

Abstracts

Mycotoxins – General

MALMAURET, L., PARENT-MASSIN, D., HARDY, J.L. and VERGER, P. 2002. **Contaminants in organic and conventional foodstuffs in France.** Food Additives and Contaminants **19**: 524–532.

Fifteen products, including meat, milk, eggs, vegetables and cereals, were tested in their organic and conventional forms for contamination by heavy metals, nitrates and nitrites, and some mycotoxins. Regulated maximum levels were exceeded for patulin in organic apples. Contamination of both conventional and organic wheat by deoxynivalenol was observed with a higher level in organic products.

TANAKA, K., GOTO, T., MANABE, M. and MATSUURA, S. 2002. Traditional Japanese fermented foods free from mycotoxin contamination. **JARQ – Japan Agricultural Research Quarterly** **36**: 45–50.

Traditional Japanese fermented foods, miso (soybean paste), shoyu (soy sauce), sake (rice wine) and katsuo-bushi (dried bonito), were examined for the presence of mycotoxins. Fungi used for the fermentation of miso, shoyu and sake are strains of *Aspergillus oryzae* and *A. sojae*. None of the strains examined produced aflatoxins. Aflatoxin contamination of rice, miso, shoyu, sake and katsuo-bushi could not be detected. Some koji moulds are known to produce kojic acid and cyclopiazonic acid. Production of these toxins was examined and the producing strains were eliminated from the commercial fermented foods. Aflatoxins, sterigmatocystin and ochratoxin A could not be detected in katsuo-bushi

SOLEDADE, M., PEDRAS, C., ZAHARIA, L.I. and WARD, D.E. 2002. **The destruxins: Synthesis, biosynthesis, biotransformation, and biological activity.** Phytochemistry **59**: 579–596.

A review with 107 references. Destruxins exhibit a wide variety of biological activities, but are best known for their insecticidal and phytotoxic activities. The great interest in destruxins derives from their potential role as virulence factors in fungi, whether such fungi are useful insect biocontrol agents or detrimental, causing plant disease epidemics. Reports on isolation, chemical structure determination, total synthesis, transformation by diverse organisms, and biological activity of destruxins and related metabolites are reviewed for the first time.

VAZQUEZ, B.I., FENTE, C.A., FRANCO, C.M., VAZQUEZ, M.J. and CEPEDA, A. 2002. **Experimental citrinin production in Spanish cheese.** Archiv für Lebensmittelhygiene **53**: 13–14.

The suitability of three typical cheeses from Galicia (NW Spain) as natural substrates for the growth of citrinin-producing strains of *Penicillium* species was examined. Citrinin-producing strains were inoculated onto slices of Arzua-Ulloa, Cebreiro and San Simon cheeses and incubated at 25°C. On soft pressed cheeses such as Arzua-Ulloa type, all strains developed well and citrinin was detectable after day 4/5. On acid coagulation cheese such as Cebreiro type, all strains grew invasively and citrinin was detected by day 2. On smoked rinded cheeses such as San Simon type, none of the strains grew.

SINHA, B.K., RANJAN, K.S. and PANDEY, T.N. 2002. **Mycotoxins, mycotoxigenic fungi and the biochemical changes in makhana (*Euryale ferox* Salisb) puffs of North Bihar.** Journal of Food Science and Technology – Mysore **39**: 38–41.

A survey was conducted in large areas of North Bihar and Ganga basin, India, for mycotoxigenic infestation in makhana puffs. About 30% of samples were contaminated with aflatoxins, ochratoxins and citrinin. Aflatoxin B₁ at up to 12 mg/kg was found in makhana puffs and significant changes were observed in their nutritional components.

ANDERSEN, B., NIELSEN, K.F. and JARVIS, B.B. 2002. **Characterization of *Stachybotrys* from water-damaged buildings based on morphology, growth, and metabolite production.** Mycologia **94**: 392–403.

One hundred and twenty-two *Stachybotrys* isolates, mainly from water-damaged buildings, were characterised and identified according to morphology, colony characteristics and metabolite production. Two different *Stachybotrys* taxa, *S. chartarum* and one undescribed species, were found in water-damaged buildings regardless of whether the buildings were in Denmark, Finland or the USA. Furthermore, two chemotypes could be distinguished in *S. chartarum*. One chemotype produced atranones and the other produced macrocyclic trichothecenes. The undescribed species produced atranones and could be differentiated from *S. chartarum* by its growth characteristics and pigment production. The results correlate with different inflammatory and toxicological properties reported for these same isolates and show that the three taxa/chemotypes should be treated separately.

The co-occurrence of these three taxa/chemotypes in water-damaged buildings may explain the inconsistent results in the literature concerning toxicity of *Stachybotrys* isolated from that environment.

ASSOULINE-DAYAN, Y., LEONG, A., SHOENFELD, Y. and GERSHWIN, M.E. 2002. **Studies of sick building syndrome. IV. Mycotoxicosis.** Journal of Asthma **39**: 191–201.

This review with 65 references discusses selected aspects of the microbiology of mycotoxin producing moulds and their potential role in human immunopathology with respect to wet building environments. One area that is attracting particular interest is the relative toxigenic potential of mycotoxins. Although exposure to moulds can produce significant mucosal irritation, there are very few data to suggest long-term ill effects. More importantly, there is no evidence in humans that mould exposure leads to non-mucosal pathology.

KIECANA, I., MIELNICZUK, E., KACZMAREK, Z., KOSTECKI, M. and GOLINSKI, P. 2002. **Scab response and moniliformin accumulation in kernels of oat genotypes inoculated with *Fusarium avenaceum* in Poland.** European Journal of Plant Pathology **108**: 245–251.

Inoculation of panicles of 14 genotypes of oats (10 cultivars and 4 lines) with a conidial suspension of *Fusarium avenaceum* caused a reduction in yield of 33% and in 1000 kernel weight (TKW) of 21%. At harvest, the level of moniliformin in kernels averaged 0.13 mg/kg. The highest reduction of yield components caused by the *F. avenaceum* inoculation was found for cultivar Santor. Cultivars Slawko, Dukat, Borys and Komes exhibited the highest resistance to the disease in terms of TKW and yield reductions after inoculation.

AZIZ, N.H. and SMYK, B. 2002. **Influence of UV radiation and nitrosamines on the induction of mycotoxins synthesis by nontoxigenic moulds isolated from feed samples.** Nahrung – Food **46**: 118–121.

The effects of UV radiation and nitrosamines on the induction of mycotoxin biosynthesis by some nontoxigenic moulds isolated from feed samples collected from Egypt and Poland were investigated. Nontoxigenic strains of *Aspergillus flavus* P-63, *A. niger* EN-200 and *A. ochraceus* P-157 synthesised mycotoxins (aflatoxins and ochratoxin A) following exposure to near UV radiation for 120–210 min. Nitrosamines (DMNA and DENA) at 30 to

1000 mg/kg induced the synthesis of aflatoxins by nontoxigenic species of *A. flavus* ES-255 and P-63 and *A. niger* EN 200.

MAGG, T., MELCHINGER, A.E., KLEIN, D. and BOHN, M. 2002. **Relationship between European corn borer resistance and concentration of mycotoxins produced by *Fusarium* spp. in grains of transgenic *Bt* maize hybrids, their isogenic counterparts, and commercial varieties.** Plant Breeding **121**: 146–154.

The European corn borer (ECB) (*Ostrinia nubilalis* Hb) is a major pest of maize in central Europe and promotes the infection of maize with *Fusarium* species. Transgenic *Bt* maize hybrids were compared with their isogenic counterparts and with commercial hybrids for their level of ECB resistance and their concentration of deoxynivalenol (DON), 15-acetylDON, 3-acetylDON, nivalenol, fusarenon-X, fumonisins and zearalenone in harvested grains. For all resistance traits, transgenic *Bt* hybrids showed significantly lower means than their corresponding isogenic counterparts and commercial hybrids. Mycotoxin concentrations varied significantly between locations, years and genotypes, but were not significantly different between infested and protected plots. Associations between ECB resistance traits and mycotoxin concentrations were not consistent across years.

JUGLAL, S., GOVINDEN, R. and ODHAV, B. 2002. **Spice oils for the control of co-occurring mycotoxin-producing fungi.** Journal of Food Protection **65**: 683–687.

The effect of different spice oils was evaluated on the growth of *Aspergillus parasiticus* and *Fusarium moniliforme* by incorporation of the oils into broth and patty cultures. Clove oil (eugenol) was the most inhibitory to the growth of the two fungi, followed by cinnamon (cinnamic aldehyde), oregano (thymol and carvacol) and mace oils (myristin). Neem and eucalyptus oil (cineole) did not affect fungal growth. The co-storing of mycotoxin contaminated grain with both whole and ground cloves markedly reduced the aflatoxin contamination of the grain.

MYERS, A.G., SIU, M. and REN, F. 2002. **Enantioselective synthesis of (–)-terpestacin and (–)-fusaproliferin: Clarification of optical rotational measurements and absolute configurational assignments establishes a homochiral structural series.** Journal of the American Chemical Society **124**: 4230–4232.

Terpestacin is a fungal metabolite that inhibits the formation of syncytia, multinucleated cell bodies that are part of the pathology of AIDS infection. Fusaproliferin is the acetic acid ester of terpestacin. The enantioselective synthesis of terpestacin and fusaproliferin is described.

Mycotoxins – Methodology

ANKLAM, E., STROKA, J. and BOENKE, A. 2002. **Acceptance of analytical methods for implementation of EU legislation with a focus on mycotoxins.** Food Control **13**: 173–183.

This paper with 68 references provides an overview on current strategies and perspectives of analytical methods as a tool for implementation of EU regulatory limits and trade specifications. Examples are given on mycotoxins that have been selected on the basis of being currently relevant contaminants in food and animal feed.

DESAEGER, S., SIBANDA, L., DESMET, A. and VANPETEGHEM, C. 2002. **A collaborative study to validate novel field immunoassay kits for rapid mycotoxin detection.** International Journal of Food Microbiology **75**: 135–142.

Kits designed to detect ochratoxin A (OA) and T-2 toxin by a membrane-based flow-through enzyme immunoassay were studied collaboratively by screening cereals including wheat, rye, maize and barley. A simple methanol-based extraction followed by filtration and dilution steps was prescribed. Reagents were successively pipetted to the membrane of the device, then colour development was evaluated visually. Limits of detection for OA and T-2 toxin were 4 and 50 mg/kg, respectively. In the second stage, four OA and four T-2 toxin kits were used by 10 collaborators to analyse 21 cereal samples. For the OA kits, the percentage of false positive and false negative results were 2% and 4%, respectively. The results of one T-2 toxin kit were outliers and when excluded, the overall percentage false positive and false negative results were 6% and 3%, respectively.

SHU, P.Y. and LIN, C.H. 2002. **Simple and sensitive determination of citrinin in *Monascus* by GC-selected ion monitoring mass spectrometry.** Analytical Sciences **18**: 283–287.

A new method for the qualitative and quantitative analysis of citrinin in *Monascus* by GC-selected ion monitoring (SIM) MS has been developed. GC separation of citrinin in *Monascus* extract was achieved without the need for chemical

derivatisation and could be detected as a single peak when the SIM mode selected 5 prominent fragmentations. The quantitative detection limit for citrinin was similar to 1 µg/kg.

MONACI, L., ARESTA, A., PALMISANO, F., VISCONTI, A. and ZAMBONIN, C.G. 2002. **Amino-bonded silica as stationary phase for liquid chromatographic determination of cyclopiazonic acid in fungal extracts.** *Journal of Chromatography A* **955**: 79–86.

A new HPLC method is described for cyclopiazonic acid (CPA) determination in fungal cultures on a propylamino-bonded stationary phase with a CH₃CN/CH₃COONH₄ buffer as mobile phase. Retention of CPA on propylamino modified silica under acidic conditions (protonated amino groups and deprotonated CPA) is governed by a mixed ion-exchange-reversed-phase mechanism. In addition to non-polar (hydrophobic) interactions, polar interactions with the surface silanols are also possible and become important as the polarity of the mobile phase decreases. A detection limit of 25 pg of CPA standard is obtained that represents an improvement of more than two orders of magnitude compared to existing HPLC procedures.

LOSITO, I., MONACI, L., ARESTA, A. and ZAMBONIN, C.G. 2002. **LC-ion trap electrospray MS-MS for the determination of cyclopiazonic acid in milk samples.** *Analyst* **127**: 499–502.

An LC-ESI-MS (negative ions) method was developed for the determination of CPA in milk samples. First, the acquisition parameters of the ESI mass spectrometer were optimised for deprotonated CPA and MS-MS measurements were performed, giving a fragmentation pattern. After this stage, LC separation was applied to milk extracts by means of an aminic column, using the selective reaction monitoring acquisition mode for MS detection. The CPA response was linear over three decades of concentration and a limit of detection of 5 µg/L was estimated.

MATEO, J.J., MATEO, R., HINOJO, M.J., LLORENS, A. and JIMENEZ, M. 2002. **Liquid chromatographic determination of toxigenic secondary metabolites produced by *Fusarium* strains.** *Journal of Chromatography A* **955**: 245–256.

Various LC methods used in the analysis of zearalenone, trichothecenes and fumonisins were compared. The results demonstrate the suitability of modern cleanup procedures employing multi func-

tional MycoSep and immunoaffinity columns although these methods are more expensive than conventional methodologies for cleanup. HPLC with both fluorescence and photodiode array detection is a suitable technique for the analysis of toxic secondary metabolites produced by *Fusarium* species.

