feed conversion of chickens fed T-2 toxin plus MYCO-AD were equal with those of the control group. (In Hungarian).

MAY, H.D., WU, Q.Z. and BLAKE, C.K. 2000. **Effects of the *Fusarium* spp. mycotoxins fusaric acid and deoxynivalenol on the growth of *Ruminococcus albus* and *Methanobrevibacter ruminantium*.** *Canadian Journal of Microbiology* **46**: 692–699.

Fusaric acid and DON were tested for antimicrobial activity against the rumen microorganisms *Ruminococcus albus* and *Methanobrevibacter ruminantium*. The growth of both organisms was inhibited by fusaric acid as low as 15 mg/L but not by DON at levels as high as 100 mg/L. No synergistic inhibitory effect was observed with DON plus fusaric acid. Neither organism

was able to adapt to fusaric acid. Inhibition of *R. albus* started before significant growth had occurred, while *M. ruminantium* doubled twice before the onset of inhibition. This is the first demonstration of fusaric acid inhibiting the growth of rumen bacteria.

Aflatoxins – General

EL-TAHAN, F.H., EL-TAHAN, M.H. and SHEBL, M.A. 2000. **Occurrence of aflatoxins in cereal grains from four Egyptian governorates.** *Nahrung* **44**: 279–280.

A total of 1387 samples of cereals were collected over the period 1996–1997 from stores of commercial feed mills located in four governorates in Egypt. AFB₁ was detected in maize, yellow corn, gluten and soybean meal in 93, 32, 35 and 3% of samples at mean concentrations of 82, 49, 49 and 11 µg/kg, respectively. AFB₂, G₁ and G₂ were not detected in any samples (detection limit was 5 µg/kg).

HELL, K., CARDWELL, K.F., SETAMOU, M. and POEHLING, H.M. 2000. **The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, west Africa.** *Journal of Stored Products Research* **36**: 365–382.

Aflatoxin levels in 300 farmers' stores in four agroecological zones in Benin, West Africa, were determined over a period of 2 years. Beninese farmers often changed their storage structures during the storage period, transferring the maize from a drying or temporary store to a more durable one. Maize samples in the southern Guinea and Sudan savannahs were associated with higher aflatoxin levels and the forest/savannah mosaic was related to lower toxin levels. Factors associated with higher aflatoxin were storage for 3–5 months, insect damage and use of *Khaya senegalensis* bark or other local plants as storage protectants. Depending on the agroecological zone, storage structures that had a higher risk of aflatoxin development were the "Ago", the "Secco", the "Zingo" or storing under or on top of the roof of the house. Lower aflatoxin levels were related to the use of storage or cotton insecticides, mechanical means or smoke to protect against pests or cleaning of stores before loading them with the new harvest. Fewer aflatoxins were found when maize was stored in the "Ago" made from bamboo or when bags were used as secondary storage containers.

ANTONACCI, L., SALVAT, A.E., FAIFER, G.C. and GODOY, H.M. 1999. **Suppression of spore germination and**

aflatoxin biosynthesis in *Aspergillus parasiticus* during and after exposure to high levels of phosphine. *Mycopathologia* **147**: 83–87.

Agar cultures of toxigenic *Aspergillus parasiticus* were exposed to phosphine (PH₃) at levels ranging from 0 to 2000 mg/kg. PH₃ at concentrations of 400 mg/kg or higher completely arrested the growth of the fungus. When PH₃ was vented and the agar plates were exposed to open air, 100% of the initial CFU developed into fully-grown colonies after PH₃ levels below 300 mg/kg, but at higher PH₃ concentrations only 50% of the colonies developed. The same strain of *A. parasiticus* was inoculated into high moisture corn. After exposure to PH₃ at 500 mg/kg, both fungal growth and aflatoxin synthesis resumed shortly after elimination of the gas, but after exposure to PH₃ levels of 1000 mg/kg and higher, there was reduced mycelial growth and almost complete absence of green pigmentation. In addition, aflatoxin synthesis was totally absent for the remainder of the experiment (20 days).

XU, H.X., ANNIS, S., LINZ, J. and TRAIL, F. 2000. **Infection and colonization of peanut pods by *Aspergillus parasiticus* and the expression of the aflatoxin biosynthetic gene, *nor-1*, in infection hyphae.** *Physiological and Molecular Plant Pathology* **56**: 185–196.

A histological study of the host-pathogen interaction between peanut, *Arachis hypogaea*, and *Aspergillus parasiticus* was performed in a system where peanuts remained attached to the plant and were inoculated without wounding. A genetically tagged strain of *A. parasiticus*, G5, was engineered to harbour the beta-glucuronidase (GUS) reporter gene under control of the *nor-1* promoter from the aflatoxin biosynthetic pathway. This strain was used to follow infection and aflatoxin production during colonisation of undamaged, drought stressed peanuts. The fungus colonised all tissues of the peanut pod and appeared to gain ingress through the corky layer of the pericarp. Two morphologically distinct types of hyphae were seen throughout the pod tissues. Statistical analysis revealed that the narrower hyphae were significantly more likely to produce GUS activity than wider ones. GUS activity was found in hyphae infecting the pericarp, embryo and cotyledons indicating expression of aflatoxin biosynthetic genes in these tissues. GUS activity was not observed in the hyphae colonising the testa.

