

Australian

Mycotoxin Newsletter

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THE LAST ISSUE

Vale *Australian Mycotoxin Newsletter*

This newsletter started life as the result of a request in 1980 from Australia's National Health and Medical Research Council (NHMRC) to the CSIRO Division of Food Research, North Ryde, to establish a centre for the collection of "experimental and toxicological data" on mycotoxins in Australia. The centre was set up in mid 1982, with some initial funding from NHMRC, and publication of this newsletter, known then as the *Australian Mycotoxin Data Centre Newsletter*, began in December, 1982. As recorded in the first issue, "Dr Paula Casey was appointed to the part-time position of coordinator, under the guidance of Dr J.I. Pitt".

The data centre was set up with three prime functions:

- to act as a repository for information on the occurrence of mycotoxins in Australia and accumulate analytical data of use to Government and Industry
- to provide information on current overseas research
- to act as a focal point for dissemination of information on all aspects of mycotoxin research.

Accordingly, the newsletter set out to publish abstracts of published papers, articles on mycotoxins, and data on mycotoxin occurrence in Australia, and to act as a forum, publishing notes and letters on topics related to mycotoxins. It was stressed that the information gathering was to be informal and not publishable elsewhere.

From the outset, the newsletter aimed to publish lucid abstracts, rewritten from the originals to convey information as succinctly as possible. Frequently the original papers were consulted to improve the information included. Subject matter was deliberately chosen to reflect major areas of mycotoxin research, but with an emphasis on occurrence and ecology — causal fungi, physiological factors influencing formation, crops affected, and geographical influences. Papers dealing with more theoretical areas, such as mechanisms of action of mycotoxins, were mostly omitted.

The first issue included an editorial, a meeting announcement, and 21 abstracts of published papers. It was typed on a fondly remembered Adler electric typewriter, in its time the most advanced machine around, with an electronic processor, interchangeable fonts, variable line and word spacing and a floppy disc drive of enormous size and minuscule capacity.

The centre and the newsletter fulfilled most functions demanded of them to a greater or lesser degree for several years. Crises arrived in the form of cessation of NHMRC funding and the need to charge a subscription rate. Small grants from rural industries granting bodies somewhat ameliorated this situation.

In March 1987, Dr Casey resigned and was replaced by Ms Joan Eyles, who has edited the newsletter ever since. Dr Ailsa Hocking also joined the masthead as an editor. The March 1987 issue included 42 abstracts.

Funding crises continued, until in March 1989 the Australian Centre for International Research (ACIAR) became a sponsor, and the newsletter became international, as ACIAR agreed to distribute it gratis with its *Postharvest Newsletter*. To accommodate these changes, the name was changed to reflect the demise of the data centre, as it was felt to be inappropriate to distribute internationally information on mycotoxins in Australia. The *Australian Mycotoxin Newsletter* became principally an information source, continuing to edit informative abstracts, but other functions more or less ceased from that time. Abstract numbers continued to increase: the March 1989 issue included 67 abstracts.

By this time, the personal computer had evolved to the point where it could be used for publications. The March 1989 issue was the first issue printed from a computer, though keying in every word by hand continued for some years after this.

Time has marched on and, after more than 20 years, production of the newsletter ceases with this issue. The decision to cease publication has not been taken lightly. A number of reasons exist. First, the newsletter in many ways has outlived its usefulness: the Internet has by now brought abstracts and journals to most people's desks, at least in the developed world. Second, the cost of the preparation of the newsletter has increased to the point of nonviability. Third, I have retired, at least nominally.

How many abstracts have we put together? Recent issues have often exceeded 120 abstracts. We have put together more than 80 issues, probably totalling some 5000 abstracts.

Finally, I wish to thank all those who have been involved in newsletter production. Dr Paula Casey, who started, and Ms Joan Eyles who continued with, the meticulous collection, collation and especially editing of abstracts. My colleague Dr Ailsa Hocking who has helped in many ways. Dr Bruce Champ and more recently Dr Greg Johnson of ACIAR, for ongoing funding, and Mr Ed Highley, of Clarus Design (originally Arawang

Information Bureau), who has been responsible for the high quality of this newsletter as published by ACIAR since 1989.

John I. Pitt
May 2003



The newsletter team at Food Science Australia, (L-R) Joan Eyles, John Pitt and Ailsa Hocking

Abstracts

Mycotoxins – General

BRERA, C., CAPUTI, R., MIRAGLIA, M., IAVICOLI, I., SALERNO, A. and CARELLI, G. 2002. **Exposure assessment to mycotoxins in workplaces: Aflatoxins and ochratoxin A occurrence in airborne dusts and human sera.** *Microchemical Journal* **73**: 167–173.

An HPLC method was used to detect aflatoxins and ochratoxin A (OA) in dust samples. A total of 44 samples of airborne particles were collected in three different workplaces in Tuscany, central Italy, where three foodstuffs susceptible to contamination (cocoa, coffee and spices) are processed. Airborne levels of OA, and aflatoxins B₁, B₂, G₁ and G₂ ranged from undetectable to 8.304, 0.038, 0.029, 0.036 and 0.131 ng/m³, respectively. OA levels in workers' sera showed much higher results (0.94–3.28 µg/L) than the mean level found in the Italian population, indicating that inhalation in the workplace can be considered a route of exposure additional to the consumption of contaminated foodstuffs.

PARK, J.W., KIM, E.K., SHON, D.H. and KIM, Y.B. 2002. **Natural co-occurrence of aflatoxin B₁, fumonisin B₁ and ochratoxin**

A in barley and corn foods from Korea. *Food Additives and Contaminants* **19**: 1073–1080.

A survey for aflatoxin B₁ (AFB₁), fumonisin B₁ (FB₁) and OA was conducted on 127 samples that included 30 food-grade barley, 32 barley foods, 18 food-grade corn and 47 corn foods, randomly collected during 1998–99 in Seoul, Korea. Mycotoxins were analysed by direct competitive ELISA and most of the positive samples from ELISA were confirmed using HPLC. In barley feeds, AFB₁, FB₁ and OA were found in 12, 6 and 12% of samples with an average of 26, 16 and 9 mg/kg, respectively. In corn foods, AFB₁ and FB₁ were detected in 8 and 19% of samples with an average of 20 and 74 mg/kg, respectively. No OA was found in any corn foods samples. AFB₁, FB₁ and OA were not detected in any food-grade barley or corn samples.

PASCALE, M., VISCONTI, A., PRONCZUK, M., WISNIEWSKA, H. and CHELKOWSKI, J. 2002. **Accumulation of fumonisins, beauvericin and fusaproliferin in maize hybrids inoculated under field conditions with *Fusarium proliferatum*.** *Mycological Research* **106**: 1026–1030.

Fifteen maize hybrids were inoculated in the field with a toxigenic strain of *Fusarium proliferatum* and ear rot severity and the accumulation of FB₁, FB₂, beauvericin (BEA) and fusaproliferin (FP) were investigated during the seasons 1996, 1997 and 1999 in Poland. Inoculated ears contained 11–71 % *Fusarium*-damaged kernels. Mycotoxins were detected in all hybrids. Mycotoxin concentrations were higher in *Fusarium*-damaged kernels (up to 361.5, 41.1, 44.3 and 10.0 mg/kg for FB₁, FB₂, BEA and FP, respectively, than in healthy-looking kernels which had up to 26.0, 2.3, 1.9, 0.3 mg/kg, respectively.

NIEMINEN, S.M., KARKI, R., AURIOLA, S., TOIVOLA, M., LAATSCH, H., LAATIKAINEN, R., HYVARINEN, A. and VON WRIGHT, A. 2002. **Isolation and identification of *Aspergillus fumigatus* mycotoxins on growth medium and some building materials.** *Applied and Environmental Microbiology* **68**: 4871–4875.

Genotoxic and cytotoxic compounds were isolated and purified from the culture medium of an indoor air mould, *Aspergillus fumigatus*. Growth of *A. fumigatus* was studied on some building materials. Strong growth of the fungus and the presence of gliotoxin were detected on spruce wood, gypsum board and chipboard under saturation conditions.

LOGRIECO, A., MULE, G., MORETTI, A. and BOTTALICO, A. 2002. **Toxicogenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe.** European Journal of Plant Pathology **108**: 597–609.

A review with 114 references. Several *Fusarium* species occurring worldwide on maize as causal agents of ear rot are capable of producing mycotoxins in infected kernels. The main groups of *Fusarium* toxins commonly found are trichothecenes, zearalenones, fumonisins and moniliformin. In addition, BEA and FP have been found in *Fusarium*-infected maize ears. Zearalenone and deoxynivalenol are commonly found in maize red ear rot, which is essentially caused by species of the Discolour section, particularly *F. graminearum*. In maize pink ear rot, which is mainly caused by *F. verticillioides*, there is increasing evidence of the wide occurrence of FB₁. This toxin is usually found in association with moniliformin, BEA and FP, both in central Europe due to the co-occurrence of *F. subglutinans*, and in southern Europe due to the widespread presence of *F. proliferatum*.

PASCALÉ, M., VISCONTI, A. and CHELKOWSKI, J. 2002. **Ear rot susceptibility and mycotoxin contamination of maize hybrids inoculated with *Fusarium* species under field conditions.** European Journal of Plant Pathology **108**: 645–651.

Several maize hybrids were investigated for *Fusarium* ear rot and accumulation of FB₁, FB₂, BEA and FP after artificial inoculation in the field with toxigenic strains of *Fusarium verticillioides* and *F. proliferatum*. Of all the hybrids tested, only Mona exhibited resistance to ear rot caused by *F. verticillioides* and produced low levels of fumonisins during three years of experiments. FB₁, FB₂, BEA and FP were detected at concentrations much higher (up to 10–20 times) in *Fusarium*-damaged kernels than in healthy-looking kernels.

FOTSO, J., LESLIE, J.F. and SMITH, J.S. 2002. **Production of beauvericin, moniliformin, fusaproliferin, and fumonisins B₁, B₂, and B₃ by fifteen ex-type strains of *Fusarium* species.** Applied and Environmental Microbiology **68**: 5195–5197.

Fifteen *Fusarium* species were analysed by HPLC for the production of six mycotoxins in corn grits cultures. Production of mycotoxins ranged from 66 to 2,500 µg/kg for FB₁, 0.6 to 1,500 mg/kg for moniliformin, 2.2 to 720 mg/kg for BEA and 12 to 130 mg/kg for FP. FB₂ was produced by two species and FB₃ was not detected in any species.

NIEMINEN, S.M., MAKI-PAKKANEN, J., HIRVONEN, M.R., ROPONEN, M. and VON WRIGHT, A. 2002. **Genotoxicity of gliotoxin, a secondary metabolite of *Aspergillus fumigatus*, in a battery of**

short-term test systems. Mutation Research – Genetic Toxicology and Environmental Mutagenesis **520**: 161–170.

Gliotoxin was purified from the culture medium of *Aspergillus fumigatus* isolated from the indoor air of a moisture problem house. Gliotoxin was found to be genotoxic in the bacterial repair assay but not in the Ames *Salmonella* assay or the SOS-chromotest. A dose-related increase in DNA damage was observed in mouse RAW264.7 macrophages exposed to gliotoxin for 2 hr in the single cell gel electrophoresis assay. However, gliotoxin did not induce any clear, dose related increase in sister-chromatid exchange in Chinese hamster ovary cells.

BOEIRA, L.S., BRYCE, J.H., STEWART, G.G. and FLANNIGAN, B. 2002. **Influence of cultural conditions on sensitivity of brewing yeasts growth to *Fusarium* mycotoxins zearalenone, deoxynivalenol and fumonisin B₁.** International Biodeterioration & Biodegradation **50**: 69–81.

The influence of cultural parameters on the sensitivity of *Saccharomyces cerevisiae* lager and ale strains to the presence of zearalenone (ZEA), deoxynivalenol (DON) and FB₁ was examined. Culture conditions appeared to affect sensitivity of yeasts to mycotoxins at 100 mg/L growth medium, but not at 2 mg/L. For both strains, cultures developing from a lower inoculum concentration were more strongly inhibited than at a higher inoculum level, except for the lager strain grown at 13°C and the ale strain at 25°C in the presence of FB₁. Growth temperature was important in sensitivity to the mycotoxins. At 25°C, the ale strain was more sensitive to ZEA and DON than the lager strain, but the lager strain was more sensitive to these two toxins at 13°C than at 25°C. In contrast, at 13°C the lager strain was more sensitive to FB₁ than at 25°C, and at 25°C it was more sensitive than the ale strain.

SIDHU, G.S. 2002. **Mycotoxin genetics and gene clusters.** European Journal of Plant Pathology **108**: 705–711.

Aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* have a complex biosynthetic pathway involving sixteen steps mediated by individual major genes. These genes, involving both regulatory and biosynthetic pathways, are clustered on the respective chromosomes. Clustering of genes in fungi indicates an evolutionary trend among genes that orchestrate gene function. Being linked together they segregate 'as a unit', thereby conferring a selective advantage to the organism. The evolution of gene clusters takes place through vertical or horizontal gene transfer. In fungi, horizontal gene transfer is most effective. Functionally, the mechanism of evolution of mycotoxin gene clusters in fungi seems to be similar to the evolution of a super-gene. The possible implications of evolutionary parallelism of gene clusters and super-genes is briefly explored.

CALVO, A.M., WILSON, R.A., BOK, J.W. and KELLER, N.P. 2002. **Relationship between secondary metabolism and fungal development.** Microbiology and Molecular Biology Reviews **66**: 447.

This review with 128 references considers the secondary metabolites produced by fungi that act as sporogenic factors to influence fungal development, are required for spore viability, or are produced at a time in the life cycle that coincides with development. Environmental and genetic factors that can influence the production of secondary metabolites are described. In the case of *Aspergillus nidulans*, the only described work that genetically links the sporulation of this fungus to the production of sterigmatocystin through a shared G-protein signalling pathway is reviewed.

PROCTOR, R.H., DESJARDINS, A.E., MCCORMICK, S.P., PLATTNER, R.D., ALEXANDER, N.J. and BROWN, D.W. 2002. **Genetic analysis of the role of trichothecene and fumonisin mycotoxins in the virulence of *Fusarium*.** European Journal of Plant Pathology **108**: 691–698.