Mycotoxicoses

TAJIMA, O., SCHOEN, E.D., FERON, V.K. and GROTEN, J.P. 2002. **Statistically designed experiments in a tiered approach to screen mixtures of *Fusarium* mycotoxins for possible interactions.** *Food and Chemical Toxicology* **40**: 685–695.

A test strategy is presented to detect interactive effects between several mycotoxins using a DNA synthesis inhibition assay in L929 cells. The joint action of T-2 toxin, DON, nivalenol, zearalenone and fumonisin B₁ was studied in a tiered approach. In the first stage the mycotoxins were tested either jointly or individually. At the highest dose level, the mixture showed a clear less than additive action when compared to the effects of the individual compounds, whereas at lower dose levels the effects were additive. In the second stage, a central composite design was used to detect interactions between specific mycotoxins. Finally, two interactions that were established in stage 2 were further studied in full factorial designs involving two mycotoxins. It was concluded that several classes of mycotoxins when present simultaneously in a mixture might show interaction. The effect of the mixture cannot be predicted solely on the basis of the effect of the individual compounds.

MIURA, K., AMINOVA, L. and MURAYAMA, Y. 2002. **Fusarenon-X induced apoptosis in HL-60 cells depends on caspase activation and cytochrome c release.** *Toxicology* **172**: 103–112.

The mode of apoptosis induced by fusarenon-X using HL-60 cell culture was investigated. When fusarenon-X at a final concentration of 0.5 mg/L was added, cell degradation was observed 5 hr after exposure, and most of the cells had fallen into apoptosis 24 hr after exposure. DNA fragmentation into 180-bp multimers was observed 5 hr after exposure. The percentage of apoptotic cells increased dose- and time-dependently after exposure. The activities of caspase-3, -8 and -9 were elevated within 2 hr after exposure. Cytochrome c release from mitochondria to cytoplasm was observed clearly, and this

release occurred caspase-independently. These findings suggest that fusarenon-X induces apoptosis in HL-60 cells by stimulating cytochrome c release followed by its downstream events including the activation of multiple caspases.

MORRISON, E., KOSIAK, B., RITIENI, A., AASTVEIT, A.H., UHLIG, S. and BERNHOFT, A. 2002. **Mycotoxin production by *Fusarium avenaceum* strains isolated from Norwegian grain and the cytotoxicity of rice culture extracts to porcine kidney epithelial cells.** *Journal of Agricultural and Food Chemistry* **50**: 3070–3075.

The secondary metabolites of 24 isolates of *Fusarium avenaceum* from Norwegian cereals have been characterised. Porcine kidney epithelial cells (PK15) were used to study the cytotoxicity of moniliformin (MON) in the extracts. The following metabolites were produced by all isolates, ranked by concentration in rice cultures: enniatin B, MON, enniatin B₁, and enniatin A. Beauvericin (BEA) was produced by eight isolates. The productions of BEA and enniatin A were significantly correlated, as was the case with enniatin B and enniatin B₁. MON production was correlated neither to any of the other toxins nor to toxicity.

LIU, B.H., YU, F.Y., CHAN, M.H. and YANG, Y.L. 2002. **The effects of mycotoxins, fumonisin B₁ and aflatoxin B₁, on primary swine alveolar macrophages.** *Toxicology and Applied Pharmacology* **180**: 197–204.

The effects of fumonisin B₁ (FB₁) and aflatoxin B₁ (AFB₁) on primary cultures of swine alveolar macrophages (AM) were examined. FB₁ at 5 mg/L for 72 hr led to a reduction in the number of viable cells to 65% of the control levels. In the presence of AFB₁ at 1.5 mg/L the viability of AM fell to less than 41% of controls after 24 hr exposure. FB₁, but not AFB₁, induced the apoptosis of swine AM with evidence of DNA laddering and nuclear fragmentation. However, both FB₁ and AFB₁ exposure induced the expression of apoptosis-related heat shock protein 72 in AM. Treatment with FB₁ at 50 µg/L and AFB₁ at 100 µg/L for 24 hr led to a reduction in phagocytic ability to approximately 55 and 36% of the control levels, respectively. FB₁ but not AFB₁ decreased the mRNA levels of interleukin-1beta and tumor necrosis factor alpha.

WALTER, S.L. 2002. **Acute penitrem A and roquefortine poisoning in a dog.** *Canadian Veterinary Journal – Revue Veterinaire Canadienne* **43**: 372–374.

Mycotoxicoses caused by penitrem A and roquefortine were diagnosed in a Labrador retriever following garbage consumption. Clinical signs of mycotoxicosis included polypnea, tachycardia and ataxia that quickly progressed to lateral recumbency and seizures. Removal of the stomach contents soon after ingestion allowed the dog to recover within 72–96 hr.

PUSCHNER, B. 2002. **Mycotoxins**. Veterinary Clinics of North America – Small Animal Practice **32**: 409.

The main fungi and mycotoxins responsible for small animal mycotoxicoses, their clinical manifestations, clinical pathology alterations, diagnostic approaches and treatment measures are presented. Aflatoxins, penitrem A and roquefortine have all resulted in natural outbreaks of mycotoxicoses.

GARALEVICIENE, D., PETERSSON, H., AUGONYTE, G., ELWINGER, K. and LINDBERG, J.E. 2001. **Effects of mould and toxin contaminated barley on laying hens performance and health**. Archives of Animal Nutrition – Archiv für Tierernährung **55**: 25–42.

Hens were fed three different diets, one supplemented with 30% of toxin-free barley and two with differently moulded barley for 7 weeks. The contaminated diets contained low to moderate concentrations of ochratoxin A, zearalenone, DON and nivalenol. Inclusion of mouldy barley in the diets had an adverse effect on feed intake, feed conversion, digestibility of nutrients, egg production and egg quality. Plasma alkaline phosphatase was increased and some biochemical blood parameters, including bilirubin, uric acid, chlorine, protein, albumin, vitamin A were also higher or changed compared to controls.

DVORSKA, J.E., SURAI, P.F., SPEAKE, B.K. and SPARKS, N.H.C. 2002. **Antioxidant systems of the developing quail embryo are compromised by mycotoxin aurofusarin**. Comparative Biochemistry and Physiology C – Toxicology Pharmacology **131**: 197–205.

Japanese quails (*Coturnix japonica*) were fed on a corn–soya diet supplemented with aurofusarin at 26.4 mg/kg feed in the form of *Fusarium graminearum* culture enriched with aurofusarin, or a control diet. Eggs obtained after seven weeks of feeding were incubated. Samples of quail tissues were collected at day 17 of embryonic development and from day-old hatchlings. Inclusion of aurofusarin in the maternal diet was associated with decreased concentrations of alpha- and gamma-tocopherols, alpha- and gamma-tocotrienols, retinol, lutein and zeaxanthin

in egg yolk. The vitamin E (tocopherols and tocotrienols) concentration in the liver and yolk sac membrane of the day 17 embryos and the hatchlings from aurofusarin-fed group was significantly decreased. Alpha-tocopherol concentration was also reduced in kidney, lung, heart, muscle and brain. In the liver, concentrations of lutein, zeaxanthin, retinol, retinyl linoleate, retinyl oleate, retinyl palmitate and retinyl stearate were also reduced. As a result of these diminished antioxidant concentrations, tissue susceptibility to lipid peroxidation was significantly increased.

Patulin

MARTINS, M.L., GIMENO, A., MARTINS, H.M. and BERNARDO, F. 2002. **Co-occurrence of patulin and citrinin in Portuguese apples with rotten spots**. Food Additives and Contaminants **19**: 568–574.

Seven different varieties of apples (Casanova, Golden Delicious, Red Delicious, Reineta, Richared, Rome Beauty, Starking) with small rotten areas were assayed for patulin and citrinin contamination. A rapid multi-detection TLC method was used. The minimum detectable concentrations of patulin and citrinin were 120–130 and 15–20 µg/kg, respectively. The percentage contamination with patulin only was higher (68.6%) than that with citrinin only (3.9%). Patulin and citrinin (19.6%) were also detected simultaneously. The highest mean patulin content was 80.50 mg/kg for the Richared variety and the lowest mean contaminations were found in Rome Beauty, Red Delicious and Reineta, ranging from 3.06 to 5.37 mg/kg. All analysed apples varieties had low citrinin contamination, ranging from 0.32 to 0.92 mg/kg.

MOODLEY, R.S., GOVINDEN, R. and ODHAV, B. 2002. **The effect of modified atmospheres and packaging on patulin production in apples**. Journal of Food Protection **65**: 867–871.

Granny Smith apples were surface sterilised and inoculated with a spore suspension of *Penicillium expansum*. The apples were packaged either in polyethylene (PE) or polypropylene (PP) and treated with three different gas combinations: 58% CO₂/42% N₂, 48% CO₂/52% N₂ or 88% CO₂/12% N₂ then incubated for 14 days at 25°C. PP did not inhibit fungal growth in any of the atmospheres tested, and it only inhibited patulin production in atmospheric gas and 58% CO₂/42% N₂. PE was very effective and inhibited fungal growth by 4- or 5-fold, depending on the modified atmo-

sphere. Patulin production in PE packaged apples was almost completely inhibited by all three gas combinations. GC analysis of the PE packaged samples before and after incubation showed that CO₂ levels dropped and N₂ levels increased for all of the atmospheres tested.

KADAKAL, C. and NAS, S. 2002. **Effect of apple decay proportion on the patulin, fumaric acid, HMF and other apple juice properties**. Journal of Food Safety **22**: 17–25.

Starking apples, classified by the decay proportion on the fruit surface as sound, 30, 60 or 100% decayed, were used in the production of apple juice. The patulin content of the juice made from sound, 30, 60 and 100% decayed apples ranged between 0–15.9, 47.1–500.3, 156.4–2257.5 and 54.9–2508.6 µg/kg, respectively.

KADAKAL, C. and NAS, S. 2002. **Effect of activated charcoal on patulin, fumaric acid and some other properties of apple juice**. Nahrung – Food **46**: 31–33.

Activated charcoal in amounts between 0.5 and 3.0 g/L was added to apple juice with a patulin content of 62.3 µg/kg obtained from a manufacturing company. Juices were then mixed for 0 to 30 min. The patulin content was reduced from 62.3 to 26.7 µg/kg by treatment with activated charcoal at 3 g/L for 30 min. In general, increasing the amount of activated charcoal decreased the patulin level in the apple juice.

MOSS, M.O. and LONG, M.T. 2002. **Fate of patulin in the presence of the yeast *Saccharomyces cerevisiae***. Food Additives and Contaminants **19**: 387–399.

The fate of [¹⁴C]-labelled patulin during the alcoholic fermentation of apple juice to produce cider was studied. Three commercial cider strains of *Saccharomyces cerevisiae* degraded patulin during active fermentative growth, but not when growing aerobically. The products of patulin degradation were more polar than patulin itself and remained in the clarified fermented cider. HPLC analysis of patulin-spiked fermentations showed the appearance of two major metabolites. Further analyses indicated these metabolites are E-ascladiol and Z-ascladiol.

Alternaria Toxins

HATTA, R., ITO, K., HOSAKI, Y., TANAKA, T., TANAKA, A., YAMAMOTO, M., AKIMITSU, K. and TSUGE, T. 2002. **A conditionally dispensable chromosome controls host-specific**

pathogenicity in the fungal plant pathogen *Alternaria alternata*. Genetics **161**: 59–70.

Alternaria alternata contains seven pathotypes which produce host specific toxins and cause diseases on different plants. In this study, three AKT homologues were isolated on a single cosmid clone from strain NAF8 of the strawberry pathotype. Transformation mediated targeting of two homologues in NAF8 produced toxin-minus, nonpathogenic mutants. All of the mutants lacked the 1.05-Mb chromosome-encoding the AKT genes. This chromosome was not essential for saprophytic growth of this pathogen. Thus, it is proposed that a conditionally dispensable chromosome controls host specific pathogenicity of this pathogen.

UEMURA, I., MIYAGAWA, H. and UENO, T. 2002. **Asymmetric total synthesis of AK-toxins.** Tetrahedron **58**: 2351–2358.

A practical total synthesis of AK-toxins (AK-toxin I, 1; II, 2), host-specific toxins against the Japanese pear, is described.

FABREGA, A., AGUT, M. and CALVO, M.A. 2002. **Optimization of the method of detection of metabolites produced by the *Alternaria* genus: Alternariol, alternariol monomethyl ether, altenuene, altertoxin I and tentoxin.** Journal of Food Science **67**: 802–806.

A simple method has been developed for the detection of alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxin I (ATX-I), tenuazonic acid (TA) and tentoxin by TLC from cultures of several strains of *Alternaria*. Metabolites were extracted with chloroform. The solvent system was benzene-methanol-acetic acid (92:6:2). TLC plates were observed under UV at 254/366 nm. *A. alternata* M 354942 produced AOH, AME, ALT, ATX-I, tentoxin and TA. *A. alternata* M 354943 produced AOH, AME and TA. *A. triticina* IMI 289962 produced tentoxin and *A. triticina* IMI 289680 produced AME, ATX-I and TA.

YEKELER, H., BITMIS, K., OZCELIK, N., DOYMAZ, M.Z. and CALTA, M. 2001. **Analysis of toxic effects of *Alternaria* toxins on esophagus of mice by light and electron microscopy.** Toxicologic Pathology **29**: 492–497.