DAS, C. and MISHRA, H.N. 2000. **In vitro degradation of aflatoxin B₁ in groundnut (*Arachis hypogaea*) meal by**

horse radish peroxidase. *Lebensmittel-Wissenschaft und-Technologie* 33: 308–312.

Finely powdered defatted groundnut meal spiked with AFB₁ was treated with commercial horseradish peroxidase and partially purified peroxidase enzyme from freshly harvested radish roots. The optimum enzyme concentration occurred when 10 IU of enzyme dissolved in 50 mM phosphate buffer at pH 6 was mixed homogeneously with 100 g of groundnut meal to react upon 1.4 mM AFB₁ at 20°C and normal pressure up to 24 hr incubation period. Hydrogen peroxide in 20 mM concentration was used as an oxidising agent in all the reactions performed. Moisture content of the reaction media was maintained at 12–15%.

DAS, C. and MISHRA, H.N. 2000. **Effect of aflatoxin B₁ detoxification on the physicochemical properties and quality of ground nut meal.** *Food Chemistry* 70: 483–487.

Finely powdered defatted groundnut meal containing AFB₁ at 1.2 mM/100 g was first detoxified up to 53% with 10 IU of horseradish peroxidase enzyme in the presence of hydrogen peroxide and then the meal containing 12–15% moisture was treated with microwave radiation at 1 kW for 15 min to achieve a final 97% detoxification. Mean weight gains of rats given the treated meals were essentially comparable to those for animals receiving aflatoxin free diets. Overall, nitrogen solubility of the enzyme treated meal increases in the pH 2–6 range. Polyacrylamide gel electrophoretic patterns of the protein did not show any notable changes. Amount of protein nitrogen in the meal increased after the enzymatic treatment.

EL-NAGERABI, S.A.F., ELSHAFIE, A.E. and ABDALLA, A.H. 2000. **Composition of mycoflora and aflatoxins in pea seeds from the Sudan.** *Kuwait Journal of Science & Engineering* 27: 109–121.

Pea (*Pisum sativum* L. “Titan”) samples were collected from the local markets of Khartoum and examined for mycoflora and mycotoxins. The genus *Aspergillus* (11 species and 5 varieties) was the most common followed by *Rhizopus*, *Alternaria*, *Fusarium*, *Emericella*, *Drechslera*, *Cladosporium* and *Penicillium*. TLC analysis of chloroform extracts showed that 3/13 samples were naturally contaminated with aflatoxins B₁, B₂, G₁ and G₂.

GRADZIEL, T., MAHONEY, N. and ABDALLAH, A. 2000. **Aflatoxin production among almond genotypes is not**

related to either kernel oil composition or *Aspergillus flavus* growth rate. *Hort-science* 35: 937–939.

Genetic differences were observed in levels of aflatoxin production following controlled inoculations of California almonds (*Prunus dulcis*, *P. communis*). Genetic variation was also observed in kernel oil composition and in susceptibility to *Aspergillus flavus*. Several almond lines resulting from the introgression of peach (*P. persica*) germplasm had very low aflatoxin levels relative to commercial cultivars tested. No correlation was detected between aflatoxin production in inoculated almond kernels and either kernel oil composition or mould growth rate on injured kernel tissue.

OLIVEIRA, C.A.F., KOBASHIGAWA, E., REIS, T.A., MESTIERI, L., ALBAQUERQUE, R. and CORREA, B. 2000. **Aflatoxin B₁ residues in eggs of laying hens fed a diet containing different levels of the mycotoxin.** *Food Additives and Contaminants* 17: 459–462.

Young laying hens were given rations containing AFB₁ at 0, 100, 300 or 500 µg/kg feed and AFB₁ residues in their eggs were determined by TLC. Egg production and average egg weights were not affected in the groups receiving AFB₁. Residues of AFB₁ were detected only in the eggs of hens given 500 µg/kg feed at levels that ranged from 0.05 to 0.16 µg/kg. The results indicate that the feed to eggs AFB₁ transmission ratio was approximately 5000:1.

FAYOKUN, C.O. and ADEGOKE, G.O. 2000. **Studies on residual aflatoxin B₁ in poultry feeds and poultry products.** *Journal of Food Science and Technology – Mysore* 37: 311–314.

The production performance of poultry fed AFB₁ at 250–1000 µg/kg did not differ significantly from that of control birds. No mortality was observed amongst poultry fed AFB₁ at 24000 µg/kg, however, samples of liver and heart of layers were enlarged with pale coloration. Cessation of egg laying by birds also occurred.

RATNAVATHI, C.V. and SASHIDHAR, R.B. 2000. **Changes in enzyme activities and aflatoxin elaboration in sorghum genotypes following *Aspergillus parasiticus* infestation.** *Journal of the Science of Food and Agriculture* 80: 1713–1721.

Six sorghum genotypes (red – AON 486, IS 620; yellow – LPJ, IS 17 779; white – SPV 86, SPV 462) were inoculated with a toxigenic strain of *Aspergillus parasiticus* and changes in the activities of various hydrolytic enzymes and aflatoxin production were evaluated. The alpha- and beta-amylase activities were positively correlated to aflatoxin production. The total aflatoxins

produced were lower in red genotypes than in yellow and white genotypes. AFB₁, B₂, G₁ and G₂ were present in five genotypes (IS 620, LPJ, IS 17 779, SPV 86 and SPV 462) at all the stages of infection but aflatoxin could not be detected in the red genotype AON 486 on day 3 after infection. White genotypes SPV 86 and SPV 462 showed maximal aflatoxin total production on day 6 after infection.