The roles of trichothecene and fumonisin mycotoxins in plant pathogenesis by *Fusarium graminearum* which causes wheat head blight and maize ear rot, and the fumonisin-producing species *F. verticillioides* which causes maize ear rot, were studied in field tests. Mutants were generated by transformation-mediated disruption of genes encoding enzymes that catalyse early steps in the biosynthesis of each toxin. Trichothecene non-producing mutants of *F. graminearum* caused less disease than the wild-type strain from which they were derived on both wheat and maize, although differences in virulence on maize were not observed under hot and dry environmental conditions. Although the analyses of virulence of fumonisin non-producing mutants of *F. verticillioides* are not complete, to date the mutants have been as virulent on maize ears as their wild-type progenitor strains.

SANTOS, I.M., ABRUNHOSA, L., VENANCIO, A. and LIMA, N. 2002. **The effect of culture preservation techniques on patulin and citrinin production by *Penicillium expansum* Link.** Letters in Applied Microbiology **35**: 272–275.

Ten strains of *Penicillium expansum* were preserved using subculture and maintenance at 4°C, mineral oil, drying on silica gel and freeze-drying. Patulin and citrinin production was assessed on yeast extract sucrose (YES) agar and grape juice (GJ) agar, using TLC before and after 0.5, 2–3, 6 and 12 months preservation. Citrinin was detected in all cultures for all preservation techniques on YES. The patulin profiles obtained differed with strain and culture media used. Citrinin production seems to be a stable character for the tested strains. Patulin production was more

consistent with silica gel storage and freeze-drying, especially when the strains were grown on GJ.

KADAKAL, C., SEBAHATTIN, N. and POYRAZOGLU, E.S. 2002. **Effect of commercial processing stages of apple juice on patulin, fumaric acid and hydroxymethylfurfural (HMF) levels.** *Journal of Food Quality* **25**: 359–368.

The effects of different stages of commercial apple juice production on the patulin, fumaric acid and hydroxymethylfurfural contents of apple juice were investigated. Heat treatment and activated charcoal were effective for the reduction of patulin, with average reductions of 13.4 and 22.9%, respectively. Statistical analysis showed no significant differences in the presence of fumaric acid between different treatments.

LARSEN, T.O., GAREIS, M. and FRISVAD, J.C. 2002. **Cell cytotoxicity and mycotoxin and secondary metabolite production by common penicillia on cheese agar.** *Journal of Agricultural and Food Chemistry* **50**: 6148–6152.

Known or potential new fungal starter culture species such as *Penicillium camemberti*, *P. roqueforti*, *P. nalgiovense*, *P. caseifulvum* and *P. solitum* were cultivated on a cheese agar medium together with the common cheese contaminants *P. commune*, *P. crustosum*, *P. discolor*, *P. atramentosum* and *P. nordicum*. Secondary metabolites were extracted and analysed by HPLC–DAD and tested for cytotoxicity using the MTT-cell culture assay. Metabolites such as cyclopiazonic acid, roquefortine C and penitrem A, previously reported from cheese, were detected together with a number of metabolites not previously reported from cheese. The two *P. nalgiovense* extracts were the most toxic in the MTT-cell culture test. None of the *P. roqueforti* extracts showed any toxicity.

HORNBOGEN, T., GLINSKI, M. and ZOCHER, R. 2002. **Biosynthesis of depsipeptide mycotoxins in *Fusarium*.** *European Journal of Plant Pathology* **108**: 713–718.

The cyclic hexadepsipeptide enniatin produced by *Fusarium* species is a phytopathogenic compound causing necrosis and wilt. Enniatins are synthesised by a 347 kDa multi-enzyme, enniatin synthetase, via a thiol template mechanism. The corresponding gene *esn1* has an open reading frame of 9393 nucleotides and harbours two modules, one responsible for D-hydroxy acid activation and one for L-amino acid activation with an integrated N-methyltransferase domain. Virulence was significantly reduced in *F. avenaceum* after disruption of the *esn1* gene.

WANG, J.F., HUANG, Y.J., FANG, M.J., ZHANG, Y.J., ZHENG, Z.H., ZHAO, Y.F. and SU, W.J. 2002. **Brefeldin A, a cytotoxin produced by *Paecilomyces* sp. and**

***Aspergillus clavatus* isolated from *Taxus mairei* and *Torreya grandis*.** *FEMS Immunology and Medical Microbiology* **34**: 51–57.

Paecilomyces sp. and *Aspergillus clavatus*, isolated from *Taxus mairei* and *Torreya grandis* from southeast China, produce toxic metabolites when grown in liquid culture. NMR, IR spectrometry, ESI–MS and X-ray analysis identified brefeldin A. This is the first report of the isolation of the cytotoxin from *Paecilomyces* sp. and *A. clavatus*.

STERGIOPOULOS, I., ZWIERS, L.H. and DE WAARD, M.A. 2002. **Secretion of natural and synthetic toxic compounds from filamentous fungi by membrane transporters of the ATP-binding cassette and major facilitator superfamily.** *European Journal of Plant Pathology* **108**: 719–734.

This review with 97 references provides an overview of members of the ATP-binding cassette and major facilitator superfamily of transporters identified in filamentous fungi. In plant pathogenic fungi, these transporters can be an important determinant of virulence on host plants by providing protection against plant defence compounds or mediating the secretion of host-specific toxins.

JELEN, H.H. 2002. **Volatile sesquiterpene hydrocarbons characteristic for *Penicillium roqueforti* strains producing PR toxin.** *Journal of Agricultural and Food Chemistry* **50**: 6569–6574.

Volatile metabolites and PR toxin were evaluated for 16 strains of *Penicillium roqueforti*. Thirteen strains produced PR toxin and all of them produced a specific set of sesquiterpene hydrocarbons including (+)-aristolochene, an intermediate in PR toxin biosynthesis. Aristolochene and the remainder of the sesquiterpene hydrocarbon profile were unique for *P. roqueforti* producing PR toxin. They were absent in nontoxicogenic *P. roqueforti* and in 40 strains of other *Penicillium* species.

MORETTI, A., BELISARIO, A., TAFURI, A., RITIENI, A., CORAZZA, L. and LOGRIECO, A. 2002. **Production of beauvericin by different races of *Fusarium oxysporum* f. sp. melonis**, the *Fusarium* wilt agent of muskmelon. *European Journal of Plant Pathology* **108**: 661–666.

Forty four strains of *Fusarium oxysporum* were isolated from plants of melon with *Fusarium* wilt symptoms. Beauvericin was produced by 36 strains in a range from 1 to 310 mg/kg. Two of the strains tested were nonpathogenic and one of these produced beauvericin at 290 mg/kg. Enniatin B was produced by 11 strains at concentrations up to 60 mg/kg. Results suggest that production of these toxins is not related to pathogenicity of *Fusarium oxysporum* f. sp. *melonis*.

SOLIMAN, K.M. and BADEAA, R.I. 2002. **Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi.** *Food and Chemical Toxicology* **40**: 1669–1675.

Essential oils of 12 medicinal plants were tested for inhibitory activity against *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*. The fungi were sensitive to the 12 essential oils, and particularly sensitive to thyme and cinnamon. The results also showed that the essential oils of thyme, cinnamon, anise and spearmint effected fungal development and subsequent mycotoxin production in wheat grains.

YAZICI, S. and VELIOGLU, Y.S. 2002. **Effect of thiamine hydrochloride, pyridoxine hydrochloride and calcium-D-pantothenate on the patulin content of apple juice concentrate.** *Nahrung – Food* **46**: 256–257.

Thiamine hydrochloride, pyridoxine hydrochloride and calcium-D-pantothenate were applied to apple juice concentrates (AJC) at various doses and their effects on patulin content were observed. AJC samples with added vitamins and containing high levels of patulin were stored at 4 or 22°C for 6 months. Patulin was fully degraded at the end of a 6 month period in samples stored at 22°C, however, other quality parameters were diminished significantly. At 4°C patulin was reduced by 35.8% after 6 months. Addition of thiamine hydrochloride (1000 mg/kg), pyridoxine hydrochloride (625 or 875 mg/kg) and calcium-D-pantothenate (1000 or 2500 mg/kg) into the samples and storage at 4°C for 6 months yielded 55.5 to 67.7% reduction in patulin content, while the other quality parameters were protected adequately.

STYRIAK, I. and CONKOVA, E. 2002. **Microbial binding and biodegradation of mycotoxins.** *Veterinary and Human Toxicology* **44**: 358–361.

A review with 64 references. An overview of literature data on the microbial binding and biodegradation of mycotoxins is presented. These data and laboratory results suggest that mycotoxin-bacterial binding or biodegradation is a realistic process and further research is warranted.

Mycotoxins – Methodology

EDWARDS, S.G., O'CALLAGHAN, J. and DOBSON, A.D.W. 2002. **PCR-based detection and quantification of mycotoxigenic fungi.** *Mycological Research* **106**: 1005–1025.

A review with 156 references. A number of polymerase chain reaction (PCR)-based methodologies have been developed for the identification of mycotoxin biosynthetic genes in different fungal genera, together with assays developed using other genes or random amplification of polymorphic DNA

(RAPD) methodologies for the identification of specific toxigenic fungi. In addition, reverse transcription (RT)-PCR, competitive PCR and Real Time quantitative PCR methodologies have also been developed for this purpose. The development of each of these techniques, their usefulness, limitations and adaptability is discussed together with descriptions of specific examples where these techniques have been utilised in different experimental settings.

ZUR, G., SHIMONI, E., HALLERMAN, E. and KASHI, Y. 2002. **Detection of *Alternaria* fungal contamination in cereal grains by a polymerase chain reaction-based assay.** *Journal of Food Protection* **65**: 1433–1440.

A PCR-based method for the detection of *Alternaria* DNA is described. PCR primers were designed to anneal to the ITS1 and ITS2 regions of the 5.8S rDNA gene of *Alternaria alternata* or *Alternaria solani* but not to other microbial or plant DNA. The sensitivity of PCR in detecting *Alternaria* DNA was compared with that of the HPLC method in detecting *Alternaria* alternariol and alternariol methyl ether toxins. In a set of commercially obtained grain samples the toxins were not detected by HPLC, while the PCR-based method and mould growth plating followed by morphological identification of *Alternaria* gave parallel, positive results for 8 of 10 samples.

ZAMBONIN, C.G., MONACI, L. and ARESTA, A. 2002. **Solid-phase microextraction-high performance liquid chromatography and diode array detection for the determination of mycophenolic acid in cheese.** *Food Chemistry* **78**: 249–254.

Solid-phase microextraction (SPME), using a carbowax/templated resin fibre, interfaced with HPLC–UV/diode array detection has been optimised for the determination of mycophenolic acid (MPA) in cheese samples. The procedure has been applied to the analysis of blue-cheese samples such as Gorgonzola and Danablu. Samples were subjected to a preliminary short sonication in bicarbonate buffer. The subsequent SPME was capable of a selective extraction of MPA, characterised by high recovery yields and detection limits of 50 and 100 µg/kg for Danablu and Gorgonzola, respectively. The method is faster and simpler than other existing methods for the extraction of MPA from cheese.

AMALFITANO, C., PENGUE, R., ANDOLFI, A., VURRO, M., ZONNO, M.C. and EVIDENTE, A. 2002. **HPLC analysis of fusaric acid, 9,10-dehydrofusaric acid and their methyl esters, toxic metabolites from weed pathogenic *Fusarium* species.** *Phytochemical Analysis* **13**: 277–282.

A simple and rapid HPLC method using a high density C₁₈ column has been developed for the quantitative analysis of fusaric and dehydrofusaric acids and their methyl esters in the methanol extract of lyophilised culture filtrates of species of *Fusarium*.

Mycotoxicoses

NAGASE, M., SHIOTA, T., TSUSHIMA, A., ALAM, M.M., FUKUOKA, S., YOSHIZAWA, T. and SAKATO, N. 2002. **Molecular mechanism of satratoxin-induced apoptosis in HL-60 cells: Activation of caspase-8 and caspase-9 is involved in activation of caspase-3.** *Immunology Letters* **84**: 23–27.

Satratoxins have been recognised as potential immunomodulatory agents in outbreaks of building related illness. Human leukaemia HL-60 cells treated with satratoxin G underwent apoptosis through the action of caspase-3 which was activated by both caspase-8 and caspase-9. Enzymic assay on IETD-AMC revealed that caspase-8 is strongly activated by exposure to satratoxin G. Furthermore, satratoxin G caused a release of cytochrome c from mitochondria into the cytosol and increased the activity of caspase-9 against LEHD-AMC. These findings indicate that satratoxin G-induced apoptosis involves activation of caspase-3 and DFF-40/CAD through both activation of caspase-8 and cytosolic accumulation of cytochrome c along with activation of caspase-9.

ATROSHI, F., RIZZO, A., WESTER-MARCK, T. and ALI-VEHMAS, T. 2002. **Antioxidant nutrients and mycotoxins.** *Toxicology* **180**: 151–167.

A review with 151 references. Several natural (vitamin, provitamins, carotenoids, chlorophyll and its derivatives, phenolics and selenium) and synthetic (butylated hydroxyanisole and butylated hydroxytoluene) compounds with antioxidant properties might be potentially very efficacious in protecting against the toxic effects of mycotoxins. The protective properties of antioxidants are probably due to their ability to act as superoxide anion scavengers, thereby protecting cell membranes from mycotoxin-induced damage and in some cases, antioxidant vitamins may play a role in preventing mycotoxicosis.

KAGAYA, N., TAGAWA, Y., NAGASHIMA, H., SAIJO, R., KAWASE, M. and YAGI, K. 2002. **Suppression of cytotoxin-induced cell death in isolated hepatocytes by tea catechins.** *European Journal of Pharmacology* **450**: 231–236.

