

## Mycotoxins – General

- ABRUNHOSA, L., PATERSON, R.R.M., KOZAKIEWICZ, Z., LIMA, N. and VENANCIO, A. 2001. **Mycotoxin production from fungi isolated from grapes.** Letters in Applied Microbiology **32**: 240–242.
- Fungi were isolated from wine producing grapes and 51 strains were assessed for their potential for producing mycotoxins. Many of the strains produced patulin and/or citrinin. Citrinin was produced by all strains grown in yeast extract sucrose medium but only one strain was able to produce citrinin in grape juice medium. Patulin was produced in the yeast extract medium by 20 strains and in grape juice medium by 33 strains. No ochratoxin producing fungi were identified.
- WEIDENBORNER, M. 2001. **Pine nuts: the mycobiota and potential mycotoxins.** Canadian Journal of Microbiology **47**: 460–463.
- A total of 1832 fungi belonging to 31 species and 15 genera was isolated from pine nuts. *Cladosporium* species dominated the mycobiota followed by *Phoma macrostoma*. Overall, 16 potentially mycotoxigenic species were present on pine nuts.
- ALMEIDA, A.P., CORREA, B., MALLOZZI, M.A.B., SAWAZAKI, E. and SOARES, L.M.V. 2000. **Mycoflora and aflatoxin/fumonisin production by fungal isolates from freshly harvested corn hybrids.** Brazilian Journal of Microbiology **31**: 321–326.
- The mycoflora of three hybrids of freshly harvested corn grains collected from three regions of the state of Sao Paulo, Brazil, was investigated. The fungal population was comprised mainly *Fusarium*, *Penicillium* and *Aspergillus* species and among the genera *Fusarium* and *Aspergillus*, the most frequently isolated species were *F. moniliforme* and *A. flavus*, respectively. All 40 isolates of *F. moniliforme* produced fumonisin B<sub>1</sub> (FB<sub>1</sub>) at 20–2168 mg/kg and/or FB<sub>2</sub> at 10–380 mg/kg. Among the 10 *A. flavus* isolates, 6 strains produced aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) at 615–30.750 µg/kg and/or AFB<sub>2</sub> at 11–22 µg/kg.
- LAWLOR, P.G. and LYNCH, P.B. 2001. **Mycotoxins in pig feeds 1: Source of toxins, prevention and management of mycotoxicosis.** Irish Veterinary Journal **54**: 117–120.
- To lessen the effect of mycotoxins on pig performance, mixing contaminated and non-contaminated feedstuffs, the use of binding agents (e.g., clays and mannanoligosaccharide) and the feeding of higher than normal levels of high molecular weight amino acids have all been used with varying degrees of success. Preventing mould growth and subsequent mycotoxin production during storage of feeds is more successful. This is achieved by storing clean grain at a moisture content less than 14% in clean, preferably insulated bins. Cereals grown in Ireland may be contaminated with deoxynivalenol, zearalenone, fusaric acid or ochratoxin. The presence of aflatoxins in animal feeds in Ireland is most likely to be due to the importation of feed ingredients from warmer climates.
- LAWLOR, P.G. and LYNCH, P.B. 2001. **Mycotoxins in pig feeds 2: clinical aspects.** Irish Veterinary Journal **54**: 172–176.
- The clinical response of pigs to mycotoxins is dependent on the concentration in feed, on the duration of feeding, on the presence or absence of other mycotoxins, and on the species, age, and health status of animal to which the mycotoxin is fed. Deoxynivalenol causes pigs to refuse feed, zearalenone affects the reproductive organs, ochratoxin causes kidney damage and aflatoxins increase susceptibility to disease through their action as immunosuppressants. Aflatoxins can also cause haemorrhages and digestive disorders.
- SCHNEWEIS, I., MEYER, K., HORMANSDORFER, S. and BAUER, J. 2001. **Metabolites of *Monascus ruber* in silages.** Journal of Animal Physiology and Animal Nutrition — Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde **85**: 38–44.
- A total of 233 silages were examined and *Monascus ruber* was found to be present in 43 samples. Citrinin was found in 14 samples at concentrations in the range 2.4–64.2 µg/kg.
- LI, F.Q., TOYAZAKI, N. and YOSHIZAWA, T. 2001. **Production of *Alternaria* mycotoxins by *Alternaria alternata* isolated from weather-damaged wheat.** Journal of Food Protection **64**: 567–571.
- The production of mycotoxins by *Alternaria alternata* isolated from Chinese weathered wheat kernels was investigated on polished rice and durum wheat grains. These mycotoxins included AOH, AME, altenuene (ALT), altertoxin I (ATX-I) and tenuazonic acid (TA). Of 25 isolates tested, all were AOH and AME producers, 21 coproduced ALT and ATX-I, and 8 produced TA in rice culture. TA was the most abundant toxin produced at a level ranging from 1,369 to 3,563 mg/kg. Average concentrations of AOH, AME, ALT and ATX-I were 54, 40, 44 and 8 mg/kg, respectively.
- GHISALBERTI, E.L., HARGREAVES, J.R., SKELTON, B.W. and WHITE, A.H. 2000. **New patulin derivatives.** Australian Journal of Chemistry **53**: 995–997.