MELLON, J.E., COTTY, P.J. and DOWD, M.K. 2000. **Influence of lipids with and without other cottonseed reserve materials on aflatoxin B₁ production by *Aspergillus flavus*.** *Journal of Agricultural and Food Chemistry* 48: 3611–3615.

Fungal utilisation of the three major cottonseed reserve materials, raffinose, triglycerides (refined cottonseed oil) and cottonseed storage protein, was monitored *in vitro* over a 7 day fermentation period. *A. flavus* rapidly converted raffinose to fructose and melibiose and then hydrolysed the melibiose. These simple sugars apparently supported initial growth and AFB₁ production. Raffinose and melibiose were nearly exhausted by day 2. Fungal hydrolysis of triglycerides began as exhaustion of carbohydrate approached. After day 2, rapid catabolism of the released fatty acids began and coincided with glucose regeneration through gluconeogenesis, which peaked on day 6. The fungus did not preferentially utilise specific fatty acids.

OATLEY, J.T., RARICK, M.D., JI, G.E. and LINZ, J.E. 2000. **Binding of aflatoxin B₁ to bifidobacteria *in vitro*.** *Journal of Food Protection* 63: 1133–1136.

One potential method for reducing human health effects due to aflatoxin ingestion is to block uptake via binding by bacteria that either make up the normal gut flora or are present in fermented foods in our diet. As bifidobacteria comprise a large fraction of the normal gut flora and are increasingly used in fermented dairy products, various strains of heat-killed bifidobacteria were tested for their ability to bind AFB₁ *in vitro*. The AFB₁ binding affinities of strains of bifidobacteria, *Staphylococcus aureus* and *Escherichia coli* were quantitated using ELISA and [³H]AFB₁ binding assays. The bacteria analysed were found to bind significant quantities of AFB₁ ranging from 25 to nearly 60% of the added toxin.

CHANG, P.K., YU, J.J., BHATNAGAR, D. and CLEVELAND, T.E. 1999. **Repressor-AFLR interaction modulates aflatoxin biosynthesis in *Aspergillus parasiticus*.** *Mycopathologia* 147: 105–112.

The aflatoxin pathway specific regulatory gene, *aflR*, produces AFLR, a zinc cluster transcription factor, which turns on or off the transcription of other aflatoxin genes. To determine if the AFLR carboxyl region (AFLRC) interacts with positive or negative acting proteins, the *Aspergillus parasiticus* *aflR* carboxyl coding region (*aflRC*) was fused to the promoter of *A. parasiticus* nitrite reductase gene (*niiA(p)::aflRC*) and transformed into *A. parasiticus* SRRC 2043. Transformants that contained two copies of *niiA(p)::aflRC*, one at the *niiA* locus and another at the *aflR* locus, overproduced aflatoxin precursors independent of the nitrogen source. The higher copy number of the integrated *niiA(p)::aflRC* correlated with increased production of aflatoxin precursors by the transformants as well as increased expression of both *aflRC* and native *aflR*. Since *aflRC* does not encode a DNA binding domain, the expressed AFLRC should not bind to the promoters of aflatoxin pathway genes and affect transcription directly. The results are consistent with AFLRC titrating out a putative repressor that interacts with AFLR under different growth conditions and modulates aflatoxin biosynthesis.

CARY, J.W., EHRlich, K.C., WRIGHT, M., CHANG, P.K. and BHATNAGAR, D. 2000. **Generation of *aflR* disruption mutants of *Aspergillus parasiticus***. Applied Microbiology and Biotechnology **53**: 680–684.

The *aflR* gene of *Aspergillus parasiticus* and *A. flavus* encodes a binuclear zinc finger, DNA binding protein, AFLR, responsible for activating the transcription of all known aflatoxin biosynthetic genes including itself. Studies to determine how environmental and nutritional factors affect *aflR* expression have been difficult to perform due to the lack of *aflR* “knockout” mutants. Transformation of an O-methylsterigmatocystin (OMST) accumulating strain of *A. parasiticus* with an *aflR-niaD* gene disruption vector resulted in clones harbouring a recombinationally inactivated *aflR* gene which no longer produced OMST or *aflR* transcript. By transformation of this *aflR* disruptant strain with constructs containing mutated versions of the *aflR* promoter, three cis-acting sites were identified that were necessary for *aflR* function: an AFLR binding site, a PacC binding site, and a G+A rich site near the transcription start site of *aflR*.

KLICH, M.A., MULLANEY, E.J., DALY, C.B. and CARY, J.W. 2000. **Molecular and physiological aspects of aflatoxin and sterigmatocystin biosynthesis by *Aspergillus tamarii* and *A. ochraceoroseus***. Applied Microbiology and Biotechnology **53**: 605–609.

