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Abstracts

Mycotoxins – General

CREWS, H., ALINK, G., ANDERSEN, R., BRAESCO, V., HOLST, B., MAIANI, G., OVESEN, L., SCOTTER, M., SOLFRIZZO, M., VANDENBERG, R., VERHAGEN, H. and WILLIAMSON, G. 2001. **A critical assessment of some biomarker approaches linked with dietary intake**. British Journal of Nutrition **86**: S5–S35.

In this review with 316 references many examples are given of the complexities involved in using some biomarkers for assessing the effects of dietary exposure to contaminants. The role of biological effect monitoring is considered for a number of dietary contaminants including fumonisins. Aflatoxins are discussed as an example of food contaminants for which the biomarker approach has been extensively studied.

OTTENEDER, H. and MAJERUS, P. 2001. [**Mycotoxins food contaminants number one? An attempt to define one's position**]. Deutsche Lebensmittel-Rundschau **97**: 334–338.

Aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, zearalenone and patulin are food contaminants which need to be monitored from the point of view of consumer protection. Data from food control authorities show that surveillance of aflatoxin limits is sufficient. A large number of monitoring investigations are conducted by food control authorities, and these should be coordinated and gathered together for evaluation. (In German).

JONSYN-ELLIS, F.E. 2001. **Seasonal variation in exposure frequency and concentration levels of aflatoxins and ochratoxins in urine samples of boys and girls**. Mycopathologia **152**: 35–40.

Urine samples from boys and girls in Sierra Leone were collected and analysed for aflatoxins and ochratoxins. Analysis of the dry season samples revealed that, with the exception of one boy, all children had detectable amounts of aflatoxins and/or ochratoxins in their urine. Similarly, with the exception of four children (two from each sex), rainy season urine samples also contained these two mycotoxins. There were significant differences in the frequency of exposure to some mycotoxins: ochratoxin A (OA), $p<0.01$; 4-hydroxyOA, $p<0.002$; aflatoxin M_1 (AFM_1), $p<0.04$; aflatoxicol, $p<0.03$; AFB_2, $p<0.04$. There were also significant differences in the levels of AFB_1 ($p<0.05$) and AFB_2 ($p<0.02$) detected in dry season samples. Stratification of these results according to season and sex indicates significant differences with respect to 4-hydroxyOA and AFB_1.

SEDMIKOVA, M., REISNEROVA, H., DUFKOVA, Z., BARTA, I. and JILEK, F. 2001. **Potential hazard of simultaneous occurrence of aflatoxin B_1 and ochratoxin A**. Veterinarni Medicina **46**: 169–174.

Samples of wheat and barley were assayed for AFB1 and OA by RIA. The average contents of AFB1 in the samples of wheat and barley were 2.4 and 2.2 mg/kg, respectively, and the average contents of OA were 3.1 and 2.4 mg/kg, respectively. Studies of the mutagenic activity of the toxins using the Ames test found that OA could increase the mutagenicity of AFB_1 when the toxins occurred together.

PINTO, V.F., PATRIARCA, A., LOCANI, O. and VAAMONDE, G. 2001. **Natural co-occurrence of aflatoxin and cyclopiazonic acid in peanuts grown in Argentina**. Food Additives and Contaminants **18**: 1017–1020.

The natural occurrence of aflatoxins and cyclopiazonic acid (CPA) contamination in peanuts grown in Argentina was investigated. Co-occurrence of CPA and aflatoxins was detected in 2/50 samples analysed. The levels of these toxins found in positive samples were 4300 and 493 mg/kg for CPA, 625 and 435 mg/kg for AFB_1, and 625 and 83 mg/kg for AFG_1. Levels of CPA contamination in the positive samples were similar to those registered in other substrates. This is the first report of natural co-occurrence of CPA and aflatoxins in Argentina.

SCOTT, P.M. 2001. **Analysis of agricultural commodities and foods for *Alternaria* mycotoxins**. Journal of AOAC International **84**: 1809–1817.

An overview with 88 references. Cleanup procedures of analytical methods for foods and foodstuffs contaminated with Alternaria toxins include solvent partition, generally used for tenuazonic acid and solid-phase extraction columns for alternariol (AOH), alternariol monomethyl ether (AME) and altertoxin I. Alternaria mycotoxins have been determined by TLC, GC and more usually LC, mainly with UV detection. Recently, atmospheric pressure chemical ionisation and electrospray LC/MS and LC–MS/MS have been applied to the determination and confirmation of AOH and AME in apple juice and other fruit beverages at sub mg/L levels. Natural occurrences of AOH, AME and other Alternaria toxins have been reported in tomatoes, olives, mandarins, melons, peppers, apples and raspberries. They have been found also in processed fruit products such as apple juice, other

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fruit beverages and tomato products, wheat and other grains, sunflower seeds, oilseed rape meal and pecans.

VARGAS, E.A., PREIS, R.A., CASTRO, L. and SILVA, C.M.G. 2001. **Co-occurrence of aflatoxins B_1, B_2, G_1, G_2, zearalenone and fumonisin B_1 in Brazilian corn**. Food Additives and Contaminants **18**: 981–986.

Unprocessed corn samples from the 1997–1998 harvest, collected at wholesale markets in different regions in Brazil, were surveyed for the occurrence of mycotoxins. Among 214 samples, AFB_1, zearalenone and fumonisin B_1 (FB_1) were found in 38.3, 30.4 and 99.1% of the samples, respectively, at levels ranging from 0.2–129, 36.8–719 and 200–6100 mg/kg, respectively. The co-occurrence of AFB_1 and FB_1 was observed in all of the 82 aflatoxin-positive samples.

LANGSETH, W., GHEBREMESKEL, M., KOSIAK, B., KOLSAKER, P. and MILLER, D. 2001. **Production of culmorin compounds and other secondary metabolites by *Fusarium culmorum* and *F. graminearum* strains isolated from Norwegian cereals**. Mycopathologia **152**: 23–34.

A total of 23 *Fusarium culmorum* and 21 *F. graminearum* isolates were studied for their ability to produce mycotoxins and other secondary metabolites when cultivated on rice. Two *F. culmorum* strains formed nivalenol and its acetylated derivatives (chemotype II), while all *F. graminearum* and the other *F. culmorum* isolates produced deoxynivalenol (DON) via 3-acetylDON (chemotype IA). 15-Hydroxy-culmorin, followed by 5-hydroxy-culmorin, were the other main metabolites produced by *F. culmorum*, while 5-, 12- and an unidentified hydroxy-culmorin, suggested to be 14-hydroxy-culmorin, were the main metabolites of *F. graminearum*.

GHEBREMESKEL, M. and LANGSETH, W. 2001. **The occurrence of culmorin and hydroxy-culmorins in cereals**. Mycopathologia **152**: 103–108.

Forty-five samples of naturally contaminated grain, barley, wheat and oats, three samples of mixed feed, and 16 samples of grain artificially inoculated with *Fusarium culmorum* during the flowering stage were analysed for DON, 3-acetylDON, culmorin and hydroxy-culmorins. The amount of each of culmorin, 5-, 12-, 14- and 15-hydroxy-culmorin and one unknown hydroxy-culmorin were determined relative to the amount of DON plus 3-acetylDON for each sample. There was a strong correlation between the amount of DON present in the grain and the amount of culmorin and hydroxy-culmorins present. The ratio of each of the culmorin compounds relative to the amount of DON compounds were in the same range in the grain artificially inoculated by *F. culmorum* as found in an earlier study for *F. culmorum* strains cultivated on rice, while the hydroxy-culmorin profile in the naturally contaminated grain was more similar to that found for the *F. graminearum* cultures in the same study.

LAIDOU, I.A., THANASSOULOPOULOS, C.C. and LIAKOPOULOU-KYRIAKIDES, M. 2001. **Diffusion of patulin in the flesh of pears inoculated with four post-harvest pathogens**. Journal of Phytopathology – Phytopathologische Zeitschrift **149**: 457–461.

Pear fruits of cv. Abate fetel were experimentally inoculated with *Penicillium expansum*, *Aspergillus flavus*, *Stemphylium vesicarium* and *Alternaria alternata*. The infected fruits were split longitudinally through the centre of lesions (5, 10, 15 and 20 mm in diameter) and were divided into four sections. The fungi were isolated in different frequencies from all sections including diseased and healthy tissues. Patulin was detected in the flesh of pears with lesions 10 mm in diameter when inoculated with *P. expansum*, *S. vesicarium* and *A. alternata*. Mycotoxin concentrations surpassed the accepted maximum residue limit in all sections, even in apparently sound tissues.

YURDUN, T., OMURTAG, G.Z. and ERSOY, O. 2001. **Incidence of patulin in apple juices marketed in Turkey**. Journal of Food Protection **64**: 1851–1853.

Apple juices consumed by the Turkish population were assayed for patulin using HPLC and TLC. From a total of 45 samples, patulin was detected in 60% of apple juices at concentrations ranging from 19.1 to 732.8 mg/L. Forty-four percent of the apple juice samples had patulin contamination levels higher than 50 mg/L, which is the allowable upper limit in Turkey.

GOKMEN, V., ARTIK, N., ACAR, J., KAHRAMAN, N. and POYRAZOGLU, E. 2001. **Effects of various clarification treatments on patulin, phenolic compound and organic acid compositions of apple juice**. European Food Research and Technology **213**: 194–199.

Clarification techniques in apple juice processing were investigated for their effects on patulin content and some other quality parameters including phenolic compounds and organic acids. The clarification techniques varied significantly in their effects for the removal of patulin. Conventional clarification using gelatin, bentonite and activated charcoal was found to be more effective than other techniques. Adsorbent resin treatment just after ultrafiltration also resulted in a remarkable decrease in patulin and brought an improvement in juice colour and clarity.

CROMEY, M.G., LAUREN, D.R., PARKES, R.A., SINCLAIR, K.I., SHORTER, S.C. and WALLACE, A.R. 2001. **Control of *Fusarium* head blight of wheat with fungicides**. Australasian Plant Pathology **30**: 301–308.

The control of *Fusarium* head blight (FHB) of wheat using fungicides was investigated in two field trials. The first trial examined the effects of tebuconazole applied at a range of crop growth stages around flowering, whereas the second trial compared nil fungicide, tebuconazole, carbendazim and azoxystrobin, applied at full ear emergence or mid anthesis. In the first trial, FHB incidence was reduced by up to 90% and yield increased by 14% following two applications of tebuconazole. Levels of *Fusarium* in harvested grain were not affected but mycotoxin levels were reduced by some treatments. In the second trial, FBH incidence was decreased and grain weight increased with all fungicides. Levels of both *Fusarium* and resulting mycotoxins were substantially reduced following treatment with tebuconazole or carbendazim but were not affected by treatment with azoxystrobin. *Fusarium graminearum* predominated in both trials.

ABBAS, H.K., TAK, H., BOYETTE, C.D., SHIER, W.T. and JARVIS, B.B. 2001. **Macrocyclic trichothecenes are undetectable in kudzu (*Pueraria montana*) plants treated with a high-producing isolate of *Myrothecium verrucaria***. Phytochemistry **58**: 269–276.

Myrothecium verrucaria was found to be an effective pathogen against kudzu grown in the greenhouse and the field. When cultured on solid rice medium, *M. verrucaria* produced large amounts of macrocyclic trichothecenes, including epiroridin E, epiisororidin E, roridin E, roridin H, trichoverrin A, trichoverrin B, verrucarin A and verrucarin J. Most of these toxins were also isolated from *M.*

verrucaria spores and mycelia grown on potato dextrose agar medium, cornsteep medium or soyflour-cornmeal broth. In contrast, no macrocyclic trichothecenes were detected by HPLC analysis of plant tissues of kudzu, sicklepod and soybean treated with aqueous suspensions of *M. verrucaria* spores. These results argue for both safety and efficacy for the use of *M. verrucaria* in biological control of kudzu and other noxious weeds, and support proceeding to animal feeding trials for further evaluation of safety.

DEALDANA, B.R.V., CIUDAD, A.G., ZABALGOGEAZCOA, I. and CRIADO, B.G. 2001. **Ergovaline levels in cultivars of *Festuca arundinacea***. Animal Feed Science and Technology **93**: 169–176.

The percentage of seeds infected with the fungal endophyte *Neotyphodium coenophialum* and the presence of ergovaline in seed and forage samples of 10 commercial cultivars of tall fescue (*Festuca arundinacea*) were determined. Infection with endophytic fungus was detected in seeds of eight cultivars. The percentage of infection in seeds of these cultivars ranged from 1 to 72%. Ergovaline contents in commercial seed lots of endophyte infected *F. arundinacea* ranged from 0.02 to 3. 71 mg/kg. There was an exponential relationship between the percentage of infection of the seed lot and ergovaline concentration across all cultivars (R-2 = 0.876).