Two new derivatives of patulin, the dimer arising from cycloaddition at the 3,4-double bond, and 3, 4-dihydroxyapatulin acetone, from attempted epoxidation with dimethyldioxirane, have been characterised.

YUKI, K., SHINDO, M. and SHISHIDO, K. 2001. **Enantioselective total synthesis of (-)-equisetin using a Me3Al-mediated intramolecular Diels-Alder reaction.** *Tetrahedron Letters* **42**: 2517–2519.

An efficient and enantioselective total synthesis of (-)-equisetin using a diastereoselective Me3Al-mediated intramolecular Diels-Alder reaction as a key reaction step is described.

HANDA, M., SUNAZUKA, T., NAGAI, R., KIMURA, R., OTOGURO, K., HARIGAYA, Y. and OMURA, S. 2001. **Determination of absolute stereochemistries of arisugacin F and territrein B, novel acetylcholinesterase inhibitors.** *Journal of Antibiotics* **54**: 386–391.

The absolute configuration of arisugacin F and territrein B were determined via the Kakisawa-Kashman modification of the Mosher NMR method.

## Mycotoxins – Methodology

DAMOTTA, S. and SOARES, L.M.V. 2000. **A method for the determination of two *Alternaria* toxins, alternariol and alternariol monomethyl ether, in tomato products.** *Brazilian Journal of Microbiology* **31**: 315–320.

A method for determining AME and AOH in tomato products was developed and evaluated. The method involves extraction with methanol, clarification with ammonium sulphate and partition to chloroform. Quantification was conducted by HPLC with diode array detector. Average recoveries were 98.7% and 84.1% for AME and AOH, respectively. The quantification limits of the method were 2.0 µg/kg for AME and 5.0 µg/kg for AOH.

## Mycotoxicoses

BEREK, L., PETRI, I.B., MESTERHAZY, A., TEREN, J. and MOLNAR, J. 2001. **Effects of mycotoxins on human immune functions *in vitro*.** *Toxicology In Vitro* **15**: 25–30.

The effects of deoxynivalenol (DON), 3-acetylDON, fusarenon-X, T-2 toxin, zearalenone, alpha-zearalenol, beta-zearalenol and nivalenol (NIV) on T and B cells in a proliferation assay, antibody-dependent cellular cytotoxicity, and natural killer (NK) cell

activity on human peripheral blood mononuclear cells were investigated. The concentrations employed, 0.2–1800 µg/L, were similar to those which can be found in normal human peripheral blood system. T-2 toxin, fusarenon X, NIV and DON exerted the highest immunosuppressing effect. Mycotoxin induced immunosuppression was manifested as depressed T or B lymphocyte activity. Furthermore, by virtue of inhibition of NK cell activity, the protection against tumour development may also be attenuated.

CASADO, J.M., THEUMER, M., MASHI, D.T., CHULZE, S. and RUBINSTEIN, H.R. 2001. **Experimental subchronic mycotoxicoses in mice: Individual and combined effects of dietary exposure to fumonisins and aflatoxin B<sub>1</sub>.** *Food and Chemical Toxicology* **39**: 579–586.

Mice were fed fumonisins (FB<sub>1</sub> at 10 mg/kg) and AFB<sub>1</sub> at 10 µg/kg in the diet, either alone or in combination, for up to 90 days. The animals fed fumonisins or combined toxins showed a significant increase in feed consumption per day compared to control animals. At 90 days, animals fed AFB<sub>1</sub> exhibited a significant decrease in the values of alkaline phosphatase and cholesterol, along with a significant increase in calcium. Mice fed fumonisins showed decreases in triglycerides, cholesterol and calcium. The activity of aspartate transaminase increased significantly in animals fed the combined toxins. Tissue specimens at 60 days showed lesions in the livers of the animals fed AFB<sub>1</sub> or fumonisins. In mice fed AFB<sub>1</sub>, fumonisins and combined toxins, the lesions were intensified in the liver at 60 days in 80, 90 and 100% of the animals, respectively.