Until recently, only three species (*Aspergillus flavus*, *A. parasiticus* and *A. nomius*) have been widely recognised as producers of aflatoxin. In this study, aflatoxin production by two other species, *A. tamarii* and *A. ochraceoroseus* was examined. Genomic DNA of these two species was probed with known aflatoxin and sterigmatocystin biosynthesis genes from *A. flavus*, *A. parasiticus* and *A. nidulans*. Under the high stringency conditions, *A. tamarii* DNA hybridised to all four of the *A. flavus* and *A. parasiticus* gene probes, indicating strong similarities in the biosynthetic pathway genes of these three species. The *A. ochraceoroseus* DNA hybridised weakly to the *A. flavus* and *A. parasiticus* *verB* gene probe, and to two of the three *A. nidulans* probes. These data indicate that, at the DNA level, the aflatoxin and sterigmatocystin biosynthetic pathway genes for *A. ochraceoroseus* are somewhat different from known pathway genes.

YU, J., CHANG, P.K., BHATNAGAR, D. and CLEVELAND, T.E. 2000. **Genes encoding cytochrome P450 and monooxygenase enzymes define one end of the aflatoxin pathway gene cluster in *Aspergillus parasiticus***. Applied Microbiology and Biotechnology **53**: 583–590.

Sequencing of the existing aflatoxin pathway gene cluster was extended in both directions, beyond the *pkcA* gene at one end and the *omtA* gene at the other. Within the 25-kb genomic DNA sequence determined at the *omtA* end of the cluster, several new gene sequences were identified including the recently reported genes, *vbs* and *orda*. Two additional genes were also found in this region, a cytochrome P450 monooxygenase encoding gene, tentatively named *cypX*, and a monooxygenase encoding gene, tentatively named *moxY*. Northern blot analysis and reverse transcriptase-polymerase chain reaction studies demonstrated that the genes, *cypX* and *moxY* are expressed concurrently with genes involved in aflatoxin biosynthesis.

NOLAND, W.E. and KEDROWSKI, B.L. 2000. **Quinone approaches toward the synthesis of aflatoxin B₂**. Organic Letters **2**: 2109–2111.

Quinones bearing electron withdrawing groups can serve as useful precursors to furobenzofuran ring systems through their reaction with 2,3-dihydrofuran. Formal racemic and stereoselective syntheses of AFB₂ are described which utilise this approach to construct the tricyclic ABC-ring core of the molecule.

JAIMEZ, J., FENTE, C.A., VAZQUEZ, B.I., FRANCO, C.M., CEPEDA, A., MAHUZIER, G. and PROGNON, P. 2000. **Application of the assay of aflatoxins by liquid chromatography with fluorescence detection in food analysis**. Journal of Chromatography A **882**: 1–10.

This review with 150 references discusses HPLC determination of aflatoxins and its application in food analyses. HPLC using fluorescence detection has several advantages over other analytical methods. Reversed phase HPLC is more popular than normal phase HPLC.

OTTA, K.H., PAPP, E. and BAGOSI, B. 2000. **Determination of aflatoxins in food by overpressured-layer chromatography**. Journal of Chromatography A **882**: 11–16.

Methods for the analysis of aflatoxins in fish, corn and wheat using overpressured-layer chromatography (OPLC) are described. Using OPLC, 10 samples could be analysed simultaneously. Quantitative evaluation of aflatoxins was accomplished by densitometry. Average recoveries from each food spiked with aflatoxins at 2–10 µg/kg were greater than 73%. The OPLC technique seems to be a rapid, reproducible and cost effective analysis for quantitative determination of aflatoxins in foods.

SIONTOROU, C.G., ANDREOU, V.G., NIKOLELIS, D.P. and KRULL, U.J. 2000. **Flow injection monitoring of aflatoxin M₁ in cheese using filter-supported bilayer lipid membranes with incorporated DNA**. Electroanalysis **12**: 747–751.

A technique for the rapid and sensitive electrochemical flow injection monitoring of AFM₁ in cheese samples is described. Stabilised filter-supported bilayer lipid membranes (BLMs) were used as detectors. Single stranded DNA oligomers terminated with alkyl chains (dT(20)-C-16) were incorporated into the membranes to control surface electrostatic properties. The incorporation of dT(20)-C-16 in BLMs lowered the detection limit for this toxin by one to four orders of magnitude as compared with the detection limit obtained in the absence of DNA. Injections of AFM₁ were made into flowing streams of a 0.1 M KCl electrolyte solution, and a transient current signal with duration of seconds reproducibly appeared in about 12 sec after exposure of the detector element to the toxin. The magnitude of this signal was linearly related to the concentration of AFM₁ with detection limits at subnanomolar level. In cheese sam-

ples, AFM₁ could be determined in continuous flowing systems with a rate of at least 3 samples per minute.

Aflatoxicoses

SAHU, S.C., CHOU, M.W., SOTOMAYOR, R.E., HINTON, D.M., BARTON, C.N. and O'DONNELL, M.W. 2000. **Effects of intermittent exposures of aflatoxin B₁ on hepatic and testicular glutathione S-transferase in rats.** *Journal of Applied Toxicology* **20**: 215–219.