BONY, S., PICHON, N., RAVEL, C., DURIX, A., BALFOURIER, F. and GUILLAUMIN, J.J. 2001. **The relationship between mycotoxin synthesis and isolate morphology in fungal endophytes of *Lolium perenne***. New Phytologist **152**: 125–137.

Variability in the fungal endophytes of 83 natural populations of *Lolium perenne* (perennial ryegrass) from Europe was assessed. Three species were found among 94 strains isolated: *Neotyphodium lolii*, *Neotyphodium* sp. (LpTG-2) and *Gliocladium*-like species. The most frequent species was *N. lolii*, which showed high variability. One-third of the isolates of *N. lolii* did not produce ergovaline whereas a few isolates did not produce lolitrem B. Ergovaline and lolitrem B-deficient strains, but not the few peramine-deficient isolates, had characteristic morphologies on PDA. No isolate was deficient for both ergovaline and lolitrem B synthesis.

WARD, D.E., GAI, Y.Z., LAZNY, R., SOLEDADE, M. and PEDRAS, C. 2001. **Probing host-selective phytotoxicity: Synthesis of destruxin B and several natural analogues**. Journal of Organic Chemistry **66**: 7832–7840.

The syntheses of the host selective phytotoxin destruxin B and the closely related natural analogues homodestruxin B, desmethyldestruxin B, hydroxydestruxin B and hydroxyhomodestruxin B, are described. In each case, the MeAla-beta Ala linkage was formed by cyclisation and the precursor linear hexadepsipeptides were formed by condensing two three-residue fragments.

Mycotoxins – Methodology

TUOMI, T., JOHNSSON, T., HINTIKKA, E.L. and REIJULA, K. 2001. **Detection of aflatoxins [G(1-2), B(1-2)], sterigmatocystin, citrinine and ochratoxin A in samples contaminated by microbes**. Analyst **126**: 1545–1550.

A method is described for the simultaneous determination of AFG_1, G_2, B_1 and B_2, and sterigmatocystin, citrinin and OA. The method was applied to a building material matrix artificially contaminated with mycotoxin-producing fungi. Aqueous methanol was used in the initial extraction and solvent partitioning and solid phase extraction in the purification of samples. The HPLC separation was run on-line with tandem mass spectrometric identification and quantification using electrospray ionisation on a quadrupole ion trap mass analyser (ESI-MS-MS). The limit of quantification was 200 ng for all compounds. The average compound and concentration dependent accuracy and precision (RSD) were 21 and 113%. respectively.

ZAMBONIN, C.G., MONACI, L. and ARESTA, A. 2001. **Determination of cyclopiazonic acid in cheese samples using solid-phase microextraction and high performance liquid chromatography**. Food Chemistry **75**: 249–254.

Solid phase micro-extraction (SPME) using a Carbowax/Templated Resin fibre was optimised for the determination of cyclopiazonic acid (CPA) and interfaced with HPLC-UV/DAD. The method was successfully applied to the analysis of white surface cheese samples. SPME was capable of a selective extraction of CPA after a short sonication step in methanol. The whole extraction gave high recovery yields and was simpler and quicker than any other existing procedure for CPA extraction from cheese. The detection limit was 7 mg/kg.

Mycotoxicoses

HUSSEIN, H.S. and BRASEL, J.M. 2001. **Toxicity, metabolism, and impact of mycotoxins on humans and animals**. Toxicology **167**: 101–134.

A review with 269 references. Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins and ergot alkaloids are the mycotoxins of greatest agro-economic importance. The economic impact of mycotoxins includes loss of human and animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce severity of the mycotoxin problem. Although efforts have continued internationally to set guidelines to control mycotoxins, practical measures have not been adequately implemented.

PAGE, E.H. and TROUT, D.B. 2001. **The role of *Stachybotrys* mycotoxins in building-related illness**. AIHAJ **62**: 644–648.

A search of NIOSHTIC (a literature database maintained by the National Institute for Occupational Safety and Health) and MEDLINE (from 1965 to present) for literature related to fungi, mycotoxins and the indoor environment was conducted. The literature review indicates that currently there is inadequate evidence supporting a causal relationship between symptoms or illness among building occupants and exposure to mycotoxins. Research involving the identification and isolation of specific fungal toxins in the environment and in humans is needed before a more definitive link between health outcomes and mycotoxins can be made.

ANINAT, C., HAYASHI, Y., ANDRE, F. and DELAFORGE, M. 2001. **Molecular requirements for inhibition of cytochrome P450 activities by roquefortine**. Chemical Research in Toxicology **14**: 1259–1265.

The interaction of roquefortine, a cyclopeptide derived from the diketopiperazine cyclo(Trp-dehydroHis), with rat and human liver cytochromes P450 was monitored by difference UV-vis spectroscopy. It was found to interact with different forms of the cytochromes, giving rise

to a type II difference spectrum, characteristic of the binding of an amino function to the heme iron. Roquefortine was compared with compounds of similar structure: cyclo(Phe-His), cyclo(Phe-dehydroHis), cyclo(Trp-His) and phenylahistin. Results indicate that the =N-imidazole moiety coordinates with the heme iron, and suggest that the dehydroHis moiety and the presence of a fused tetracycle play an important part in roquefortine inhibitory power.

NAGASHIMA, H., NAKAMURA, K. and GOTO, T. 2001. **Hepatotoxin rubratoxin B induced the secretion of TNF-alpha, IL-8, and MCP-1 in HL60 cells**. Biochemical and Biophysical Research Communications **287**: 829–832.

Induction of cytokine secretion by rubratoxin B was investigated using HL60 cells. Tumor necrosis factor alpha and interleukin 8 were secreted from cells treated with rubratoxin B at 40 and 80 mg/L. In cells treated with rubratoxin B at 20 and 40 mg/L monocyte chemotactic protein 1 was released. These rubratoxin B induced cytokines are known to promote liver myelocytic cell infiltration and activate cytokine-recruited cells. As a result, recruited myelocytic cells are considered to contribute to hepatic injury. Results of investigations into the effects of tyrosine kinase inhibitors, genistein and emodin, on rubratoxin B treated cells suggested that rubratoxin B exerts its toxicity using two or more signal transduction pathways.

LUSKY, K., GOBEL, R., TESCH, D., DOBERSCHUTZ, K.D., BERNAU, K.L. and HAIDER, W. 2001. [**Sole and combined administration of the mycotoxins OTA, ZEA and DON. Investigations on animal health and residue behaviour of pigs**]. Fleischwirtschaft **81**: 98–102.

The effect of the administration of OA, zearalenone (ZEA) and DON, either alone or in combination, on the health of pigs and residue behaviour in organs and tissues of pigs was investigated over 90 days. The administration of DON at 1000 mg/kg feed or ZEA at 250 mg/kg feed, either alone or in combination with other mycotoxins, resulted in no detectable residues of these mycotoxins in organs and tissues. However, the simultaneous administration of ZEA and DON with OA did have an effect on the metabolism and secretion of OA. (In German).

HARVEY, R.B., EDRINGTON, T.S., KUBENA, L.F., ROTTINGHAUS, G.E., TURK, J.R., GENOVESE, K.J. and NISBET, D.J. 2001. **Toxicity of moniliformin from *Fusarium fujikuroi* culture material to growing barrows**. Journal of Food Protection **64**: 1780–1784.

Growing barrows were fed diets containing moniliformin at 25–200 mg/kg feed for 28 days. Diets of 100 or 200 mg/kg feed reduced body weight, body weight gain and feed consumption. Serum biochemical analytes were affected by 100 and 200 mg/kg feed diets while haematologic values were affected by moniliformin at 50–200 mg/kg feed. The most consistent sign of moniliformin toxicity in barrows appeared to be death induced within 2 to 5 days by the 100–200 mg/kg feed diets.

BONY, S., DURIX, A., LEBLOND, A. and JAUSSAUD, P. 2001. **Toxicokinetics of ergovaline in the horse after an intravenous administration**. Veterinary Research **32**: 509–513.

The toxicokinetics of ergovaline, an ergopeptine mycotoxin present in some grasses infected with endophytic fungus of the genus Neotyphodium, were studied after iv administration of a single dose of 15 mg/kg body weight in four gelding horses. The elimination half-life and the total clearance of ergovaline were found to be 56.83 ± 13.48 min and 0.020 ± 0.004 L/min/kg, respectively. According to the toxicological data previously reported in the horse, and in spite of the very low dose administered, clinical signs were observed, including excessive coolness of the ears and the nose, excessive sweating and prostration.

DVORSKA, J.E., SURAI, P.F., SPEAKE, B.K. and SPARKS, N.H.C. 2001. **Effect of the mycotoxin aurofusarin on the antioxidant composition and fatty acid profile of quail eggs**. British Poultry Science **42**: 643–649.

The effect of aurofusarin on the antioxidant composition and fatty acid profile of quail eggs was investigated. Japanese quails were fed on a maize–soya diet supplemented with aurofusarin at 26.4 mg/kg feed in the form of *Fusarium graminearum* culture enriched with aurofusarin. Aurofusarin caused a significant decrease in vitamins E, A, total carotenoid, lutein and zeaxanthin concentrations and significantly increased egg yolk susceptibility to lipid peroxidation. There was a significant decrease in the docosahexaenoic acid proportion in the phospholipid, cholesteryl ester and free fatty acid fractions of the egg yolk. At the same time the proportion of linoleic acid in the phospholipid, free fatty acid and triacylglycerol fractions significantly increased.

Ochratoxins – General

PERAICA, M., DOMIJAN, A.M., MATASIN, M., LUCIC, A., RADIC, B., DELAS, F., HORVAT, M., BOSANAC, I., BALIJA, M. and GRGICEVIC, D. 2001. **Variations of ochratoxin A concentration in the blood of healthy populations in some Croatian cities**. Archives of Toxicology **75**: 410–414.

Human blood samples were collected randomly from blood donors for blood banks in several Croatian cities between June, September and December 1997, and March 1998. OA was measured in a total of 983 samples using an HPLC technique with fluorescence detection. Samples containing OA above the detection limit (0.2 mg/L of plasma) were found in all populations at all collection periods. The highest frequency (59%) of samples containing OA above the detection limit and the highest mean concentration (0.39 mg/L) were found in June. Both the frequency and the mean concentration were lowest in all samples in December (36% and 0.19 mg/L, respectively). The estimated daily intake of OA is 0.40 ng/kg body weight (bw) which is much lower than that proposed by World Health Organization as the tolerable daily intake (16.0 ng/kg bw).

THUVANDER, A., PAULSEN, J.E., AXBERG, K., JOHANSSON, N., VIDNES, A., ENGHARDT-BARBIERI, H., TRYGG, K., LUND-LARSEN, K., JAHRL, S., WIDENFALK, A., BOSNES, V., ALEXANDER, J., HULT, K. and OLSEN, M. 2001. **Levels of ochratoxin A in blood from Norwegian and Swedish blood donors and their possible correlation with food consumption**. Food and Chemical Toxicology **39**: 1145–1151.

Blood levels of OA were determined in Scandinavian blood donors (206 from Oslo, Norway, and 200 from Visby on the island of Gotland, Sweden) using an HPLC method. Individual dietary information relevant to OA exposure was also collected. The mean plasma level of OA was 0.18 mg/L in Oslo and 0.21 mg/L in Visby. There was no correlation between plasma levels of OA and the estimated total dietary intake of OA based on consumption data and levels in food (retrieved from the literature). However, consumption of several foods, including

cereal products, wine, beer and pork, were to some minor degree related to high plasma levels of OA. The strongest correlations were observed for women in relation to the consumption of beer or medium brown bread.

GILBERT, J., BRERETON, P. and MACDONALD, S. 2001. **Assessment of dietary exposure to ochratoxin A in the UK using a duplicate diet approach and analysis of urine and plasma samples**. Food Additives and Contaminants **18**: 1088–1093.

A method to assess dietary exposure to OA by the analysis of human plasma and urine samples has been developed. Composite duplicate diet samples from 50 individuals and corresponding plasma and urine samples were obtained over 30 days. Analysis of the foods indicated OA levels contributing to an average intake in the range 0.26–3.54 ng/kg bw/day over the 30 days. OA was found in all plasma samples and in 46 urine samples. The correlation between the plasma OA levels and OA consumption was not significant. However, a significant correlation was found between OA consumption and the urine OA concentration expressed as the total amount excreted.

KOTOWSKI, K., GRABARKIEWICZ-SZCZESNA, J., WASKIEWICZ, A., KOSTECKI, M. and GOLINSKI, P. 2000. **Ochratoxin A in porcine blood and in consumed feed samples**. Mycotoxin Research **16**: 66–72.