Mice were fed AME or TA daily for 10 months and at the end of this period, the effects of the toxins on the oesophageal mucosa were determined. By light microscopic examination, moderate and severe dysplasia characterised by loss of polarity,

nuclear pleomorphism and hyperchromasia were observed in TA-treated animals. Electron-microscopic examination of TA-treated mucosal epithelial cells revealed pyknosis in some nuclei, granulation and increase in chromatin mass, irregularities in the nuclear contours, vacuolisation in nucleoplasm and marked pleomorphism in the nuclei.

Ochratoxins – General

GAUCHI, J.P. and LEBLANC, J.C. 2002. **Quantitative assessment of exposure to the mycotoxin ochratoxin A in food.** Risk Analysis **22**: 219–234.

The methodology and simulation results for the quantitative assessment of dietary exposure to OA in France is presented. The possibility of providing reliable calculations of exposure to OA using the conjugate means of a nonparametric-type method of simulation, a parametric-type method of simulation, and the use of bootstrap confidence intervals, is demonstrated. In the context of the Monte Carlo simulation, the nonparametric method takes into account the consumptions and the contaminations in the simulations only via the raw data whereas the parametric method depends on the random samplings from distribution functions fitted to consumption and contamination data.

ALBERT, I. and GAUCHI, J.P. 2002. **Sensitivity analysis for high quantiles of ochratoxin A exposure distribution.** International Journal of Food Microbiology **75**: 143–155.

Using available data from a consumption survey and contamination data on OA in food, a sensitivity analysis (SA) for high quantiles (95th and 99th quantiles) of OA exposure distribution was carried out, obtained by a Monte Carlo simulation in French children. As these exposure distributions are generally very asymmetrical, it is difficult to obtain relevant and stable high quantiles in such a context. After some preliminary trials, a quadratic polynomial regression model for the quantiles of OA exposure distribution in view of undertaking this SA is postulated. This regression model comprises 32 main factors, their 496 two-factor interactions and their 32 quadratic terms. On the basis of these analyses, validated and useful models for each desired quintile are obtained showing a major influence of the parameters of 'Cereals' (consumption and contamination) and slightly less so for parameter of 'Pork' consumption in the sensitivity of the quintiles.

BAYMAN, P., BAKER, J.L., DOSTER, M.A., MICHAELIDES, T.J. and MAHONEY, N.E. 2002. **Ochratoxin production by the *Aspergillus ochraceus* group and *Aspergillus alliaceus*.** Applied and Environmental Microbiology **68**: 2326–2329.

Fungi in the *Aspergillus ochraceus* group (Section *Circumdati*) and *A. alliaceus* were isolated from tree nut orchards, nuts and figs in California. A total of 72 isolates were tested for the production of OA. Among the isolates, *A. ochraceus* and *A. melleus* were the most common species. No field isolates of *A. ochraceus* or *A. melleus* produced OA above the level of detection (0.01 mg/L). All *A. alliaceus* isolates produced OA, up to 30 mg/L. Fifty-thousand figs were examined for fungal infections and OA was measured in figs with visible fungal colonies. Pooled figs infected with *A. alliaceus* contained OA, figs infected with the *A. ochraceus* group had little or none. These results suggest that the little-known species *A. alliaceus* is an important ochratoxin-producing fungus in California.

ROSA, C.A.D., PALACIOS, V., COMBINA, M., FRAGA, M.E., REKSON, A.D., MAGNOLI, C.E. and DALCERO, A.M. 2002. **Potential ochratoxin A producers from wine grapes in Argentina and Brazil.** Food Additives and Contaminants **19**: 408–414.

Fifty grapes samples from Malbec and Chardonnay varieties were collected in Argentina and Brazil during the 1997–98 harvest. The most frequent genera of filamentous fungi isolated were *Aspergillus*, *Penicillium* and *Botrytis*. Of 48 *Aspergillus niger* strains isolated from Argentinean grapes, eight strains produced OA. Sixteen of 53 *A. niger* strains from Brazilian grapes produced OA.

CZERWIECKI, L., CZAJKOWSKA, D. and WITKOWSKA-GWIAZDOWSKA, A. 2002. **On ochratoxin A and fungal flora in Polish cereals from conventional and ecological farms. Part 1: Occurrence of ochratoxin A and fungi in cereals in 1997.** Food Additives and Contaminants **19**: 470–477.

Over 200 samples of Polish cereal grain from the 1997 harvest obtained from conventional and ecological farms were tested for the presence of OA. OA contamination of rye from ecological farms was over six times more frequent than that from conventional cultivation. The OA content in wheat and barley samples from ecological farms was also higher. No wheat sample from conventional farms contained the mycotoxin. The OA levels ranged from 0.2 to 57 µg/kg. Fungi were isolated from sam-

ples containing detectable amounts of OA and those producing OA under laboratory conditions were identified as *Penicillium cyclopium*, *P. viridicatum*, *P. chrysogenum* and also *Aspergillus alliaceus*, *A. versicolor*, *A. glaucus* and *A. flavus*.

JORGENSEN, K. and PETERSEN, A. 2002. **Content of ochratoxin A in paired kidney and meat samples from healthy Danish slaughter pigs.** Food Additives and Contaminants **19**: 562–567.

Paired samples of kidney and meat were taken from 300 healthy Danish pigs and analysed for OA. The concentrations of OA in kidney ranged from 0 to 15 µg/kg (mean 0.50 µg/kg, median 0.18 µg/kg) and in meat from 0 to 2.9 µg/kg (mean 0.12 µg/kg, median 0.03 µg/kg). The data, together with the Danish control data, show that today the pig industry in Denmark has no problem keeping the content of OA in pigs at very low levels even in years with wet harvest conditions.

Ochratoxins – Methodology

SANTOS, E.A. and VARGAS, E.A. 2002. **Immunoaffinity column clean-up and thin layer chromatography for determination of ochratoxin A in green coffee.** Food Additives and Contaminants **19**: 447–458.

An immunoaffinity cleanup-based method for determining OA in green coffee aiming at one-dimensional TLC analysis was established. OA was extracted with a mixture of methanol and aqueous sodium hydrogen carbonate solution, purified through an immunoaffinity column, separated on normal or reversed-phase TLC plates and detected and quantified by visual and densitometric analysis. The linear equation of the standard calibration curve by densitometric analysis gave $R^2 > 0.999$ (0.04–84 ng). The mean recovery of OA from spiked samples by densitometric and visual analyses were 98.4 and 103.8%, respectively. The relative standard deviations for densitometric and visual analysis varied from 1.1 to 24.9% and from 0.0 to 18.8%, respectively. The correlation between HPLC and densitometry, and between visual and densitometric analysis for spiked samples were greater than 0.99. The limit of detection was 0.5 µg/kg for normal TLC.

CHIAVARO, E., LEPIANI, A., COLLA, F., BETTONI, P., PARI, E. and SPOTTI, E. 2002. **Ochratoxin A determination in ham by immunoaffinity clean-up and a quick fluorometric method.** Food Additives and Contaminants **19**: 575–581.

A simple and rapid method for the determination of OA in ham was developed using a basic methanolic extraction, immunoaffinity column cleanup and fluorometric determination. The mean recovery of OA from ham samples spiked at levels from 0.7 to 9.7 µg/kg was $83 \pm 6\%$ using the fluorometric method, with a detection limit of 0.7 µg/kg. Recovery data were compared statistically with those obtained using RP-HPLC with acetonitrile–water–acetic acid (99:99:2) as mobile phase and fluorescence detection, commonly used for OA determination in food. A good correlation between the two analytical techniques was obtained. Both methods were successfully applied to 42 ham samples. Twenty-seven samples showed an OA contamination level of less than 1.0 µg/kg. The maximum contamination level found was 2.3 µg/kg.

Ochratoxicoses

HELLER, M., ROSNER, H., BURKERT, B., MOLLER, U., HINSCHING, A., ROHRMANN, B., THIERBACH, S. and KOHLER, H. 2002. **[In vitro studies into the influence of ochratoxin A on the production of tumor necrosis factor alpha by the human monocytic cell line THP-1].** Deutsche Tierärztliche Wochenschrift **109**: 200–205.

The influence of pure OA and an *Aspergillus ochraceus* crude toxin on the intracellular expression and the secretion of tumor necrosis factor (TNF) alpha by the monocytic cell line THP-1 was studied. After 4 hr exposure, the secretion of TNF alpha was inhibited to 50% by pure OA at 400 µg/L and by crude toxin at 100 µg/L. The intracellular expression of TNF alpha was not disturbed by pure OA at the concentrations tested, whereas crude toxin showed an inhibitory effect. (In German).

STOEV, S.D., PASKALEV, M., MACDONALD, S. and MANTLE, P.G. 2002. **Experimental one year ochratoxin A toxicosis in pigs.** Experimental and Toxicologic Pathology **53**: 481–487.

Mild mycotoxic nephropathy was induced in six pigs by a diet containing OA at 800 µg/kg, several times higher than that naturally encountered in some feed for pig production in Bulgaria. The nephropathy was expressed only as slightly hypertrophied kidneys with a faintly mottled surface, discernible at the end of the experiment to a skilled observer but probably not recognisable in routine slaughterhouse processing. Histological examination showed two types of changes: degenerative changes affecting epithelial

cells in some proximal tubules of pigs after six months, and proliferative changes in the interstitium which predominated after one year of exposure to OA. The renal lesions were similar to those described for classical mycotoxic porcine nephropathy formerly encountered in Denmark, but they were rather different from the porcine nephropathy which occurs spontaneously in Bulgaria.

LEBRUN, S. and FOLLMANN, W. 2002. **Detection of ochratoxin A-induced DNA damage in MDCK cells by alkaline single cell gel electrophoresis (comet assay).** Archives of Toxicology **75**: 734–741.

The induction of DNA damage by OA and the subsequent DNA repair was investigated by alkaline single cell gel electrophoresis (comet assay) in Madin-Darby canine kidney (MDCK) cells. OA induced single-strand breaks in a concentration dependent manner. When an external metabolising enzyme system (S9-mix from rat liver) was added, this genotoxic effect was significantly stronger. By co-incubation with methotrexate or with citrinin, a substrate of the organic anion transporter, the adverse effect of OA was inhibited. A further culture of the damaged cells in the absence of any supplement resulted in a complete repair of the DNA damage within 2 hr.

MULLER, H.M., MULLER, K. and STEINGASS, H. 2001. **Effect of feeding regime on the metabolism of ochratoxin A during the in vitro incubation in buffered rumen fluid from cows.** Archives of Animal Nutrition – Archiv für Tierernährung **54**: 265–279.

Pure OA was added to buffered rumen fluid collected from two fistulated cows and incubated under anaerobic conditions. Both animals were fed six diets containing grass, grass silage or hay, and two different amounts of concentrate consisting of barley and soybean meal. The concentration of OA declined exponentially to a very low or non-detectable level under all conditions examined, with half-lives of 0.51 to 2.76 hr. The disappearance of OA was accompanied by the appearance of ochratoxin alpha (O alpha) with an average amount of O alpha formed relative to the disappearance of OA near 100%, independent of diet and animal. The disappearance of OA mostly was accelerated by replacing grass silage or hay with fresh grass and by increasing the content of concentrate from 10 to 50% of dry matter.

HONG, J.T., LEE, M.K., PARK, K.S., JUNG, K.M., LEE, R.D., JUNG, H.K., PARK, K.L., YANG, K.H. and CHUNG, S.Y. 2002. **Inhibitory effect of peroxisome proliferator-activated receptor**

gamma agonist ochratoxin A-induced cytotoxicity and activation of transcription factors in cultured rat embryonic midbrain cells. *Journal of Toxicology and Environmental Health – Part A* **65**: 407–418.

The effects of 15-deoxy-Delta(12,14)-prostaglandin I-2 (15-deoxyPGI(2)) on OA induced neurotoxicity and on the activation of transcription factors activator protein-1 (AP-1) and nuclear factor-kappa B (NF-kappaB) were investigated in cultured rat embryonic midbrain cells. OA caused concentration dependent reductions in neurite outgrowth and cell number, and induced AP-1 and NF-kappaB activation. Cotreatment with 15-deoxyPGI(2) blocked OA-induced decrease in neurite outgrowth and cell number and inhibited AP-1 and NF-kappaB activation.

STOEV, S.D., KOYNARSKY, V. and MANTLE, P.G. 2002. **Clinicopathological studies in chicks fed ochratoxin A while simultaneously developing coccidiosis.** *Veterinary Research Communications* **26**: 189–204.

The progression of coccidiosis and the resultant mortality were followed in chicks fed a diet containing OA and infected with *Eimeria tenella*. The intensity of the clinical signs, the impairment of kidney function, macroscopic and histopathological changes, deviations in the weight of some organs and general depression in growth were greater when chicks infected with *E. tenella* were also given OA than in chicks given a toxin free diet.

BIRO, K., SOLTI, L., BARNA-VETRO, I., BAGO, G., GLAVITS, R., SZABO, E. and FINK-GREMMELS, J. 2002. **Tissue distribution of ochratoxin A as determined by HPLC and ELISA and histopathological effects in chickens.** *Avian Pathology* **31**: 141–148.

Broiler chicks were exposed to a total of 0.5 mg OA per week for each of 4 weeks. There was an accumulation of OA in plasma and a wide distribution into all organs, with high levels in the liver and the kidney. Microscopical changes that could primarily be associated with toxin exposure were glomerulonephrosis, tubulonephrosis, focal tubular epithelial cell proliferation and multiple, adenoma-like structures in the renal parenchyma.