The protective effects of (–)-epigallocatechin-3-gallate (EGCG), the major component of green tea catechin, together with other catechins, against the hepatotoxins, bromobenzene and rubratoxin B, were examined in primary cultures of rat hepatocytes. EGCG and (–)-epigallocatechin-3-(3"-O-methyl)gal-

late (EGCG-3"-OMe) prevented apoptotic cell death caused by rubratoxin B. In treated cells, both catechins were found to suppress the activation of caspase-3 by rubratoxin B. These results suggest that EGCG and EGCG-3"-OMe are potent hepatoprotective agents.

BILMEN, J.G., WOOTTON, L.L. and MICHELANGELI, F. 2002. **The mechanism of inhibition of the sarco/endoplasmic reticulum Ca²⁺ ATPase by paxilline.** *Archives of Biochemistry and Biophysics* **406**: 55–64.

Paxilline, an indole alkaloid mycotoxin from *Penicillium paxilli*, is an inhibitor of the sarco/endoplasmic reticulum Ca²⁺ ATPase (SERCA). Paxilline inhibited differing isoforms of SERCA with IC₅₀s between 5 and 50 µM. Studies suggest that paxilline has two effects on the Ca²⁺ ATPase. At lower concentrations (5–10 µM), paxilline inhibits the ATP-dependent acceleration of Ca²⁺ release from the phosphoenzyme and/or phosphoenzyme decay. At higher concentrations, paxilline inhibits phosphoenzyme formation.

BOYSEN, S.R., ROZANSKI, E.A., CHAN, D.L., GROBE, T.L., FALLON, M.J. and RUSH, J.E. 2002. **Tremorgenic mycotoxicosis in four dogs from a single household.** *Journal of the American Veterinary Medical Association* **221**: 1441–1444.

Penitrem A and roquefortine are tremorgenic mycotoxins that are rarely identified but should be considered as a cause of continual tremors and seizures in dogs, especially if there is exposure to mouldy foods.

SHARMA, C., AULERICH, R.J., RENDER, J.A., REIMERS, T., ROTTINGHAUS, G.E., KIZILKAYA, K. and BURSIAAN, S.J. 2002. **Reproductive toxicity of ergot alkaloids in mink.** *Veterinary and Human Toxicology* **44**: 324–327.

The reproductive toxicity of ergot alkaloids derived from ergot contaminated oats was investigated in mink. Four groups of 12 female mink each were fed diets containing ergot alkaloids at 0, 3, 6 or 12 mg/kg from 2 weeks prior to the breeding season until the kits were approximated 33 days old (133 days). Females were mated with untreated males. The gestation period of the mink in the 6 mg/kg group was longer compared to controls. The number of mink whelping varied significantly with nine mink whelping each in the control and 3 mg/kg groups compared to four mink in the 6 mg/kg group and one in the 12 mg/kg group. Ergot alkaloids had a significant effect on kit survivability with no kits surviving in the 12 mg/kg group.

VERMA, J., SWAIN, B.K. and JOHRI, T.S. 2002. **Effect of various levels of aflatoxin and ochratoxin A and combinations thereof on protein and energy utilisation in broilers.** *Journal of the Science of Food and Agriculture* **82**: 1412–1417.

Protein and energy utilisation were evaluated in broilers fed diets containing aflatoxin at 0, 0.5, 1 and 2 mg/kg and OA at 0, 1, 2 and 4 mg/kg, either singly or in combination. Total protein efficiency (TPE) was reduced by 50.97, 76.52 and 132.75% at 2 mg/kg of aflatoxin, and 2 and 4 mg/kg of OA, respectively. When the toxins were combined, 1 mg/kg of aflatoxin plus 2 mg/kg of OA and 2 mg/kg of aflatoxin plus 4 mg/kg of OA, reductions in TPE of 78.58 and 127.43%, respectively, were observed. Aflatoxins at all three levels and OA at 2 and 4 mg/kg caused significant decreases in net protein utilisation (NPU). Co-toxicity at all three levels led to significantly lower NPU. The reduction in NPU ranged from 18.68% at 0.5 mg/kg of aflatoxin to 75.12% at 2 mg/kg of aflatoxin plus 4 mg/kg of OA.

DIAZ, G.J. 2002. **Evaluation of the efficacy of a feed additive to ameliorate the toxic effects of 4,15-diacetoxyscirpenol in growing chicks.** *Poultry Science* **81**: 1492–1495.

The possible protective effect of a feed additive, Mycofix[®], against the toxic effects of 4,15-diacetoxyscirpenol (DAS) in growing broiler chickens was investigated. Chicks were fed diets containing DAS at 1 or 2 mg/kg diet, with or without the addition of Mycofix[®] at 0.75 or 1.5 g/kg diet. When no feed additive was included, both levels of dietary DAS significantly decreased body weight and feed intake, and caused oral lesions, with the effect of 2 mg/kg being more severe. With DAS at 1 mg/kg, Mycofix[®] at both levels of inclusion protected against the adverse effects of DAS on feed intake and body weight. However, no protection against oral lesions was obtained by Mycofix[®] supplementation. With DAS at 2 mg/kg, only partial protection on body weight and feed intake was obtained by Mycofix[®] supplementation.

GANASSI, S., MORETTI, A., PAGLIAI, A.M.B., LOGRIECO, A. and SABATINI, M.A. 2002. **Effects of beauvericin on *Schizaphis graminum* (Aphididae).** *Journal of Invertebrate Pathology* **80**: 90–96.

Aphids (*Schizaphis graminum*) were reared on wheat leaves inserted into a sandy substratum wetted with a solution of beauvericin. Ingestion of this solution through leaves did not significantly decrease the lifespan of females of all generations as compared to controls. However, the mean number of offspring from the third generation of treated females was significantly smaller than those in controls. Furthermore, treated second and third generation females produced a greater number of abortive embryos.

VEY, A., MATHA, V. and DUMAS, C. 2002. **Effects of the peptide mycotoxin destruxin E on insect haemocytes and on dynamics and efficiency of the multicellular immune reaction.** *Journal of Invertebrate Pathology* **80**: 177–187.

The effects of destruxin E on haemocytes, immunocompetent insect cells, and on the dynamics and efficacy of the multicellular defence of insect hosts have been investigated. Ultrastructural alterations have been observed in circulating plasmatocytes and granular haemocytes, and in attached haemocytes of *Galleria mellonella* larvae treated with a toxic dose of destruxin E (LC₅₀). These changes appear as a consequence of disturbances induced in the cellular calcium balance. Morphological studies of haemocytic capsules formed *in vivo* revealed disturbances of the multicellular defence mechanism after toxin treatment. However, an attempt to establish if these changes were significant was unsuccessful. In contrast, comparative assays regarding the effect of toxin treatment on the efficacy of the antifungal effect of encapsulation has given conclusive results. The germination of injected *Aspergillus niger* spores became slightly but significantly increased, and when the granuloma were incubated the fungus escaped more easily from the haemocytic envelope.

Ochratoxins – General

CABANES, F.J., ACCENSI, F., BRAGULAT, M.R., ABARCA, M.L., CASTELLA, G., MINGUEZ, S. and PONS, A. 2002. **What is the source of ochratoxin A in wine?** *International Journal of Food Microbiology* **79**: 213–215.

During a microvinification trial using natural mouldy grapes, OA contaminated white wine was obtained. The mycobiota of grape samples used in this microvinification process was assessed. Only the *Aspergillus carbonarius* isolates were found to produce OA.

BATTILANI, P. and PIETRI, A. 2002. **Ochratoxin A in grapes and wine.** *European Journal of Plant Pathology* **108**: 639–643.

Field trials were conducted in Italy during 1999 and 2000 to study fungi associated with grapes and their ability to produce ochratoxin. In both years, 95% of strains colonising grapes belonged to the genus *Aspergillus*. *A. niger* aggregate was dominant, with about 50% of the ochratoxin producing strains identified as *A. carbonarius*. This species is very invasive and colonises and penetrates berries, even without skin damage. Temperature, rain and relative humidity are the main factors that influence ochratoxin production in grapes.

DECERAIN, A.L., GONZALEZ-PENAS, E., JIMENEZ, A.M. and BELLO, J. 2002. **Contribution to the study of ochratoxin A in Spanish wines.** *Food Additives and Contaminants* **19**: 1058–1064.

Forty wines (28 red and 12 white) obtained from grapes cultivated in three different places of the northern Spanish region of Navarra in 1997 and 1998 were assayed for OA. Among samples collected in 1997, 85%

showed OA levels above the detection limit of 0.05 µg/L (range 0.056–0.316 µg/L). Of samples collected in 1998, 15% showed OA levels above the detection limit (range 0.074–0.193 µg/L). These differences in OA contamination from the two different years were attributed to the bad climate in 1997. Further analyses showed that OA is stable in wine for at least one year.

LOMBAERT, G.A., PELLAERS, P., CHETTIAR, M., LAVALEE, D., SCOTT, P.M. and LAU, B.P.Y. 2002. **Survey of Canadian retail coffees for ochratoxin A.** *Food Additives and Contaminants* **19**: 869–877.

Samples of coffee were gathered from retail outlets across Canada and analysed for OA. OA was quantified by LC with fluorescence detection after cleanup either by immunoaffinity column chromatography or by a combination of solid-phase extraction and immunoaffinity column chromatography. The minimum quantifiable level was 0.1 µg/kg. OA was present in 42 (59%) of 71 roasted beans and roasted ground coffee and in 20 (67%) of 30 instant coffees. The mean OA levels in the positive samples were 0.6 and 1.1 µg/kg, respectively.

RIZZO, A., ESKOLA, M. and ATROSHI, F. 2002. **Ochratoxin A in cereals, food-stuffs and human plasma.** *European Journal of Plant Pathology* **108**: 631–637.

Five new isolates of *Aspergillus*, *A. albertensis*, *A. auricomus*, *A. wentii*, *A. fumigatus* and *A. versicolor*, were found to produce OA. Data on the occurrence and the concentration levels of OA in European food of vegetable and animal origin are reported. Furthermore, data on the concentration of OA in blood of citizens of Western Europe are compared with those of some areas where Balkan endemic nephropathy is endemic.

ABOUZIED, M.M., HORVATH, A.D., PODLESNY, P.M., REGINA, N.P., METODIEV, V.D., KAMENOVA-TOZEVA, R.M., NIAGOLOVA, N.D., STEIN, A.D., PETROPOULOS, E.A. and GANEV, V.S. 2002. **Ochratoxin A concentrations in food and feed from a region with Balkan Endemic Nephropathy.** *Food Additives and Contaminants* **19**: 755–764.

OA contamination in 165 samples of home-produced food (beans, potatoes, corn, wheat, flour) and feed from households in villages from the Balkan Endemic Nephropathy (BEN) region (Vratza district) of north-western Bulgaria were investigated. Samples were collected from BEN villages and from BEN households and BEN-free households within the villages, and from BEN-free villages and therein BEN-free households. BEN households consistently had a higher proportion of OA positive samples than BEN-free households within the BEN villages but similar or lower proportions compared to BEN-free vil-

lage households. OA exposure estimates showed an OA intake in BEN households of 1.21 µg/day, compared to 1.03 µg/day in BEN-free village households and 0.71 µg/day in BEN-free households within BEN villages. The results indicate that OA alone may not cause BEN, but might act synergistically with other environmental toxicants and/or predisposing genotypes.

CZERWIECKI, L., CZAJKOWSKA, D. and WITKOWSKA-GWIAZDOWSKA, A. 2002. **On ochratoxin A and fungal flora in Polish cereals from conventional and ecological farms. Part 2: Occurrence of ochratoxin A and fungi in cereals in 1998.** Food Additives and Contaminants **19**: 1051–1057.

Over 200 samples of Polish cereal grain from the 1998 harvest obtained from conventional and ecological farms were investigated for the presence of OA. The frequency of contamination of rye and barley grains from conventional and ecological farms was similar in most cases. However, samples from ecological farms had higher maximum concentrations of OA (35 µg/kg; range 1.4–35.3 µg/kg) for both cereals rye and barley in comparison with rye and barley from conventional farms (maximum levels of 8.8 and 9.7 µg/kg; respectively). Wheat grain from conventional farms showed OA concentrations in a wide range from 0.6 to 1024 µg/kg with an average frequency of contaminated samples of about 48%. In contrast, in wheat samples from ecological farming OA ranged from 0.8 to 1.6 µg/kg and the frequency of contamination was 23%.

DALCERO, A., MAGNOLI, C., HALLAK, C., CHIACCHIERA, S.M., PALACIO, G. and ROSA, C.A.R. 2002. **Detection of ochratoxin A in animal feeds and capacity to produce this mycotoxin by *Aspergillus* section *Nigri* in Argentina.** Food Additives and Contaminants **19**: 1065–1072.

The natural occurrence of OA in poultry, pig and rabbit feeds collected in Argentina over 8 months was assayed. OA was found in 38% of the poultry feed samples tested with levels ranging from 25 to 30 µg/kg. From rabbit feed samples, 25% contained OA and the levels ranged from 18.5 to 25 µg/kg. Only 13% of the pig feed samples were contaminated with similar levels of toxins. Among 94 black *Aspergillus* strains isolated from feed-stuffs, 46% were producers of OA, with levels ranging from 13 to 25 µg/L culture medium.

PETZINGER, E. and WEIDENBACH, A. 2002. **Mycotoxins in the food chain: the role of ochratoxins.** Livestock Production Science **76**: 245–250.

Recent studies have documented significant effects of OA on immune responses even at OA concentrations far below the doses used in carcinogenicity studies. This report deals with OA food contamination, consumer

exposure and recent toxicological data relating to the effect of OA on cytokine release in the liver.

VARGA, J., RIGO, K., LAMPER, C., TEREN, J. and SZABO, G. 2002. **Kinetics of ochratoxin A production in different *Aspergillus* species.** Acta Biologica Hungarica **53**: 381–388.

OA production kinetics were examined in a number of ochratoxin producing isolates representing different sections of the genus *Aspergillus*. All isolates were found to produce the highest amounts of ochratoxin A after 7–10 days of incubation. Production varied between 0.03 and 500 mg/L.

Ochratoxins – Methodology

SIBANDA, L., DESAEGER, S., BARNA-VETRO, I. and VAN-PETEGHEM, C. 2002. **Development of a solid-phase cleanup and portable rapid flow-through enzyme immunoassay for the detection of ochratoxin A in roasted coffee.** Journal of Agricultural and Food Chemistry **50**: 6964–6967.