The occurrence of OA in feeds and the metabolite residues in porcine blood serum in Poland were investigated. Altogether 40 and 45 samples of feed and porcine blood serum, respectively, were analysed for OA. In winter and spring, the percentages of feeds contaminated with OA were 47.6 and 26.3%, respectively, and for porcine serum they were 66.7 and 50.0%, respectively. In 25% of cases OA was present in both feed and blood, whereas in 27.5% of samples OA was detected in blood only, and in 7.5% in feeds only.

VANDERSTEGEN, G.H.D., ESSENS, P.J.M. and VANDERLIJN, J. 2001. **Effect of roasting conditions on reduction of ochratoxin A in coffee**. Journal of Agricultural and Food Chemistry **49**: 4713–4715.

A commercial lot of green coffee, naturally contaminated with OA, was roasted under various conditions and the effects on its final OA content were determined. Roasting time varied from 2.5 to 10 min and the roast colour varied from light medium to dark. The differences in OA reduction between the different levels of roasting times and colours did not reach statistical significance. However, for all roasting conditions, the reduction was highly significant, being 69% reduction over the combined results. Three different explanations are available for this reduction: physical removal of OA with chaff, isomerisation at the C-3 position into another diastereomer, and thermal degradation with possible involvement of moisture. All three explanations may play a partial role in the OA reduction during coffee roasting.

OBERHEU, D.T. and DABBERT, C.B. 2001. **Exposure of game birds to ochratoxin A through supplemental feeds**. Journal of Zoo and Wildlife Medicine **32**: 136–138.

Thirty randomly selected game bird feeders were sampled at 25- to 33-day intervals from November 1996 to March 1997 to quantitate OA concentrations in supplemental feed. OA concentrations from individual feeders ranged from 5 to 109.9 μg/kg, with a monthly mean of 8.3 μg/kg. These levels that have not been demonstrated to negatively affect game birds in a laboratory environment.

HARRIS, J.P. and MANTLE, P.G. 2001. **Biosynthesis of ochratoxins by *Aspergillus ochraceus***. Phytochemistry **58**: 709–716.

Shaken liquid fermentation of an isolate of *Aspergillus ochraceus* showed growth-associated production of OA and OB, followed by production of a related polyketide diaporthin. Later, between 150 and 250 hr, mellein accumulated transitorily. In contrast, shaken solid substrate fermentation over 14 days produced mainly OA and OB. In these systems, experiments with [^{14}C]-labelled precursors and putative intermediates revealed temporal separation of early and late stages of the ochratoxin biosynthetic pathway, but did not support an intermediary role for mellein. The pentaketide intermediate ochratoxin beta was biotransformed very efficiently into both ochratoxins A and B, 14 and 19%, respectively. The already chlorinated ochratoxin alpha was only biotransformed significantly (4.85%) into OA, indicating that chlorination is mainly a penultimate biosynthetic step in the biosynthesis of OA.

LARSEN, T.O., PETERSEN, B.O. and DUUS, J.O. 2001. **Lumpidin, a novel biomarker of some ochratoxin A producing penicillia**. Journal of Agricultural and Food Chemistry **49**: 5081–5084.

The novel compound lumpidin has been isolated as a major compound from an isolate of *Penicillium nordicum*. The fact that lumpidin has been detected from only 3/16 isolates of *P. nordicum* indicates that lumpidin producing isolates might represent a separate and third OA producing *Penicillium* species.

ACCENSI, F., ABARCA, M.L., CANO, J., FIGUERA, L. and CABANES, F.J. 2001. **Distribution of ochratoxin A producing strains in the *A. niger* aggregate**. Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology **79**: 365–370.

Ninety-two isolates belonging to the *Aspergillus niger* aggregate were tested for OA production. All the isolates were grouped into the two proposed species, *A. niger* and *A. tubingensis*, according to their ITS-5.8S rDNA RFLP patterns. Six out of the 92 isolates studied produced OA. All the OA producing strains were classified as pattern N (corresponding to *A. niger*) while none of the isolates classified as pattern T (corresponding to *A. tubingensis*) produced OA.

JUNG, K.Y., TAKEDA, M., KIM, D.K., TOJO, A., NARIKAWA, S., YOO, B.S., HOSOYAMADA, M., CHA, S.H., SEKINE, T. and ENDOU, H. 2001. **Characterization of ochratoxin A transport by human organic anion transporters**. Life Sciences **69**: 2123–2135.

The characteristics of OA transport by multispecific human organic anion transporters (hOAT1 and hOAT3, respectively) using the second segment of proximal tubule (S-2) cells from mice stably expressing hOAT1 and hOAT3 (S-2 hOAT1 and S-2 hOAT3) were investigated. S-2 hOAT1 and S-2 hOAT3 exhibited a time- and dose-dependent, and a saturable increase in uptake of [^{3}H]-OA. The inhibition of OA uptake by several substrates for the OATs were examined. Results indicate that hOAT1, as well as hOAT3, mediates a high-affinity transport of OA on the basolateral side of the proximal tubule, but hOAT1- and hOAT3-mediated OA transport are differently influenced by the substrates for the OATs.

Ochratoxins – Methodology

VISCONTI, A., PASCALE, M. and CENTONZE, G. 2001. **Determination of ochratoxin A in wine and beer by immunoaffinity column cleanup and liquid chromatographic analysis with fluorometric detection: Collaborative study**. Journal of AOAC International **84**: 1818–1827.

The accuracy, repeatability and reproducibility characteristics of an LC method for the determination of OA in white wine, red wine and beer were established in a collaborative study involving 18 laboratories in 10 countries. Samples were diluted with a solution containing polyethylene glycol and sodium hydrogen carbonate, and the diluted samples were filtered and cleaned up on an immunoaffinity column. OA was eluted with methanol and quantified by reversed phase LC with fluorometric detection. Average recoveries from white wine, red wine and beer ranged from 88.2 to 105.4%, 84.3 to 93.1% and 87.0 to 95.0%, respectively. Relative standard deviations for within-laboratory repeatability (RSD_r) for white wine, red wine and beer ranged from 6.6 to 10.8%, 6.5 to 10.8% and 4.7 to 16.5%, respectively. HORRAT values were less than or equal to 0.4 for the 3 matrixes.

PASCALE, M. and VISCONTI, A. 2001. **Rapid method for the determination of ochratoxin A in urine by immunoaffinity column clean-up and high-performance liquid chromatography**. Mycopathologia **152**: 91–95.

A rapid and accurate method to quantify OA at ng/L levels in urine has been developed. The method uses commercial immunoaffinity columns for cleanup and reversed phase HPLC with fluorescence detector for quantification. Average recoveries of OA from human urine spiked at levels from 0.05 to 1.0 mg/L ranged from 88% to 93%, with relative standard deviations (RSDs) between 1 and 8%. The detection limit was 0.005 mg/L. Of 41 human urine samples, 25 were found positive to OA.

SIBANDA, L., DESAEGER, S., BAUTERS, T.G.M., NELIS, H.J. and VANPETEGHEM, C. 2001. **Development of a flow-through enzyme immunoassay and application in screening green coffee samples for ochratoxin A with confirmation by high-performance liquid chromatography**. Journal of Food Protection **64**: 1597–1602.

A flow through enzyme immunoassay for the screening of green coffee bean for OA has been developed. The test has a sensitivity of 8 mg/kg, and calculated recoveries ranged from 70 to 89% and from 86 to 95% for spiked and naturally contaminated samples, respectively. Green coffee samples (15 Arabica and 7 Robusta) were analysed for intrinsic fungal contamination, screened for OA, and subsequently confirmed by HPLC. Results of the screening procedure found that 4/22 samples were contaminated with OA at 8 mg/kg or higher. The HPLC method found that OA levels ranged from 27 to 168 mg/kg. A fifth sample, which was shown to be negative during screening, had an OA concentration of 4 mg/kg. There were no false negatives or positives recorded and the flow-through enzyme immunoassay results correlated with those obtained by HPLC.

BURDASPAL, P., LEGARDA, T.M. and GILBERT, J. 2001. **Determination of ochratoxin A in baby food by immunoaffinity column cleanup with liquid chromatography: Interlaboratory study**. Journal of AOAC International **84**: 1445–1452.

An interlaboratory study (13 laboratories in eight different European countries) was performed to evaluate the effectiveness of an immunoaffinity column cleanup LC method for the determination of OA in baby food at a possible future European regulatory limit of 0.1 mg/kg. The test portion is extracted with tert-butyl methyl ether after addition of a phosphoric acid-sodium chloride solution. The extract is centrifuged and redissolved in a mixture of phosphate buffered saline solution and methanol. After removal of lypophilic substances with hexane, the extract is applied to an immunoaffinity column containing antibodies specific to OA. The column is washed with water and the purified OA is eluted with methanol. The separation and determination of OA is performed by reversed phase LC and detected by fluorescence after postcolumn derivatisation with ammonia. The average recovery for the spiked blank baby food was 108%. The relative standard deviation for repeatability (RSDr) ranged from 18 to 36%. The relative standard deviation for reproducibility (RSDr) ranged from 29 to 63% and HORRAT values of between 0.4 and 0.9 were obtained.

Ochratoxicoses

O'BRIEN, E., HEUSSNER, A.H. and DIETRICH, D.R. 2001. **Species-, sex-, and cell type-specific effects of ochratoxin A and B**. Toxicological Sciences **63**: 256–264.

Assessment of the toxic effects of OA including antiproliferative, apoptotic and necrotic effects, in rat and porcine continuous cell lines and in primary cells from humans and pigs of both sexes, have displayed a similar sex- and species-sensitivity rank order to that observed in previous *in vivo* experiments. These toxic effects were observed at nM concentrations in the presence of serum *in vitro*, thus closely mimicking the *in vivo* situation. These effects were reversible in all cell types except in human primary epithelial cells of both sexes and did not appear to be primarily dependent on the amount of OA taken up. The results presented here support the continued use of primary renal epithelial cells for the investigation of the mechanism of OA induced carcinogenesis and nephropathy and provide an as-yet preliminary data set that supports the existence of a causal relationship between OA exposure and human nephropathy.

MARESCA, M., MAHFOUD, R., PFOHL-LESZKOWICZ, A. and FANTINI, J. 2001. **The mycotoxin ochratoxin A alters intestinal barrier and absorption functions but has no effect on chloride secretion**. Toxicology and Applied Pharmacology **176**: 54–63.

Two human epithelial intestinal cell lines, HT-29-D4 and Caco-2-14 cells, widely used as *in vitro* models for the intestinal epithelium, were incubated with OA. The main effects observed were an inhibition of cellular growth and a dramatic decrease of transepithelial resistance in both cell lines. Since transepithelial resistance reflects the organisation of tight junctions over the cell monolayer, these data may suggest that OA could potentiate its own absorption through paracellular pathways. OA induced a 60% decrease of sodium-dependent glucose absorption but increased the absorption of fructose and L-serine in HT-29-D4 cells. Moreover, the mycotoxin did not inhibit the cAMP-dependent chloride secretion through the cystic fibrosis transmembrane conductance regulator channel.

MEKI, A.R.M.A. and HUSSEIN, A.A.A. 2001. **Melatonin reduces oxidative stress induced by ochratoxin A in rat liver and kidney**. Comparative Biochemistry and Physiology C – Toxicology & Pharmacology **130**: 305–313.

Rats were administered melatonin at 5 mg/kg bw or OA at 250 mg/kg bw, singly and in combination. After 4 weeks of treatment the levels of malondialdehyde, a lipid peroxidation product (LPO) were measured in serum and homogenates of liver and kidney. In OA treated rats, the levels of LPO in serum and in both liver and kidney were significantly increased compared to levels in controls. Concomitantly, the levels of glutathione and enzyme activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in both liver and kidney were significantly decreased in comparison with controls. In rats receiving melatonin plus OA, melatonin showed a protective effect against OA toxicity through an inhibition of the oxidative damage and stimulation of GST activities.

Fumonisins – General

DOWD, P.F. 2001. **Biotic and abiotic factors limiting efficacy of Bt corn in indirectly reducing mycotoxin levels in commercial field**s. Journal of Economic Entomology **94**: 1067–1074.

Incidence of insect damage and association of insect damage with mycotoxigenic fungi and mycotoxins was examined in commercial fields of Bt and non-Bt hybrids of different backgrounds. Levels of total fumonisins were generally less (15- to 1.8-fold) in Bt versus non-Bt hybrids at the same site, with some significant differences. There were several instances where there were no significant differences in fumonisin levels between low or no Bt kernel hybrids and Bt hybrids that produced high levels of protein in the kernel and silk tissue. However, significant correlations were often noted between numbers of insect damaged kernels and total fumonisin levels, suggesting in these cases that reducing insect damage was still reducing fumonisin levels. Although reductions in fumonisins in Bt hybrids were more limited than reported in the past, planting the Bt hybrids still appears to be a useful method for indirectly reducing mycotoxins in corn ears.