UMESH, H., GANESH, B. and REDDY, C.S. 2000. **Secalonic acid D alters the expression and phosphorylation of the transcription factors and their binding to cAMP response element in developing murine secondary palate.** *Journal of Craniofacial Genetics and Developmental Biology* **20**: 173–182.

Secalonic acid D (SAD), a cleft palate inducing mycotoxin, reduces palatal cyclic AMP (cAMP) levels. cAMP relays its signals via the transcription factors (TF) such as cAMP response element (CRE) binding protein (CREB), CRE modulator (CREM) and activator transcription factor-1 (ATF-1) to CRE-containing genes. Electrophoretic mobility shift assays, supershift/ablation assays and Western analyses showed that the cAMP signalling pathway is functional in the palate and that SAD alters CREB phosphorylation and inhibits its binding to CRE, leading to altered expression of genes involved in cell proliferation, an event critical for normal palate development.

SKLAN, D., KLIPPER, E., FRIEDMAN, A., SHELLY, M. and MAKOVSKY, B. 2001. **The effect of chronic feeding of diacetoxyscirpenol, T-2 toxin, and aflatoxin on performance, health, and antibody production in chicks.** *Journal of Applied Poultry Research* **10**: 79–85.

The effects of T-2 toxin, diacetoxyscirpenol (DAS) and AFB<sub>1</sub> at levels up to 1,000 µg/kg diet on performance, health and immune response of enterally and parenterally immunised chicks were examined. T-2 toxin and DAS, fed singly and in combination for 35 days, had no effect on growth or feed efficiency. AFB<sub>1</sub> at concentrations above 800 µg/kg resulted in decreased growth and feed efficiency after 4 weeks. Feeding T-2 toxin and DAS resulted in oral lesions and mild intestinal inflammation, but no other pathological or histopathological lesions. AFB<sub>1</sub> caused enlargement and discoloration of liver and kidneys and mild intestinal inflammation. No effects of T-2, DAS or AFB<sub>1</sub> were observed on antibody production to antigens administered by enteral or parenteral routes.

CARLSON, D.B., WILLIAMS, D.E., SPITSBERGEN, J.M., ROSS, P.F., BACON, C.W., MEREDITH, F.I. and RILEY, R.T. 2001. **Fumonisin B<sub>1</sub> promotes aflatoxin B<sub>1</sub> and N-methyl-N'-nitro-nitrosoguanidine-initiated liver tumors in rainbow trout.** *Toxicology and Applied Pharmacology* **172**: 29–36.

In fish fed diets containing FB<sub>1</sub> at 0, 3.2, 23 or 104 mg/kg for 34 weeks, no tumours were observed in any tissue in the absence of a known initiator. FB<sub>1</sub> promoted AFB<sub>1</sub>-initiated liver tumours in fish fed FB<sub>1</sub> greater than or equal to 23 mg/kg for 42 weeks. In N-methyl-N'-nitro-nitrosoguanidine-initiated fish, liver tumours were promoted in the 104 mg/kg treatment, but FB<sub>1</sub> did not promote tumours in any other tissue. The FB<sub>1</sub> promotional activity in AFB<sub>1</sub>-initiated fish was correlated with disruption of sphingolipid metabolism, suggesting that alterations in associated sphingolipid signalling pathways are potentially responsible for the promotional activity of FB<sub>1</sub> in AFB<sub>1</sub>-initiated fish.

RUSSELL, B.M., KAY, B.H. and SHIPTON, W. 2001. **Survival of *Aedes aegypti* (Diptera: culicidae) eggs in surface and subterranean breeding sites during the northern Queensland dry season.** *Journal of Medical Entomology* **38**: 441–445.

The effect of a protracted dry season on the viability of *Aedes aegypti* (L.) eggs was examined in Townsville, northern Queensland, Australia. Eggs were placed in several different surface and subterranean larval habitats. After four dry season

months, only 1–10% of eggs remained viable in the surface and subterranean sites, respectively. *Periplaneta americana* was the most significant cause of egg predation in subterranean breeding sites but fungi, especially *Penicillium citrinum*, covered egg latches within 15 days. Mycotoxins produced by the spores of *P. citrinum* are believed to have killed embryonating eggs.