A membrane-based flow-through enzyme immunoassay for the detection of OA in roasted coffee was developed. First, an extraction and solid-phase cleanup method was developed. A high partition coefficient for OA in the mobile phase was achieved by using methanol/5% aqueous NaHCO₃ as the sample extraction and cleanup solvent. The solid-phase (aminopropyl) cleanup was developed to chromatographically elute OA but retain cross-reacting compounds. The limit of detection was 4 µg/kg of spiked roasted coffee.

ABOUL-ENEIN, H.Y., KUTLUK, O.B., ALTIOKKA, G. and TUNCEL, M. 2002. **A modified HPLC method for the determination of ochratoxin A by fluorescence detection.** Biomedical Chromatography **16**: 470–474.

An HPLC method with fluorescence detection is described for the determination of OA. A mobile phase consisting of acetonitrile:water:acetic acid (99:99:2; v/v/v) was used for the resolution of OA on a C₁₈ Hypersil column. The relative standard deviation (RSD) was 1.70 and linear in the range of 2.5 x 10⁻⁹ to 1.5 x 10⁻⁸ M OA. The limit of detection and limit of quantification were 2.5 x 10⁻¹⁰ M corresponding to 0.1 µg/L and 8.2 x 10⁻¹⁰ M corresponding to 3.3 µg/L, respectively. The method was applied for analysis of OA in wheat, corn, red pepper, cheese and wine.

Ochratoxicoses

SCHAAF, G.J., NIJMEIJER, S.M., MAAS, R.F.M., ROESTENBERG, P., DEGROENE, E.M. and FINK-GREM-

MELS, J. 2002. **The role of oxidative stress in the ochratoxin A-mediated toxicity in proximal tubular cells.** Biochimica et Biophysica Acta – Molecular Basis of Disease **1588**: 149–158.

OA predominantly affects the kidney and is known to accumulate in the proximal tubule (PT). Primary rat PT cells and LLC-PK1 cells, which express characteristics of the PT, were used to investigate the OA-mediated oxidative stress response. OA exposure of these cells resulted in a concentration dependent elevation of reactive oxygen species (ROS) levels, depletion of cellular glutathione (GSH) levels and an increase in the formation of 8-oxoguanine. The OA-induced ROS response was significantly reduced following treatment with alpha-tocopherol (TOCO), a chain breaking antioxidant. However, TOCO did not reduce the cytotoxicity of OA and was unable to prevent the depletion of total GSH levels. In contrast, pre-incubation of the cell with N-acetyl-L-cysteine completely prevented the OA-induced increase in ROS levels, the formation of 8-oxoguanine, limited GSH depletion, and completely protected against the cytotoxicity of OA.

STOEV, S.D., DJUVINOV, D., MIRTICHEVA, T., PAVLOV, D. and MANTLE, P. 2002. **Studies on some feed additives giving partial protection against ochratoxin A toxicity in chicks.** Toxicology Letters **135**: 33–50.

Water extract of artichoke, sesame seed, Roxazyme-G and L-beta phenylalanine, gave significant protective effects against growth inhibitory effects and associated pathomorphological changes in chicks treated with OA. Whereas OA induced strong degenerative changes and an increase in weight of kidneys and liver as well its a decrease of the weight of lymphoid organs, the additives variously gave protection against these changes. The protection of Roxazyme-G and sesame seed was better expressed in kidneys and liver, whereas phenylalanine better protected the weight changes in gizzard, heart and the changes in differential WBC count. Sesame seed gave strong protection against OA-induced suppression of humoral immune response.

Fumonisin – General

ONO, E.Y.S., SASAKI, E.Y., HASHIMOTO, E.H., HARA, L.N., CORREA, B., ITANO, E.N., SUGIURA, T., UENO, Y. and HIROOKA, E.Y. 2002. **Post-harvest storage of corn: Effect of beginning moisture content on mycoflora and fumonisin contamination.** Food Additives and Contaminants **19**: 1081–1090.

The effect of storage on mycoflora profile was monitored bimonthly in 36 corn samples dried to 11 and 14% moisture content. In corn dried to 11%, the fumonisin content was anal-

ysed at the initial stage (freshly harvested) and at the end of 12-month storage. Fumonisin were detected in all freshly harvested corn at a mean concentration of 9.9 ± 6.0 mg/kg (range 0.74–22.6 mg/kg). These values did not change in the 12-month stored corn (mean of 9.9 ± 5.8 mg/kg; range 0.81–23.7 mg/kg).

ASRAN, M.R. and BUCHENAUER, H. 2002. **Virulence of *Fusarium moniliforme* isolates on maize plants in relation to fumonisin and ergosterol levels.** Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz – Journal of Plant Diseases and Protection **109**: 491–505.

Six isolates of *Fusarium moniliforme* obtained from naturally infected maize plants were examined for possible correlations between maize seedling blight severity and fumonisin production as well as ergosterol levels in the infected tissues. While in the most and least virulent isolates fumonisin and ergosterol contents in the seedlings was associated with disease severity, in the isolates with middle virulence no relations between these parameters were found.

SHEPHARD, G.S., LEGGOTT, N.L., STOCKENSTROM, S., SOMDYALA, N.I.M. and MARASAS, W.F.O. 2002. **Preparation of South African maize porridge: effect on fumonisin mycotoxin levels.** South African Journal of Science **98**: 393–396.

During the preparation of a typical South African stiff porridge, fumonisin levels in naturally contaminated maize meal were reduced during cooking. A mean reduction in FB_1 levels of 23% was observed. A survey of available maize consumption data from around the world indicated that the highest levels of maize consumption are found in the general Mexican population and in the rural population of the Transkei region of South Africa.

REYNOSO, M.M., TORRES, A.M., RAMIREZ, M.L., RODRIGUEZ, M.L., CHULZE, S.N. and MAGAN, N. 2002. **Efficacy of antioxidant mixtures on growth, fumonisin production and hydrolytic enzyme production by *Fusarium verticillioides* and *F. proliferatum* in vitro on maize-based media.** Mycological Research **106**: 1093–1099.

The effects of antioxidants, alone and in combination, on the lag phase prior to growth, growth rate, hydrolytic enzyme production and fumonisin production by *Fusarium verticillioides* and *F. proliferatum* was evaluated on maize-based media at 25°C under different water activity (a_w) conditions. For both species, propyl paraben (PP) alone or in combination with butylated hydroxyanisole (BHA), at concentrations of 0.5 and 1 mM reduced the growth rates by 85% at the three a_w levels tested (0.995, 0.98 and 0.95). PP plus butylated hydroxytoluene or trihydroxybutyrophenone were less effective in

controlling growth. Combinations of PP+BHA reduced the fumonisin concentrations produced by both species at 0.995 and 0.98 a_w , significantly. However, at low concentrations of antioxidants (0.5 mM) some stimulation in fumonisin production was observed with some treatments.

CORTEZ-ROCHA, M.O., TRIGO-STOCKLI, D.M., WETZEL, D.L. and REED, C.R. 2002. **Effects of extrusion processing on fumonisin B_1 and hydrolyzed fumonisin B_1 in contaminated alkali-cooked corn.** Bulletin of Environmental Contamination and Toxicology **69**: 471–478.

The effects of moisture content and die configuration during extrusion processing on FB_1 and hydrolysed FB_1 (H FB_1) contaminated alkali-cooked corn were investigated. About one-third of the initial fumonisin contamination was removed by the steep water. Of the 61% of the original FB_1 remaining in the nixtamal, 72% was present as H FB_1 . Only 17% of the FB_1 remained unchanged during the alkali cooking of the grain. The recoverable FB_1 and H FB_1 were affected significantly by the die configuration during extrusion processing. The tapered angular die reduced the fumonisin levels more than the tapered circular die. With the tapered angular die, the amount of moisture during processing also significantly affected the FB_1 and H FB_1 levels with reductions of 90–99% obtained by extruding at moisture contents of 24, 27 and 30%.

DESJARDINS, A.E., MUNKVOLD, G.P., PLATTNER, R.D. and PROCTOR, R.H. 2002. ***FUMI-A* gene required for fumonisin biosynthesis but not for maize ear rot and ear infection by *Gibberella moniliformis* in field tests.** Molecular Plant–Microbe Interactions **15**: 1157–1164.

Fumonisin-nonproducing mutants of *Fusarium verticillioides* were obtained by disrupting *FUMI* (previously *FUM5*), the gene encoding a polyketide synthase required for fumonisin biosynthesis. Maize ear rot, ear infection and fumonisin contamination were assessed after silk-channel injection and also by spray application onto maize silks, injection into maize stalks, and application onto maize seeds at planting. The presence of applied strains in kernels was determined by analysis of recovered isolates for genetic markers and fumonisin production. Two independent fumonisin-nonproducing (*fum1-3* and *fum1-4*) mutants were similar to their respective fumonisin-producing (*FUMI-1*) progenitor strains in ability to cause ear rot following silk-channel injection and also were similar in ability to infect maize ears following application by all four methods tested. This evidence confirms that fumonisins are not required for maize ear rot and ear infection by *F. verticillioides*.

Fumonisin – Methodology

SEEFELDER, W., SCHWERDT, G., FREUDINGER, R., GEKLE, M. and HUMPF, H.U. 2002. **Liquid chromatography/electrospray ionisation-mass spectrometry method for the quantification of sphingosine and sphinganine in cell cultures exposed to fumonisins.** Journal of Chromatography B – Analytical Technologies in the Biomedical and Life Sciences **780**: 137–144.

A new column liquid chromatography/electrospray ionisation-mass spectrometry (LC-ESI-MS) method is described for the rapid, simultaneous and quantitative determination of sphingosine (So) and sphinganine (Sa) in cell cultures of immortalised human kidney epithelial cells (IHKE cells). For sample preparation, cell lysates were only diluted, centrifuged and directly used for LC-MS measurements. Detecting the protonated molecule $[M+H]^+$ signals of So (m/z 300) and Sa (m/z 302) in the selected ion monitoring mode, detection limits of 10 pg for So (signal-to-noise ratio S/N=3:1) and 25 pg for Sa (S/N=3:1) were established. The average recovery for So and Sa was higher than 90% for control IHKE-cells.

Fumonisin – Toxicology

ZOMBORSZKY-KOVACS, M., KOVACS, F., VETESI, F., REPA, I., TORNYOS, G. and TOTH, A. 2002. **Investigations into the time- and dose-dependent effect of fumonisin B_1 in order to determine tolerable limit values in pigs.** Livestock Production Science **76**: 251–256.

Culture material of *Fusarium moniliforme* was added to the diet of weaned piglets so that the FB_1 exposure was 0–40 mg/kg feed for 4 weeks; 0–10 mg/kg for 8 weeks; or 0–10 mg/kg for 20 weeks. None of the treatments had any significant effect on feed consumption, body weight gain or feed conversion of weaned pigs. The haematological parameters proved to be within the range of the physiological limit values. FB_1 at 1 mg/kg diet was the lowest concentration that failed to increase the Sa/So ratio significantly. FB_1 at 10–40 mg/kg fed for 4 weeks caused mild or severe pulmonary oedema, while even lower doses (1–10 mg/kg) fed for 2–20 weeks caused irreversible fibrosis in the lung.

HE, Q.R., BHANDARI, N. and SHARMA, R.P. 2002. **Fumonisin B_1 alters sphingolipid metabolism and tumor necrosis factor alpha expression in heart and lung of mice.** Life Sciences **71**: 2015–2023.

The effects of FB_1 on free sphingoid bases and expression of tumor necrosis factor alpha (TNF α) were examined in mouse heart and lung, organs that are not targets. Mice were treated with 5-daily sc injections of FB_1 at 2.25 mg/kg. A significant increase in free

sphingoid bases was observed in both heart and lung of treated mice. The magnitude of increase in both organs of female mice was much higher than that in males. TNF α expression was increased in the lung of male mice and in the heart of female mice, whereas the expression of interferon gamma was unaltered.

VOSS, K.A., HOWARD, P.C., RILEY, R.T., SHARMA, R.P., BUCCI, T.J. and LORENTZEN, R.J. 2002. **Carcinogenicity and mechanism of action of fumonisin B₁: A mycotoxin produced by *Fusarium moniliforme* (= *F. verticillioides*).** *Cancer Detection and Prevention* **26**: 1–9.

FB₁ was fed to rats and mice for 2 years or, in separate studies, given to rats or mice for up to 4 weeks. Kidney tubule adenomas and carcinomas were found in male rats fed FB₁ at greater than or equal to 50 mg/kg, whereas liver adenomas and carcinomas were found in female mice fed greater than or equal to 50 mg/kg for 2 years. In the short-term studies, increases in tissue concentrations of Sa and Sa/So ratio were correlated with apoptosis. Further, hepatotoxicity was ameliorated in mice lacking either the TNFR1 or the TNFR2 TNF α receptors.

SHARMA, R.P., HE, Q.R., MEREDITH, F.I., RILEY, R.T. and VOSS, K.A. 2002. **Paradoxical role of tumor necrosis factor alpha in fumonisin induced hepatotoxicity in mice.** *Toxicology* **180**: 221–232.

The effect of fumonisin treatment on mice lacking either TNF α or both TNF α receptors was compared their wild type (WT) counterparts. The FB₁-induced increase in circulating liver enzymes was enhanced by deletion of TNF α or unchanged in mice lacking both TNF α receptors. These findings corresponded with the degree of toxicity as established by microscopic examination of liver. FB₁ induced the expression of TNF α in the liver of all strains, except the animals with a deleted TNF α gene. The FB₁-mediated increases in liver So or Sa paralleled the hepatotoxic responses. It is apparent that the presence of TNF α is not necessary for FB₁-induced hepatotoxicity in mice and a lack of the function of this cytokine may aggravate the hepatotoxic responses to fumonisins, perhaps by preventing repair mechanisms or by expression of other signaling molecules.

DRESDEN-OSBORNE, C. and NOBLET, G.P. 2002. **Fumonisin B₁ affects viability and alters nitric oxide production of a murine macrophage cell line.** *International Immunopharmacology* **2**: 1087–1093.