Fumonisin – General

RHEEDER, J.P., MARASAS, W.F.O. and VISMER, H.F. 2002. **Production of fumonisin analogs by *Fusarium* species.** *Applied and Environmental Microbiology* **68**: 2101–2105.

This review with 72 references focuses on the *Fusarium* species that produce fumonisins and the fumonisin analogues produced by each of these species. The 28 fumonisin analogues that have been characterised can be separated into four main groups, identified as the fumonisin A, B, C and P series. Fumonisin B analogues are the most abundant naturally occurring fumonisins. Some of the other analogues may occur in naturally contaminated maize at relatively low levels (less than 5% of total fumonisins present). These lesser known fumonisins are not detected with most analytical techniques due to the derivatisation process, but can be detected with the use of LC–MS with electrospray ionisation.

SROBAROVA, A., MORETTI, A., FERACANE, R., RITIENI, A. and LOGRIECO, A. 2002. **Toxicogenic *Fusarium* species of *Liseola* section in pre-harvest maize ear rot, and associated mycotoxins in Slovakia.** *European Journal of Plant Pathology* **108**: 299–306.

The occurrence of *Fusarium* species of *Liseola* section and related toxins was investigated for two years (1996 and 1998) on maize ear rot samples collected in the most important areas for maize growing in Slovakia. The species most frequently isolated was *F. verticillioides*, followed by *F. proliferatum* in 1996 and *F. subglutinans* in 1998. *F. graminearum* was also frequently recovered in both years. Toxin analysis of maize ears showed that 21/22 samples were contaminated with at least one toxin. All 29 strains of *F. verticillioides*, 2/3 strains of *F. proliferatum* and 0/8 strains of *F. subglutinans* isolated from samples produced FB₁ in culture. Two strains of *F. subglutinans*, two of *F. proliferatum* and 10 of *F. verticillioides* produced beauvericin. Fusaproliferin was produced by two strains of *F. proliferatum*, seven of *F. subglutinans* and three of *F. verticillioides*. This is the first report on fusaproliferin production by *F. verticillioides*.

VELLUTI, A., MARIN, S., SANCHIS, V. and RAMOS, A.J. 2001. **Note. Occurrence of fumonisin B₁ in Spanish corn-based foods for animal and human consumption.** *Food Science and Technology International* **7**: 433–437.

Corn based food samples from Spain destined for human (228 samples) and animal (58 samples) consumption were assayed for FB₁. FB₁ was detected in 86% of samples for animal consumption in amounts ranging from 89 to 8757 µg/kg, and in 23% of samples for human consumption in amounts ranging from 16 to 938 µg/kg. The most contaminated sam-

ples were the wholemeal corn flours, with a range of contamination that varied from 299–938 µg/kg.

ETCHEVERRY, M., TORRES, A., RAMIREZ, M.L., CHULZE, S. and MAGAN, N. 2002. **In vitro control of growth and fumonisin production by *Fusarium verticillioides* and *F. proliferatum* using antioxidants under different water availability and temperature regimes.** *Journal of Applied Microbiology* **92**: 624–632.

The effect of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), trihydroxybutyphenone and propylparaben (PP) at concentrations of 1–20 mmol/L on growth of and fumonisin production by Argentinian strains of *Fusarium verticillioides* and *F. proliferatum* were examined. The lag phase prior to growth and growth rates were significantly decreased by PP and BHA at 10 and 20 mmol/L, regardless of the temperature or water activity (a_w) level tested. Total fumonisin production was higher at 0.98 a_w and decreased by about 45–50% at 0.995 and 0.95 a_w. Overall, BHT only inhibited fumonisin production at 0.95 a_w at 10 and 20 mmol/L, while BHA was effective at most a_w levels tested at 10 and 20 mmol/L. Propylparaben completely inhibited fumonisin production by both *F. verticillioides* and *F. proliferatum* at more than 1 mmol/L, regardless of the temperature or a_w level.

TRIGO-STOCKLI, D.M., CORTEZ-ROCHA, M.O., SANCHEZ-MARINEZ, R.I. and REED, C.R. 2002. **Fumonisin B₁ levels in 1995 and 1996 Kansas corn.** *Bulletin of Environmental Contamination and Toxicology* **68**: 720–724.

Fumonisin producing *Fusarium* species were common in Kansas corn with *F. moniliforme* the most common species isolated. However, no significant correlation ($r = 0.215$, $p = 0.02$) occurred between FB₁ contamination and level of invasion by *F. moniliforme*. Presently, no tolerance limits exist for fumonisins. Because of effects on animals, the American Association of Veterinary Laboratory Diagnosticians has issued guidance levels for FB₁ in livestock feed. The limits are 5 mg/kg for horses, 10 mg/kg for swine and 50 mg/kg for poultry and beef cattle. Results indicate that approximately 65% of Kansas corn samples in 1995 and 1996 exceeded the limit for swine.

Fumonisin – Methodology

BIRD, C.B., MALONE, B., RICE, L.G., ROSS, P.F., EPPLEY, R. and ABOUZIED, M.M. 2002. **Determination of total fumonisins in corn by competitive direct enzyme-linked immunosorbent assay: Collaborative study.** Journal of AOAC International **85**: 404–410.

A collaborative study among 13 laboratories in the USA was conducted to evaluate the effectiveness of a competitive direct (cd)-ELISA for the determination of total fumonisins in corn. The test portion was extracted with methanol–water (7 + 3), filtered, diluted and tested on the cd-ELISA. Average recoveries of FB₁, FB₂ and FB₃ were 120, 100 and 90%, respectively. The relative standard deviations for repeatability ranged from 13.3 to 23.3% and the relative standard deviations for reproducibility ranged from 15.8 to 30.3% across all levels tested. HORRAT values, calculated for each individual sample, ranged from 1.24 to 1.94.

DOWELL, F.E., PEARSON, T.C., MAGHIRANG, E.B., XIE, F. and WICKLOW, D.T. 2002. **Reflectance and transmittance spectroscopy applied to detecting fumonisin in single corn kernels infected with *Fusarium verticillioides*.** Cereal Chemistry **79**: 222–226.

Reflectance and transmittance visible and near-infrared spectroscopy were used to detect fumonisin in single corn kernels infected with *Fusarium verticillioides*. Kernels with fumonisin levels greater than 100 mg/kg and less than 10 mg/kg could be classed accurately as fumonisin positive or negative, respectively. Classification results were generally better for oriented kernels than for kernels that were randomly placed in the spectrometer viewing area. Generally, models based on reflectance spectra have higher correct classification than models based on transmittance spectra. Results show that this technology can be used to rapidly and non-destructively screen single corn kernels for the presence of fumonisin and may be adaptable to on-line detection and sorting.

SEEFELDER, W., GOSSMANN, M. and HUMPF, H.U. 2002. **Analysis of fumonisin B₁ in *Fusarium proliferatum*-infected asparagus spears and garlic bulbs from Germany by liquid chromatography electrospray ionization mass spectrometry.** Journal of Agricultural and Food Chemistry **50**: 2778–2781.

The occurrence of FB₁ in asparagus spears infected with *Fusarium proliferatum* was investigated using an

LC–ESI–MS method. FB₁ was detected in 9/10 samples in amounts ranging from 36.4 to 4513.7 µg/kg dry weight. In further experiments, garlic was cultured in soil contaminated with *F. proliferatum* and the bulbs were screened for infection with *F. proliferatum* and for the occurrence of fumonisins by LC–MS. *F. proliferatum* was detectable in the garlic tissue and all samples contained FB₁ (26.0–94.6 µg/kg).

KIM, E.K., SCOTT, P.M., LAU, B.P.Y. and LEWIS, D.A. 2002. **Extraction of fumonisins B₁ and B₂ from white rice flour and their stability in white rice flour, cornstarch, cornmeal, and glucose.** Journal of Agricultural and Food Chemistry **50**: 3614–3620.

For the extraction of FB₁ and FB₂ from Thai white rice flour, different solvent mixtures, temperatures, pH values and addition of enzymes or Na₂EDTA were examined. Three extractions with 0.1M Na₂EDTA achieved the highest recoveries. Initial recoveries of fumonisins added to white rice flour, cornstarch, cornmeal and glucose varied with commodity. Fumonins disappeared in Thai white rice flour after 12 hr, but 55% remained in another white rice flour. With cornstarch 20–30% fumonisins remained after 24 hr. Fumonins were stable in cornmeal for 24 hr but only similar to 50% remained after 30 days. With glucose, 25% of FB₁ and FB₂ remained 24 hr after addition.

KIM, E.K., SHON, D.H., CHUNG, S.H. and KIM, Y.B. 2002. **Survey for fumonisin B₁ in Korean corn-based food products.** Food Additives and Contaminants **19**: 459–464.

Seventy-six corn based foods collected in Seoul, Korea, were analysed for the occurrence of FB₁ by cd-ELISA and HPLC. The average recoveries of FB₁ from corn flakes samples spiked in the range 5–1000 µg/kg were 104 and 82% by cd-ELISA and HPLC, respectively, and the limits of detection were approximately 5 and 20 µg/kg, respectively. By cd-ELISA, the incidences and mean levels of FB₁ from corn flakes, corn snack, corn starch, corn for popping, roasted corn for tea and other corn products were 73.3, 78.6, 50, 58.3, 17.6 and 40% and 41.8, 67.9, 114, 256, 172 and 22 µg/kg, respectively. No FB₁ was found in canned sweet corn. The results obtained by cd-ELISA were correlated to those by HPLC ($r(2) = 0.992$).

BROGGI, L.E., RESNIK, S.L., PACIN, A.M., GONZALEZ, H.H.L., CANO, G. and TAGLIERI, D. 2002. **Distribution of fumonisins in dry-milled corn fractions in Argentina.** Food Additives and Contaminants **19**: 465–469.

Corn samples and different dry-milled fractions collected from an industrial mill in Argentina were analysed for fumonisins. Average contaminations for FB₁, FB₂ and FB₃ in whole corn were 1540, 716 and 152 µg/kg; in corn grits 135, 39.1 and 10.2 µg/kg; in 'C' flour 358, 122 and 45.9 µg/kg; in corn meal 148, 52.5 and 28.3 µg/kg; and in germ and bran together 4210, 2010 and 447 µg/kg, respectively. Taking into account the distribution of fumonisins in commercial dry-milled corn fractions and corn meal consumption in Argentina, a theoretical whole corn level of 6640 µg/kg maximum of total fumonisins could be processed to obtain products considered safe for human health.

Fumonisin – Toxicoses

EHRlich, V., DARROUDI, F., UHL, M., STEINKELLNER, H., ZSIVKOVITS, M. and KNASMUELLER, S. 2002. **Fumonisin B₁ is genotoxic in human derived hepatoma (HepG2) cells.** Mutagenesis **17**: 257–260.

FB₁ was tested in micronucleus (MN) and single cell gel electrophoresis (SCGE) assays in human derived hepatoma (HepG2) cells and caused a pronounced dose dependent genotoxic effect at exposure concentrations greater than or equal to 25 mg/L. In contrast, no induction of his(+) revertants was found in *Salmonella* microsome assays with strains TA98, TA100, TA102, TA1535 and TA1537 upon addition of HepG2-derived enzyme (S9) mix in liquid incubation assays with identical exposure concentrations. Taken together, our results indicate that FB₁ is clastogenic in human derived cells.

HE, Q.R., RILEY, R.T. and SHARMA, R.P. 2002. **Pharmacological antagonism of fumonisin B₁ cytotoxicity in porcine renal epithelial cells (LLC-PK1): A model for reducing fumonisin-induced nephrotoxicity in vivo.** Pharmacology & Toxicology **90**: 268–277.

Pharmacological approaches for intervening in FB₁ toxicity were investigated using the pig kidney epithelial cell (LLC-PK1) model. The toxicity of FB₁ was assayed using cell viability and lactate dehydrogenase release. Pretreatment of cells with myriocin, preventing sphinganine accumulation, prevented the FB₁ induced decrease in cell viability and increased lactate dehydrogenase release. Likewise, silymarin pretreatment prevented the FB₁-induced effects on cell viability and lactate dehydrogenase release. Myriocin, but not silymarin, blocked the accumulation of sphinganine in FB₁ treated cells. Silymarin, unlike myriocin,

induced expression of TNF alpha to an extent similar to FB₁, but pretreatment with silymarin decreased the FB₁-induced TNF alpha. Results suggest that silymarin potentially prevents FB₁-induced toxicity by modulating TNF alpha expression or signals downstream of the inhibition of ceramide synthase.

SMITH, G.W., CONSTABLE, P.D., FOREMAN, J.H., EPPLEY, R.M., WAGGONER, A.L., TUMBLESON, M.E. and HASCHEK, W.M. 2002. **Cardiovascular changes associated with intravenous administration of fumonisin B₁ in horses.** American Journal of Veterinary Research **63**: 538–545.

Eleven healthy horses were given daily iv injections of FB₁ at 0, 0.01 or 0.20 mg/kg for 7 to 28 days. When neurologic signs consistent with leukoencephalomalacia were evident, horses were anaesthetised and catheters were inserted for evaluation of the cardiovascular system. Fumonisin treated horses with clinical signs of neurologic disease had evidence of cardiovascular dysfunction manifested as decreases in heart rate, cardiac output, right ventricular contractility, coccygeal artery pulse pressure, and pH and base excess in venous blood as well as increases in systemic vascular resistance, compared with values for control horses. Fumonisin treated horses with and without clinical signs of neurologic disease also had higher serum and right ventricular sphinganine and sphingosine concentrations than control horses.