MEISTER, U. 2001. **Investigations on the change of fumonisin content of maize during hydrothermal treatment of maize. Analysis by means of HPLC methods and ELISA**. European Food Research and Technology **213**: 187–193.

The effects of extrusion cooking, gelatinisation and cornflaking on the stability of fumonisins in artificially contaminated maize grits spiked with FB_1 and FB_2 at levels of 2 and 0.6 mg/kg, respectively, was investigated. All the samples showed significant decreases in the fumonisin levels. If analysed according to AOAC–HPLC method, cooking extrusion and gelatinisation reduced fumonisin levels to approximately 30–55%, cooking the grits for flaking to approximately 20–65%, and roasting the flakes to approximately 6–35% (depending on the selected technological parameters). With ELISA the fumonisin contents were 15–50% and after alkaline hydrolysis 19–380% higher than with the AOAC–HPLC method.

SOLFRIZZO, M., VISCONTI, A., AVANTAGGIATO, G., TORRES, A. and CHULZE, S. 2001. ***In vitro*** **and** ***in vivo*** **studies to assess the effectiveness of cholestyramine as a binding agent for fumonisins**. Mycopathologia **151**: 147–153.

Several adsorbent materials were tested at 1 g/L for their capacity to adsorb FB_1 from aqueous solutions. Cholestyramine showed the best adsorption capacity (85% from a solution containing FB_1 at 200 mg/L) followed by activated carbon (62%). Cholestyramine was tested *in vivo* to evaluate its capacity to reduce the bioavailability of fumonisins in rats fed diet contaminated with toxigenic *Fusarium verticillioides* culture material containing FB_1 plus FB_2 at 6 or 20 mg/kg with or without the addition of cholestyramine at 20 g/kg. The addition of cholestyramine consistently reduced the effect of fumonisins by reducing significantly both urinary and renal sphinganine/sphingosine ratios.

Fumonisins – Methodology

VISCONTI, A., SOLFRIZZO, M. and DEGIROLAMO, A. 2001. **Determination of fumonisins B_1 and B_2 in corn and corn flakes by liquid chromatography with immunoaffinity column cleanup: Collaborative study**. Journal of AOAC International **84**: 1828–1837.

An LC method for the determination of FB_1 and FB_2 in corn and corn flakes was collaboratively studied by 23 laboratories. The method involved double extraction with acetonitrile–methanol–water (25 + 25 + 50), cleanup through an immunoaffinity column and LC determination of the fumonisins after derivatisation with *o*-phthaldialdehyde. Relative standard deviations for the intralaboratory repeatability (RSD_r) of the corn analyses for FB_1 and FB_2 ranged from 19 to 24 and 19 to 27%, respectively, and for corn flakes from 9 to 21% and 8 to 22%, respectively. Mean recoveries of FB_1 and FB_2 from spiked corn were 76 and 72%, respectively, and for corn flakes were 110 and 97%, respectively. HORRAT ratios for corn ranged from 1.44 to 1.53 for FB_1 and from 0.96 to 1.48 for FB_2, whereas for corn flakes they ranged from 1.60 to 1.82 and 1.39 to 1.68, respectively.

Fumonisins – Toxicology

ABNET, C.C., BORKOWF, C.B., QIAO, Y.L., ALBERT, P.S., WANG, E., MERRILL, A.H., MARK, S.D., DONG, Z.W., TAYLOR, P.R. and DAWSEY, S.M. 2001. **Sphingolipids as biomarkers of fumonisin exposure and risk of esophageal squamous cell carcinoma in China**. Cancer Causes & Control **12**: 821–828.

Ecologic studies of oesophageal squamous cell carcinoma (ESCC) have reported an association with consumption of maize contaminated with *Fusarium verticillioides*, which produces fumonisins. A prospective nested case-control study to examine the relationship between serum sphingolipids and ESCC incidence was conducted in Linxian, People's Republic of China among 98 cases of ESCC and 185 randomly selected controls. No significant associations were found between serum sphingosine (So), sphinganine (Sa) or the Sa/So ratio and ESCC incidence in conditional and unconditional logistic regression models with adjustment for age, sex, tobacco use and alcohol use.

NORRED, W.P., RILEY, R.T., MEREDITH, F.I., POLING, S.M. and PLATTNER, R.D. 2001. **Instability of *N*-acetylated fumonisin B_1 (FA_1) and the impact on inhibition of ceramide synthase in rat liver slices**. Food and Chemical Toxicology **39**: 1071–1078.

N-acetylated FB_1 (FA_1) has been reported previously to prevent ceramide synthase inhibition by FB_1, but impure

preparations of FA_1 can contain a contaminant with the ability to inhibit ceramide synthase. It has now been shown that FA_1 spontaneously rearranges to O-acetylated analogues. These rearrangement products are putative inhibitors of ceramide synthase. Rat liver slices exposed to impure FA_1 containing O-acetylated FB_1 had Sa/So ratios of 1.15–1.64. Control slices had Sa/So ratios of 0.07–0.24. Cleanup to remove the O-acetylated FB_1 yielded purified FA_1 which produced Sa/So ratios in liver slices of 0.08–0.18. After storage for approximately one year as either a dry powder in a desiccator, or as a dried film at 4°C, the purified FA_1 again contained O-acetylated FB_1, and was capable of ceramide synthase inhibition. As FA_1 is considerably less cytotoxic than FB_1, these results provide additional support for the conclusion that a primary amino group is necessary for both ceramide synthase inhibition and toxicity.

BHANDARI, N., HE, Q.R. and SHARMA, R.P. 2001. **Gender-related differences in subacute fumonisin B_1 hepatotoxicity in BALB/c mice**. Toxicology **165**: 195–204.

Male and female BALB/c mice were injected sc with FB_1 at 2.25 mg/kg/day for 5 days. One day after the last injection, females showed a greater increase in circulating alanine aminotransferase and greater number of apoptotic cells in liver than males, indicating greater hepatotoxicity. Peripheral leukocytic counts, including neutrophils, were increased in females but not in males. The increased toxicity in females correlated with a greater increase of Sa and So levels in liver compared to males. In males, FB_1 treatment caused increased expression of TNF alpha, IL-12 p40, interferon gamma (IFN gamma), IL-1 beta, IL-6 and IL-10. In females, FB_1 treatment caused an increased expression of IL-6 only, and a downward modulation of IFN gamma, indicating gender differences in cytokine pathways in liver activated by FB_1.

JOHNSON, V.J. and SHARMA, R.P. 2001. **Gender-dependent immunosuppression following subacute exposure to fumonisin B_1**. International Immunopharmacology **1**: 2023–2034.

Female and male mice BALB/c mice received five daily sc injections of FB_1 at 2.25 mg/kg/day and on the following day tissues were collected for immunological examinations. FB_1 treatment dramatically reduced relative spleen and thymus weights in females, whereas there was no effect on organ weights in males. Exposure to FB_1 reduced splenic cellularity and the basal rate of lymphocyte proliferation in females only. In addition, phytohemagglutinin-induced T-lymphocyte and LPS-induced B-lymphocyte proliferation were reduced in female mice. Splenocytes from female mice showed a reduced expression of IL-2 mRNA. These changes occurred in the absence of alterations in tumor necrosis factor alpha or IL-1 beta mRNA expression.

KIM, M.S., LEE, D.Y., WANG, T. and SCHROEDER, J.J. 2001. **Fumonisin B_1 induces apoptosis in LLC-PK1 renal epithelial cells via a sphinganine- and calmodulin-dependent pathway**. Toxicology and Applied Pharmacology **176**: 118–126.

FB_1 applied to LLC-PK1 renal kidney epithelial cells produced morphological changes and time-dependent increases in DNA fragmentation demonstrating that the toxin induces apoptosis. Simultaneously, FB_1 blocked sphingolipid biosynthesis and caused accumulation of Sa. The role of Sa in FB_1-induced apoptosis was further investigated using beta-fluoroalanine (betaFA) to inhibit serine palmitoyltransferase, which catalyses an earlier step in the sphingolipid biosynthetic pathway. betaFA blocked Sa accumulation and prevented FB_1-induced DNA fragmentation, confirming that apoptosis induced by FB_1 is dependent upon accumulation of Sa. To examine gene expression, differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) was applied to RNA isolated after 16 hr of exposure to FB_1. Differential expression in response to FB_1 of a gene identified as calmodulin was verified by Northern analysis. Sa appears to mediate the effect because betaFA reduces induction of calmodulin mRNA by FB_1. The results establish a sequence of events for FB_1-induced apoptosis involving initial disruption of sphingolipid metabolism and accumulation of Sa (or a metabolite), which, in turn, induces expression of calmodulin.

Trichothecenes – General

AL-JULAIFI, M.Z. and AL-FALIH, A.M. 2001. **Detection of trichothecenes in animal feeds and foodstuffs during the years 1997 to 2000 in Saudi Arabia**. Journal of Food Protection **64**: 1603–1606.

A total of 843 commercial animal feed and foodstuff samples from all over the Kingdom of Saudi Arabia were collected during the years 1997 to 2000 and analysed for type A and type B trichothecenes. Levels of DON ranged from <2 to 4,000 mg/kg, fusarenon-X from 3.25 to 500 mg/kg, nivalenol from 3.13 to 600 mg/kg, diacetoxyscirpenol from 3.13 to 50 mg/kg, neosolaniol from 6.25 to 200 mg/kg, HT-2 toxin from 3.13 to 18.75 mg/kg and T-2 toxin 6.25 mg/kg.

ESKOLA, M., RIZZO, A. and SOUPAS, L. 2001. **Occurrence and amounts of some *Fusarium* toxins in Finnish cereal samples in 1998**. Acta Agriculturae Scandinavica Section B – Soil and Plant Science **50**: 183–186.

The amounts of Fusarium toxins in Finnish cereals, particularly wheat intended for human consumption, were determined. Although the growing season of 1998 was very rainy, cool and favourable for fungi production, the amounts of Fusarium toxins in the samples were low. Among 47 cereal samples the mean concentrations of DON, 3-acetylDON, nivalenol, HT-2 toxin and ZEA in contaminated samples were 60, 30, 42, 43 and 5 mg/kg, respectively. T-2 toxin was detected only once (23 mg/kg) and fusarenon-X and diacetoxyscirpenol were not detected. However, 85% of the analysed cereal samples were contaminated with Fusarium toxins.

KEBLYS, M., FLAOYEN, A. and LANGSETH, W. 2001. **The occurrence of type A and B trichothecenes in Lithuanian cereals**. Acta Agriculturae Scandinavica Section B – Soil and Plant Science **50**: 155–160.

Samples of winter wheat (n = 84), winter rye (46) and barley (29) were collected from the larger family farms and from partnerships in Lithuania just after the 1998 harvest. The levels of the *Fusarium* toxins DON, 3-acetylDON, 15-acetylDON, nivalenol (NIV), fusarenon-X, T-2 toxin, HT-2 toxin, 4,5-diacetoxyscirpenol, 1,5-monoacetoxyscirpenol and scirpentriol in the grain were determined by GC-MS. DON was most often detected in the wheat and rye samples and NIV in the barley samples. The concentrations found were lower than those causing acute or chronic toxic effects in livestock or humans. No fusarenon-X or 15-acetylDON was detected, and only small amounts of other trichothecenes were present.

MARTINS, M.L. and MARTINS, H.M. 2001. **Determination of deoxynivalenol in wheat-based breakfast cereals marketed in Portugal**. Journal of Food Protection **64**: 1848–1850.

A total of 88 commercially available samples of wheat based breakfast cereals were randomly collected from different supermarkets in Lisbon, Portugal. The samples were analysed using immunoaffinity column, and DON was quantified by LC. The detection limit was 100 mg/kg. Average recovery of DON was 80%. Of 88 analysed samples, 72.8% contained levels of DON between 103 and 6040 mg/kg, with mean level of 754 mg/kg.

SCHAAFSMA, A.W., TAMBURIC-ILINIC, L., MILLER, J.D. and HOOKER, D.C. 2001. **Agronomic considerations for reducing deoxynivalenol in wheat grain**. Canadian Journal of Plant Pathology **23**: 279–285.

Wheat fields under an array of agronomic practices were studied during harvest across southern and eastern Ontario. Mature wheat grain samples were harvested by hand and analysed for DON. The amount of variation in DON levels associated with year and agronomic effects was calculated from simple linear models. The largest factor associated with variation in DON levels was the year. Year effects accounted for 48% of the variation in DON levels across all fields during four years of the survey, followed by cultivar (27%), and the crop one year previous to wheat (14–28% depending on the year). No effect on DON could be detected from other agronomic factors including tillage system, crops planted three years before wheat, or type of nitrogen fertiliser applied in the spring.