FB₁ at 1–100 μ M decreased viability in the murine macrophage cell line RAW264.7 in a dose dependent manner. When cells exposed to FB₁ were stimulated with lipopolysaccharide (LPS), a dose dependent increase in production of nitric oxide (NO) was observed, but only at FB₁ concentrations

(10–50 μ M) that induced significant cytotoxicity. Stimulation of cells with phorbol myristate acetate resulted in increased NO production at 50 μ M FB₁, but induced a variable NO response at 1–10 μ M FB₁.

GELDERBLUM, W.C.A., MORITZ, W., SWANEVELDER, S., SMUTS, C.M. and ABEL, S. 2002. **Lipids and Delta 6-desaturase activity alterations in rat liver microsomal membranes induced by fumonisin B₁.** *Lipids* **37**: 869–877.

Detailed analyses of lipids in liver microsomal fractions of rats exposed to different dietary levels of FB₁ over a period of 21 days indicated an increase in PC, PE, PI, and cholesterol (Chol). These changes decreased the PC/PE and increased the total phospholipid/Chol ratios. The quantities of total FA increased in the major phospholipid fractions as a result of the increased phospholipid levels. However, when considering the relative levels of specific FA, the monounsaturated FA (16:1n-7 and 18:1n-9) and 18:2n-6 increased, whereas the long-chain PUFA decreased in the main phospholipid fractions. Disruption of microsomal lipid metabolism at different levels by FB₁ could play an important role in the alteration of growth regulatory effects in the liver.

SADLER, T.W., MERRILL, A.H., STEVENS, V.L., SULLARDS, M.C., WANG, E. and WANG, P. 2002. **Prevention of fumonisin B₁-induced neural tube defects by folic acid.** *Teratology* **66**: 169–176.

Neurulating mouse embryos were exposed to fumonisin or folic acid in whole embryo culture and assessed for effects on growth and development. Fumonisin exposure inhibited sphingolipid synthesis, reduced growth and caused cranial neural tube defects in a dose dependent manner. Supplemental folic acid ameliorated the effects on growth and development, but not inhibition of sphingolipid synthesis.

Trichothecenes – General

LEBLANC, J.C., MALMAURET, L., DELOBEL, D. and VERGER, P. 2002. **Simulation of the exposure to deoxynivalenol of French consumers of organic and conventional foodstuffs.** *Regulatory Toxicology and Pharmacology* **36**: 149–154.

The exposure to DON in contaminated wheat of French consumers of organic and conventional products was estimated using a probabilistic method. A product consumption frequency questionnaire was completed by consumers of organic products and data on the consumption of conventional products were obtained from the French 'INCA' survey. Data on DON levels in wheat came from a previous study. The results of exposure simulations using the Monte-Carlo sampling

method showed that 10% of those consuming organic wheat containing DON may be exposed to the toxin at levels above the provisional maximum tolerable daily intake.

MUTHOMI, J.W., OERKE, E.C., MUTITU, E.W., SCHADE-SCHUETZE, A. and DEHNE, H.W. 2002. **Variation among *Fusarium* species and isolates infecting wheat ears based on aggressiveness, mycotoxin production and RAPD-PCR analysis.** *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz – Journal of Plant Diseases and Protection* **109**: 462–477.

Fusarium species from Germany and Kenya were investigated for their variation in mycotoxin production, aggressiveness on wheat ears and PCR-based DNA fingerprints in order to characterise species involved in the head scab complex of wheat in different regions. Nineteen isolates of *F. graminearum* from Kenya were screened for aggressiveness and trichothecene production on wheat ears and *in vitro* mycotoxin production. Isolates *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. poae* were compared for their virulence and aggressiveness on four winter wheat cultivars. Isolates of *F. graminearum* varied markedly in aggressiveness and mycotoxin production. Variation among isolates was also evident in RAPD-PCR profiles. The high genetic variability for aggressiveness and mycotoxin production among isolates of *Fusarium* species within small regions considerably complicates the forecasting of the effects of head scab on yield quantity and quality.

BIRZELE, B., MEIER, A., HINDORF, H., KRAMER, J. and DEHNE, H.W. 2002. **Epidemiology of *Fusarium* infection and deoxynivalenol content in winter wheat in the Rhineland, Germany.** *European Journal of Plant Pathology* **108**: 667–673.

Results of a long-term research programme studying the epidemiology of *Fusarium* species and mycotoxin production, mainly on winter wheat (*Triticum aestivum*), are summarised. During 1995–1998, 2–15% of grains were infected at three climatologically differing localities of the Rhineland, Germany. Disease progress was accelerated by rainfall during the flowering season. The species most frequently isolated were *Fusarium avenaceum*, *F. poae*, *F. culmorum* and *F. graminearum*. The mean DON content varied from 19 μ g/kg (1995) to 310 μ g/kg (1998) and was not always correlated with disease severity. Organic farming systems showed lower rates of infection with ear blight and lower mycotoxin contamination than conventional farming systems.

BOTTALICO, A. and PERRONE, G. 2002. **Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe.** *European Journal of Plant Pathology* **108**: 611–624.

The most frequently encountered mycotoxins in *Fusarium* head blight (FHB) of small grains in Europe are DON and ZEA produced by *F. graminearum* and *F. culmorum*, with the former more common in southern (warmer) and the latter in northern (colder) European areas. Nivalenol was usually found associated with DON and its derivatives (mono-acetylDONs), together with fusarenone-X, formed by *F. graminearum*, *F. cerealis*, *F. culmorum* and, in northern areas, by *F. poae*. Moreover, from central to northern European countries, moniliformin has been consistently reported, as a consequence of the widespread distribution of *F. avenaceum*, whereas the occurrence of T-2 toxin derivatives, such as T-2 toxin and HT-2 toxin, and DAS have been recorded in conjunction with sporadic epidemics of *F. sporotrichioides* and *F. poae*. Finally, BEA and various enniatins have recently been found in Finnish wheat colonised by *F. avenaceum* and *F. poae*.

TOMCZAK, M., WISNIEWSKA, H., STEPIEN, L., KOSTECKI, M., CHELKOWSKI, J. and GOLINSKI, P. 2002. **Deoxynivalenol, nivalenol and moniliformin in wheat samples with head blight (scab) symptoms in Poland (1998–2000)**. European Journal of Plant Pathology **108**: 625–630.

Fusarium head blight epidemics of wheat occurred in Zulawy (Northern Poland) during 1998 and in Wielkopolska (West) and in Southern regions of Poland in 1999. Four species were identified in wheat heads with scab symptoms: *F. culmorum*, *F. graminearum*, *F. avenaceum* and *Microdochium nivale*. DON, nivalenol and moniliformin in amounts up to 24.3, 14.2 and 1.72 mg/kg, respectively, were identified in kernels samples.

KANG, Z. and BUCHENAUER, H. 2002. **Studies on the infection process of *Fusarium culmorum* in wheat spikes: Degradation of host cell wall components and localization of trichothecene toxins in infected tissue**. European Journal of Plant Pathology **108**: 653–660.

After single spikelet inoculation of wheat, the infection process of *Fusarium culmorum* and spread of fungal hyphae in the spike tissues were studied by scanning and transmission electron microscopy. While hyphal growth on outer surfaces of the spike was scanty and no successful penetration was observed, the fungus developed a dense mycelium on the inner surfaces and effectively invaded the lemma, glume, palea and ovary by penetration pegs. Localisation studies of trichothecenes indicated that toxins could be detected in host tissues at an early stage of infection.

MESTERHAZY, A. 2002. **Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in**

resistance to *Fusarium* head blight. European Journal of Plant Pathology **108**: 675–684.

The data available indicate that aggressiveness of *Fusarium graminearum* and *F. culmorum* depends on their DON and nivalenol producing capacity. However, the resistance of a cultivar can influence DON production significantly. In the most resistant genotypes, toxin contamination remained near zero, whereas the same isolates and inoculum produced very high toxin levels in susceptible cultivars. As toxin levels were correlated with the ratio of *Fusarium*-damaged kernels (FDK) and this ratio is very low in highly resistant cultivars, the conclusion is that the level of resistance level is more important in governing DON accumulation in a given cultivar than is the aggressiveness of an isolate. In different years, the same FDK values were associated with different DON concentrations and this depended very much on the precipitation towards the end of May, the time of inoculation.

URREA, C.A., HORSLEY, R.D., STEFFENSON, B.J. and SCHWARZ, P.B. 2002. **Heritability of *Fusarium* head blight resistance and deoxynivalenol accumulation from barley accession CIho 4196**. Crop Science **42**: 1404–1408.

In developing an efficient breeding strategy for developing *Fusarium* head blight (FHB) resistant cultivars of barley, F-4:5 and F-4:6 families from the cross between the FHB susceptible six-rowed cultivar Foster and the resistant two-rowed accession CIho 4196 were studied. Heritability of FHB severity and DON accumulation was 0.65 and 0.46, respectively. A moderately strong positive association between FHB severity and DON accumulation was observed ($r = 0.62$). FHB severity and DON accumulation were negatively associated with plant height, days to heading, spike angle and spike density. The selection differentials calculated between the top F-4:6 families selected for low FHB severity and the unselected F-4:5 families were moderately high for FHB severity, DON accumulation and days to heading.

ABRAMSON, D., MCCALLUM, B., SMITH, D.M. and TEKAUZ, A. 2002. **Moniliformin in barley inoculated with *Fusarium avenaceum***. Food Additives and Contaminants **19**: 765–769.

Moniliformin (MON) production was assessed in inoculation trials with four barley cultivars and local isolates of *Fusarium avenaceum* at two different experimental farms in southern Manitoba, Canada, during 1997–99. In 1997, MON was detected in 11/16 barley rows and *F. avenaceum* infection ranged from 10 to 57% in rows where the toxin was detected. In 1998, MON was detected in only 3/16 barley rows and *F. avenaceum* infection between 16 and 39%. In 1999, MON was detected in 11/16 barley rows with *F. avenaceum* infection between 37 and 76%. The data

suggest that in years of high rainfall and *F. avenaceum* infection, MON is likely to be found in Manitoba barley.

SCHAAFSMA, A.W., HOOKER, D.C., BAUTE, T.S. and ILLINCIC-TAMBURIC, L. 2002. **Effect of *Bt*-corn hybrids on deoxynivalenol content in grain at harvest**. Plant Disease **86**: 1123–1126.

Concentrations of DON and FB₁ in grain were compared among *Bt*-transformed corn hybrids and their non-*Bt* isolines on 102 commercial corn fields across Ontario from 1996 to 1999. Intensities of naturally occurring populations of *Ostrinia nubilalis* were assessed from tunnelling measurements in the stalks of non-*Bt* isolines. The effect of *Bt* hybrids on reducing concentrations of DON was mainly dependent on the intensity of *O. nubilalis* in each field. Where a high intensity (stalk injury) of *O. nubilalis* was observed, the use of *Bt* hybrids reduced concentrations of DON by an average of 59% compared to concentrations in the non-*Bt* isolate. Where the intensity of *O. nubilalis* was low, concentrations of DON were not different among *Bt* and non-*Bt* hybrids. A quadratic relationship was developed showing that the concentration of DON increased with intensity of *O. nubilalis* feeding. Relationships between the concentration of FB₁ and intensity of *O. nubilalis* could not be determined because the concentrations of FB₁ were below the lower limit of detection in most fields.

PINEDA-VALDES, G., RYU, D., JACKSON, D.S. and BULLERMAN, L.B. 2002. **Reduction of moniliformin during alkaline cooking of corn**. Cereal Chemistry **79**: 779–782.

The incidence of MON-producing *Fusarium* species in selected corn samples from Mexico and the United States, and the effects of alkaline cooking and the tortilla manufacturing processes on the reduction of MON, were determined. A 100% reduction of MON was observed when a naturally contaminated corn sample containing MON at 1.4 mg/kg was used in a pilot-scale alkaline cooking and tortilla manufacturing process. In a companion laboratory-scale study using a cultured corn sample containing MON at 17.6 mg/kg, a 71% reduction of the toxin was observed during the process.

MARTINS, M.L. and MARTINS, H.M. 2002. **Influence of water activity, temperature and incubation time on the simultaneous production of deoxynivalenol and zearalenone in corn (*Zea mays*) by *Fusarium graminearum***. Food Chemistry **79**: 315–318.

The production of DON and ZEA by *Fusarium graminearum* was studied under different culture conditions of water activity a_w , temperature and incubation time. The maximum levels of both toxins were obtained at the 35th day of incubation. The culture conditions that gave higher yields of DON

were at 22 and 28°C after 35 days. The highest level of ZEA was obtained at 28°C for 16 days, followed by incubation at 12°C. *Fusarium graminearum* did not produce DON and ZEA at 37°C.

ERIKSEN, G.S., PETTERSSON, H., JOHNSEN, K. and LINDBERG, J.E. 2002. **Transformation of trichothecenes in ileal digesta and faeces from pigs.** Archives of Animal Nutrition – Archiv für Tierernährung **56**: 263–274.

The capacity of pig gastrointestinal microflora to metabolise 3-acetylDON and nivaleol (NIV) was investigated. 3-acetylDON was deacetylated to DON in anaerobic incubations with pig faeces collected at different pig farms. Furthermore, both 3-acetylDON and NIV were metabolised to the corresponding deoxy metabolite in these incubates. Faeces from pigs with a known de-epoxidation ability was spread out in the pens of five pigs in which the gastrointestinal microflora lacked the ability to transform 3-acetylDON and NIV, and left for 24 hr. One week after the faeces had been spread out in the pens, the de-epoxidation ability was found in faecal incubations from 4/5 experimental pigs. This change in metabolic ability of the intestinal de-epoxidation ability was not accompanied by any detectable changes in the DNA profiles of the bacterial community.

MAGAN, N., HOPE, R., COLLEATE, A. and BAXTER, E.S. 2002. **Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment.** European Journal of Plant Pathology **108**: 685–690.