## Ochratoxins – General

SKAUG, M.A., HELLAND, I., SOLVOLL, K. and SAUGSTAD, O.D. 2001. **Presence of ochratoxin A in human milk in relation to dietary intake.** Food Additives and Contaminants **18**: 321–327.

Human milk samples were collected from 80 Norwegian women. The usual food intake during the last year was recorded using a quantitative food frequency questionnaire. Seventeen out of 80 human milk samples contained ochratoxin A (OA) in the range 10–182 ng/L. Women with a high dietary intake of liver paste (liverwurst, liver pate) and cakes (cookies, fruitcakes, chocolate cakes, etc.) were more likely to have OA contaminated milk. The risk of OA contamination was also increased by the intake of juice (all kinds). In addition, the results indicate that breakfast cereals, processed meat products and cheese could be important contributors to dietary OA intake. OA contamination of the milk was unrelated to smoking, age, parity and anthropometric data other than body weight.

OTTENEDER, H. and MAJERUS, P. 2001. **Ochratoxin A (OTA) in coffee: Nation-wide evaluation of data collected by German Food Control 1995–1999.** Food Additives and Contaminants **18**: 431–435.

In a nation-wide evaluation of data collected by German Food Control 1995–1999, a total of 613 samples of coffee were analysed for OA. The median concentrations for green coffee, roasted coffee, decaffeinated roasted coffee together with low-acid decaffeinated roasted coffee, and for soluble coffee were 0.4, 0.6, 0.4 and 0.7 µg/kg, respectively. The result is a mean daily total intake per consumer of 9 ng OA.

JOOSTEN, H.M.L.J., GOETZ, J., PIT-TET, A., SCHELLENBERG, M. and BUCHELI, P. 2001. **Production of ochratoxin A by *Aspergillus carbonarius* on coffee cherries.** International Journal of Food Microbiology **65**: 39–44.

Robusta coffee cherries collected before and during sun drying from two coffee farms in Thailand were examined for moulds producing OA. *Aspergillus ochra-*

*ceus* was detected in one sample and *Aspergillus carbonarius* was isolated from 7/14 samples. On gamma-irradiated coffee cherries, each of the six tested *A. carbonarius* strains produced OA. More than 4800 µg/kg of toxin were detected under optimal conditions (25°C, water activity (aw) 0.99). OA production was strongly reduced (230 µg/kg) at an aw of 0.94.

## Ochratoxins – Methodology

MARKAKI, P., DELPONT-BINET, C., GROSSO, F. and DRAGACCI, S. 2001. **Determination of ochratoxin A in red wine and vinegar by immunoaffinity high-pressure liquid chromatography.** Journal of Food Protection **64**: 533–537.

A method is described for the determination of OA in red wine and vinegar using an acidic chloroform extraction, an immunoaffinity cleanup step, and HPLC determination with fluorescence detection. The detection limit was estimated at 2 mg/L. The mean recovery factors were found at 91.3 and 96.6% for wine and vinegar, respectively. Thirty-one samples of red wine originating from Mediterranean Sea countries and 15 samples of vinegar were examined for the presence of OA. All red wine samples contained OA. Seventy-two percent of these samples were found to be contaminated with more than 0.1 µg/L. All 15 vinegar samples showed the presence of OA. The most contaminated ones were three balsamic vinegar samples containing 0.156, 0.102 and 0.252 µg/L.

ENTWISLE, A.C., WILLIAMS, A.C., MANN, P.J., RUSSELL, J., SLACK, P.T. and GILBERT, J. 2001. **Combined phenyl silane and immunoaffinity column cleanup with liquid chromatography for determination of ochratoxin A in roasted coffee: Collaborative study.** Journal of AOAC International **84**: 444–450.

A collaborative study was conducted to evaluate an LC method for OA using sequential phenyl silane and immunoaffinity column cleanup. The test portion was extracted with methanol and sodium bicarbonate by shaking. The extract was filtered, centrifuged and then cleaned up on a phenyl silane column before being eluted from the washed column with methanol–water. The eluate was diluted with phosphate buffered saline and applied to an OA immunoaffinity column, which was washed with water. OA was eluted with methanol, the solvent was evaporated and the residue was redissolved in injection solvent and applied to a reversed phase LC apparatus followed by fluorescence detection. In samples spiked with OA at 4 µg/kg recoveries ranged from 65 to

97%. The relative standard deviation for repeatability (RSD<sub>r</sub>) ranged from 2 to 22% and the relative standard deviation for reproducibility (RSD<sub>R</sub>) ranged from 14 to 26%. The method showed acceptable within and between laboratory precision, as evidenced by HORRAT values, at the low level of determination for OA in roasted coffee.