EUDES, F., COMEAU, A., RIOUX, S. and COLLIN, J. 2001. **Impact of trichothecenes on *Fusarium* head blight (*Fusarium graminearum*) development in spring wheat (*Triticum aestivum*)**. Canadian Journal of Plant Pathology **23**: 318–322.

Fusarium head blight pathogens produce trichothecenes that have been demonstrated to play a role in the pathogenesis. The impact of trichothecenes on a broad range of genotypes was tested in 18 spring wheat lines (*Triticum aestivum*) inoculated with two *Fusarium graminearum* strains, the genetically modified GzT40 strain, which could not produce trichothecenes, and the wild parental Gz3639 strain. The two fungal strains showed extreme differences in aggressiveness in all but three of the wheat genotypes tested. While the GzT40 mutant did not spread into the rachis, the wild-type strain quickly spread in the spike, supporting earlier findings that trichothecenes are a principal determinant of *F. graminearum* aggressiveness on most spring wheat cultivars.

BAKAN, B., PINSON, L., CAHAGNIER, B., MELCION, D., SEMON, E. and RICHARD-MOLARD, D. 2001. **Toxigenic potential of *Fusarium culmorum* strains isolated from French wheat**. Food Additives and Contaminants **18**: 998–1003.

Sixty *Fusarium culmorum* strains were isolated from wheat grains collected from different wheat growing areas in France and from different cultivars. The isolates were grown on autoclaved wheat grain to assess their ability to produce trichothecenes and ZEA. All the isolates produced ZEA in the range 0.39–1660 mg/kg. Thirty-five of 60 strains produced NIV, 12/60 produced fusarenon-X, 5/60 produced 15-acetylDON, 13/60 produced 3-acetylDON and 24/60 produced DON. The distribution of the different chemotypes as well as the high and the low mycotoxin producing *Fusarium* strains could not be associated to geographical origin.

WIDESTRAND, J. and PETTERSSON, H. 2001. **Effect of time, temperature and solvent on the stability of T-2 toxin, HT-2 toxin, deoxynivalenol and nivalenol calibrants**. Food Additives and Contaminants **18**: 987–992.

The influence of solvent, storage time and temperature on the stability of T-2 toxin, HT-2 toxin, DON and NIV was investigated. Toxins in acetonitrile, ethyl acetate or as thin film were stored in sealed glass ampoules at –18, 4, 25 and 40°C for up to 24 months. The results show that acetonitrile was the preferred solvent and no significant decomposition occurred for any of the four trichothecenes when stored for 24 months at 25°C or 3 months at 40°C. T-2 toxin and HT-2 toxin in ethyl acetate or as thin film were also stable under the same conditions. DON and NIV in ethyl acetate or as thin film were stable for up to 24 months at –18°C. There was significant decomposition of DON and NIV in ethyl acetate was observed after 24 months at 4°C and after 12 months at 25°C.

SAMAR, M.M., NEIRA, M.S., RESNIK, S.L. and PACIN, A. 2001. **Effect of fermentation on naturally occurring deoxynivalenol (DON) in Argentinean bread processing technology**. Food Additives and Contaminants **18**: 1004–1010.

The stability of naturally occurring DON was evaluated during the fermentation stage of the bread-making process on a pilot scale. French bread and Vienna bread were prepared with wheat flour naturally contaminated with DON at 150 mg/kg. Dough was fermented at 30, 40 and 50°C according to the standard procedures employed in Argentinean low-technology bakeries. When the dough was fermented at 50°C, the maximum reduction in DON was 56% for the Vienna bread and 41% for French bread.

LAUREN, D.R. and SMITH, W.A. 2001. **Stability of the *Fusarium* mycotoxins nivalenol, deoxynivalenol and zearalenone in ground maize under typical cooking environments**. Food Additives and Contaminants **18**: 1011–1016.

The effects of moisture, pH and heat on the stability of NIV, DON and ZEA in naturally contaminated ground maize were measured for different periods. Standard solution tests to measure pH, salt and temperature effects on NIV and DON were also performed. The solution tests showed NIV and DON to be relatively stable in buffer solutions over the pH range 1–10. Conditions of pH 12, high salt concentration, 80°C and prolonged exposure were needed to give substantial breakdown. In the ground maize substrate, these toxins were further stabilised relative to the solution tests. NIV and DON were both reduced (range 60–100%) by treatment with aqueous bicarbonate solution at 10, 20 or 50% of the ground maize dry weight, and subsequent heating at 80 or 110°C for 2 and 12 days. ZEA content was not reduced even by 12 days of heating at 110°C after treatment with a sodium bicarbonate solution.

KANG, Z., HUANG, L.L. and BUCHENAUER, H. 2001. **Ultrastructural and cytochemical studies of effects of the fungicide metconazole on *Fusarium culmorum in vitro***. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz – Journal of Plant Diseases and Protection **108**: 419–432.

The effects of metconazole, a systemic fungicide from the triazol group, were examined on morphology, structure, cell wall components and toxin production of *Fusarium culmorum*. Immunocytochemical labelling with antiserum against DON revealed that *Fusarium* toxins, DON, 3-acetylDON and 15-acetylDON, are localised in the cell walls, cytoplasm, mito-

chondria and vacuoles of control and fungicide treated hyphae. However, the labelling density for the toxin in the fungicide treated hyphae was markedly lower compared to the control hyphae.

D'MELLO, J.P.F., MACDONALD, A.M.C. and BRIERE L. 2000. **Mycotoxin production in a carbendazim-resistant strain of *Fusarium sporotrichioides*.** Mycotoxin Research **16**: 101–111.

Mycelial yield and production of T-2 toxin, diacetoxyscirpenol (DAS) and neosolaniol (NEO) were compared in control and carbendazim resistant strains of *Fusarium sporotrichioides*. Each strain was exposed to carbendazim at 1, 2 and 4 mg/L media for 2, 5 and 7 days under shake culture conditions at 25°C. Carbendazim inhibited T-2 toxin production by control isolates in a dose related manner. In contrast, resistant strains exposed to carbendazim at 2 mg/L significantly increased T-2 toxin production. With carbendazim at 1 and 4 mg/L, T-2 toxin inhibition occurred but the effect was less marked than in the control series. DAS and NEO production were also higher in resistant than in control strains. Results suggest that carbendazim resistance induces changes in the terminal rather than initial phases of trichothecene biosynthesis.

D'MELLO, J.P.F., MACDONALD, A.M.C. and RINNA, R. 2001. **Effects of azoxystrobin on mycotoxin production in a carbendazim-resistant strain of *Fusarium sporotrichioides*.** Phytoparasitica 29: 431–440.

Carbendazim resistant and control strains of Fusarium sporotrichioides were exposed to graded concentrations of azoxystrobin at 1–100 mg/L under shake culture conditions at 25°C for 2, 3, 4 and 8 days. Significant strain-fungicide interactions were recorded in trichothecene production following exposure to azoxystrobin. At 4 and 8 days of incubation, azoxystrobin at 10 mg/L stimulated T-2 toxin synthesis only in resistant cultures. In contrast, T-2 toxin enhancement in control cultures occurred only on day 8 but at a lower level of azoxystrobin (1 mg/L). As with T-2 toxin, DAS production in control strains was stimulated only at low exposure levels of azoxystrobin. In the case of NEO, however, the main effect of strain was significant, with control strains producing consistently more of the mycotoxin than resistant strains on day 4 of the experiment. At this point, the NEO:T-2 toxin ratio was also higher in controls (0.63) than in resistant strains (0.12). The present investigation has shown for the first time that the development of resistance to one fungicide can affect trichothecene production in F sporotrichioides on exposure to a second fungicide.

MURAKAMI, Y., OKUDA, T. and SHINDO, K. 2001. **Roridin L, M and Verrucarin M, new macrocyclic trichothecene group antitumor antibiotics, from *Myrothecium verrucaria*.** Journal of Antibiotics **54**: 980–983.

Three novel macrocyclic trichothecene antibiotics, roridin L, M and verrucarin M, have been isolated from the culture broth of Myrothecium verrucaria. The fermentation, purification and structural elucidation are reported. The IC50 values against P388 (murine leukaemia) were 1.6, 4.6 and 4.8 mg/L, respectively.

TAG, A.G., GARIFULLINA, G.F., PEPLOW, A.W., AKE, C., PHILLIPS, T.D., HOHN, T.M. and BEREMAND, M.N. 2001. **A novel regulatory gene, *Tri10*, controls trichothecene toxin production and gene expression**. Applied and Environmental Microbiology **67**: 5294–5302.

The characterisation of *Tri10*, a novel regulatory gene within the trichothecene gene cluster, is reported. Disruption of *Tri10* in *Fusarium sporotrichioides* abolished T-2 toxin production and dramatically decreased the transcript accumulation for four trichothecene genes (*Tri4*, *Tri5*, *Tri6* and *Tri101*) and an apparent farnesyl pyrophosphate synthetase (Fpps) gene. Conversely, homologous integration of a disruption vector by a single upstream crossover event significantly increased T-2 toxin production and elevated the transcript accumulation of the trichothecene genes and Fpps. A model is suggested in which *Tri10* acts upstream of the cluster-encoded transcription factor TRI6 and is necessary for full expression of both the other trichothecene genes and the genes for the primary metabolic pathway that precedes the trichothecene biosynthetic pathway, as well as for wild-type levels of trichothecene self-protection.

ERMOLENKO, M.S. 2001. **A new carbohydrate-based synthetic approach to trichothecenes. Synthesis of a bicyclic BC core of verrucarol from D-galactose**. Tetrahedron Letters **42**: 6679–6682.

An advanced bicyclic BC intermediate towards the total synthesis of verrucarol has been prepared from *D*-galactose via an intramolecular aldehyde-allylstannane reaction.

Trichothecenes – Methodology

KRSKA, R., BAUMGARTNER, S. and JOSEPHS, R. 2001. **The state-of-the-art in the analysis of type-A and -B trichothecene mycotoxins in cereals**. Fresenius Journal of Analytical Chemistry **371**: 285–299.

A review with 119 references. Current screening tests and quantitative methods for the most prevalent type A and B trichothecenes, HT-2 and T-2-toxin as well as DON, are reviewed. Results from recent intercomparison studies of the determination of DON are also discussed. Experience gained during these intercomparisons clearly shows the need for further improvement in the determination of trichothecenes, to obtain more accurate and comparable results. For both A and B trichothecenes there is still a lack of simple and reliable screening methods enabling the rapid detection of these mycotoxins at low cost.

NIELSEN, K.F. and THRANE, U. 2001. **Fast methods for screening of trichothecenes in fungal cultures using gas chromatography-tandem mass spectrometry**. Journal of Chromatography A **929**: 75–87.

A fast method for trichothecene profiling and chemotaxonomic studies in species of *Fusarium*, *Stachybotrys*, *Trichoderma* and *Memnoniella* is presented. Micro scale extracted crude *Fusarium* extracts were derivatised using pentafluoropropionic anhydride and analysed by GC with simultaneous full scan and tandem mass spectrometric detection. It was possible to monitor up to four compounds simultaneously, making detection of acetyl T-2 toxin, T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, NEO, iso-NEO, scirpentriol, 4,15-DAS, 15-acetoxyscirpenol, 4-acetoxyscirpentriol, NIV, fusarenon-X, DON, 15-acetylDON and 3-acetylDON possible during a 23 minute GC run. A slightly modified method could detect trichothecenes produced by *Stachybotrys*, *Memnoniella* and *Trichoderma.*

FAZEKAS, B. and TAR, A. 2001. **Determination of zearalenone content in cereals and feedstuffs by immunoaffin-**

ity column coupled with liquid chromatography. Journal of AOAC International **84**: 1453–1459.

The ZEA content of maize, wheat, barley, swine feed and poultry feed samples was determined by immunoaffinity column cleanup followed by LC (IAC–LC). Samples were extracted in methanol–water (8 + 2, v/v) solution. The filtered extract was diluted with distilled water and applied to immunoaffinity columns. ZEA was eluted with methanol, dried by evaporation and dissolved in acetonitrile–water (3 + 7, v/v). ZEA was separated by isocratic elution of acetonitrile–water (50 + 50, v/v) on reversed phase C18 column. The quantitative analysis was performed by fluorescence detector and confirmation was based on the UV spectrum obtained by a diode array detector. The mean recovery rate of ZEA was 82–97%. The limit of detection of ZEA by fluorescence was 10 mg/kg. The specificity of the method was increased by using fluorescence detection in parallel with UV detection.