Field studies suggest that fungicides such as tebuconazole and metconazole give good control of both *Fusarium* infection of cereals and control of DON production. However, azoxystrobin and related fungicides are less effective, and grain from treated crops has sometimes been found to have increased concentrations of DON and NIV. Studies of isolates of *Fusarium culmorum* from different parts of Europe showed that complex interactions occur between environmental factors, fungicide type and isolate in relation to growth inhibition and DON production. These studies confirmed the ineffectiveness of azoxystrobin and suggest that environmental stress factors, particularly water availability and temperature, and low fungicide doses may stimulate mycotoxin production by *Fusaria in vitro* and in wheat grain.

MOON, Y. and PESTKA, J.J. 2002. **Vomitoxin-induced cyclooxygenase-2 gene expression in macrophages mediated by activation of ERK and p38 but not JNK mitogen-activated protein kinases.** Toxicological Sciences **69**: 373–382.

DON induction of cyclooxygenase-2 (COX-2) gene expression in macrophages and its regulation at the level of mitogen-activated protein kinases was investigated. Expo-

sure of the murine macrophage cell line RAW 264.7 to DON at 50–250 µg/L for 24 hr markedly enhanced the production of prostaglandin E-2, a major COX-2 metabolite. Prostaglandin E-2 elevation was preceded by increases in COX-2 mRNA (2 hr) and COX-2 protein (15 hr). Results of further studies indicate that DON induced prostaglandin E-2 production and COX-2 expression by elevating transcriptional activity and mRNA stability. Enhanced transcriptional activity was modulated by ERK and p38 signalling pathways, whereas mRNA stability was promoted exclusively by DON-activated p38 phosphorylation. These data provide insight into possible general mechanisms by which DON and other trichothecenes upregulate proinflammatory genes and impart immunotoxicity.

Trichothecenes – Methodology

BAKAN, B., GIRAUD-DELVILLE, C., PINSON, L., RICHARD-MOLARD, D., FOURNIER, E. and BRYGOO, Y. 2002. **Identification by PCR of *Fusarium culmorum* strains producing large and small amounts of deoxynivalenol.** Applied and Environmental Microbiology **68**: 5472–5479.

Thirty DON-producing *Fusarium culmorum* strains isolated from wheat grains were analysed for trichothecene production. Strains were divided into two groups, one group produced more than 1 mg/kg of DON and acetylDON and were considered high DON-producing strains, and a second group producing less than 0.07 mg/kg of trichothecenes and were considered low DON-producing strains. For all strains, a 550-base portion of the trichodiene synthase gene (*tri5*) was amplified and sequenced. According to the *tri5* data, the *F. culmorum* strains tested clustered into two groups that correlated with *in vitro* DON production. The *tri5-tri6* intergenic region was then sequenced which confirmed the two separate clusters within the *F. culmorum* strains. Specific PCR primers were then designed to allow differentiation of high-producing from low-producing *F. culmorum* strains.

VANBENNEKOM, E.O., BROUWER, L., LAURANT, E.H.M., HOOLJERINK, H. and NIELEN, M.W.F. 2002. **Confirmatory analysis method for zearanol, its metabolites and related mycotoxins in urine by liquid chromatography-negative ion electrospray tandem mass spectrometry.** Analytica Chimica Acta **473**: 151–160.

The determination of the banned anabolic substance zearanol and the metabolites taleranol and zearalanone in bovine urine is complicated by the occurrence of the structurally related ZEA and the corresponding zearalenol metabolites. A liquid chromatography-negative ion electrospray tandem mass spectrometric method is presented for the

confirmatory analysis of all six resorcylic acid lactones in urine samples. The method was validated as a confirmatory method for bovine urine samples according to new draft EU guidelines and showed good precision and linearity.

RODRIGUES-FO, E., MIROCHA, C.J., XIE, W.P., KRICK, T.P. and MARTINELLI, J.A. 2002. **Electron ionization mass spectral fragmentation of deoxynivalenol and related trichothecenes.** Rapid Communications in Mass Spectrometry **16**: 1827–1835.

Analysis of the electron impact mass spectral data obtained for the trimethylsilyl ethers of known trichothecene mycotoxins of the DON group permitted the construction of a database useful for the identification of these mycotoxins directly from a GC-MS run. Structures of the ions at *m/z* 103, 117, 147 and 191 were elucidated by high-resolution MS and a fragmentation scheme was suggested. A new mycotoxin of this group, 3-acetylNIV, was tentatively identified by using MS data interpretation only.

PALLARONI, L., VONHOLST, C., ESKILSSON, C.S. and BJORKLUND, E. 2002. **Microwave-assisted extraction of zearalenone from wheat and corn.** Analytical and Bioanalytical Chemistry **374**: 161–166.

A microwave-assisted extraction method has been developed for determination of ZEA in wheat and corn by LC-MS with an atmospheric pressure chemical ionisation interface. Matrix effects were minimised by use of matrix-matched standard curves for quantification of the analyte. The limit of quantification of the method is 30 µg/kg in wheat and 20 µg/kg in corn. The rapid LC-MS method enabled analysis of the extracts without cleanup, thereby reducing analyte losses, the time required for the analytical procedure and costs.

Trichothecenes – Toxicoses

PILLAY, D., CHUTURGOON, A.A., NEVINES, E., MANICKUM, T., DEPPE, W. and DUTTON, M.F. 2002. **The quantitative analysis of zearalenone and its derivatives in plasma of patients with breast and cervical cancer.** Clinical Chemistry and Laboratory Medicine **40**: 946–951.

The quantity of ZEA and its congeners, alpha-zearalenol and beta-zearalenol, present in the plasma of patients with breast and cervical carcinoma were compared with levels in patients presenting with other diagnoses and healthy volunteers. There were no significant differences between the groups in the levels of ZEA and its congeners, using analysis of covariance. These results suggest that the presence of ZEA in blood does not indicate causal relationship between exposure to this exogenous mycooestrogen and the subse-

quent biological effect in this study population but may be used as an indicator of exposure.

MARESCA, M., MAHFOUD, R., GARMY, N. and FANTINI, J. 2002. **The mycotoxin deoxynivalenol affects nutrient absorption in human intestinal epithelial cells.** *Journal of Nutrition* **132**: 2723–2731.

The effect of DON on the uptake of different classes of nutrients, including sugars, amino acids and lipids was studied using the human epithelial intestinal cell line HT-29-D4 as an *in vitro* model. At low concentrations (10 µmol/L), DON selectively modulated the activities of intestinal transporters: the (D)-glucose/(D)-galactose sodium-dependent transporter (SGLT1) was strongly inhibited by the mycotoxin (50% inhibition), followed by the (D)-fructose transporter GLUT5 (42% inhibition), active and passive (L)-serine transporters (30 and 38% inhibition, respectively). The passive transporters of (D)-glucose (GLUT) were slightly inhibited by DON (15% inhibition at 1 µmol/L), whereas the transport of palmitate was increased by 35% at 10 µmol/L. At high concentrations (100 µmol/L), SGLT1 activity was inhibited by 76%, whereas the activities of all other transporters were increased.

ALM, K., DAHLBOM, M., SAYNAJARVI, M., ANDERSSON, M.A., SALKINOJA-SALONEN, M.S. and ANDERSSON, M.C. 2002. **Impaired semen quality of AI bulls fed with moldy hay: a case report.** *Theriogenology* **58**: 1497–1502.

At a Finnish artificial insemination (AI) bull station semen quality dropped suddenly in autumn 1998. During five consecutive months, the number of rejected ejaculates and discarded frozen semen batches due to poor motility increased, and the number of all forms of abnormal spermatozoa increased. The summer of 1998 in Finland was rainy and the hay used in the AI station was visibly mouldy. GC-MS detected HT-2 toxin and T-2 toxin, but no ZEA in the hay. Occurrence of T-2 toxin and HT-2 toxin in the mouldy hay coincided with, and may have been responsible for, the impaired semen quality in AI bulls.

DANICKE, S., GADEKEN, D., UEBERSCHAR, K.H., MEYER, U. and SCHOLZ, H. 2002. **Effects of *Fusarium* toxin contaminated wheat and of a detoxifying agent on performance of growing bulls, on nutrient digestibility in wethers and on the carry over of zearalenone.** *Archives of Animal Nutrition – Archiv für Tierernährung* **56**: 245–261.

The effects of a *Fusarium* contaminated wheat and of a detoxifying agent, Mycofix® Plus, on the growing performance of bulls, carryover of ZEA and its metabolites into body fluids and tissues, and on nutrient

digestibility in wethers was investigated. For growing bulls the diet contained approximately 2.2 mg DON and 0.1 mg ZON per kg complete ration at a reference dry matter content of 88%. ZEA and its metabolites were not detected in edible tissues. The most striking effects of feeding the *Fusarium* toxin contaminated wheat on carcass characteristics were a reduced dressing percentage, an increased weight of the emptied gastrointestinal tract and a reduced weight of the testicles. Feeding of *Fusarium* toxin contaminated wheat did not adversely affect nutrient digestibility in wethers. No effect of the detoxifying agent was seen for these parameters.

DANICKE, S. 2002. **Effects of *Fusarium* toxin contaminated wheat grain and of a detoxifying agent on rumen physiological parameters and *in sacco* dry matter degradation of wheat straw and lucerne hay in wethers.** *Journal of Animal and Feed Sciences* **11**: 437–451.

Wethers equipped with a rumen fistulae were fed rations containing *Fusarium*-contaminated wheat grain (DON and ZEA at concentrations of approximately 10 and 0.76 mg/kg dry matter, respectively) and pasture hay at a ratio of 1:1 on a dry matter basis, with or without the addition of a detoxifying agent Mycofix® Plus. Parameters of rumen fermentation such as the molar ratios of short chained volatile fatty acids and ammonia concentration in rumen fluid remained unchanged in response to dietary treatments whereas the detoxifying agent exerted a rumen pH-buffering effect. This effect was independent of the mycotoxin contamination of the wheat. The kinetic profile of the *in sacco* dry matter degradation revealed a reduced degradation rate for wheat straw incubated in wethers fed the mycotoxin contaminated rations whereas no changes were obvious when lucerne hay was incubated.

PESTKA, J.J. and ZHOU, H.R. 2002. **Effects of tumor necrosis factor type 1 and 2 receptor deficiencies on anorexia, growth and IgA dysregulation in mice exposed to the trichothecene vomitoxin.** *Food and Chemical Toxicology* **40**: 1623–1631.

The role of TNFalpha on the nutritional and immunological effects of DON in mice was investigated. Feed intake, weight gain, serum IgA levels and kidney mesangial IgA deposition in mice homozygous for targeted disruption of the two known TNFalpha cell surface receptors, TNFR1(p55) or TNFR2(p75), were compared to effects in corresponding C57BL/6J wild-type mice with normal receptor function. Results suggest that while DON-mediated anorexic and growth effects were largely independent of TNFalpha, DON-induced dysregulation of IgA production was dependent, in part, on the interaction of TNFalpha with TNFR1.

SZKUDELSKA, K., SZKUDELSKI, T. and NOGOWSKI, L. 2002. **Short-time deoxynivalenol treatment induces metabolic disturbances in the rat.** *Toxicology Letters* **136**: 25–31.

The effect of DON on blood insulin, glucagon, leptin and metabolic parameters in growing Wistar rats was studied. Animals were injected sc with DON at 1 mg/kg body weight. After 3 days a significant increase in blood insulin, glucose and free fatty acids were observed in comparison to the control group. DON treatment caused an increment in glycogen depots and a reduction in triglycerides content in the muscle. In isolated adipocytes DON at 20 µmol/L slightly stimulated basal lipogenesis, whereas insulin-induced lipid synthesis and lipolysis were unchanged.

DANICKE, S., UEBERSCHAR, K.H., HALLE, I., VALENTA, H. and FLACHOWSKY, G. 2002. **Excretion kinetics and metabolism of zearalenone in broilers in dependence on a detoxifying agent.** *Archives of Animal Nutrition – Archiv für Tierernährung* **55**: 299–313.

Male broilers were given a single feed of wheat naturally contaminated with ZEA (approximately 6 µg/kg body weight) and the excretion kinetics of ZEA and its metabolites and their occurrence in blood plasma and bile fluid were determined. ZEA was administered either in the absence or presence of a detoxifying agent, Mycofix® Plus. Excretion of ZEA and alpha-zearalenol as the only detectable metabolite of ZEA peaked at approximately 6.5 hr after administration. Cumulative excretion of both substances amounted to approximately 58% of ZEA intake after 48 hr, when a plateau was achieved. Mycofix® Plus supplementation seemed to have only minor or no effects on the parameters examined.

DANICKE, S., UEBERSCHAR, K.H., HALLE, I., MATTHES, S., VALENTA, H. and FLACHOWSKY, G. 2002. **Effect of addition of a detoxifying agent to laying hen diets containing uncontaminated or *Fusarium* toxin-contaminated maize on performance of hens and on carryover of zearalenone.** *Poultry Science* **81**: 1671–1680.

Laying hens were fed a diet which included 70% *Fusarium* toxin-contaminated maize containing DON at 17,630 µg/kg and ZEA at 1,580 µg/kg, with or without the addition of Mycofix® Plus, a detoxifying agent. Nutrient digestibility and metabolisability of gross energy were slightly depressed by feeding the contaminated maize diet and improved by addition of Mycofix® Plus. Feeding of the contaminated maize diets resulted in a significant decrease in serum titres to Newcastle disease virus and to an increase in yolk titres to antigen K88. No residues of ZEA or of its metabolites were found in yolk, albumen, abdominal fat, breast meat, follicles greater than 1cm in diameter, ovaries

including follicles smaller than 1cm in diameter, magnum and serum. No residues of ZEA or its metabolites were found in eggs.

SUGIMOTO, Y., AHMED, N.E., YASUDA, N. and INANAGA, S. 2002. **Trichothecene inhibitors of *Striga hermonthica* germination produced by *Fusarium solani*.** *Weed Science* **50**: 658–661.

Metabolites of *Fusarium solani* inhibited germination of the parasitic weed *Striga hermonthica*. The active principles were identified as acuminatin, neosolaniol, 8-acetylneosolaniol and tetraacetoxy T-2 tetraol (neosolaniol diacetate). Inhibitory activity of the four trichothecenes against *Striga* germination increased with acetylation of the hydroxyl moieties. The most abundant inhibitor produced by the fungus, 8-acetylneosolaniol, completely inhibited *Striga* germination at 24 μ M. The toxin did not affect the germination of sorghum, a host crop, but retarded root and shoot elongation of the seedlings by 60 and 30%, respectively, at the same concentration.