## Ochratoxicoses

OZCELIK, N., KOSAR, A. and SOYSAL, D. 2001. **Ochratoxin A in human serum samples collected in Isparta, Turkey from healthy individuals and individuals suffering from different urinary disorders.** Toxicology Letters **121**: 9–13.

OA levels in human serum samples collected from 40 healthy individuals and 93 individuals suffering from different urinary disorders in Isparta, Turkey, are presented. Four different kinds of urinary disorders were represented: chronic renal failure treated by haemodialysis, chronic renal failure treated by peritoneal dialysis, patients with bladder cancer and patients with renal stones. The mean concentration of OA in the healthy group was 0.4 ± 0.28 µg/L. The highest mean concentration was found in the group of patients treated by haemodialysis, 2.1 ± 1.2 µg/L. The mean concentrations of OA in all patients groups were higher compared to the control group. A higher level of OA in dialysis groups compared to the control, renal stones and bladder cancer groups could probably be explained by the reduced glomerular filtration rate of these patients.

DORTANT, P.M., PETERS-VOLLEBERG, G.W.M., VANLOVEREN, H., MARQUARDT, R.R. and SPEIJERS, G.J.A. 2001. **Age-related differences in the toxicity of ochratoxin A in female rats.** Food and Chemical Toxicology **39**: 55–65.

Age related differences, especially in nephro- and immunotoxicity of OA, were investigated in young adult (aged 12 weeks) and old (aged 27–30 months) female SPF Wag rats treated by gavage with OA at 0.07, 0.34 or 1.68 mg/kg body weight for 4 weeks. In both age groups, survival was significantly decreased in the highest dose group. OA induced primarily nephropathy. Old rats were more sensitive to induction of tubular karyomegaly and vacuolation/necrosis. In young rats, OA induced a dose related thickening of the basement membrane and reduction in splenic T-cell fraction. Decreased IgG levels were seen at 0.34 mg/kg (young and old rats) and 1.68 mg/kg (young rats). Vacuolation of the white brain matter (cerebellar medulla and ventral parts

of the brain stem) was significantly increased in young rats at 0.34 and 1.68 mg/kg and in old rats at 0.07 and 0.34 mg/kg.

GAUTIER, J.C., HOLZHAUSER, D., MARKOVIC, J., GREMAUD, E., SCHILTER, B. and TURESKY, R.J. 2001. **Oxidative damage and stress response from ochratoxin A exposure in rats.** *Free Radical Biology and Medicine* **30**: 1089–1098.

Several chemical and biological markers associated with oxidative stress response were measured in rats to determine if this process is involved in OA mediated toxicity. Male rats dosed with OA at up to 2 mg/kg (24 hr exposure) did not increase the formation of biomarkers of oxidative damage such as the lipid peroxidation marker malondialdehyde in rat plasma, kidney and liver, or the DNA damage marker 8-oxo-7,8-dihydro-2-deoxyguanosine in kidney DNA. However, OA treatment at 1 mg/kg did result in a 22% decrease in alpha-tocopherol plasma levels and a 5-fold increase in the expression of the oxidative stress responsive protein haem oxygenase-1, specifically in the kidney.

## Fumonisin – General

MARASAS, W.F.O. 2001. **Discovery and occurrence of the fumonisins: A historical perspective.** *Environmental Health Perspectives* **109**: 239–243.

This article describes the events leading to the discovery of the fumonisins in South Africa in 1988 and highlights the first 10 years (1988–1998) of fumonisin research. The predominant fungus isolated from mouldy corn implicated in a field outbreak of equine leukoencephalomalacia (ELEM) in South Africa in 1970 was *Fusarium verticillioides* (*F. moniliforme*). This fungus was also prevalent in mouldy home-grown corn consumed by people in high-incidence areas of oesophageal cancer in the Transkei region of South Africa. Culture material of *F. verticillioides* strain MRC 826, which was isolated from mouldy corn in Transkei, was shown to cause ELEM in horses, porcine pulmonary oedema syndrome in pigs and liver cancer in rats. A short-term cancer initiation/promotion assay in rat liver was used to purify the carcinogen(s) in the culture material and FB<sub>1</sub> and FB<sub>2</sub> were finally isolated from culture material of *F. verticillioides* MRC 826.