The effects of intermittent exposures to AFB₁ on hepatic and testicular glutathione S-transferase (GST) in rats was evaluated. Male Fischer 344 rats were fed diets containing AFB₁ at 0.01, 0.04, 0.4 and 1.6 mg/kg intermittently at 4-week intervals up to 20 weeks. Rats consuming diets with 0.01 mg/kg did not show the induction of hepatic or testicular GST activity. Exposures to AFB₁ at concentrations of 0.04–1.6 mg/kg significantly increased the GST activities. The increase of the enzyme activity was proportional to the dose and length of AFB₁ exposure.

ALLAMEH, A., FARAHANI, M. and ZARGHI, A. 2000. **Kinetic studies of aflatoxin B₁-glutathione conjugate formation in liver and kidneys of adult and weanling rats.** *Mechanisms of Ageing and Development* **115**: 73–83.

AFB₁-GSH conjugation is the major pathway for the detoxification of aflatoxin metabolites. This reaction is catalysed by GST. Measurement of cytosolic GST showed that the enzyme activity is initially lower in weanling tissues compared to that of adults. Nevertheless hepatic and renal cytosolic GST activity is increased significantly in growing rats pretreated with AFB₁. Kinetic studies of AFB₁-GSH conjugate formation in kidneys and livers of immature and adult rats treated with a single ip dose of AFB₁ at 400 µg/kg body weight revealed that at the end of 24 hr of AFB₁ administration the rate of the conjugate formation in kidneys of immature rats was approximately twice of that measured in adults. Age related differences in the GST activity as well as AFB₁-GSH conjugation was more pronounced in kidneys.

LARSSON, P. and TJALVE, H. 2000. **Intranasal instillation of aflatoxin B₁ in rats: Bioactivation in the nasal mucosa and neuronal transport to the olfactory bulb.** *Toxicological Sciences* **55**: 383–391.

Female Sprague-Dawley rats were given [³H]AFB₁ at 0.2, 1 or 20 µg intranasally and were sacrificed at various intervals up to 20 days. Tissues were examined autoradio-

graphically or histopathologically. The data obtained indicated that intranasal administration of AFB₁ resulted in formation of tissue-bound metabolites in sustentacular cells, in some cells of Bowman's glands and in a population of neuronal cells in the olfactory mucosa, whereas in the respiratory nasal mucosa, there was selective bioactivation of AFB₁ in mucous cells. Intranasal instillation of 20 µg AFB₁ resulted in disorganised undulating olfactory epithelium, with injured neuronal and sustentacular cells. In the respiratory epithelium, there was selective destruction of mucous cells. beta-Spectrometry and autoradiography indicated transport of AFB₁ and/or AFB₁ metabolites along the axons of the primary olfactory neurons to their terminations in the glomeruli of the olfactory bulb.

ABDEL-HAQ, H., GIACOMELLI, S., PALMERY, M., LEONE, M.G., SASO, L. and SILVESTRI, B. 2000. **Aflatoxins inhibit prolactin secretion by rat pituitary cells in culture.** *Drug and Chemical Toxicology* **23**: 381–386.

The effects of specific aflatoxins on prolactin secretion by rat pituitary cells in culture was studied. AFB₁ and AFQ₁ (1 × 10⁻⁴M) reduced the stimulating effect of dimethyl sulfoxide on prolactin secretion. The mechanism responsible for this action is unknown, but it may be a specific toxic effect, because AFB₁ at the same concentration did not significantly alter cell viability, as indicated by the Trypan blue dye-exclusion test.

BARTON, C.C., HILL, D.A., YEE, S.B., BARTON, E.X., GANEY, P.E. and ROTH, R.A. 2000. **Bacterial lipopolysaccharide exposure augments aflatoxin B₁-induced liver injury.** *Toxicological Sciences* **55**: 444–452.

Male Sprague-Dawley rats were treated with AFB₁ at 1 mg/kg ip and 4 hr later with *E. coli* lipopolysaccharide (LPS) (7.4 × 10⁶ EU/kg iv). Liver injury was assessed between 6 and 96 hr after AFB₁ administration. Hepatic parenchymal cell injury was evaluated as increased alanine aminotransferase and aspartate aminotransferase activities in serum and from histologic examination of liver sections. At all times and for all markers, injury in rats treated with either AFB₁ or LPS alone was absent or modest. In the AFB₁/LPS co-treated group, hepatic parenchymal cell injury was pronounced by 24 hr and had returned to control values by 72 hr. Furthermore, changes in serum markers indicative of biliary tract alterations were evident by 12 hr and had returned to control values by 72 hr. Results suggest that LPS potentiated the effects of AFB₁ on both parenchymal and bile duct epithelial cells.

MOON, E.Y. and PYO, S. 2000. **Aflatoxin B₁ inhibits CD14-mediated nitric oxide production in murine peritoneal macrophages.** *International Journal of Immunopharmacology* **22**: 237–246.

The effects of AFB₁ on CD14 mediated nitric oxide (NO) production in murine peritoneal macrophages was investigated. When macrophages were stimulated with LPS, either at the same time or after AFB₁ treatment, NO production decreased in a dose dependent manner. In contrast, when macrophages were treated with AFB₁ after LPS stimulation, NO production was unchanged. DNA, RNA and protein synthesis were reduced by AFB₁ pretreatment of macrophages. The addition of anti-CD14 antibodies to the cultures decreased NO production further. FACS analysis showed that the binding of anti-CD14 antibodies to the macrophages was suppressed by AFB₁ but only after LPS stimulation. The results indicate that the reduced NO production in murine peritoneal macrophages by AFB₁ pretreatment is related to the suppressed expression of CD14 on macrophage membrane and to the increased secretion of it to culture medium after LPS stimulation.