BANCZEROWSKI-PELYHE, I., VILAGI, I., DETARI, L., DOCZI, J., KOVACS, F. and KUKORELLI, T. 2002. **In vivo and in vitro electrophysiological monitoring of rat neocortical activity after dietary fumonisin exposure.** Mycopathologia **153**: 149–156.

Rats were fed corn pellets containing FB₁ at 30 mg/kg bw/day. Spontaneous and evoked potentials were measured in the neocortex both *in vivo* and *in vitro* after five days of treatment. Auditory evoked potentials recorded *in vivo* on freely moving animals revealed a highly significant 20–60% decrease in the primary and mid-latency components. Cortex slices *in vitro* showed a reduced excitability both in standard artificial cerebrospinal fluid solution and in a 4-aminopyridine induced epilepsy model. Spontaneous epileptic discharges after FB₁ exposure had an increased latency, decreased frequency, longer duration and modified signal forms. Altered excitability and seizure susceptibility of the neocortex after fumonisin exposure are suspected to be associated with modified signal transmission.

GALVANO, F., CAMPISI, A., RUSSO, A., GALVANO, G., PALUMBO, M., RENIS, M., BARCELLONA, M.L., PEREZ-POLO, J.R. and VANELLA, A. 2002. **DNA damage in astrocytes exposed to fumonisin B₁.** Neurochemical Research **27**: 345–351.

FB₁ is an inhibitor of ceramide synthase, a key enzyme involved in *de novo* sphingolipid biosynthesis and in the reacylation of free sphingoid bases derived from sphingolipid turnover. However, little is known on the down stream effectors activated by these sphingolipids in the cell death signalling pathway. Rat astrocytes were exposed to FB₁ with the aim of evaluating the involvement of oxygen free radicals and of some other biochemical pathways such as caspase-3 activity and DNA damage. Results indicate that FB₁ treatment does not affect cell viability. Conversely, FB₁ induced DNA damage and an enhancement of caspase-3 activity. In addition, FB₁ increased the expression of heat shock protein 70. These results indicate that the genotoxic potential of FB₁ has probably been underestimated.

HARD, G.C., HOWARD, P.C., KOVATCH, R.M. and BUCCI, T.J. 2001. **Rat kidney pathology induced by chronic exposure to fumonisin B₁ includes rare variants of renal tubule tumor.** Toxicologic Pathology **29**: 379–386.

In a 2-year feeding study, Fischer F344 rats were fed FB₁ at 50–150 mg/kg in the diet. Kidneys were examined to characterise the various histopathological changes associated with renal tumour induction. In all high-dose (150 mg/kg) and mid-dose (50 mg/kg) male rats, and to a lesser extent in high-dose (100 mg/kg) female rats, there was evidence of sustained nephrotoxicity manifested as basophilia, apoptosis, cell regeneration and simple tubule hyperplasia, affecting proximal convoluted tubules in the deep cortex, extending into the outer region of the outer stripe of outer medulla. The continued presence of nephrotoxicity throughout chronic exposure to FB₁ suggested that renal tumour development may have been an outcome of sustained cell loss and compensatory regeneration. In some cases, preneoplastic tubules or incipient renal tumours presented an immature or foetal form in association with interstitial hyalinisation. The renal tubule tumours induced by FB₁ were typified by a rare, highly malignant, anaplastic variant capable of growth by infiltration.

BHANDARI, N., ENONGENE, E.N., RILEY, R.T., MEREDITH, F.I. and SHARMA, R.P. 2002. **Temporal expression of fumonisin B₁-induced tumor necrosis factor-alpha and interferon gamma in mice.** Comparative Biochemistry and Physiology C – Toxicology & Pharmacology **131**: 113–122.

The time course of TNF alpha production and signalling was investigated in mice treated subcutaneously (sc) or per os (po) with FB₁ at 25 mg/kg and sacrificed at 0–24 hr after treatment. The TNF alpha expression was increased in liver and kidney after both routes of FB₁ exposure without any alterations in spleen. The po treatment caused greater hepatotoxicity compared to the sc route, as depicted by increased alanine aminotransferase and aspartate aminotransferase level in plasma, observed only after po FB₁ treatment. The increase in enzymes at 8 hr after po treatment correlated with the highest TNF alpha expression, also noted at 8 hr after po treatment, thus further confirming the involvement of TNF alpha in FB₁ toxicity. The interferon (IFN) gamma expression was increased in liver at 4 hr after po treatment, suggesting a possible combined role of TNF alpha and IFN gamma in their induction and hepatotoxicity.

BHANDARI, N. and SHARMA, R.P. 2002. **Fumonisin B₁-induced alterations in cytokine expression and apoptosis signaling genes in mouse liver and kidney after an acute exposure.** Toxicology **172**: 81–92.

The time related induction of cytokines and genes involved in apoptosis signalling was investigated in male BALB/c mice administered orally with FB₁ at 25 mg/kg and sampled 4 or 8 hr after treatment. Expression of various genes was analysed by ribonuclease protection assay. FB₁ treatment caused increased expression of TNF alpha and interleukin (IL)-1beta in both liver and kidney, whereas IL-1alpha and IL-1 receptor antagonist expression was induced only in the liver. Expression of TNF alpha, signalling molecules, TNF receptor 55 and receptor interacting protein, was increased in liver and kidney. Caspase 8 expression was increased only in liver. FB₁ treatment induced expression of Fas in liver and kidney with no alterations in Fas signalling molecules, Fas ligand, Fas-associated death domain and Fas-associated protein factor. FB₁ increased the expression of B-Myc, c-Myc and Max, oncogenic transcription factors in the kidney. FB₁ toxicity caused induction of cytokine network in liver with involvement of TNF alpha signalling pathway.

BHANDARI, N. and SHARMA, R.P. 2002. **Modulation of selected cell signaling genes in mouse liver by fumonisin B₁**. *Chemico-Biological Interactions* **139**: 317–331.

BALB/c mice were exposed to a sub-chronic dose of FB₁ and gene alterations were observed. Male and female mice were injected sc with FB₁ at 2.25 mg/kg/day for 5 days. FB₁ caused increased expression of TNF alpha, IL-1alpha, IL-1beta, IL-1 receptor antagonist (IL-1Ra), IL-6, IL-10, IL-12, p40, IL-18 and IFN gamma in male liver, with a similar increase in females except for IL-1beta and IL-18. Control females showed higher basal levels of IL-1alpha, IL-1Ra, IL-10, IL-12, p40 and IFN gamma compared with males. Expression of TNF receptor 55 and TNF receptor associated death domain was increased, with no changes in Fas signalling molecules, Fcγ, Fas ligand, Fas associated death domain and Fas-associated protein factor. Expression of oncogenic transcription factors, c-Myc, B-Myc, Max and Mad, and apoptotic genes, namely Bcl-22, Bax and Bad, was increased after FB₁ treatment. FB₁ caused an activation of cytokine network in liver, particularly the TNF alpha signalling pathway.

ENONGENE, E.N., SHARMA, R.P., BHANDARI, N., MILLER, J.D., MEREDITH, F.I., VOSS, K.A. and RILEY, R.T. 2002. **Persistence and reversibility of the elevation in free sphingoid bases induced by fumonisin inhibition of ceramide synthase**. *Toxicological Sciences* **67**: 173–181.

The time course for sphingoid base elevation in the small intestines, liver and kidney of mice following oral dosing with FB₁ was studied. After a single dose of FB₁ at 25 mg/kg bw, there was an increase in sphinganine in intestinal cells and liver that peaked at 4 to 12 hr and declined to near the control level by 48 hr. In kidney, sphinganine peaked at 6–12 hr but remained elevated until 72 hr, approaching control levels at 96–120 hr. Oral administration of FB₁ at 0.03 mg/kg bw (low dose) for 5 days had no effect on the sphingoid bases in kidney. However, following an initial high dose, daily administration of the low dose prolonged the elevation in kidney sphinganine compared to mice receiving a single high dose. In cultured renal cells, FB₁ was rapidly eliminated, but elevated sphinganine was persistent. This persistence in renal cells was rapidly reversed in the presence of the serine palmitoyltransferase inhibitor, indicating that the persistence was due to differences in the rates of sphinganine biosynthesis and degradation.

FERRANTE, M.C., MELI, R., RASO, G.M., ESPOSITO, E., SEVERINO, L., DICARLO, G. and LUCISANO, A. 2002. **Effect of fumonisin B₁ on structure and function of macrophage plasma membrane**. *Toxicology Letters* **129**: 181–187.

The modifications of fluidity, endocytosis and peroxidative damage of plasma membrane induced by FB₁ in macrophage cell line J774A were investigated. FB₁ enhanced membrane fluidity and increased, time-dependently, the horseradish peroxidase endocytosis. This effect was concentration dependent and reverted by IFN gamma. Moreover, FB₁ induced a membrane peroxidative damage as evidenced by the increase of malondialdehyde production.

RUMORA, L., KOVACIC, S., ROZGAJ, R., CEPELAK, I., PEPELJNJAK, S. and GRUBISIC, T.Z. 2002. **Cytotoxic and genotoxic effects of fumonisin B₁ on rabbit kidney RK13 cell line**. *Archives of Toxicology* **76**: 55–61.

Exposure of rabbit kidney RK13 cells to FB₁ caused a significant increase in micronucleus frequency in a concentration and in a time dependent manner. Nanomolar concentrations of FB₁ decreased cell viability after 24 hr and even more so after 48 hr of exposure. The morphological changes observed suggested that an increased number of RK13 cells were dying by the process of apoptosis. However, FB₁ also induced impairments of cell and mitochondrial membrane integrity, as assessed by lactate dehydrogenase and glutamate dehydrogenase leakage.

Trichothecenes – General

PERKOWSKI, J. and BASINSKI, T. 2002. **Natural contamination of oat with group A trichothecene mycotoxins in Poland**. *Food Additives and Contaminants* **19**: 478–482.

Naturally contaminated oat grain samples were collected in 12 plant breeding stations in different parts of Poland. T-2 toxin, HT-2 toxin and diacetoxyscirpenol (DAS) levels were determined by GC–MS. HT-2 toxin was the major toxin with an incidence of 24% and its average level in positive samples was 21 µg/kg. The incidence of T-2 toxin and DAS was 15 and 12%, and their average levels were 60 and 23 µg/kg, respectively. Sixty-five of 99 samples were free of detectable amounts of the toxic metabolites analysed.

GILBERT, J., ABRAMSON, D., MCCALLUM, B. and CLEAR, R. 2002. **Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness,**

vegetative compatibility, and production of ergosterol and mycotoxins. *Mycopathologia* **153**: 209–215.

Fifteen Canadian isolates of *Fusarium graminearum* were highly diverse in terms of vegetative compatibility grouping and varied for production of ergosterol and mycotoxin production in rice culture. Disease severity, ergosterol production and mycotoxin development were not correlated. DON was produced by all isolates in rice culture, at levels between 0.2 and 249 mg/kg. 15-acetylDON was produced by 14/15 isolates at levels between 0.4 and 44.6 mg/kg.

MUTHOMI, J.W., OERKE, E.C., DEHNE, H.W. and MUTITU, E.W. 2002. **Susceptibility of Kenyan wheat varieties to head blight, fungal invasion and deoxynivalenol accumulation inoculated with *Fusarium graminearum***. *Journal of Phytopathology – Phytopathologische Zeitschrift* **150**: 30–36.

Fifteen wheat varieties commercially grown in Kenya were tested for their susceptibility to *Fusarium* head blight (FHB) and mycotoxin accumulation after inoculation with *Fusarium graminearum* in pot experiments. All varieties were found to be moderately to highly susceptible to FHB. However, the varieties differed in FHB susceptibility, fungal colonisation and the resulting DON contamination. The kernels of highly susceptible varieties had higher mycotoxin and ergosterol contents. However, the kernels of some varieties contained more fungal mycelium (ergosterol) without the corresponding high amounts of DON, suggesting that they possess some resistance to DON accumulation.

ZOLLNER, P., JODLBAUER, J., KLEINOVA, M., KAHLBACHER, H., KUHN, T., HOCHSTEINER, W. and LINDNER, W. 2002. **Concentration levels of zearalenone and its metabolites in urine, muscle tissue, and liver samples of pigs fed with mycotoxin-contaminated oats**. *Journal of Agricultural and Food Chemistry* **50**: 2494–2501.

The content of zearalenone (ZEA) and its metabolites in urine and tissue samples from pigs fed ZEA contaminated oats was determined. Analysis of the urine samples revealed that approximately 60% of ZEA was transformed *in vivo* to alpha-zearalenol and its epimer beta-zearalenol in a mean ratio of 3:1. Zearanol and taleranol as further metabolites could only be detected in trace amounts. In contrast, liver samples contained predominantly alpha-zearalenol, and to a minor extent beta-zearalenol and ZEA, with a mean ratio of alpha- to beta-zearalenol of 2.5:1, while zearanol, tal-

eranol and ZEA were not detected. The degree of glucuronidation was established for ZEA as 27% in urine and 62% in liver; for alpha-zearalenol as 88% in urine and 77% in liver, and for beta-zearalenol as 94% in urine and 29% in liver. Analyses of muscle tissue revealed relatively high amounts of nonglucuronidated zearanol and alpha-zearalenol together with traces of taleranol and ZEA.