MITTERBAUER, R. and ADAM, G. 2002. ***Saccharomyces cerevisiae* and *Arabidopsis thaliana*: Useful model systems for the identification of molecular mechanisms involved in resistance of plants to toxins.** *European Journal of Plant Pathology* **108**: 699–703.

In the yeast model, resistance mechanisms against DON were identified: (1) reduced toxin uptake due to the ABC transporter protein Pdr5p (molecular efflux pump); (2) detoxification by the acetyltransferase Ayt1p; and (3) modification of the ribosomal target by amino acid changes in the ribosomal protein L3 (Rpl3p). *PDR5*-like genes exist in plant genomes as large gene families and could play an important role as a first line of defence against a broad range of toxic metabolites. Amino acid alterations in the highly conserved *RPL3* genes could likewise play a role in trichothecene resistance in plants.

ZONNO, M.C. and VURRO, M. 2002. **Inhibition of germination of *Orobanche ramosa* seeds by *Fusarium* toxins.** *Phytoparasitica* **30**: 519–524.

Eighteen toxins produced by *Fusarium* species were tested at different concentrations on *Orobanche ramosa* seeds to evaluate their effectiveness in inhibiting germination. Seven toxins: fusarenon X, NIV, DON, T-2 toxin, HT-2 toxin, DAS and neosolaniol, were highly active at 10 μ M, causing 100% inhibition of germination. T-2 toxin, HT-2 toxin, NIV, neosolaniol and diacetoxyscirpenol were still able to cause total inhibition when assayed at 1 μ M. The results show that fungal culture extracts could be an interesting source of new compounds acting as natural and original herbicides.

Aflatoxins – General

GUNSEN, U. and BUYUKYORUK, I. 2002. **Aflatoxins in retail food products in Bursa, Turkey.** *Veterinary and Human Toxicology* **44**: 289–290.

Random samples of foods collected from traditional retail markets with insufficient chilling facilities in Bursa, Turkey, were assayed for AFB₁ and AFM₁. Samples included 25 cacao hazelnut cream and 15 dried apricot samples, and 130 cheese samples (35 full fatty Turkish white cheeses, 35 fresh kashars, 25 old kashars, 20 Gravyer cheeses and 15 cream cheeses). Mean AFB₁ and AFM₁ in the cacao hazelnut cream, dried apricot and cheese were 1076.5 \pm 194.4 ng/kg, 1441.3 \pm 331.9 ng/kg and 142.2 \pm 18.7 ng/kg, respectively. Of the cheese samples, 15.45% exceeded the Turkish AFM₁ tolerance limit of 250 ng/kg.

PRUDENTE JR, A.D. and KING, J.M. 2002. **Efficacy and safety evaluation of ozonation to degrade aflatoxin in corn.** *Journal of Food Science* **67**: 2866–2872.

The efficacy and safety of ozonation in degrading aflatoxin in corn was investigated. Ozonation (10 to 12 wt%) reduced aflatoxin levels by 92% and no reversion to the parent compound was observed. Ozonation had minimal effect on fatty acids of uncontaminated corn, but had significant effect on fatty acids of contaminated corn.

AZIZ, N.H., EL-ZEANY, S.A. and MOUSSA, L.A.A. 2002. **Influence of gamma-irradiation and maize lipids on the production of aflatoxin B₁ by *Aspergillus flavus*.** *Nahrung – Food* **46**: 327–331.

The effect of gamma-irradiation and maize lipids on AFB₁ production by *Aspergillus flavus* inoculated into sterilised maize at reduced water activity (a_w 0.84) was investigated. The total viable population of *A. flavus* decreased with increasing irradiation and the fungus was completely inhibited at 3.0 kGy. The amounts of AFB₁ were enhanced at dose levels of 1.0 and 1.5 kGy in both full-fat maize and defatted maize media, and no AFB₁ production at 3.0 kGy gamma-irradiation over 45 days of storage was observed. The ability of *A. flavus* to grow at a_w 0.84 and produce AFB₁ is related to the lipid composition of maize. The enhancement of AFB₁ at low doses was correlated to the enhancement of fungal lipase activity.

ALY, S.E. 2002. **Distribution of aflatoxins in product and by-products during glucose production from contaminated corn.** *Nahrung – Food* **46**: 341–344.

The fate and distribution of aflatoxin in contaminated corn during the wet-milling process, and aflatoxin destruction during starch conversion to glucose syrup, were studied. Up to 54.4% of the aflatoxin in con-

taminated corn was removed in the steep water. After wet-milling, 8.7% of the initial aflatoxin was found in the starch fraction, 25.3% in the gluten fraction, and 11.6% in the fibre and germ fractions. The glucose syrup produced from contaminated starch was found to be free of aflatoxin.

CHEN, Z.Y., BROWN, R.L., DAMANN, K.E. and CLEVELAND, T.E. 2002. **Identification of unique or elevated levels of kernel proteins in aflatoxin-resistant maize genotypes through proteomic analysis.** *Phytopathology* **92**: 1084–1094.

Aflatoxin-resistant maize genotypes have been identified, but the incorporation of resistance into commercial lines has been slow due to the lack of selectable markers. Potential markers have now been identified in resistant maize lines using a proteomics approach. Kernel embryo proteins from each of two resistant genotypes have been compared with those from a composite of five susceptible genotypes using large format two-dimensional gel electrophoresis. Through these comparisons, both quantitative and qualitative differences have been identified. Protein spots have been sequenced, and are categorised as follows: storage proteins (globulin 1 and globulin 2), late embryogenesis abundant (LEA) proteins related to drought or desiccation (LEA3 and LEA 14), water- or osmo-stress related proteins (WSI18 and aldose reductase), and heat-stress related proteins (HSP16.9). Results of this study point to a correlation between host resistance and stress tolerance.

WILLIAMS, W.P., BUCKLEY, P.M. and WINDHAM, G.L. 2002. **Southwestern corn borer (*Lepidoptera* : *Crambidae*) damage and aflatoxin accumulation in maize.** *Journal of Economic Entomology* **95**: 1049–1053.

Crossed maize germplasm lines with aflatoxin resistance were tested for their resistance to southwestern corn borer, *Diatraea grandiosella* Dyar. Differences in ear damage among southwestern corn borer infested hybrids were significant. Estimates of general combining ability effects indicated that the lines Mp80:04, Mp420 and Mp488 contributed to reduced ear damage, and SC213 and T165 contributed to greater damage when used in hybrids. Crosses that included lines selected for aflatoxin resistance as parents (Mp,80:04 and Mp313E) exhibited lower levels of aflatoxin contamination both with and without southwestern corn borer infestation. Only the experimental line Mp80:04 contributed significantly to both reduced southwestern corn borer damage and reduced aflatoxin contamination.

CONZANE, R.S., STENZEL, W.R. and KROH, L.W. 2002. **[Reducing the aflatoxin content in peanuts].** *Deutsche Lebensmittel-Rundschau* **98**: 321–325.

The development of a cheap method for the chemical treatment of peanuts contaminated with aflatoxins, which is technically applicable in Mozambique, was investigated. A reduction in aflatoxins was achieved in both artificially and naturally contaminated peanuts under acid conditions after 60 minutes in a boiling water bath with a 10% H₂O₂ solution. The sensory properties of the peanuts were not affected by this decontamination method. (In German).

AYCICEK, H., YARSAN, E., SARIMEH-METOGLU, B. and CAKMAK, O. 2002. **Aflatoxin M₁ in white cheese and butter consumed in Istanbul, Turkey.** *Veterinary and Human Toxicology* **44**: 295–296.

The occurrence of AFM₁ in 183 sample of white cheese and butter in Istanbul, Turkey, in 2001 was investigated. The incidence of AFM₁ in white cheese and butter samples was as high as 65 and 81%, respectively.

ROUSSI, V., GOVAIS, A., VARAGOULI, A. and BOTSOGLOU, N.A. 2002. **Occurrence of aflatoxin M₁ in raw and market milk commercialized in Greece.** *Food Additives and Contaminants* **19**: 863–868.

From December 1999 to May 2000, samples of pasteurised, ultra-high temperature (UHT) and concentrated milk were collected in supermarkets, and raw milk samples from cow, sheep and goat were obtained from different milk producers all over Greece. Sample collection was repeated from December 2000 to May 2001. A total of 297 samples were analysed for AFM₁. In the first sampling, the incidence rates of AFM₁ contamination in pasteurised, UHT, concentrated and cow, sheep and goat raw milk were 85.4, 82.3, 93.3, 73.3, 66.7 and 40%, respectively, with only one cow raw milk and two concentrated milk samples exceeding the EU limit of 50 ng/L. In the second sampling, the incidence rates of AFM₁ contamination in pasteurised, bulk-tank and cow, sheep and goat raw milk were 79.6, 78.3, 64.3, 73.3 and 66.7%, respectively, with only one cow and one sheep raw milk samples exceeding the EU limit.

GOVARIS, A., ROUSSI, V., KOIDIS, P.A. and BOTSOGLOU, N.A. 2002. **Distribution and stability of aflatoxin M₁ during production and storage of yoghurt.** *Food Additives and Contaminants* **19**: 1043–1050.

Yoghurt from cows' milk artificially contaminated with AFM₁ at levels of 0.050 and 0.100 g/L was fermented to reach pH 4.0 and pH 4.6. Yoghurts were stored at 4°C for up to 4 weeks. The percentage loss of the initial amount of AFM₁ in milk was estimated at about 13 and 22% by the end of the fermentation, and 16 and 34% by the end of storage, for yoghurts with pH 4.6 and pH 4.0, respectively. Growth of culture lactic acid bacteria was not affected in the AFM₁ contaminated yoghurts with the exception of *Streptococcus*

thermophilus which showed a significantly lower increase in the yoghurt containing AFM₁ at high concentration.

SHENASI, M., AIDOO, K.E. and CANDLISH, A.A.G. 2002. **Microflora of date fruits and production of aflatoxins at various stages of maturation.** *International Journal of Food Microbiology* **79**: 113–119.

Twenty-five varieties of dates (*Phoenix dactylifera*) were examined at different maturation stages for total microbial counts, aflatoxins and aflatoxigenic *Aspergillus* sp. and lactic acid bacteria. Microbial counts were high at the first stage of maturation (Kimri) and increased sharply at the second stage (Rutab), then decrease significantly at the final dried stage of maturation (Tamr). Aflatoxins were detected in 12% of the samples although aflatoxigenic *Aspergillus* were detected in 40% of the varieties examined, all at Kimri stage only. No aflatoxins or aflatoxigenic *Aspergillus* were detected at the final edible stage of maturation.

ELIAS-OROZCO, R., CASTELLANOS-NAVA, A., GAYTAN-MARTINEZ, M., FIGUEROA-CARDENAS, J.D. and LOARCA-PINA, G. 2002. **Comparison of nixtamalization and extrusion processes for a reduction in aflatoxin content.** *Food Additives and Contaminants* **19**: 878–885.

Traditional nixtamalisation and an extrusion method for making the dough (masa) for corn tortillas that requires using lime and hydrogen peroxide were evaluated for the detoxification of aflatoxins. The traditional nixtamalisation process reduced levels of AFB₁, AFM₁ and AFB₁ 8,9-dihydrodiol by 94, 90 and 93%, respectively. The extrusion process reduced levels by 46, 20 and 53%, respectively. Inactivation of the toxins in the extrusion process using lime together with hydrogen peroxide showed higher elimination of AFB₁ than treatments with lime or hydrogen peroxide alone. The extrusion process with 0.3% lime and 1.5% hydrogen peroxide was the most effective process to detoxify aflatoxins in corn tortillas, but a high level of those reagents negatively affected the taste and aroma of the corn tortilla as compared with tortillas prepared by the traditional nixtamalisation process.

SHUKLA, R.S., VERMA, R.J. and MEHTA, D.N. 2002. **Kinetic and mechanistic investigations on reductions of aflatoxins by lactic acid.** *Bioorganic & Medicinal Chemistry Letters* **12**: 2737–2741.

The kinetics of reduction of AFB₁ to AFB₂, and AFG₁ to AFG₂ by lactic acid has been investigated in dilute aqueous acidic solutions (pH 3.35–4.50) at 37°C. The rate of the reaction was found to be first order with respect to the concentrations of lactic acid and aflatoxins and independent of hydrogen ion concentration.

PACKIYASOTHY, E.V. and KYLE, S. 2002. **Antimicrobial properties of some herb essential oils.** *Food Australia* **54**: 384–387.

The essential oils of sage, turmeric, thyme, mustard and fenugreek showed antimicrobial activity against *Salmonella typhimurium*, *Bacillus cereus*, *Escherichia coli* and *Aspergillus flavus*, turmeric exhibiting high potency and fenugreek only weak activity. The four active turmeric components, identified as borneol, cymene, cuparene and careen, each exhibited strong antifungal and anti-aflatoxigenic activity in the range 50–100 g/L.

HORN, B.W. and DORNER, J.W. 2002. **Effect of competition and adverse culture conditions on aflatoxin production by *Aspergillus flavus* through successive generations.** *Mycologia* **94**: 741–751.

Three aflatoxin-producing strains of *Aspergillus flavus* were serially transferred using conidia for 20 generations on potato dextrose agar at 30°C. The rate of degeneration of aflatoxin producing ability was compared to that of cultures grown in the presence of competing fungi (*A. terreus*, *Penicillium funiculosum* and the yeast, *Pichia guilliermondii*) and under adverse conditions of elevated temperature, reduced a_w, low pH and nutrient deprivation. Formation of morphological variants and the associated loss of aflatoxin production over generations varied considerably according to strain and the generation line within each strain. In the strain most sensitive to degeneration on potato dextrose agar, aflatoxin-producing ability was maintained to varying degrees under adverse culture conditions, but not when *A. flavus* was competing with other fungi.

TUBAJIKA, K.M. and DAMANN, K.E. 2002. **Glufosinate-ammonium reduces growth and aflatoxin B₁ production by *Aspergillus flavus*.** *Journal of Food Protection* **65**: 1483–1487.