ABDEL-HAQ, H., PALMERY, M., LEONE, M.G., SASO, L. and SILVESTRI, B. 2000. **Relaxant effects of aflatoxins on isolated guinea pig trachea.** *Toxicological Sciences* **55**: 162–170.

Dyspnea is one of the symptoms of acute aflatoxicosis. Contrary to expectations, it was observed that naturally occurring aflatoxins, AFB₁, B₂, G₁ and G₂, and their major metabolites, AFM₁, M₂, P₁, Q₁ and G_{2a}, relaxed carbachol precontracted guinea pig trachea to different degrees. The efficacies but not the potencies of AFB₁, B₂, G₁ and G₂ were similar to that of isoprenaline, whose activity was potentiated by the aflatoxins. Studies indicated that the symptoms of acute aflatoxicosis do not seem to be due to a direct activity on the tracheal muscle, but rather, to the well-known pro-inflammatory activity of the aflatoxins, which are capable of releasing arachidonic acid from cell membranes.

ZHANG, Y.J., CHEN, S.Y., TSAI, W.Y., AHSAN, H., LUNN, R.M., WANG, L.Y., CHEN, C.J. and SANTELLA, R. 2000. **Expression of cytochrome P450 1A1/2 and 3A4 in liver tissues of hepatocellular carcinoma cases and controls from Taiwan and their relationship to hepatitis B virus and aflatoxin B₁- and 4-aminobiphenyl-DNA adducts.** *Biomarkers* **5**: 295–306.

Cytochrome P450 enzymes play a major role in the metabolism of several of the chemical carcinogens involved in the development of hepatocellular carcinoma (HCC).

Polyclonal antisera and immunoperoxidase staining were used to detect the expression of CYP1A1/2 and 3A4 in 37 surgical control tissues and 105 tumour and adjacent non-tumour tissues of HCC cases from Taiwan. Overall there was no relationship between CYP1A1/2 or CYP3A4 and AFB₁-DNA adducts in control tissues. Staining intensity for CYP1A1/2 and 3A4 followed the order: tumour tissues <control tissues <adjacent non-tumour tissues. For CYP3A4, in contrast to control tissues, there was a significant association with AFB₁-DNA adducts in tumour and adjacent non-tumour tissue of HCC cases. These results suggest that one factor influencing carcinogen-DNA adducts is levels of specific P450 enzymes.

SOHN, S., JAITOVITCH-GROISMAN, I., BENLIMAME, N., GALIPEAU, J., BATIST, G. and ALAOUJ-JAMALI, M.A. 2000. **Retroviral expression of the hepatitis B virus × gene promotes liver cell susceptibility to carcinogen-induced site specific mutagenesis.** *Mutation Research – DNA Repair* **460**: 17–28.

The effect of the hepatitis B virus (HBV) × protein (HBx) on carcinogen induced cytotoxicity and AGG to AGT mutation in codon 249 of the p53 gene in the human liver cell line CCL13 was studied. Expression of HBx, as revealed by its transactivation function, results in enhanced cell susceptibility to cytotoxicity induced by the AFB₁ active metabolite, AFB₁-8,9-epoxide. Exposure to AFB₁-8,9-epoxide alone induces a low frequency of AGG to AGT mutation in codon 249 of the p53 gene. However, expression of HBx enhances the frequency of AFB₁-epoxide-induced AGG to AGT mutation compared to control cells.

LEE, Y.I., LEE, S., DAS, G.C., PARK, U.S., PARK, S.M. and LEE, Y.I. 2000. **Activation of the insulin-like growth factor II transcription by aflatoxin B₁ induced p53 mutant 249 is caused by activation of transcription complexes; implications for a gain-of-function during the formation of hepatocellular carcinoma.** *Oncogene* **19**: 3717–3726.

AFB₁ induced mutation of the p53 gene at codon 249 (p53mt249) is critical during the formation of HCC following HBV infection. p53mt249 markedly increases insulin-like growth factor II (IGF-II) transcription largely from promoter 4, accumulating the foetal form of IGF-II. Modulation of the transcription factor binding to IGF-II P4 by wild-type p53 and p53mt249 was identified. Wild-type p53 inhibited binding of transcription factors Sp1 and TBP on the P4 promoter, while p53mt249 enhanced the

formation of transcriptional complexes through enhanced DNA-protein (Sp1 or TBP) and protein-protein (Sp1 and TBP) interactions. p53mt249 stimulates transcription factor Sp1 phosphorylation which might be a cause of increased transcription factor binding on the P4 promoter while wild-type p53 does not.

BANERJEE, R., CARUCCIO, L., ZHANG, Y.J., MCKERCHER, S. and SANTELLA, R.M. 2000. **Effects of carcinogen-induced transcription factors on the activation of hepatitis B virus expression in human hepatoblastoma HepG2 cells and its implication on hepatocellular carcinomas.** *Hepatology* **32**: 367–374.