HOOKE, D.C., SCHAAFSMA, A.W. and TAMBURIC-ILINCIC, L. 2002. **Using weather variables pre- and post-heading to predict deoxynivalenol content in winter wheat.** *Plant Disease* **86**: 611–619.

The concentration of DON in wheat from 399 farm fields in southern Ontario, Canada, was measured. Weather variables, such as daily rainfall, daily minimum and maximum air temperatures, and hourly relative humidity, were estimated for each field from nearby weather stations and were normalised to the date of 50% head emergence. DON was responsive to weather in three critical periods around heading. In the first period, 4 to 7 days before heading, DON generally increased with the number of days with greater than 5 mm of rain and decreased with the number of days of less than 10°C. In the second period, 3 to 6 days after heading, DON increased with the number of days of rain greater than 3 mm and decreased with days exceeding 32°C. In the third period, 7 to 10 days after heading, DON increased with number of days with more than 3 mm of rain. Overall, 73% of the variation in the concentration of DON was explained by using weather from all three critical periods.

BAI, G.H., DESJARDINS, A.E. and PLATTNER, R.D. 2002. **Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes.** *Mycopathologia* **153**: 91–98.

In previous studies, the trichodiene synthase gene (*Tri5*) in *Fusarium graminearum* strain GZ3639 was disrupted to produce the DON-nonproducing strain GZT40. In this report, the virulence of strains GZ3639 and GZT40 was tested on wheat cultivars with various resistance levels to FHB. Under field and greenhouse conditions, strain GZ3639 produced significantly more disease symptoms and reduced more yield than strain GZT40 in all wheat cultivars tested. Conidia of strain GZT40 germinated and infected inoculated spikelets, but disease symptoms were limited to inoculated spikelets without spread to uninoculated spikelets. The results confirm that DON production plays a significant

role in the spread of FHB within a spike, and are the first report that DON production is not necessary for initial infection by the fungus.

FUCHS, E., BINDER, E.M., HEIDLER, D. and KRŠKA, R. 2002. **Structural characterization of metabolites after the microbial degradation of type A trichothecenes by the bacterial strain BBSH 797.** *Food Additives and Contaminants* **19**: 379–386.

The bacterial strain BBSH 797 can transform DON into its non-toxic deepoxide metabolite, DOM-1. Analogous to the microbial degradation of DON, the transformation of six different type A trichothecenes was observed. The metabolites appearing were characterised by GC–MS after derivatisation with TRISIL(R)TBT. The major finding was that scirpentriol was completely transformed into its non-toxic metabolite deepoxy scirpentriol, while T-2 triol underwent a more complicated metabolism being degraded to its non-toxic deepoxy form and into T-2 tetraol which was then further metabolised to deepoxy T-2 tetraol.

CHU, Y.M., JEON, J.J., YEA, S.J., KIM, Y.H., YUN, S.H., LEE, Y.W. and KIM, K.H. 2002. **Double-stranded RNA mycovirus from *Fusarium graminearum*.** *Applied and Environmental Microbiology* **68**: 2529–2534.

Double stranded RNAs (dsRNA) were isolated from 13 of 256 field strains of *Fusarium graminearum* isolated from maize in Korea. One of these strains, DK21, had pronounced morphological changes, including reduction in mycelial growth, increased pigmentation, reduced virulence towards wheat and decreased (60-fold) production of trichothecene mycotoxins. The presence or absence of the 7.5-kb dsRNA was correlated with the changes in pathogenicity and morphology. The dsRNA could be transferred to virus-free strains by hyphal fusion, and the recipient strain acquired the virus-associated phenotype of the donor strain. The dsRNA was transmitted to approximately 50% of the conidia, and only colonies resulting from conidia carrying the mycovirus had the virus-associated phenotype.

LEE, T., HAN, Y.K., KIM, K.H., YUN, S.H. and LEE, Y.W. 2002. ***Tri13* and *Tri7* determine deoxynivalenol- and nivalelol-producing chemotypes of *Gibberella zeae*.** *Applied and Environmental Microbiology* **68**: 2148–2154.

A *Tri13* homologue from each chemotype of *Gibberella zeae* was cloned and sequenced. The *Tri13* from a nivalelol

(NIV) chemotype strain (88-1) is located in the trichothecene gene cluster and carries an open reading frame similar to that of *Fusarium sporotrichioides*, whereas the *Tri13* from a DON chemotype strain (H-11) carries several mutations. To confirm the roles of the *Tri13* and *TK7* genes in trichothecene production by *G. zeae*, toxin production in 88-1 and H-11 was genetically altered. In transgenic strains, the targeted deletion of *Tri13* from the genome of 88-1 caused production of DON rather than NIV. Heterologous expression of the 88-1 *Tri13* gene alone or in combination with the 88-1 *Tri7* gene conferred on H-11 the ability to synthesise NIV; in the latter case, 4-acetylNIV also was produced. These results suggest that *Tri13* and *Tri7* are required for oxygenation and acetylation of the oxygen at C-4 during synthesis of NIV and 4-acetylNIV in *G. zeae*.

JURGENSON, J.E., BOWDEN, R.L., ZELLER, K.A., LESLIE, J.F., ALEXANDER, N.J. and PLATTNER, R.D. 2002. **A genetic map of *Gibberella zeae* (*Fusarium graminearum*).** *Genetics* **160**: 1451–1460.

A genetic linkage map of *Gibberella zeae* was constructed by crossing complementary nitrate-nonutilising mutants of *G. zeae* strains R-5470 and Z-3639. The total map length is similar to 1300 cM with an average interval of 2.8 map units between loci. Loci governing trichothecene toxin amount and type (DON or NIV) map on linkage groups IV and I, respectively. The locus governing the type of trichothecene produced (NIV or DON) co-segregated with the *Tri5* gene (which encodes trichodiene synthase) and probably maps in the trichothecene gene cluster.

ALVI, K.A., RABENSTEIN, J., WOODARD, J., BAKER, D.D., BERGTHOLD, J.D., LYNCH, J., LIEU, K.L. and BRAUDE, I.A. 2002. **14'-hydroxymyotoxin B and 16-hydroxyroridin E, two new cytotoxic trichothecenes from *Myrothecium roridum*.** *Journal of Natural Products* **65**: 742–744.

Two new trichothecenes, 14'-hydroxymyotoxin B and 16-hydroxyroridin E, were isolated from a fermentation extract of *Myrothecium roridum*. Both compounds showed potent cytotoxic activity against primary soft tissue sarcoma cells.

Trichothecenes – Methodology

MARAGOS, C.M., JOLLEY, M.E. and NASIR, M.S. 2002. **Fluorescence polarization as a tool for the determination of deoxynivalenol in wheat.** *Food Additives and Contaminants* **19**: 400–407.

A fluorescence polarisation (FP) immunoassay using a previously described monoclonal antibody for DON was developed. The assay was based on the competition of unlabelled DON from a sample with a fluorescently tagged DON, DON-fluorescein (DON-FL), for a DON-specific monoclonal antibody in solution. The assays were very simple to perform, requiring only mixing of an aqueous extract of wheat with the DON-FL and antibody. Samples of wheat naturally contaminated with DON were evaluated by FP and by an HPLC-UV method, with a good correlation ($r(2) = 0.97$). Although the FP method tended to overestimate DON slightly in the wheat samples, by similar to 20%, the assay was easy to use and very useful for the screening of wheat.

MARAGOS, C.M. and PLATTNER, R.D. 2002. **Rapid fluorescence polarization immunoassay for the mycotoxin deoxynivalenol in wheat.** *Journal of Agricultural and Food Chemistry* **50**: 1827–1832.

A rapid test for DON in wheat was developed using the principle of FP immunoassay. The assay was based on the competition between DON and a novel DON-fluorescein tracer (DON-FL2) for a DON-specific monoclonal antibody in solution. The method, which is a substantial improvement over a previous DON FP immunoassay, combined a rapid (3 min) extraction step with a rapid (2 min) detection step. A series of naturally contaminated wheat and maize samples were analysed by both FP immunoassay and HPLC-UV. The HPLC-UV and FP methods for wheat agreed well (linear regression $r(2) = 0.936$), but not for maize ($r(2) = 0.849$).

SCHNEWEIS, I., MEYER, K., ENGELHARDT, G. and BAUER, J. 2002. **Occurrence of zearalenone-4-beta-D-glucopyranoside in wheat.** *Journal of Agricultural and Food Chemistry* **50**: 1736–1738.

An LC-MS method was developed for the analysis of ZEA-4-beta-D-glucopyranoside and ZEA in wheat. The limit of determination for both substances was 10 µg/kg. The recovery rates were 69 and 89% at a concentration of 100 µg/kg for ZEA-4-beta-D-glucopyranoside and ZEA, respectively. Of 24 Bavarian wheat samples, 22 were positive for ZEA with the

levels ranging from 11 to 860 µg/kg. ZEA-4-beta-D-glucopyranoside was found in 10 of the ZEA positive samples with the levels ranging from 17 to 104 µg/kg. The amounts of ZEA-4-beta-D-glucopyranoside were correlated to those of ZEA ($r(2) = 0.86$, $b = 0.10$).

Trichothecenes – General

GARCIA, C.C., ROSSO, M.L., BERTONI, M.D., MAIER, M.S. and DAMONTE, E.B. 2002. **Evaluation of the antiviral activity against junin virus of macrocyclic trichothecenes produced by the hypocrealean epibiont of *Baccharis coridifolia*.** *Planta Medica* **68**: 209–212.

Roridin A, roridin E, verrucarins A and verrucarins J were evaluated for their inhibitory activity against the arenavirus Junin (JUNV), the etiological agent of Argentine haemorrhagic fever. The trichothecenes achieved a dose dependent inhibition of JUNV multiplication at concentrations not affecting cell viability. The 50% inhibitory concentration values determined by a virus yield inhibition assay were in the range 1.2–4.9 µg/L. The most active compound was verrucarins J which reduced JUNV yield more than 2 log units and had a similar effect against the arenavirus Tacaribe.

MURATA, H., SHIMADA, N. and YOSHIOKA, M. 2002. **Suppressive effect of zearalenone, an estrogenic mycotoxin, on bovine neutrophil chemiluminescence.** *Veterinary and Human Toxicology* **44**: 83–86.

The effects of ZEA on bovine neutrophils were investigated *in vitro* using chemiluminescence, a bactericidal parameter. ZEA suppressed luminol-dependent, phorbol 12-myristate 13-acetate (PMA)-elicited chemiluminescence in a dose dependent manner at concentrations of 10^{-4} and 10^{-5} M. The possible mode of action of ZEA on the cell activity was investigated with special reference to intracellular Ca^{2+} release and oestrogen receptors. The 10^{-4} M ZEA treatment significantly impaired Ca^{2+} release. When pretreated with a low dose (10^{-6} M) of PMA, the cells resisted the ZEA induced chemiluminescence suppression. However, pretreatment of the cells with oestrogen receptor blockers did not annul the suppressive ZEA action, therefore not supporting the suggestion that ZEA affects cells via oestrogen receptor pathways.

VILA, B., JARADAT, Z.W., MARQUARDT, R.R. and FROHLICH, A.A. 2002. **Effect of T-2 toxin on *in vivo* lipid**

peroxidation and vitamin E status in mice. *Food and Chemical Toxicology* **40**: 479–486.

The effects of an acute administration of T-2 toxin on vitamin E status and the corresponding degree of lipid peroxidation, as determined by the plasma and organ content of malondialdehyde (MDA), was studied in mice. T-2 toxin was administered in doses ranging from 1 to 6.25 mg/kg bw while the dietary content of vitamin E ranged from near 0 to 5000 IU/kg. T-2 toxin treatment of mice increased lipid peroxidation in the liver as measured by MDA production. This process was maximal after 48 hr of T-2 toxin challenge, and decreased thereafter. Plasma alpha-tocopherol levels decreased as soon as 6 hr after the toxin challenge, while MDA did not increase until there was a severe depletion of vitamin E. These changes were accompanied by decrease in weight of spleen and thymus.

YANG, G.H. and PESTKA, J.J. 2002. **Vomitoxin (deoxynivalenol)-mediated inhibition of nuclear protein binding to NRE-A, an IL-2 promoter negative regulatory element, in EL-4 cells.** *Toxicology* **172**: 169–179.

IL-2 gene expression is superinduced by DON in primary and cloned murine T cells. Binding of the transcription factor ZEB (NIL-2-a) to the negative regulatory element NRE-A plays a critical role in silencing IL-2 expression in the EL-4 cell line, a murine Th1-like lymphoma. This study tested the hypothesis that DON impairs NRE-A-binding activity in the EL-4 T cells model. Results indicate that the capacity of DON to up-regulate IL-2 expression may relate, in part, to its capacity to decrease ZEB binding to the negative regulatory element within this cytokine's promoter. The inability to associate decreased NRE-A binding with impaired ZEB transcription or translation suggests that post-translational modification of this negative transcription factor may be requisite for DON down-regulatory effects.

ISLAM, Z., MOON, Y.S., ZHOU, H.R., KING, L.E., FRAKER, P.J. and PESTKA, J.J. 2002. **Endotoxin potentiation of trichothecene-induced lymphocyte apoptosis is mediated by up-regulation of glucocorticoids.** *Toxicology and Applied Pharmacology* **180**: 43–55.