The herbicide glufosinate-ammonium (GA) [butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)-ammonium salt] was tested at concentrations from 2 to 2,000 mg/L for activity against growth and AFB₁ production by *Aspergillus flavus*. Reduction in mycelial dry weight and AFB₁ production in response to GA application ranged from 17.2 to 97.1% and from 39.1 to 90.1%, respectively, over the concentration range tested.

LEE, L.W., CHIOU, C.H. and LINZ, J.E. 2002. **Function of native OmtA in vivo and expression and distribution of this protein in colonies of *Aspergillus parasiticus*.** *Applied and Environmental Microbiology* **68**: 5718–5727.

To determine if OmtA is necessary and sufficient to catalyse the conversion of the aflatoxin pathway intermediate sterigmatocystin to O-methylsterigmatocystin *in vivo* and if this reaction is necessary for aflatoxin

synthesis, an *Aspergillus parasiticus omtA*-null mutant LW1432 and a maltose binding protein-OmtA fusion protein expressed in *Escherichia coli* were generated. Feeding studies conducted with LW1432 demonstrated a critical role for OmtA, and the reaction catalysed by this enzyme, in aflatoxin synthesis. A novel time-dependent colony fractionation protocol was developed to analyse the accumulation and distribution of OmtA in fungal colonies grown on a solid medium that supports both toxin synthesis and conidiation. OmtA was found to be evenly distributed among different cell types and is not concentrated in conidiophores. These data suggest that OmtA is present in newly formed fungal tissue and then is proteolytically cleaved as cells in that section of the colony age.

Aflatoxins – Methodology

VONHOLST, C., STROKA, J. and ANKLAM, E. 2002. **Correction of analytical results for recovery: A comparison of the method performance characteristics from recent collaborative trials studies for aflatoxin quantification using conventional and robust statistics.** Food Additives and Contaminants **19**: 701–708.

Results from recently conducted collaborative trials on the determination of AFB₁ in various matrices have been evaluated to establish whether the use of recovery data would result in a distinct change of the relative between-laboratory standard deviation (RSDR) of the corrected data compared with the uncorrected data. In addition, conventional and robust statistics were applied to evaluate whether the impact of the use of recovery data on the estimation of RSDR depended on the statistical method applied for data analysis. The study revealed that applying conventional and robust statistics in general led to comparable estimates for RSDR. The comparison about the use of recovery data showed that in most cases, the RSDR obtained from the analysis of AFB₁ decreased after correction of the results for recovery. This tendency was similar when the comparison was done using robust or conventional statistics.

PAPP, E., H-OTTA, K., ZARAY, G. and MINCSOVICS, E. 2002. **Liquid chromatographic determination of aflatoxins.** Microchemical Journal **73**: 39–46.

This review with 59 references discusses the overpressured-layer chromatographic (OPLC) and HPLC methods most often used for the analysis of aflatoxins. Emphasis is placed on summarising the OPLC methods developed for determination of aflatoxins in maize, wheat, fish meat, peanut samples, rice and sunflower seeds spiked with aflatoxins which were developed in the authors' laboratory.

Aflatoxicoses

SUN, C.A., WU, D.M., WANG, L.Y., CHEN, C.J., YOU, S.L. and SANTELLA, R.M. 2002. **Determinants of formation of aflatoxin-albumin adducts: A seven-township study in Taiwan.** British Journal of Cancer **87**: 966–970.

In a cross-sectional study to evaluate determinants of the formation of aflatoxin covalently bound to albumin (AFB₁-albumin adducts), a total of 474 subjects who were free of liver cancer and cirrhosis and were initially selected as controls for previous case-control studies of aflatoxin-induced hepatocarcinogenesis in Taiwan, were examined. The detection rate of AFB₁-albumin adducts was significantly higher in males (42.5%) than in females (21.6%). The formation of detectable albumin adducts was moderately higher in hepatitis B surface antigen carriers (42.8%) than in non-carriers (36.6%). In addition, the detection rate of AFB₁-albumin adducts tended to increase with the increasing number of null genotypes of glutathione S-transferase (GST) M1-1 and GST T1-1.

LOPEZ, C., RAMOS, L., BULACIO, L., RAMADAN, S. and RODRIGUEZ, F. 2002. **Aflatoxin B₁ content in patients with hepatic diseases.** Medicina – Buenos Aires **62**: 313–316.

AFB₁ was determined in serum samples obtained from 20 patient volunteers with hepatic disease. AFB₁ was detected in only one patient and the concentration was 0.47 ng/mL.

KENSLER, T.W., EGNER, P.A., WANG, J.B., ZHU, Y.R., ZHANG, B.C., QIAN, G.S., KUANG, S.Y., GANGE, S.J., JACOBSON, L.P., MUNOZ, A. and GROOPMAN, J.D. 2002. **Strategies for chemoprevention of liver cancer.** European Journal of Cancer Prevention **11**: S58–S64.

This review with 40 references highlights the findings of recent randomised clinical trials with oltipraz and chlorophyllin conducted in individuals exposed to dietary aflatoxins and at high risk for development of liver cancer. Both chemopreventive agents modulated levels of aflatoxin biomarkers in the study participants in manners consonant with protection.

MING, L., THORGEIRSSON, S.S., GAIL, M.H., LU, P.X., HARRIS, C.C., WANG, N.J., SHAO, Y.F., WU, Z.Y., LIU, G.T., WANG, X.H. and SUN, Z.T. 2002. **Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China.** Hepatology **36**: 1214–1220.

The separate and combined effects of hepatitis B virus (HBV), hepatitis C virus (HCV) and aflatoxin in causing hepatocellular carcinoma (HCC) in Qidong, China, were

assessed. A consecutive series of 181 pathologic-diagnosed HCC cases were studied for hepatitis B surface antigen (HBsAg), antibodies to HBV core antigens, HBV X gene sequence, antibodies to HCV, the 249ser-p53 mutation, and chronic hepatitis pathology. Each of the 181 incident HCC cases had markers for HBV infection and hepatitis pathology; only 6 of 119 cases were co-infected with HCV. The 249ser-p53 mutation was found in 54% of HCC cases and in all 7 cases with tissue for analysis from the hepatitis cohort but in none of 42 matched cases from Beijing. Follow-up data through 13.25 years on a cohort of 145 men with chronic HBV hepatitis showed that the relative risk from aflatoxin exposure was 3.5 (1.5–8.1). A similar relative risk was found using 249ser-p53 mutation as a marker for aflatoxin exposure. The 249ser-p53 mutation appears to result from co-exposure to aflatoxin and HBV infection.

WILLIAMS, G.M., IATROPOULOS, M.J. and JEFFREY, A.M. 2002. **Anticarcinogenicity of monocyclic phenolic compounds.** European Journal of Cancer Prevention **11**: S101–S107.

Butylated hydroxyanisole and butylated hydroxytoluene at 100–125 mg/kg in the diet of rats inhibited the initiation phase of AFB₁ hepatocarcinogenesis. These monocyclic phenolics appear to be anticarcinogenic through a mechanism different from that of most other chemopreventive agents, possibly involving interception of the reactive chemical species of the carcinogen.

MADDEN, C.R., FINEGOLD, M.J. and SLAGLE, B.L. 2002. **Altered DNA mutation spectrum in aflatoxin B₁-treated transgenic mice that express the hepatitis B virus X protein.** Journal of Virology **76**: 11770–11774.

Double transgenic mice (ATX mice) that express the HBV X protein (HBx) and possess a bacteriophage lambda transgene were used to evaluate the *in vivo* effect of HBx expression on AFB₁-induced DNA mutations. The expression of HBx correlated with a 24% increase in mutation frequency overall and an approximately two-fold increase in the incidence of G/C to T/A transversion mutations following AFB₁ exposure.

LU, H. and LI, Y. 2002. **Effects of bicyclol on aflatoxin B₁ metabolism and hepatotoxicity in rats.** Acta Pharmacologica Sinica **23**: 942–945.

The effect of a new antihepatitis drug, bicyclol, on the metabolism and hepatotoxicity of AFB₁ in rats was investigated. Rats were given bicyclol at 300 mg/kg/day for 3 days and then injected ip with AFB₁ at 1.5 mg/kg. Bicyclol pretreatment provided protection against AFB₁ hepatotoxicity as evidenced by the decrease of AFB₁-elevated serum aminotransferase and hepatic malondialdehyde in rats. Bicyclol pretreatment

slightly increased the production of the less toxic metabolite AFQ₁. Bicyclol increased liver cytochrome P450 content, CYP 2B1-mediated 7-pentoxoresorufin O-dealkylase activity, cytosolic GSH level and GST activities.

TOWNER, R.A., MASON, R.P. and REINKE, L.A. 2002. **In vivo detection of aflatoxin-induced lipid free radicals in rat bile.** *Biochimica et Biophysica Acta – General Subjects* 1573: 55–62.

Free radical intermediates from the hepatic metabolism of AFB₁ were investigated in rats. Rat bile ducts were cannulated and rats were treated simultaneously with AFB₁ and the spin trapping agent 4-POBN (alpha-(4-pyridyl-1-oxide)-N-tert-butyl nitron). Bile was collected over a period of 2 hr at 20 minute intervals. ESR spectroscopy was used to detect a carbon-centred radical adduct of 4-POBN in bile. There was a significant decrease in free radical formation by pretreatment with the metabolic inhibitors deferoxamine mesylate, an iron chelator, and SKF 525A, a cytochrome P-450 inhibitor. This indicates that oxidation of AFB₁ generates free radical species via CYP metabolism and an iron-mediated redox mechanism.

WANG, C., BAMMLER, T.K. and EATON, D.L. 2002. **Complementary DNA cloning, protein expression, and characterization of alpha-class GSTs from *Macaca fascicularis* liver.** *Toxicological Sciences* 70: 20–26.

In contrast to rodents, constitutively expressed human hepatic alpha-class GSTs have little or no AFB₁-8,9-epoxide (AFBO) detoxifying activity. The nonhuman primate, *Macaca fascicularis*, has significant constitutive hepatic GST activity toward AFBO and most of this activity belongs to mu-class GSTs. To determine if any alpha-class GSTs in *Macaca* liver have AFBO activity, a cDNA library from a male *Macaca* liver was constructed and screened using the human alpha-class Gsta1 cDNA as a probe. In contrast to rodents but similar to humans, alpha-class GSTs from the nonhuman primate have little conjugating activity toward AFBO.

LUZI, A., COMETA, M.F. and PALMERY, M. 2002. **Acute effects of aflatoxins on guinea pig isolated ileum.** *Toxicology In Vitro* 16: 525–529.

The acute gastrointestinal effects of aflatoxins on isolated guinea pig ileum were studied. AFB₁ and AFB₂ contracted isolated guinea pig ileum in a dose dependent manner, whereas AFG₁ and AFG₂ evoked no contractions. Atropine antagonised AFB₁-induced contractions while pretreatment with the nicotinic ganglionic blocker, hexamethonium, left AFB₁-induced contractions unchanged. Tetrodotoxin blocked AFB₁ contractile activity. The two inhibitors of acetylcholine release, morphine and clonidine, antagonised AFB₁-induced contractions, and apamin, a drug that

increases neuronal excitability, facilitated the AFB₁-induced contractile effect. The choline uptake blocker, hemicholinium markedly reduced AFB₁-induced contractions. These results suggest that aflatoxins induce their contractile effect indirectly through the cholinergic system by stimulating acetylcholine release from the postganglionic parasympathetic nerve endings.

ATEF, M., YOUSSEF, S.A.H., EL-EANNA, H.A. and EL-MAAZ, A.A. 2002. **Influence of aflatoxin B₁ on the kinetic disposition, systemic bioavailability and tissue residues of doxycycline in chickens.** *British Poultry Science* 43: 528–532.

Disposition kinetics of doxycycline (doxy) was studied in chickens dosed with AFB₁ by iv, oral or im injection, in a single dose of 15 mg/kg body weight. The tissue distribution and residual pattern of the drug were also determined. The maximum serum concentrations of doxy were reached 1.97 and 2.37 hr after oral, and 1.57 and 2.92 hr after im dosage in healthy and toxin-treated birds, respectively. The volumes of distribution and total body clearances were higher in toxin-treated birds (1.75 L/kg and 14.61 mL/kg/min) than in healthy chickens (0.93 L/kg and 4.6 mL/kg/min). The highest concentration of doxy residues were present in liver, kidney and serum followed by heart and muscles. Doxy residue concentrations in edible tissues was below the EEC limit 6 days after cessation of oral or im medication with 15 mg/kg body weight twice daily for 5 successive days.

OGUZ, H., KURTOGLU, F., KURTOGLU, V. and BIRDANE, Y.O. 2002. **Evaluation of biochemical characters of broiler chickens during dietary aflatoxin (50 and 100 ppb) and clinoptilolite exposure.** *Research in Veterinary Science* 73: 101–103.

Day-old Ross broiler chicks were fed diets containing aflatoxin at 50 or 100 µg/kg body weight, with and without the addition of clinoptilolite at 15 g/kg diet, from days 1 to 42. Aflatoxin treatment significantly increased the serum Na levels and the aspartate amino transferase and alanine amino transferase enzyme activities, while total protein, albumin, total cholesterol uric acid and K levels were not significantly different between groups.

TUAN, N.A., GRIZZLE, J.M., LOVELL, R.T., MANNING, B.B. and ROTTINGHAUS, G.E. 2002. **Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B₁.** *Aquaculture* 212: 311–319.

Nile tilapia were fed semi-purified diets containing AFB₁ at 0–100 mg/kg diet for 8 weeks. Weight gain and hematocrit of fish fed AFB₁ at 0.25 mg/kg were not significantly different from that of the control, however, diets containing higher levels of AFB₁ had significantly reduced weight gain and hematocrit. Histologically, livers of fish fed with diets containing AFB₁ at 10 mg/kg contained excess lipofuscin and irregularly sized hepatocellular nuclei. Diets containing 100 mg/kg caused weight loss and severe hepatic necrosis and 60% of the fish in this treatment died by the end of the 8-week feeding period.

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