ESKOLA, M., BOONZAAIJER, G., VAN OSENBRUGGEN, W.A., RIZZO, A. and TIJMENSEN, G. 2000. **A study of the suitability of gas chromatography-electron capture detection for the analysis of deoxynivalenol in cereals**. Mycotoxin Research **16**: 73–90.

A GC-electron capture detection (GC-ECD) method for the analysis of DON in cereals was investigated. The sample was extracted with a mixture of acetonitrile–water and purified with a MycoSep #225 column. The silylation was performed with Tri-Sil-TBT reagent, followed by dilution with hexane and a washing step with buffer. By using Tri-Sil-TBT reagent, no double peaks were observed for DON in the gas chromatograms, in comparison with two other silylation reagents, TMSI and TriSil-Z. Trichothecolone was used as an internal standard for DON and 1,1-bis-(4-chlorophenyl)-2,2-dichloroethylene (DDE), was used as a GC standard.

Trichothecenes – Methodology

ALBARENQUE, S.M., SUZUKI, K., NAKAYAMA, H. and DOI, K. 2001. **Kinetics of cytokines mRNAs expression in the dorsal skin of WBN/ILA-Ht rats following topical application of T-2 toxin**. Experimental and Toxicologic Pathology **53**: 271–274.

The kinetics of cytokines mRNAs expression was examined in the dorsal skin of Wistar-derived hypotrichotic WBN/ILA-Ht rats topically applied with T-2 toxin. After application of T-2 toxin solution, the total mRNA was obtained from skin biopsies at 3–24 hr after treatment. Reverse transcription-polymerase chain reaction was carried out with pairs of oligonucleotide primers corresponding to the cDNA sequences of rat tumor necrosis factor alpha (TNF-alpha), IL-1 alpha, IL-1 beta, IL-6 and IL-10 cytokines. The level of TNF-alpha mRNA showed marked elevation at 3 hr and decreased toward 24 hr, but it remained significantly higher even at 24 hr. In addition, the level of IL-1 beta mRNA expression showed a slight but significant elevation at 3 and 24 hr. No significant differences were observed in other cytokines mRNAs expression.

NAGATA, T., SUZUKI, H., ISHIGAMI, N., SHINOZUKA, J., UETSUKA, K., NAKAYAMA, H. and DOI, K.N. 2001. **Development of apoptosis and changes in lymphocyte subsets in thymus, mesenteric lymph nodes and PEYER'S patches of mice orally inoculated with T-2 toxin**. Experimental and Toxicologic Pathology **53**: 309–315.

Development of apoptosis and changes in lymphocyte subsets were examined mainly by flow cytometer in thymus, mesenteric lymph nodes and PEYER'S patches of mice up to 24 hr after oral inoculation with T-2 toxin at 10 mg/kg. The degree of lymphocyte apoptosis was prominent in the thymus, moderate in the PEYER'S patches and somewhat mild in the mesenteric lymph nodes, suggesting the difference in lymphocyte population susceptible to T-2 toxin. In lymphocyte subsets, CD4(+) CD8(+) T cells were most sensitive to T-2 toxin, and CD4(+) CD8(–) T cells were more severely depressed than CD4(–) CD8(+) T cells in the thymus. In the mesenteric lymph nodes, CD3(+) cells was more clearly affected than CD19(+) cells, and the numbers of CD4(+) and CD8(+) cells were similarly decreased. In the PEYER'S patches, the numbers of CD3(+), CD19(+), CD4(+) and CD8(+) cells were unexceptionally decreased.

NAGASE, M., ALAM, M.M., TSUSHIMA, A., YOSHIZAWA, T. and SAKATO, N. 2001. **Apoptosis induction by T-2 toxin: Activation of caspase-9, caspase-3, and DFF-40/CAD through cytosolic release of cytochrome c in HL-60 cells**. Bioscience Biotechnology and Biochemistry **65**: 1741–1747.

In human leukaemia HL60 cells the rank order of the potency of trichothecene mycotoxins to induce internucleosomal DNA fragmentation was found to be T-2 toxin, satratoxin G, roridin A >> DAS > baccharin B-5 >> NIV, DON, 3-acetyl-DON, fusarenon-X, baccharin B-4 = control. Western blot analysis of caspase-3 in T-2 toxin treated cells clearly indicated the appearance of its catalytically active fragment of 17-kDa. Next, cells exposed to T-2 toxin led to cleavage of PARP from its native 116-kDa form to the 85-kDa product. Moreover, DFF-45/ICAD were cleaved to give a 12.5-kDa fragment via T-2 treatment. These data indicate that T-2 toxin induced apoptosis involves activation of caspase-3 and DFF-40/CAD through cytosolic accumulation of cytochrome c along with caspase-9 activation.

JUHASZ, J., NAGY, P., REICZIGEL, J., KULCSAR, M., SZIGETI, G. and HUSZENICZA, G. 2001. [**Effect of certain mycotoxins contaminating feedstuff (zearalenone, T-2 toxin) and dexamethasone on the reproductive function of horses. Summary report of a research program**]. Magyar Allatorvosok Lapja **123**: 475–482.

After an entire oestrous cycle, mares were given 7 mg of ZEA per os daily or 7 mg of T-2 toxin per os daily. Toxin administration had no effect on the length of the interovulatory interval, the luteal and follicular phases. ZEA did not influence significantly peripheral plasma progesterone profiles, follicular activity and uterine oedema. T-2 toxins had no effect on peripheral plasma progesterone profiles and on follicular kinetics. (In Hungarian).

BERNHOFT, A., BEHRENS, G.H.G., INGEBRIGTSEN, K., LANGSETH, W., BERNDT, S., HAUGEN, T.B. and GROTMOL, T. 2001. **Placental transfer of the estrogenic mycotoxin zearalenone in rats**. Reproductive Toxicology **15**: 545–550.

Sprague Dawley rats were treated with a single dose of ZEA at 0.74 mg/kg bw on day 12 or day 18 of pregnancy, or intragastrically on day 18 of pregnancy. Samples of placenta, foetus and maternal liver and spleen were collected for chemical analyses. Tissue distribution of ZEA was studied by means of whole body autoradiography at 4 and 24 hr after treatment with tritiated ZEA. ZEA and alpha-zearalenol were transferred into the foetus

on both gestational days. However, a delay in distribution into the foetus, relative to the maternal tissue, was observed. beta-Zearalenol was below the detection limit in the foetus. No specific site of foetal accumulation of ZEA or its metabolites was apparent. In the maternal tissues, the highest levels of ZEA and of alpha- and beta-zearalenol were found in the liver.

MINERVINI, F., DELLAQUILA, M.E., MARITATO, F., MINOIA, P. and VISCONTI, A. 2001. **Toxic effects of the mycotoxin zearalenone and its derivatives on *in vitro* maturation of bovine oocytes and 17 beta-estradiol levels in mural granulosa cell cu**ltures. Toxicology In Vitro **15**: 489–495.

ZEA, alpha-zearalenol and zearalanone were tested, at levels ranging from 0.3 to 30 mg/L, in order to evaluate their effects on the *in vitro* maturation (IVM) rate of bovine oocytes and on the formation of 17 beta-estradiol in supernatants of mural granulosa cell cultures. Maturation of oocytes to metaphase II was inhibited in oocytes cultured in the presence of each toxin at 30 mg/L, with a significant increase in chromatin abnormalities occurring in the presence of ZEA and alpha-zearalenol. In preliminary trials on 17 beta-estradiol formation at the same testing concentration, higher levels of 17 beta-estradiol were found in the presence of alpha-zearalenol with respect to ZEA and zearalanone.

Aflatoxins – General

OTSUKI, T., WILSON, J.S. and SEWADEH, M. 2001. **What price precaution? European harmonisation of aflatoxin regulations and African groundnut exports**. European Review of Agricultural Economics **28**: 263–283.

The effects of varying regulatory standards on trade in agricultural products are at the forefront of policy debate. A gravity model was employed to estimate the impact of changes in aflatoxin standards on trade flows of groundnut products using trade data for Europe and Africa during 1989–1998. Results suggest that a new European Union regulation on aflatoxins will result in a trade flow that is 63% lower than when the Codex Alimentarius international standards are followed.

OTSUKI, T., WILSON, J.S. and SEWADEH, M. 2001. **Saving two in a billion: Quantifying the trade effect of European food safety standards on African exports**. Food Policy **26**: 495–514.

A gravity model was used to estimate the impact of changes caused by a new harmonised aflatoxin standard set by the EU on food exports from Africa. The analysis is based on trade and regulatory survey data for 15 European countries and nine African countries between 1989 and 1998. Our results suggest that the implementation of the new aflatoxin standard in the EU will have a negative impact on African exports of cereals, dried fruits and nuts to Europe. The new EU standard, which would reduce health risk by approximately 1.4 deaths per billion a year, will decrease these African exports by 64% or US$670 million, in contrast to regulation set through an international standard.

STENZEL, W.R. 2001. [**Hygienic risks caused by chemico-physical contaminants in food citing aflatoxins as an example**]. Archiv fur Lebensmittelhygiene **52**: 97–100.

Due to changed conditions in food production, risk analysis has become an essential requirement. Using the example of aflatoxin contamination of nonroasted peanuts from Mozambique, it is shown that only the strict elimination of these mycotoxins from the food chain results in safe products. (In German).

EI-SAYED, A.M.A.A., NEAMATALLAH, A.A. and SOHER, E.A. 2000. **Situation of mycotoxins in milk, dairy products and human milk in Egypt**. Mycotoxin Research **16**: 91–100.

Milk and dairy products purchased at Egyptian markets and breast milk from lactating mothers in Cairo and Giza governorates were analysed for some mycotoxins. Three of 15 cows milk samples were found positive for AFM1 with mean value 6.3 mg/kg. Two of 10 hard cheese samples contained detectable levels of AFM1 and one sample contained AFB1 and AFG1. For breast milk, 2/10 samples were positive for AFM1 with mean value 2.75 mg/kg, while 3/10 samples were positive for OA.

HUSEYIN, H. and SONAL, S. 2001. **Determination of aflatoxin M_1 levels in cheese and milk consumed in Bursa, Turkey**. Veterinary and Human Toxicology **43**: 292–293.

AFM_1 was determined by ELISA in 57 cheese and 10 milk samples collected from supermarkets and street milkmen in Bursa Province, Turkey. The highest AFM_1 concentration found was 810 ng/kg in full fatty white cheese. The incidence of AFM_1 in cheese was 89.47% and in milk it was 10%. The level of AFM_1 in 7/87 cheese samples exceeded the Turkish AFM_1 tolerance limits of 250 ng/kg, but none of the milk samples exceeded the FAO/WHO European Union and Turkish tolerance limit of 50 ng/L.

SRIVASTAVA, V.P., BU-ABBAS, A., ALAA-BASUNY, AL-JOHAR, W., AL-MUFTI, S. and SIDDIQUI, M.K.J. 2001. **Aflatoxin M_1 contamination in commercial samples of milk and dairy products in Kuwait**. Food Additives and Contaminants **18**: 993–997.

Fifty-four samples of fresh full cream and skimmed milk, powdered milk, yoghurt and infant formula collected in Kuwait were analysed for AFM1. Among the samples, 28% were contaminated with AFM1 with 6% being above the maximum permissible limit of 0.2 mg/L. Three fresh cow milk samples collected from a private local producer showed the highest level of 0.21 mg/L. There was no contamination with AFM1 in powdered milk and infant formulas.

PELTONEN, K., EL-NEZAMI, H., HASKARD, C., AHOKAS, J. and SALMINEN, S. 2001. **Aflatoxin B_1 binding by dairy strains of lactic acid bacteria and bifidobacteria**. Journal of Dairy Science **84**: 2152–2156.

The binding of AFB_1 from contaminated solution by 20 strains of lactic acid bacteria and bifidobacteria was studied. The selected strains are used in the food industry and comprised 12 *Lactobacillus*, five *Bifidobacterium* and three *Lactococcus* strains. Bacteria and AFB_1 were incubated for 24 hr at 37°C and the amount of unbound AFB1 was quantitated by HPLC. Between 5.6 and 59.7% AFB_1 was bound from solution by these strains. Two *Lactobacillus amylovorus* strains and one *Lactobacillus rhamnosus* strain removed more than 50% AFB_1. Binding was reversible and AFB_1 was released by repeated aqueous washes.

CHEN, Z.Y., BROWN, R.L., CLEVELAND, T.E., DAMANN, K.E. and RUSSIN, J.S. 2001. **Comparison of constitutive and inducible maize kernel proteins of genotypes resistant or susceptible to aflatoxin production**. Journal of Food Protection **64**: 1785–1792.