The mechanisms by which bacterial endotoxin (lipopolysaccharide, LPS) potentiates lymphoid tissue depletion in B6C3F1 mice exposed to DON was investigated. DNA fragmentation and flow cytometric analysis demonstrated that apoptosis in thymus, Peyer's patches and bone marrow was marked in mice 12 hr after

administering *Escherichia coli* LPS (0.1 mg/kg bw ip) concurrently with DON (12.5 mg/kg bw po), whereas apoptosis in control mice or mice treated with either toxin alone was minimal. Based on observed increases in TNF alpha and IL-6 serum concentrations following LPS and DON co-treatment, the roles of these cytokines in apoptosis potentiation were assessed. Injection with rolipram, an inhibitor of TNF alpha expression, or use of IL-6 knockout mice was ineffective at impairing thymic apoptosis induction by the toxin co-treatment, suggesting that these cytokines did not mediate LPS potentiation. Indomethacin, a broad spectrum inhibitor of cyclooxygenases, failed to block thymus apoptosis. RU 486, a glucocorticoid receptor antagonist, significantly abrogated apoptosis in thymus, Peyer's patches and bone marrow following LPS plus DON exposure. These results, and the known capacity of glucocorticoids to cause apoptosis, indicate that hypothalamic-pituitary-adrenal axis plays a key role in LPS potentiation of trichothecene induced lymphocyte apoptosis.

MITAK, M., GOJMERAC, T., CVRTELA, D. and CVETNIC, Z. 2002. **Effect of atrazine and zearalenone on the number of receptor binding sites for H-3-estradiol in the rat uterus cytosol.** *Veterinarni Medicina* **47**: 12–16.

The effect of a daily dose of atrazine at 14 mg and/or ZEA at 2.5 mg, administered to rats during 5 days of oestrous cycle, on the number of receptor binding sites for [³H]-estradiol in uterine cytosol was investigated. In animals administered ZEA, a decline in the number of receptor binding sites for [³H]-estradiol in uterine cytosol was observed. On day 4, the number of binding sites was significantly greater in this group than in the control group, whereafter it gradually decreased to a significantly lower value as compared to the controls on day 6. In animals administered a mixture of atrazine and ZEA, the number of receptor binding sites did not decrease but showed a similar pattern as in the control group of animals, however, at a statistically significantly higher level.

POAPOLATHEP, A., OHTSUKA, R., KIATIPATTANASAKUL, W., ISHIGAMI, N., NAKAYAMA, H. and DOI, K. 2002. **Nivalenol-induced apoptosis in thymus, spleen and Peyer's patches of mice.** *Experimental and Toxicologic Pathology* **53**: 441–446.

ICR:CD-1 male mice were orally administered with NIV at 5, 10 and 15 mg/kg bw, and examined at 12, 24 and 48 hr after inoculation (HAI), respectively. The number of apoptotic lymphocytes evalu-

ated by *in situ* detection for fragmented DNA showed a dose dependent increase at 12 hr in both the thymus and the Peyer's patch, while it began to increase at 24 hr in the spleen. Dead lymphocytes in the thymus, spleen and Peyer's patch showed ultrastructural characteristics of apoptosis. Moreover, the DNA ladder was first detected at 12 hr in the thymus of the 15 mg/kg treated group. The results clearly indicate that NIV is able to induce apoptosis in the lymphoid tissues of mice.

MORRISON, E., RUNDBERGET, T., KOSIAK, B., AASTVEIT, A.H. and BERNHOFT, A. 2002. **Cytotoxicity of trichothecenes and fusarochromanone produced by *Fusarium equiseti* strains isolated from Norwegian cereals.** *Mycopathologia* **153**: 49–56.

The cytotoxicity and secondary metabolites of 28 Norwegian strains of *Fusarium equiseti* have been characterised. In rice culture extracts the following metabolites were found in all isolates: fusarochromanone, NIV, scirpentriol, 4-acetylNIV (fusarenon-X), 15-acetylNIV, and DAS. 4,15-DiacetylNIV was found in five isolates. Descriptive statistics and factor analysis of the identified secondary metabolites show that their main metabolites were fusarochromanone, NIV, scirpentriol, DAS and 15-acetylNIV, consecutively. Porcine kidney epithelial cells (PK15) were exposed to rice culture extracts. Stepwise multiple regression analysis of cytotoxicity and metabolite profiles of rice culture extracts ascribed the toxicity mainly to a combination of fusarochromanone and 15-acetylNIV, though scirpentriol or DAS are agents in the combined toxicity as well.

Aflatoxins – General

ABDULRAZZAQ, Y.M., OSMAN, N. and IBRAHIM, A. 2002. **Fetal exposure to aflatoxins in the United Arab Emirates.** *Annals of Tropical Paediatrics* **22**: 3–9.

Aflatoxin levels were measured in umbilical cord blood from 201 women delivered consecutively in Tawam and Al Ain hospitals, United Arab Emirates. Aflatoxins were detected in 110 (54.7%) samples, 27 of which were positive for AFB₁, 106 for AFM₁ and 31 for AFM₂. There was a significant negative correlation (p<0.001) between birth weight and levels of aflatoxin.

NESCI, A. and ETCHEVERRY, M. 2002. ***Aspergillus* Section *Flavi* populations from field maize in Argentina.** *Letters in Applied Microbiology* **34**: 343–348.

Populations of *Aspergillus* Section *Flavi* were studied from a commercial field of maize in Rio Cuarto, Cordoba, Argentina. *Aspergillus* species were isolated from soil, debris and insects during three periods: pre-planting, growing maize and postharvest. Aflatoxigenic strains were present in lower levels in each component of the agroecosystem studied. All the strains that produced sclerotia were L strains. In this field agroecosystem, the only strains with a high probability for transfer to the storage agroecosystem were L strains with low toxigenic potential.

CARDWELL, K.F. and COTTY, P.J. 2002. **Distribution of *Aspergillus* Section *Flavi* among field soils from the four agroecological zones of the Republic of Benin, West Africa.** *Plant Disease* **86**: 434–439.

The distribution of *Aspergillus* Section *Flavi* across the four different agroecologies of Benin Republic was determined. The four agroecological zones range from humid equatorial tropics in the south to the dry savanna near the Sahara desert in the north. Incidence of *A. flavus* strain isolations varied from south to north, with greater number of colony forming units (CFU) of L strain isolates in southern latitudes and greater numbers of CFU of S strain isolates found in the north. The L strain isolates occurred in 81/88 samples, whereas S strain isolates were in 41/88 soil samples. Of 96 L strain isolates tested, 44% produced aflatoxins, with only B toxins being produced. All S strain isolates produced both B and G aflatoxins. *A. parasiticus* and *A. tamarii* were present in less than 10% of the fields and were not associated with any particular agroecological zone.

GUNSEN, U. and YAROGLU, T. 2002. **Aflatoxin in dog and horse feeds in Turkey.** *Veterinary and Human Toxicology* **44**: 113–114.

Aflatoxin levels were determined by ELISA in 18 dog and 20 horse feed samples collected from different farms in Turkey. In dog feeds, aflatoxins ranged from less than 1.75 to 20 µg/kg and in horse feeds from less than 1.75 to 14 µg/kg. Among the samples, 16.7% of dog feed and 10% of horse feed samples exceeded the Turkish tolerance limit of 10 µg/kg.

CHANG, P.K., BENNETT, J.W. and COTTY, P.J. 2002. **Association of aflatoxin biosynthesis and sclerotial development in *Aspergillus parasiticus*.** *Mycopathologia* **153**: 41–48.

Introduction of the aflatoxin pathway-specific regulatory gene, *aflR*, and *aflJ*, which encodes a putative co-activator, into

an *O*-methylsterigmatocystin (OMST) accumulating strain, *A. parasiticus* SRRC 2043, resulted in elevated levels of accumulation of major aflatoxin precursors. Total amounts of these aflatoxin precursors, norsolorinic acid, averantin, versicolorin A and OMST, produced by the *aflR* plus *aflJ* transformants was 2- to 3-fold that produced by the *aflR* transformants. This increase indicated a synergistic effect of *aflR* and *aflJ* on the synthesis of aflatoxin precursors. Increased production of the aflatoxin precursors was associated with progressive decrease in sclerotial size, alteration in sclerotial shape and weakening in the sclerotial structure of the transformants. The results showed that sclerotial development and aflatoxin biosynthesis are closely related. It is proposed that competition for a common substrate, such as acetate, by the aflatoxin biosynthetic pathway could adversely affect sclerotial development in *A. parasiticus*.

BURGOS-HERNANDEZ, A., PRICE, R.L., JORGENSEN-KORNMAN, K., LOPEZ-GARCIA, R., NJAPAU, H. and PARK, D.L. 2002. **Decontamination of aflatoxin B₁-contaminated corn by ammonium persulphate during fermentation.** Journal of the Science of Food and Agriculture **82**: 546–552.

The decontamination of AFB₁ contaminated corn by an ammoniation-fermentation integrated process was studied. This process could be used for the production of fuel ethanol from aflatoxin containing corn. Different concentrations of ammonium persulphate from 0.25 to 2.0% w/w were tested. In order to increase the decontamination of corn, 0.5 and 1.0% (w/w) azodicarbonamide, benzoyl peroxide and hydrogen peroxide were tested. Peroxides were added at three different stages of the fermentation process: liquefaction, saccharification and fermentation. Addition of 2.0% (w/w) ammonium persulphate caused the highest level of decontamination without affecting ethanol production. Addition of peroxides did not significantly increase ethanol production or significantly improve the decontamination process. The best processes for decontamination of corn and for ethanol production included the addition of 2.0% (w/w) ammonium persulphate for both, and of 1.0 and 0.5% (w/w) benzoyl peroxide, respectively. All treated corn samples showed no mutagenic potential.

BUSER, M.D. and ABBAS, H.K. 2002. **Effects of extrusion temperature and dwell time on aflatoxin levels in cottonseed.** Journal of Agricultural and Food Chemistry **50**: 2556–2559.

Whole cottonseed was extruded to determine if the temperature and dwell time (multiple stages of processing) associated with the process affected aflatoxin levels. To estimate the aflatoxin reductions due to extrusion temperature and dwell time, the least mean fits obtained for the individual studies were combined. Total estimated reductions of 55% (three stages of processing at 104°C), 50% (two stages of processing at 132°C), and 47% (one stage of processing at 160°C) were obtained from the combined equations. If the extreme conditions (four stages of processing at 160°C) of the evaluation studies are applied to the combined temperature and processing equation, the resulting aflatoxin reduction would be 76%.

ALLAMEH, A., ABYANEH, M.R., SHAMS, M.R., REZAEI, M.B. and JAIMAND, K. 2002. **Effects of neem leaf extract on production of aflatoxins and activities of fatty acid synthetase, isocitrate dehydrogenase and glutathione S-transferase in *Aspergillus parasiticus*.** Mycopathologia **154**: 79–84.

The activity of isocitrate dehydrogenase (IDH) was higher in non-toxicogenic strains of *Aspergillus parasiticus* as compared to toxicogenic strains. In contrast, the activities of fatty acid synthase (FAS) as well as glutathione S-transferase (GST) were higher in toxicogenic strains than in non-toxicogenic strains. Aflatoxin production was inhibited by greater than 90% in fungi grown in presence of 50% v/v neem (*Azadirachta indica*) leaf extract, while there were no significant changes in FAS and IDH activities. Unlike FAS and IDH, GST was readily inhibited (similar to 67%) in mycelia treated with 1.56% v/v of the neem extract. The inhibition reached to maximum of 80% in samples exposed to 6.25–12.5% of the extract.

UDWARY, D.W., CASILLAS, L.K. and TOWNSEND, C.A. 2002. **Synthesis of 11-hydroxyl *O*-methylsterigmatocystin and the role of a cytochrome P-450 in the final step of aflatoxin biosynthesis.** Journal of the American Chemical Society **124**: 5294–5303.

Previous experiments have suggested that two successive monooxygenase reactions are required to convert OMST to aflatoxin, a process shown here to be mediated by a single cytochrome P-450, OrdA, in *Aspergillus parasiticus* in accord with findings in *A. flavus*. The first oxidative cycle is proposed to result in the formation of 11-hydroxyOMST (HOMST), while the second entails aryl ring cleavage, demethylation, dehydration, decarboxylation and rearrangement to give aflatoxin. To test this hypothesis, HOMST has been

synthesised by an alkyl nitrilium variant of the Houben-Hoesch reaction. Expression of *orda* in a yeast membrane preparation enabled the intermediacy of HOMST both to be detected in the conversion of OMST to aflatoxin and to be established directly in the biosynthesis of the mycotoxin.

CHANG, P.K. and YU, J. 2002. **Characterization of a partial duplication of the aflatoxin gene cluster in *Aspergillus parasiticus* ATCC 56775.** Applied Microbiology and Biotechnology **58**: 632–636.

A partial duplication of the complete aflatoxin gene cluster containing homologues of *aflR-aflJ-adhA-estA-norA-ver1* and *omtB* was identified from *Aspergillus parasiticus*. One-hybrid assays showed that the duplicated *aflR* gene (*aflR2*) encoded a protein that could activate transcription just as that encoded by *aflR1*, the *aflR* gene in the complete gene cluster. Deletion of *aflR1* resulted in the loss of production of aflatoxin precursors, which suggested that *aflR2* could not completely replace the function of *aflR1*. Point mutations found in *adhA2*, pre-termination in *ver1B* and *norA2*, and a large deletion in *omtB2* probably render these duplicated genes to become nonfunctional. A close examination of the history of isolates reported to have a partial duplication suggested that duplication of the aflatoxin cluster is not a prevalent event.