The influence of AFB₁ on the induction of various transcription factors in human hepatoblastoma HepG2 cells was investigated. DNA electrophoretic mobility shift assays were performed with nuclear extracts from HepG2 cells treated with AFB₁ at 10 μmol/L for 6 and 24 hr. Eight-fold increases in nuclear transcription factor kappa B (NF-kappa B) and 5-fold increases in activated protein (AP-1) transcription factor were observed after 24 hr. Four-fold activation of stress protein was detected by a consensus heat shock factor sequence binding probe. DNA adducts were observed by immunoassays in HepG2 cells treated with AFB₁. Increased HBV surface antigen (HBsAg) synthesis was detected in AFB₁ treated HepG2 cells following transfection with recircularised HBV DNA.

WANG, C., BAMMLER, T.K., GUO, Y.Y., KELLY, E.J. and EATON, D.L. 2000. **Mu-class GSTs are responsible for aflatoxin B₁-8,9-epoxide-conjugating activity in the nonhuman primate *Macaca fascicularis* liver.** *Toxicological Sciences* **56**: 26–36.

The primate *Macaca fascicularis* (Mf) exhibits significant constitutive hepatic glutathione S-transferase (GST) activity towards AFB₁-8,9-epoxide. These GSTs were purified from liver tissue, characterised and mu-class GST cDNAs were cloned by reverse transcriptase-coupled polymerase chain reaction (RT-PCR) to determine which specific GST isoenzyme(s) are responsible for this activity. Two distinct mu-class GST cDNAs, mfaGSTM1 and mfaGSTM2, were generated by RT-PCR. Results indicate that, in contrast to rodents, mu-class GSTs are responsible for the majority of AFB₁-8,9-epoxide conjugating activity in the liver of *M. fascicularis*.

KIM, J.G., LEE, Y.W., KIM, P.G., ROH, W.S. and SHINTANI, H. 2000. **Reduction of aflatoxins by Korean soybean**

paste and its effect on cytotoxicity and reproductive toxicity. Part I. Inhibition of growth and aflatoxin production of *Aspergillus parasiticus* by Korean soybean paste (doen-jang) and identification of the active component. *Journal of Food Protection* **63**: 1295–1298.

The inhibitory effect of methanol extract of Korean soybean paste on mould growth and aflatoxin production of a strain of *Aspergillus parasiticus* was studied. Reduction of mycelial weight as a result of addition of the extract was observed to range from 1.5 to 12.9% while reduction of aflatoxin production ranged from 14.3 to 41.7%. The main active component was identified by GC-MS as linoleic acid.

TOWNER, R.A., HASHIMOTO, H. and SUMMERS, P.M. 2000. **Non-invasive *in vivo* magnetic resonance imaging assessment of acute aflatoxin B₁ hepatotoxicity in rats.** *Biochimica et Biophysica Acta – General Subjects* **1475**: 314–320.

Male rats were dosed ip with AFB₁ at 3 mg/kg body weight and hepatotoxicity was assessed *in vivo* 24 hr later using magnetic resonance imaging. Regions of damage, characterised by increased proton signal intensities in T2-weighted images, were observed in the vicinity of the hepatic portal vein and in the right medial regions of the liver. Histopathological assessment was characterised by portal/central vein/artery congestion, sinusoid congestion, nuclear pyknosis and karyolysis, and hepatocyte vacuolation. Electron microscopy examination indicated nuclear debris, swollen cytoplasmic compartments, vacuolation and the disappearance of the smooth endoplasmic reticulum.

PREMALATHA, B. and SACHDANAN-DAM, P. 2000. **Potency of *Semecarpus anacardium* Linn. nut milk extract against aflatoxin B₁-induced hepatocarcinogenesis: Reflection on microsomal biotransformation enzymes.** *Pharmacological Research* **42**: 161–166.

The effect of *Semecarpus anacardium* nut milk extract on the host detoxification system in AFB₁ induced HCC was studied in male albino rats. Cancer-bearing animals showed an overall decrease of liver microsomal cytochrome P450, cytochrome b5, NADPH-cytochrome P450 reductase, NADH-cytochrome b5 reductase and aniline hydroxylase with a subsequent decrease of phase II enzymes, GST and UDP-glucuronyl transferase. Oral administration of nut extract at 200 mg/kg body weight per day for 14 days was found to be highly effective in inducing phase I and phase II biotransformation enzymes to near normal levels.

KUILMAN, M.E.M., MAAS, R.F.M. and FINK-GREMMELS, J. 2000. **Cytochrome P450-mediated metabolism and cytotoxicity of aflatoxin B₁ in bovine hepatocytes.** *Toxicology In Vitro* **14**: 321–327.

The time and dose dependent rate of AFB₁ metabolism in bovine hepatocytes was investigated. AFM₁ is the most prominent metabolite formed within the first 2–8 hr of incubation, whereas AFB₁-dhd is detectable in medium mainly after a prolonged incubation period. The delayed formation of AFB₁-dhd corresponds to the cytotoxicity demonstrated by the MTT assay. alpha-Naphthoflavone and ketoconazole, inhibitors of CYP1A and CYP3A, respectively in humans, inhibited ethoxyresorufin O-deethylation and testosterone 6 beta-hydroxylation also in bovine hepatocytes. Both inhibitors reduced AFM₁ and AFB₁-dhd formation concentration dependently, suggesting that both enzyme groups contribute to the formation of these metabolites.