APSIMON, J.W. 2001. **Structure, synthesis, and biosynthesis of fumonisin B<sub>1</sub> and related compounds.** *Environmental Health Perspectives* **109**: 245–249.

The absolute stereochemical description of FB<sub>1</sub> and presumably of its congeners is now secure. This article summarises studies leading to this conclusion and outline the biosynthetic and synthetic studies of FB<sub>1</sub>.

SCOTT, P.M. (Ed.) 2001. **Abstracts. Fumonisin Risk Assessment Workshop, 10–12 January 2000.** University of Maryland, College Park, USA. *Food Additives and Contaminants* **18**: 187–210.

Abstracts of 42 papers given at the Fumonisin Risk Assessment Workshop are presented. Topics covered include occurrence, analytical methods, toxicity and risk assessment of fumonisins and control of fumonisin production.

PETERSEN, A. and THORUP, I. 2001. **Preliminary evaluation of fumonisins by the Nordic countries and occurrence of fumonisins (FB<sub>1</sub> and FB<sub>2</sub>) in corn-based foods on the Danish market.** *Food Additives and Contaminants* **18**: 221–226.

The presence of FB<sub>1</sub> and FB<sub>2</sub> in corn based food on the Danish retail market was surveyed. A total of 70 samples were analysed and 37% contained FB<sub>1</sub> and 21% contained FB<sub>2</sub>. No fumonisins were found in sweet corn (canned or frozen), corn-on-the-cob, corn starch or gruel powder for babies. FB<sub>1</sub> was found in about half of the corn flakes, corn snack and popcorn samples, whereas FB<sub>2</sub> was seen to a lesser extent. Both FB<sub>1</sub> and FB<sub>2</sub> were found in 75% or more of the corn flour, tacos and polenta samples. In general, the content of FB<sub>1</sub> was in the range 1–1000 µg/kg and the content of FB<sub>2</sub> was in the range 4–250 µg/kg.

CHELULE, P.K., GOALENI, N., DUTTON, M.F. and CHUTURGOON, A.A. 2001. **Exposure of rural and urban populations in KwaZulu Natal, South Africa, to fumonisin B<sub>1</sub> in maize.** *Environmental Health Perspectives* **109**: 253–256.

Households in rural and urban areas of KwaZulu Natal, South Africa, were surveyed to assess the exposure of the inhabitants to FB<sub>1</sub>. Of the 50 rural maize samples examined, 32% had levels of FB<sub>1</sub> ranging from 0.1 to 22.2 mg/kg, whereas 29% of the 28 cooked maize (phutu) samples contained FB<sub>1</sub> ranging from 0.1 to 0.4 mg/kg. The incidence and levels of FB<sub>1</sub> in faeces were 33% and 0.5–39.0 mg/kg, respectively. Of the 49 urban maize samples analysed 6.1% contained FB<sub>1</sub> in the range 0.2–0.5 mg/kg, whereas 3/44 faecal samples contained FB<sub>1</sub> in the range 0.6–16.2 mg/kg. No FB<sub>1</sub> was detected in urban phutu samples.

TSENG, T.C. and LIU, C.Y. 2001. **Occurrence of fumonisin B<sub>1</sub> in maize imported into Taiwan.** *International Journal of Food Microbiology* **65**: 23–26.

Samples of maize imported into Taiwan during 1997–1998 were collected and analysed for FB<sub>1</sub>. Eight of 118 samples were found to contain FB<sub>1</sub> and values ranged from 334 to 1614 µg/kg. The frequency of FB<sub>1</sub> found in maize samples imported from Australia was 20%, followed by Thailand (10%) and USA (5.1%). Only four samples contained FB<sub>1</sub> in excess of 300 µg/kg.

CAMARGOS, S.M., SOARES, L.M.V., SAWAZAKI, E., BOLONHEZI, D., CASTRO, J.L. and BORTOLLETO, N. 2001. **Fumonisin in corn cultivars in the state of Sao Paulo.** *Brazilian Journal of Microbiology* **31**: 226–229.