Aflatoxins – Methodology

CHEN, R.S., TSAY, J.G., HUANG, Y.F. and CHIOU, R.Y.Y. 2002. **Polymerase chain reaction-mediated characterization of molds belonging to the *Aspergillus flavus* group and detection of *Aspergillus parasiticus* in peanut kernels by a multiplex polymerase chain reaction.** Journal of Food Protection **65**: 840–844.

A polymerase chain reaction (PCR)-mediated method of detecting the aflatoxin synthesising genes was developed. Nineteen strains of the *Aspergillus flavus* group, including *A. flavus*, *A. parasiticus*, *A. oryzae*, *A. sojae* and *A. niger*, were subjected to PCR testing in an attempt to detect four genes encoding for norsolorinic acid reductase (*nor-1*), versicolorin A dehydrogenase (*ver-1*), sterigmatocystin *O*-methyltransferase (*omt-1*) and a regulatory protein (*apa-2*), involved in aflatoxin biosynthesis. All seven aflatoxigenic strains possessed the four DNA fragments, and five strains bearing less than the four DNA fragments did not produce aflatoxin. When peanut kernels were artificially contaminated with *A. parasiticus* and *A. niger*,

the contaminant DNA was extractable from a piece of cotyledon, and when subjected to multiplex PCR testing using the four pairs of primers coding for the above genes, they were successfully detected. The target DNA fragments were detected in the kernels infected with *A. parasiticus* but not in the uninoculated kernels or in the kernels infected with *A. niger*.

PAPADOPOULOU-BOUROAOUI, A., STROKA, J. and ANKLAM, E. 2002. **Comparison of two post-column derivatization systems, ultraviolet irradiation and electrochemical determination, for the liquid chromatographic determination of aflatoxins in food.** Journal of AOAC International **85**: 411–416.

Two post-column derivatisation (PCD) techniques, ultraviolet (UV) irradiation (PCDuv) and electrochemical bromination (PCDEC), for the determination of aflatoxins by fluorescence detection after LC separation were compared. Photochemical fluorescence enhancement was obtained with two different commercially available systems (PCDUV1 and PCDUV2). An electrochemical bromination apparatus was used for bromination. Analyses of naturally contaminated or spiked food samples showed that neither of the techniques resulted in derivatisation-specific matrix interferences for any of the matrixes under study. The response ratios PCDUV/PCDEC for AFB₁, B₂, G₁ and G₂ were 0.86, 0.96, 0.70, and 0.96, respectively, for PCDUV1, and 0.82, 0.95, 0.60, and 0.90, respectively, for PCDUV2. The relative standard deviation obtained for each of the devices ranged from 0.3 to 1.8% for PCDUV1, from 0.8 to 1.3% for PCDUV2, and from 0.9 to 2.0% for PCDEC.

STROKA, J. and ANKLAM, E. 2002. **New strategies for the screening and determination of aflatoxins and the detection of aflatoxin-producing moulds in food and feed.** TRAC – Trends in Analytical Chemistry **21**: 90–95.

This article with 33 references describes the current analytical approaches for the detection and determination of aflatoxins in food. Emphasis is on the development of simplified and cost-effective but efficient analytical methods for fast screening or for precise determination in order to comply with legislative requirements. An overview is also given of methods for the detection of aflatoxin producing moulds.

HO, J.A.A. and WAUCHOPE, R.D. 2002. **A strip liposome immunoassay for aflatoxin B₁.** Analytical Chemistry **74**: 1493–1496.

A technique has been developed for the preparation of AFB₁-tagged liposomes encapsulating a visible dye. A method using the liposomes for detecting AFB₁ is described. Samples containing AFB₁ are allowed to migrate by capillary action along a simple plastic-backed nitrocellulose strip into a zone containing immobilised antibodies. AFB₁-tagged liposomes are allowed to migrate into the same area, filling any remaining antibody sites. The liposomes binding to the antibody zone exhibit an intense purplish pink colour and the optical density is inversely proportional to the aflatoxin concentration in the sample. The device is capable of detecting AFB₁ at levels down to 20 ng and could serve as a rapid procedure for visual screening of agricultural and food samples for AFB₁ or, with densitometry, as an inexpensive quantitative assay.

SCHATZKI, T.F. and HADDON, W.F. 2002. **Rapid, non-destructive selection of peanuts for high aflatoxin content by soaking and tandem mass spectrometry.** Journal of Agricultural and Food Chemistry **50**: 3062–3069.

A rapid, non-destructive method is presented for selecting undamaged, aflatoxin-contaminated nuts from among peanut lots. The method is based on dipping nuts into extraction fluid and examining the resulting fluid by tandem MS without preliminary cleanup. This method has been applied to examine over 65,000 nuts, yielding approximately 120 nuts, each containing aflatoxin at more than 250–43000 µg/kg, depending on process stream.

NASIR, M.S. and JOLLEY, M.E. 2002. **Development of a fluorescence polarization assay for the determination of aflatoxins in grains.** Journal of Agricultural and Food Chemistry **50**: 3116–3121.

A simple, portable and rapid fluorescence polarisation (FP) assay for aflatoxin determination in grains is presented. The immunoassay is field portable, homogeneous and without any washing and cleaning steps. The assay is based upon the competition between free aflatoxin and an aflatoxin–fluorescein tracer for an aflatoxin specific monoclonal antibody in solution. FP results of naturally contaminated corn, sorghum, peanut butter and peanut paste samples correlated well with HPLC ($r(2) = 0.97$). FP analysis of spiked popcorn samples gave a good correlation with spiked values ($r(2) = 0.99$). However, FP consistently underestimated the aflatoxin contents, perhaps due to low cross-reactivity of the antibody used toward B₂, G₁ and G₂ aflatoxins.

NILUFER, D. and BOYACIOGLU, D. 2002. **Comparative study of three different methods for the determination of aflatoxins in tahini.** Journal of Agricultural and Food Chemistry **50**: 3375–3379.

Tahini, a sesame butter, was spiked with aflatoxins and analysed using three different methods, HPLC, fluorometry and ELISA. An immunoaffinity column was used for cleanup and purification of extracts prior to detection by HPLC and fluorometry. All methods were statistically evaluated for accuracy, precision and simple correlations. The fluorometric determination method involving an immunoaffinity column cleanup step was found to be highly correlated with the HPLC method ($r = 0.978$). Both methods were found to be effective due to their high recoveries and low variance for the prediction of total aflatoxin contamination in tahini samples. The ELISA method, due to its high variation in replicates, was found to be applicable only as a screening method.

Aflatoxicoses

RICORDY, R., GENSABELLA, G., CACCI, E. and AUGUSTI-TOCCO, G. 2002. **Impairment of cell cycle progression by aflatoxin B₁ in human cell lines.** Mutagenesis **17**: 241–249.

The effect of AFB₁ on cell cycle progression in three human cell lines, HepG2, SK-N-MC and SK-N-SH derived from liver and nervous tissue tumours, was investigated. The cell cycle, DNA synthesis and expression of *p21* and *p53* in the presence and absence of AFB₁ were analysed. In all cell lines, exposure to a 24 hr toxin treatment causes a dose dependent accumulation in S phase, however, the ability to recover from impairment to traverse S phase varied. SK-N-MC cells appear more prone to resume DNA synthesis when the toxin is removed, while the other two cell lines maintain a significant inhibition of DNA synthesis. The level of *p53* and *p21* expression in the three cell lines was examined by western blot analysis and significant differences were detected. The ready resumption of DNA synthesis displayed by SK-N-MC cells could possibly be related to the absence of *p53* control of cell cycle progression.

SMELA, M.E., HAMM, M.L., HENDERSON, P.T., HARRIS, C.M. and HARRIS, T.M. 2002. **The aflatoxin B₁ formamidopyrimidine adduct plays a major role in causing the types of mutations observed in human hepatocellular carcinoma.** Journal of Agricultural and Food Chemistry **50**: 3375–3379.

noma. Proceedings of the National Academy of Sciences of the United States of America **99**: 6655–6660.

The primary DNA adduct of AFB₁ is 8,9-dihydro-8-(N-7-guanyl)-9-hydroxy-AFB₁ (AFB₁-N7-Gua) which is converted naturally to two secondary lesions, an apurinic site and an AFB₁-formamidopyrimidine (AFB₁-FAPY) adduct. AFB₁-FAPY is detected at near maximal levels in rat DNA days to weeks after AFB₁ exposure. The present study reveals two striking properties of this DNA adduct: firstly, AFB₁-FAPY was found to cause a G to T mutation frequency in *Escherichia coli* approximately 6 times higher than that of AFB₁-N7-Gua, and secondly, one proposed rotamer of AFB₁-FAPY is a block to replication, even when the efficient bypass polymerase MucAB is used by the cell. Taken together, these characteristics make the FAPY adduct the prime candidate for both the genotoxicity of aflatoxin, because mammalian cells also have similar bypass mechanisms for combating DNA damage, and the mutagenicity that ultimately may lead to liver cancer.

MARIN, D.E., TARANU, I., BUNACIU, R.P., PASCALE, F., TUDOR, D.S., AVRAM, N., SARCA, M., CUREU, I., CRISTE, R.D., SUTA, V. and OSWALD, I.P. 2002. **Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin.** Journal of Animal Science **80**: 1250–1257.

Weanling piglets were fed diets containing aflatoxins at 140 or 280 µg/kg *ad libitum* for 4 weeks. A dose related decrease in weight gain was observed in treated animals. Ingestion of aflatoxins at either level had no effect on total red blood cell numbers or on their relative number of lymphocytes, monocytes, neutrophils, basophils and eosinophils in blood. Likewise, aflatoxins did not alter globulin, albumins or total protein concentrations in serum, nor the expression of regulatory cytokines produced by either Th1 (IL-2) or Th2 (IL-4) lymphocyte subsets. By contrast, the low dose (140 µg/kg) decreased the total number of white blood cells, whereas the high dose (280 µg/kg) had the opposite effect. Aflatoxin also increased the concentration of gamma-globulin in the serum. A reduced immune response induced by *Mycoplasma agalactiae* in the 280 µg/kg group was also observed. Cytokine mRNA expression in phytohaemagglutinin-stimulated blood cells indicated that aflatoxins decreased proinflammatory (IL-1beta, TNF alpha) and increased anti-inflammatory (IL-10) cytokine mRNA expression.

VERMA, R.J. and NAIR, A. 2002. **Effect of aflatoxins on testicular steroidogenesis and amelioration by vitamin E.** Food and Chemical Toxicology **40**: 669–672.

Oral administration of aflatoxin at 25 and 50 µg/animal/day for 4–5 days to adult mice caused a dose dependent significant rise in cholesterol content. The activities of 3-beta- and 17-beta-hydroxysteroid dehydrogenases and serum testosterone levels were significantly reduced in aflatoxin treated mice as compared with the controls. Pretreatment with vitamin E at 2 mg/animal/day significantly ameliorated aflatoxin induced changes.

TAKAHASHI, Y., NAKATSURU, Y., ZHANG, S., SHIMIZU, Y., KUME, H., TANAKA, K., IDE, F. and ISHIKAWA, T. 2002. **Enhanced spontaneous and aflatoxin-induced liver tumorigenesis in xeroderma pigmentosum group A gene-deficient mice.** Carcinogenesis **23**: 627–633.

Xeroderma pigmentosum (XP) is an autosomal recessive hereditary disease featuring defective nucleotide excision repair. The development of spontaneous and AFB₁ induced liver tumours in XP group A (XPA) deficient congenic mice, originally created by repeated back-crosses with inbred C3H/HeN mice, was analysed. The incidence of, and average number of, spontaneous tumours per mouse were significantly higher in XPA^{-/-} than in XPA^{+/+} and +/- mice. Similarly, F₁₀ XPA^{-/-} mice receiving ip injection of AFB₁ at 0.6 or 1.5 mg/kg bw at 7 days of age demonstrated more liver tumours than their heterozygous or homozygous positive counterparts when examined at month 11.

TOMKOVA, I., SEVCIKOVA, Z., LEVKUT, M., REVAJOVA, V., CONKOVA, E., LACIAKOVA, A. and LENHARDT, L. 2002. **Effect of aflatoxin B₁ on CD3 T cells and alkaline phosphatase in the intestine of mice.** Mycopathologia **154**: 15–19.

The effect of repeated applications of AFB₁ on immunocompetent cells (CD3 T cells) and alkaline phosphatase in the intestinal mucosa was studied. Mice were orally treated with AFB₁ for 24 days. The mucosa of the intestine showed a significant decrease in the number of CD3 T cells and a significantly lower level activity of alkaline phosphatase on day 24. Similarly, with changes in the small intestine, qualitative haematological parameters were modified in systemic immunity as lymphopenia, and neutropenia, monocytopenia.

GUPTA, A. and GOPAL, M. 2002. **Aflatoxin production by *Aspergillus flavus* isolates pathogenic to coconut insect pests.** World Journal of Microbiology & Biotechnology **18**: 325–331.

Aspergillus flavus isolated from naturally infected leaf-eating caterpillar (*Opisina arenosella* W.), lace bug (*Stephanitis typica* D.) and plant hopper (*Proutista moesta* Westwood), insect pests of the coconut palm, were tested for aflatoxin production. Only two isolates of *A. flavus*, originally isolated from *O. arenosella* and *P. moesta*, produced aflatoxins. Aflatoxin production was not necessary for pathogenicity in the insects.

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