PARK, U.S., SU, J.J., BAN, K.C., QIN, L.L., LEE, E.H. and LEE, Y.I. 2000. **Mutations in the p53 tumor suppressor gene in tree shrew hepatocellular carcinoma associated with hepatitis B virus infection and intake of aflatoxin B₁.** *Gene* **251**: 73–80.

The tree shrew (*Tupaia belangeri chinensis*) is a useful animal model for the development of HCC after human HBV infection or AFB₁ treatment. Tree shrew wild-type p53 sequence showed 91.7 and 93.4% homologies with human p53 nucleotide and amino acids sequences, respectively, while it showed 77.2 and 73.7% homologies in mice. Examination of HCC and normal liver tissue showed that tree shrews exposed to AFB₁ and/or HBV had neither codon 249 mutations nor significant levels of other mutations in the p53 gene, as is the case with humans.

ABDEL-HAQ, H., PALMERY, M., LEONE, M.G., SASO, L. and SILVESTRI, B. 2000. **Stimulation of guinea pig isolated atria by aflatoxins.** *Toxicology In Vitro* **14**: 193–197.

The effects of aflatoxins on isolated guinea pig atria was investigated. Isoprenaline at 4×10^{-9} M, AFB₁ at 3×10^{-6} M and AFG1 at 3×10^{-6} M contracted the isolated guinea pig atria, leaving the preparation hyperresponsive to isoprenaline.

OGUZ, H., KECECI, T., BIRDANE, Y.O., ONDER, F. and KURTOGLU, V. 2000. **Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis.** *Research in Veterinary Science* **69**: 89–93.

The ability of clinoptilolite, a natural zeolite, to reduce the deleterious effects of aflatoxins in the diet of broiler chickens was investigated. Chickens were fed total aflatoxins at 2.5 mg/kg diet, with or without the addition of clinoptilolite at 1.5 or 2.5%, from 1 to 21 days of age. Aflatoxin treatment significantly decreased serum total protein, albumin, inorganic phosphorus, uric acid, total cholesterol and the values of haematocrit, red blood cell counts, mean corpuscular volume, haemoglobin, thrombocyte counts, percentage of monocyte counts; increased values of white blood cell and heterophil counts. The addition of CLI to the diet reduced the adverse effects of aflatoxins.

QUIST, C.F., BOUNOUS, D.I., KILBURN, J.V., NETTLES, V.F. and WYATT, R.D. 2000. **The effect of dietary aflatoxin on wild turkey poults.** *Journal of Wildlife Diseases* **36**: 436–444.

The effects of aflatoxins on the wild turkey (*Meleagris gallopova silvestris*) are of concern because the conspecific domestic turkey is highly susceptible to aflatoxins. Wild turkeys were fed diets containing aflatoxin at 100, 200 or 400 µg/kg feed for 2 weeks. Aflatoxin fed poults had decreased feed consumption and weight gains as compared with controls. Decreased liver to body weight ratios, liver enzyme alterations, slightly altered blood coagulation patterns and mild histologic changes indicated low level liver damage. Compromise of cell mediated immunity was indicated by decreased lymphoblast transformation. The effects were apparent in all treatment groups but significant differences most often were found at 400 µg/kg feed.

KLEIN, P.J., BUCKNER, R., KELLY, J. and COULOMBE, R.A. 2000. **Biochemical basis for the extreme sensitivity of turkeys to aflatoxin B₁.** *Toxicology and Applied Pharmacology* **165**: 45–52.

The biochemical mechanisms for the extreme sensitivity of poultry to AFB₁ were investigated by measuring microsomal activation of AFB₁ to the AFB₁-8,9-epoxide as well as cytosolic GST mediated detoxification of AFB₁-8,9-epoxide, in addition to other hepatic phase I and phase II enzyme activities in 3-week-old male Oorlop strain turkeys. Cytosol prepared from turkey livers exhibited no measurable GST mediated detoxification of microsomally activated AFB₁, indicating that turkeys are deficient in the most crucial AFB₁ detoxification pathway. In total, the data indicate that the extreme sensitivity of turkeys to AFB₁ may be attributed to a combination of efficient AFB₁ activation and deficient detoxification by phase II enzymes, such as GSTs.

QUEZADA, T., CUELLAR, H., JARAMILLO-JUAREZ, F., VALDIVIA, A.G. and REYES, J.L. 2000. **Effects of aflatoxin B₁ on the liver and kidney of broiler chickens during development.** *Comparative Biochemistry and Physiology C – Pharmacology Toxicology & Endocrinology* **125**: 265–272.

The influence of age on the toxic effects of AFB₁ on plasma, renal and hepatic enzymes in adult and in developing Arbor-Acres chickens was studied. Four-week-old chickens received AFB₁ at 0.5, 1.0 or 2.0 mg/kg feed and 1-week-old chickens received AFB₁ at 2 mg/kg feed. Birds were slaughtered between 7 and 28 days of treatment and body, hepatic and renal weights, succinate dehydrogenase (SDH) and glutamate dehydrogenase (GluDH) in plasma and liver were measured. Results suggest that serum proteins SDH and GluDH are sensitive early indicators of toxicity that was more severe in developing chickens. Decrease in serum albumin might be used as an early and suitable indicator of the deleterious effect of this mycotoxin in developing chickens.

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