Samples of corn belonging to 19 cultivars with distinct types of germplasms, endosperm and length of vegetative cycle, were analysed for FB<sub>1</sub> and B<sub>2</sub>. The cultivars were grown in experimental fields in three locations within the State of Sao Paulo, Brazil, during the 1997/1998 crop. All 23 samples were contaminated with fumonisins, with concentrations of FB<sub>1</sub> ranging from 1.63 to 25.69 mg/kg with an average of 5.61 mg/kg and of FB<sub>2</sub> from 0.38 to 8.60 mg/kg with an average of 1.86 mg/kg. In terms of fumonisins, these high levels put the corn cultivated in Sao Paulo among the most contaminated in the world reported to date.

MILLER, J.D. 2001. **Factors that affect the occurrence of fumonisin.** *Environmental Health Perspectives* **109**: 321–324.

The two important *Fusarium* ear rots of corn, *Gibberella* ear rot (*Fusarium graminearum*, formally *F. moniliforme* and allied species) and *Fusarium* ear rot (*F. verticillioides* and allied species) grow under different environmental conditions. *F. graminearum* grows well only between 26 and 28°C and requires rain both at silking and during disease progression. *F. verticillioides* grows well at higher temperatures, and ear rot and fumonisin accumulation are associated with drought and insect stress and growing hybrids outside their areas of adaptation. The best available strategies for reducing the risk of fumonisin contents of maize are to ensure that hybrids are adapted to the environment and to limit drought stress and insect herbivory. It may also be necessary to make use of alternative strategies such as producing hybrids that contain enzymes to degrade fumonisin as it is produced.

BACON, C.W., YATES, I.E., HINTON, D.M. and MEREDITH, F. 2001. **Biological control of *Fusarium moniliforme* in maize**. Environmental Health Perspectives **109**: 325–332.

*Fusarium moniliforme* is a facultative fungal endophyte and during the biotrophic endophytic association with maize, as well as during saprophytic growth, it produces fumonisins. The fungus is transmitted vertically and horizontally to the next generation of plants via clonal infection of seeds and plant debris. A biological control system using an endophytic bacterium, *Bacillus subtilis*, has been developed that shows great promise for reducing mycotoxin accumulation during the endophytic (vertical transmission) growth phase. Because this bacterium occupies the identical ecological niche within the plant, it is considered an ecological homologue to *F. moniliforme*, and the inhibitory mechanism, regardless of the mode of action, operates on the competitive exclusion principle. In addition to this bacterium, an isolate of a species of the fungus *Trichoderma* shows promise in the post-harvest control of the growth and toxin accumulation from *F. moniliforme* on corn in storage.

SAUNDERS, D.S., MEREDITH, F.I. and VOSS, K.A. 2001. **Control of fumonisin: Effects of processing**. Environmental Health Perspectives **109**: 333–336.

Studies on the effects of wet-milling on fumonisin residues in corn found these residues were not detectable in cornstarch, the starting material for high-fructose corn syrup, and most other wet-milled food ingredients. Similar effects were noted for the dry-milling process. Fumonisin residues were not detectable or quite low in dry flaking grits and corn flour, higher in corn germ, and highest in corn bran. Extrusion of dry-milled products reduced fumonisin concentrations by 30–90% for mixing-type extruders and 20–50% for nonmixing extruders. Cooking and canning generally had little effect on fumonisin content. In the masa process, measurable fumonisin was reduced following the cooking, soaking and washing steps, with little conversion of fumonisin to the hydrolysed form. Sheeting, baking and frying at commercial times and temperatures generally had no effect. Studies have shown no fumonisin residues present in food products or ingredients.

DEGIROLAMO, A., SOLFRIZZO, M. and VISCONTI, A. 2001. **Effect of processing on fumonisin concentration in corn flakes**. Journal of Food Protection **64**: 701–705.

The stability of FB<sub>1</sub> and B<sub>2</sub> during processing of corn flakes was investigated using three different methods for analysis of

the naturally contaminated raw material (corn flour), intermediate product (extruded corn flakes) and final product (roasted corn flakes). Only one method, using immunoaffinity column cleanup, provided reliable results in the determination of fumonisins in corn flake samples at the intermediate and final steps of processing. About 60–70% of the initial amount of fumonisins were lost during the entire cycle of corn flake processing, with less than 30% losses occurring during the intermediate extrusion step (70–170°C for 2 to 5 min). The effect of different additives commonly present in commercial products (sodium chloride, sucrose and ferrous sulphate heptahydrate) on the reliability of fumonisin analysis was also investigated. The presence of sodium chloride strongly reduced fumonisin recovery when strong anion-exchange columns were used for the cleanup step, whereas the other additives appeared to have little or no effect on the accuracy of fumonisin analysis.