Maize genotypes resistant or susceptible to aflatoxin production or contamination were compared for differences in both constitutive and inducible proteins. Five additional constitutive proteins were found to be associated with resistance in over 8 of the 10 genotypes examined. Among these, the 58- and 46-kDa proteins were identified as globulin-1 and globulin-2, respectively. Differences in the ability to induce specific antifungal proteins, such as the higher synthesis of the 22-kDa zeamatin in resistant genotypes, were also observed between resistant and susceptible kernels. Both constitutive and inducible proteins appear to be necessary for kernel resistance.

GEMBEH, S.V., BROWN, R.L., GRIMM, C. and CLEVELAND, T.E. 2001. **Identification of chemical components of corn kernel pericarp wax associated with resistance to *Aspergillus flavus* infection and aflatoxin production**. Journal of Agricultural and Food Chemistry **49**: 4635–4641.

Kernel pericarp wax of the corn breeding population GT-MAS:gk has been associated with resistance to *Aspergillus flavus* infection and aflatoxin production. GT-MAS:gk wax was compared to wax of a group of susceptible lines. Wax separation by TLC demonstrated a unique GT-MAS:gk band and a unique "susceptible" band. Only GT-MAS:gk wax inhibited the growth of *A. flavus.* GC–MS analysis of kernel whole wax showed a higher percentage of phenol-like compounds in wax from GT-MAS:gk than in waxes from the susceptible lines. The GT-MAS:gk unique band contained phenol-like compounds and ethyl-hexadecanoate. Butyl-hexadecanoate was pre-eminent in most of the "susceptible bands".

GUO, B.Z., LI, R.G., WIDSTROM, N.W., LYNCH, R.E. and CLEVELAND, T.E. 2001. **Genetic variation within maize population GT-MAS:gk and the relationship with resistance to *Aspergillus flavus* and aflatoxin production**. Theoretical and Applied Genetics **103**: 533–539.

Maize population GT-MAS:gk has been released for use as a resistance source for *Aspergillus flavus* infection and aflatoxin production. The genetic variation in this population was surveyed using RAPD analysis. This study showed that the maize population is heterogeneous and individuals are different in resistance to *A. flavus* and aflatoxin production. Resistance should be confirmed through progeny testing before further development.

HENKE, S.E., GALLARDO, V.C., MARTINEZ, B. and BAILEY, R. 2001. **Survey of aflatoxin concentrations in wild bird seed purchased in Texas**. Journal of Wildlife Diseases **37**: 831–835.

Bags of wild bird seed (n = 142) were purchased from grain cooperatives, grocery stores and pet shops from various locations in Texas during spring and summer 1999. Aflatoxin concentrations in bird seed ranged from undetectable to 2,780 mg/kg. Overall, 17% of samples had aflatoxin concentrations greater than 100 mg/kg, of which 83% contained corn as an ingredient. Average concentrations of aflatoxin were greater from bags of bird seed purchased from grain cooperatives, followed by pet shops then grocery stores.

PARK, D.L. and PRICE, W.D. 2001. **Reduction of aflatoxin hazards using ammoniation**. Reviews of Environmental Contamination and Toxicology **171**: 139–175.

This review with 110 references provides: a legal history of aflatoxins and current FDA action levels; information of the levels of aflatoxins in domestic surveillance samples of feed during fiscal years 1989–1998; efficacy of the ammoniation process for reducing aflatoxin levels in contaminated feedstuffs; benefits and limitations shown in animal feeding studies; effect of ammonia on the aflatoxin molecule and the formation of reaction products; relative toxic/mutagenic potentials and safety of aflatoxin and its reaction products in ammoniated aflatoxin contaminated feeds, and; an estimate of the action level for aflatoxin, including ammoniation reaction products in ammoniated aflatoxin contaminated feeds and specifications for ammoniation of aflatoxin contaminated feeds.

LEE, S.E., CAMPBELL, B.C., MOLYNEUX, R.J., HASEGAWA, S. and LEE, H.S. 2001. **Inhibitory effects of naturally occurring compounds on aflatoxin B_1 biotransformation**. Journal of Agricultural and Food Chemistry **49**: 5171–5177.

Among 77 naturally occurring plant compounds tested for their effects on biotransformation of AFB_1, anthraquinones, coumarins and flavone-type flavonoids were shown to be potent inhibitors of AFB_1-8,9-epoxide formation. Addition of the flavonoids galangin, rhamnetin and flavone strongly inhibited mouse liver microsomal conversion of AFB_1 to AFB_1-8,9-epoxide. In contrast to these results, addition of isoflavonoids, catechins, terpenes, alkaloids and quinones to mouse liver microsomes did not inhibit formation of AFB_1-8,9-epoxide. Formation of the AFB_1 reductase product, aflatoxicol, by chicken liver cytosols was strongly inhibited by curcumin. Curcumin analogues also showed inhibitory effects, and a structure-activity study established that beta-diketone groups linked with two benzyl moieties were essential for inhibition of aflatoxicol formation.

SCHATZKI, T.F. and ONG, M.S. 2001. **Dependence of aflatoxin in almonds on the type and amount of insect damage**. Journal of Agricultural and Food Chemistry **49**: 4513–4519.

The aflatoxin distribution in insect damaged almonds has been measured. In Nonpareil almonds, separate distributions were obtained for pinhole, insect (feeding) and gross damage. Only a low level of aflatoxin contamination was found for pinhole-only damaged nuts. The distributions for insect and gross damage did not differ, but did differ significantly from the distribution previously obtained for gross damaged Ne Plus almonds. Aflatoxin lot averages of 31.7 and 3.47 mg/kg were obtained for 100% insect damaged Ne Plus and Nonpareil almonds, respectively. The distribution functions were used to compute the seller's risk of nonacceptance of lots in the European Union. To obtain a 95% acceptance rate, AFB1 levels of 0.12 and 0.22 mg/kg would be required, which would correspond to 3.8 and 1.2% (feeding and gross) insect damage in Nonpareil and Ne Plus almond lots, respectively.

LEMKE, S.L., OTTINGER, S.E., MAYURA, K., AKE, C.L., PIMPUKDEE, K., WANG, N. and PHILLIPS, T.D. 2001. **Development of a multi-tiered approach to the *in vitro* prescreening of clay-based enterosorbents**. Animal Feed Science and Technology **93**: 17–29.

Three previously established *in vitro* methods: single-concentration sorption, isotherms and chemisorption index (C-alpha) were compared as methods for predicting the adsorption of AFB_1 from solution by four sorbents: HSCAS, charcoal, clinoptilolite and sand. In addition, a gastrointestinal (GI) model was utilised to measure adsorption. Finally, maize was included in modified isotherm studies to examine interactions. HSCAS proved efficacious in all testing methods. Bind-

ing of AFB_1 by charcoal was comparable to HSCAS but was hindered in the presence of maize. Under GI conditions, clinoptilolite demonstrated catalytic activity not observed in the other methods. Results indicate that the GI model is a rapid and more physiologically relevant method of screening sorbents.

CRISEO, G., BAGNARA, A. and BISIGNANO, G. 2001. **Differentiation of aflatoxin-producing and non-producing strains of *Aspergillus flavus* group**. Letters in Applied Microbiology **33**: 291–295.

Three conventional methods and a multiplex PCR procedure with a set of four primers (Quadruplex-PCR) were used to differentiate between aflatoxin-producing and non-producing strains of the *Aspergillus flavus* group. By combining sets of primers for *aflR*, *nor-1*, *ver-1* and *omt-A* genes of the aflatoxin biosynthetic pathway, Quadruplex-PCR showed that aflatoxigenic strains gave a quadruplet pattern, indicating the presence of all the genes involved in the aflatoxin biosynthetic pathway which encode for functional products. Non-aflatoxigenic strains gave varying results with one, two, three or four banding patterns. A banding pattern in three non-aflatoxigenic strains resulted in non-differentiation between these and aflatoxigenic strains.

VANDENBROEK, P., PITTET, A. and HAJJAJ, H. 2001. **Aflatoxin genes and the aflatoxigenic potential of Koji moulds**. Applied Microbiology and Biotechnology **57**: 192–199.

Sixty-four *Aspergillus* isolates, 54 of which originated from food fermentations, were identified and screened for the presence of aflatoxin genes *aflR* and *omt-1*. Among the Koji moulds, not only *A. oryzae* but also *A. flavus* strains were found. Furthermore, 27% of *A. oryzae* and 93% of *A. flavus* strains lacked either *aflR* or both *aflR* and *omt-1*. A selection of 29 strains was also checked for the presence of *pksA* and *nor-l*. This revealed large deletions in the aflatoxin gene cluster of some strains. In the 73% of *A. oryzae* strains showing no apparent deletions using the *aflR* and *omt-1* probes, no AFB_1 production was detected after incubation in aflatoxin inducing media.

DEZOTTI, N.O.C.R. and ZUCCHI, T.M.A.D. 2001. **Identification of *Aspergillus nidulans* genes essential for the accumulation of sterigmatocystin**. Fungal Genetics and Biology **34**: 93–105.

Aspergillus nidulans was used as a genetic model for the identification of genes required for efficient accumulation of sterigmatocystin (STG). Genetic analysis included studies of the sexual and parasexual cycles. The allelic segregation rates and recombination frequencies between linked and nonlinked genetic markers were determined by the crossing of the strains UT448 (*stc*) to UT196 (stc^+) and UT448 (*stc*) to UT184 (*stc*). Based on results, a regulation model for STG production is proposed.

HITCHMAN, T.S., SCHMIDT, E.W., TRAIL, F., RARICK, M.D., LINZ, J.E. and TOWNSEND, C.A. 2001. **Hexanoate synthase, a specialised type I fatty acid synthase in aflatoxin B_1 biosynthesis**. Bioorganic Chemistry **29**: 293–307.

Incorporation studies in aflatoxin and genetic analyses of STG and aflatoxin biosynthesis strongly suggest that their biosyntheses begin with the production of a C-6 fatty acid by a specialised fatty acid synthase. The genes encoding the alpha (hexA) and beta (hexB) subunits of this hexanoate synthase (HexS) from the aflatoxin pathway in *Aspergillus parsiticus* SU-1 were cloned and both their gDNAs and cDNAs were sequenced and their transcriptional ends analysed. Phylogenetic predictions and an analysis of GC-bias in aflatoxin and STG pathway genes compared with primary metabolic *Aspergillus* genes were used as a basis to propose a route for the evolution of the aflatoxin and STG clusters.

Aflatoxins – Methodology

AKIYAMA, H., GODA, Y., TANAKA, T. and TOYODA, M. 2001. **Determination of aflatoxins B_1, B_2, G_1 and G_2 in spices using a multifunctional column clean-up**. Journal of Chromatography A **932**: 153–157.

A rapid and simple method using a multifunctional column which contains lipophilic and charged active sites was developed to analyse aflatoxins in various spices. After extraction by acetonitrile–water (9:1) and cleanup using MultiSep #228 column, the aflatoxins and aflatoxin-TFA derivatives are determined using LC with fluorescence detection. Recoveries of each AFB1, B2, G1 and G2 spiked to red pepper, white pepper, black pepper, nutmeg and tear grass at 10 mg/kg were over 80–85% in all instances. The minimum detectable concentration for aflatoxins in red pepper was 0.5 mg/kg.

RASTOGI, S., DAS, M. and KHANNA, S.K. 2001. **Quantitative determination of aflatoxin B_1-oxime by column liquid chromatography with ultraviolet detection**. Journal of Chromatography A **933**: 91–97.

Liquid chromatography with UV detection was used for the quantification of AFB_1-oxime. The yield of AFB_1-oxime in the reaction mixture was 89%, and after purification on silica gel it was 72%. LC analysis of the reaction mixture after silica gel fractionation revealed a retention time of 0.84 min for AFB_1-oxime and 8.42 min for AFB_1. UV-visible analysis of the reaction mixture after silica gel fractionation showed a lambda (max) of 269 and 361 nm for AFB_1-oxime and 263 and 360 nm for AFB_1. Excitation and emission wavelengths were found to be 269 and 368/438 nm for AFB_1-oxime and 359/424 nm for AFB_1.

CHIAVARO, E., DALLASTA, C., GALAVERNA, G., BIANCARDI, A., GAMBARELLI, E., DOSSENA, A. and MARCHELLI, R. 2001. **New reversed-phase liquid chromatographic method to detect aflatoxins in food and feed with cyclodextrins as fluorescence enhancers added to the eluent**. Journal of Chromatography A **937**: 31–40.

The effect of succynil-beta-cyclodextrin (beta-CD-Su), dimethyl-beta-cyclodextrin (DIMEB) and beta-cyclodextrin (beta-CD) on the fluorescence of aflatoxins was studied. beta-CD-Su promoted the largest fluorescence enhancement for AFB1 and AFM1 while DIMEB showed better results for AFG1. On the basis of the fluorescence enhancement, a new RP–HPLC method for detecting aflatoxins was developed using cyclodextrins directly dissolved in the LC eluent. AFB1, B2, G1 and G2 were resolved using a MICRA NPS ODS-1 column using methanol–water as mobile phase to which 6×10^{-3} M beta-CD-Su or beta-CD were added. Chromatographic responses of AFB1 and G1 achieved using beta-CD were enhanced 8 and 12 times, respectively, and 10 and 15 times with beta-CD-Su. Detection limits lower than 0.3 mg/kg were achieved for all the four aflatoxins. AFM1 was analysed using a Spherisorb S3 ODS-2 Narrow Bore column and methanol–water as mobile phase with added 2×10^{-3} M beta-CD-Su. An area enhancement of 1.5 was achieved with a detection limit lower than 0.5 ng/kg.

FENTE, C.A., ORDAZ, J.J., VAZQUEZ, B.I., FRANCO, C.M. and CEPEDA, A. 2001. **New additive for culture media for rapid identification of aflatoxin-producing *Aspergillus* strains**. Applied and Environmental Microbiology **67**: 4858–4862.

A new reliable, fast and simple method for the detection of aflatoxigenic Aspergillus strains, consisting of the addition of a cyclodextrin (a methylated beta-cyclodextrin derivative) to common media used for testing mycotoxin production ability, was developed. The production of aflatoxins coincided with the presence of a bright blue or blue-green fluorescent area surrounding colonies when observed under long wavelength (365 nm) UV light after 3 days of incubation at 28°C.

MIRGHANI, M.E.S., MAN, Y.B.C., JINAP, S., BAHARIN, B.S. and BAKAR, J. 2001. **A new method for determining aflatoxins in groundnut and groundnut cake using Fourier transform infrared spectroscopy with attenuated total reflectance**. Journal of the American Oil Chemists Society **78**: 985–992.

A new analytical method was developed for the determination of aflatoxins in groundnut and groundnut cakes by Fourier transform infrared (FTIR) spectroscopy using horizontal attenuated total reflectance technique. A partial least square regression was used to derive the calibration models for each toxin. The coefficients of determination (R-2) of the calibration model were computed for the FTIR spectroscopy predicted values vs. actual values of aflatoxins in parts per billion. The R-2 was found to be 0.9911, 0.9859, 0.9986 and 0.9789 for AFB_1, B_2, G_1 and G_2, respectively. Standard errors of calibration for groundnut samples were found to be 1.80, 2.03, 1.42, and 2.05 for AFB_1, B_2, G_1 and G_2, respectively. With its speed and ease of data manipulation by computer software, it is a possible alternative to the standard wet chemical methods for a rapid and accurate routine determination of aflatoxin levels in food and feed.

KORDE, A., BANERJEE, S., PILLAI, M.R.A. and VENKATESH, M. 2001. **Preparation and evaluation of I-125-aflatoxin B_1**. Journal of Radioanalytical and Nuclear Chemistry **250**: 231–237.

$[^{125}I]$-AFB_1 is a key reagent for development of radioimmunoassay (RIA) which exhibits less interference and better sensitivity than other immunoassays. Since AFB_1 lacks suitable functional groups for radiolabelling, an oxime derivative of AFB_1 was synthesised and evaluated by UV spectrophotometry and $[^1H]$-NMR spectroscopy. $[^{125}I]$-histamine was conjugated to AFB_1-oxime by mixed anhydride method and purified by solvent extraction followed by TLC. The tracer obtained was immunoreactive, stable as ethanolic solution and could be used in RIA.

GARDEN, S.R. and STRACHAN, N.J.C. 2001. **Novel colorimetric immunoassay for the detection of aflatoxin B_1**. Analytica Chimica Acta **444**: 187–191.

A rapid colorimetric sequential injection immunoassay (SIIA) system utilising a jet ring flow cell packed with a solid phase of polymethylmethacrylate beads was used for the detection of AFB1. This method is proven in its ability to detect AFB1 down to a level of 0.2 mg/L in artificially contaminated food materials, which is comparable to the sensitivity of microtitre plate ELISA. The automated SIIA system takes under 10 min per sample and uses a methanol–water and hexane extraction procedure that takes approximately 15 min to perform.

SUJATHA, N., SURYAKALA, S. and RAO, B.S. 2001. **Enzyme immunoassay for aflatoxin B_1-lysine adduct and its validation**. Journal of AOAC International **84**: 1465–1474.

A simple procedure was developed for *in vitro* synthesis and characterisation of AFB_1-lysine adduct using AFB_1, N-alpha-acetyl lysine and *m*-chloroperbenzoic acid (MCPBA). At a molar ratio of 1:16, AFB_1:N-alpha-cetyl lysine, the recovery of adduct was 62%. Analysis of the adduct by TLC showed a single spot. Absorption spectra of the adduct showed 2 peaks at 275 and 335 nm. LC analysis of the AFB_1-lysine adduct showed a relative retention time of 2.1 min. Using the same epoxidation procedure, BSA-AFB_1 adduct and ovalbumin-AFB_1 adduct were synthesised for production of antibodies and as coating antigen, respectively.

SHARMA, M. and MARQUEZ, C. 2001. **Determination of aflatoxins in domestic pet foods (dog and cat) using immunoaffinity column and HPLC**. Animal Feed Science and Technology **93**: 109–114.

Thirty-five samples of commercially available domestic pet foods (dog and cat) in Mexico were assayed for aflatoxins. A reversed phase HPLC method with fluorometric detection is described for the determination and quantification of AFB_1, B_2, G_1, G_2, M_1, M_2, P_1 and aflatoxicol after an immunoaffinity column cleanup. Mean average recoveries of different aflatoxins and aflatoxicol spiked at levels of 0.5–8.0 mg/kg ranged from 83–87% for AFG1 and B2 with relative standard deviation of <7% for AFP_1. Detection limits were 3–7 mg/kg. AFB_1 was the mycotoxin found with higher frequency (0.885) and was at high concentration in six samples. Two of the samples had total aflatoxin concentrations of 72.4 and 59.7 mg/kg.

Aflatoxicoses

AHSAN, H., WANG, L.Y., CHEN, C.J., TSAI, W.Y. and SANTELLA, R.M. 2001. **Variability in aflatoxin–albumin adduct levels and effects of hepatitis B and C virus infection and glutathione S-transferase M1 and T1 genotype**. Environmental Health Perspectives **109**: 833–837.

AFB_1-albumin adduct levels among 264 healthy male residents of Taiwan were assayed to determine intra-individual variability in AFB_1–albumin adducts and whether the baseline or followup adduct levels and the intraindividual variability in adduct levels are modified by endogenous and environmental factors. The study measured adduct levels at two different time points within the range 1.00–3.17 years. There was a generalised reduction in the adduct levels, with the median values being 22.1 pmol/mg at time 1 and 14.3 pmol/mg at time 2. This intraindividual variability in adduct levels was inversely associated with the age of subjects and the time interval between the two blood draws. No significant association was observed for the intraindividual variability with regard to the season when blood was drawn, hepatitis B surface antigen, anti-hepatitis C virus, glutathione S-transferase (GST) M1 or GSTT1 status.

CHEN, S.Y., CHEN, C.J., CHOU, S.R., HSIEH, L.L., WANG, L.Y., TSAI, W.Y., AHSAN, H. and SANTELLA, R.M. 2001. **Association of aflatoxin B_1–albumin adduct levels with hepatitis B surface antigen status among adolescents in Taiwan**. Cancer Epidemiology Biomarkers & Prevention **10**: 1223–1226.

The association between chronic hepatitis B virus (HBV) infection and AFB_1–albumin adduct level was assessed in 200 junior high school adolescents in Taiwan by measuring hepatitis B surface antigen (HBsAg) and AFB_1–albumin

adducts. The mean AFB_1–albumin adduct level was higher in HBsAg-positive compared with HBsAg-negative subjects. The association between HBsAg status and AFB_1–albumin adducts remained after multivariate adjustment. This finding additionally supports the synergetic interaction between HBV and AFB_1.

PALANEE, T., DUTTON, M.F. and CHUTURGOON, A.A. 2001. **Cytotoxicity of aflatoxin B_1 and its chemically synthesised epoxide derivative on the A549 human epithelioid lung cell line**. Mycopathologia **151**: 155–159.

The A549 human epithelioid lung cell line and the methylthiazol tetrazolium (MTT) bioassay were used to investigate the cytotoxicity of AFB_1 and its chemically synthesised epoxide (AFBO) *in vitro*. Statistical analysis of the MTT results indicated that there were overall significant differences between the control and both the AFB_1 treated and AFBO treated cells. However, there was no significant difference between AFB_1 and AFBO treated cells when the entire range of concentrations were assessed against each other.

AGNES, V.F. and AKBARSHA, M.A. 2001. **Pale vacuolated epithelial cells in epididymis of aflatoxin-treated mice**. Reproduction **122**: 629–641.

The responses of the mouse epididymal epithelium to subchronic doses of AFB_1 were investigated in a histological study. Either few and large or small and profuse vacuoles containing an amorphous to dense periodic acid-Schiff-positive material were observed in the epithelium of all the segments of the epididymis. Resin-embedded semi-thin sections and transmission electron microscopy indicated that these vacuoles were intracellular. The cells that contained these vacuoles were quite different in organization and electron density from the cell types already established in the epididymal epithelium and are designated as pale vacuolated epithelial cells. The development of pale vacuolated epithelial cells may be a protective device preventing an autoimmune response to sperm antigens in the context of toxicant-induced degeneration of the principal cells of the epididymal epithelium.

ORTATATLI, M. and OGUZ, H. 2001. **Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis**. Research in Veterinary Science **71**: 59–66.

Broiler chickens were fed aflatoxin at 2.5 mg/kg diet with or without the addition of clinoptilolite at 1.5 or 2.5% diet from 1 to 21 days of age. Aflatoxin treated chickens showed increases in the relative weights of liver and kidney and gross histopathologic hepatic lesions. Glumerular hypertrophy, increases in the number of mesengial cells and hydropic degeneration of tubuler epithelium in kidneys was also observed. The addition of clinoptilolite at 1.5 or 2.5% to the aflatoxin-containing diet moderately (significantly in some cases) decreased the number of affected broilers and/or the severity of lesions.

LIM, H.A., NG, W.K., LIM, S.L. and IBRAHIM, C.O. 2001. **Contamination of palm kernel meal with *Aspergillus flavus* affects its nutritive value in pelleted feed for tilapia, *Oreochromis mossambicus***. Aquaculture Research **32**: 895–905.

An assessment of the nutritive value of palm kernel meal (PKM) and aflatoxin contaminated PKM (obtained by fermenting PKM with *Aspergillus flavus*) as a dietary ingredient in pelleted feed for tilapia, Oreochromis mossambicus, was carried out in a 12-week feeding trial. AFB1 levels in the fermented PKM based diets ranged from 75 to 100 mg/kg diet. The growth performance and feed utilisation efficiency of tilapia fed fermented PKM based diets were significantly lower than in fish fed the control diet at all inclusion levels. The addition of 0.5% Sorbatox, a commercial aflatoxin adsorber, did not produce any beneficial or negative effects to the growth of tilapia.

VERMA, R.J. and NAIR, A. 2001. **Ameliorative effect of vitamin E on aflatoxin-induced lipid peroxidation in the testis of mice**. Asian Journal of Andrology **3**: 217–221.

Adult male albino mice were orally dosed with AFB_1 at 25 or 50 mg/day. There was a dose dependent significantly higher lipid peroxidation in the testis of aflatoxin treated mice than in the controls. The levels of non-enzymatic antioxidants such as glutathione, total and reduced ascorbic acid, as well as the activities of enzymatic antioxidants were significantly lower in the testis of aflatoxin treated mice. Pretreatment with vitamin E at 2 mg/day per animal significantly ameliorates the aflatoxin induced lipid peroxidation.

ZAB, S.G., SADEK, M.M. and CRAILSHEIM, K. 2001. **Protein metabolism in larvae of the cotton leaf-worm *Spodoptera littoralis* (Lepidoptera: *Noctuidae*) and its response to three mycotoxins**. Environmental Entomology **30**: 817–823.

The effects of orally administered AFB_1, B_2 and G_1 on protein metabolism of *Spodoptera littoralis* larvae were investigated. The incorporation of phenylalanine into protein was determined in penultimate and final instar larvae and pupae. Both AFB_1 and G_1 at concentrations of 2.5 and 4 mg/kg, respectively, had profound inhibitory effects on the incorporation of phenylalanine into protein in both larvae and pupae, whereas AFB_2 at 4 mg/kg had only a slight effect.

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Compiled and edited by J.C. Eyles, A. Hocking, and J.I. Pitt, Food Science Australia, Sydney. Typesetting by Clarus Design, Canberra.

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

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