SPOTTI, M., CALONI, F., FRACCHIOLLA, L., POMPA, G., VIGO, D. and MAFFEO, G. 2001. **Fumonisin B<sub>1</sub> carry-over into milk in the isolated perfused bovine udder**. Veterinary and Human Toxicology **43**: 109–111.

The carry-over of FB<sub>1</sub> into bovine milk was investigated using the isolated perfused bovine udder. Two mg of FB<sub>1</sub> was injected into the perfusion blood of three udders, and milk and perfused serum levels were determined for 150 minutes. FB<sub>1</sub> passed through the mammary barrier into the milk but in such low concentrations as to present a negligible risk for consumers.

SHIM, W.B. and WOLOSHUK, C.P. 2001. **Regulation of fumonisin B<sub>1</sub> biosynthesis and conidiation in *Fusarium verticillioides* by a cyclin-like (C-type) gene, *FCCI***. Applied and Environmental Microbiology **67**: 1607–1612.

A mutant of *Fusarium verticillioides*, FT536, carrying a disrupted gene named *FCCI* (for *Fusarium* cyclin C1) resulting in altered FB<sub>1</sub> biosynthesis has been generated. *FCCI* contains an open reading frame of 1,018 bp, with one intron, and encodes a putative 319-amino-acid polypeptide. When strain FT536 was grown on corn kernels or on defined minimal medium at pH 6, conidiation was reduced and *FUM5*, the polyketide synthase gene involved in FB<sub>1</sub> biosynthesis, was not expressed. However, when the mutant was grown on a defined minimal medium at pH 3, conidiation was restored, and the blocks in expression of *FUM5* and FB<sub>1</sub> production were suppressed. Our data suggest that *FCCI* plays an important role in signal transduction regulating

secondary metabolism (fumonisin biosynthesis) and fungal development (conidiation) in *F. verticillioides*.

HARTL, M. and HUMPF, H.U. 2001. **Combined synthetic/CD strategy for the stereochemical assignment of the tricarballic acid side chains of fumonisin B<sub>1</sub>**. Journal of Organic Chemistry **66**: 3678–3681.

The circular dichroism exciton chirality method was employed for the stereochemical assignment of the tricarballic acid (TCA) side chains of FB<sub>1</sub>. Using 2-naphthoate for chromophoric derivatisation of the reduced TCA moieties, the absolute configuration was demonstrated.

## Fumonisin – Methodology

SOLFRIZZO, M., DEGIROLAMO, A. and VISCONTI, A. 2001. **Determination of fumonisins B<sub>1</sub> and B<sub>2</sub> in cornflakes by high performance liquid chromatography and immunoaffinity clean-up**. Food Additives and Contaminants **18**: 227–235.

An accurate method for the determination of FB<sub>1</sub> and B<sub>2</sub> in cornflakes has been developed. Samples were extracted with acetonitrile-methanol-water (25:25:50), diluted with phosphate buffered saline (PBS) and applied to a FumoniTest(TM) immunoaffinity column. After washing with PBS, fumonisins were eluted from the column with methanol and reacted with *o*-phthalaldehyde/2-mercaptoethanol to form fluorescent derivatives. Fumonisin derivatives were analysed by reversed phase HPLC with fluorometric detection. The average recoveries for FB<sub>1</sub> and FB<sub>2</sub> spiked in the ranges 0.33–2.80 and 0.17–1.40 mg/kg were 102.6 and 95.1%, respectively, with average relative standard deviations of 9 and 8%, respectively. The limit of quantification for FB<sub>1</sub> and FB<sub>2</sub> was 0.005 mg/kg. In 18 cornflakes and cornflake cereals samples tested, all but one sample were found to be contaminated, with mean concentrations of FB<sub>1</sub> and FB<sub>2</sub> of 0.157 and 0.036 mg/kg, respectively.

SEEFELDER, W., HARTL, M. and HUMPF, H.U. 2001. **Determination of N-(carboxymethyl) fumonisin B<sub>1</sub> in corn products by liquid chromatography/electrospray ionization-mass spectrometry**. Journal of Agricultural and Food Chemistry **49**: 2146–2151.

N-(carboxymethyl)FB<sub>1</sub> (NCM-FB<sub>1</sub>) is the reaction product of FB<sub>1</sub> and reducing sugars. In model experiments, corn grits were spiked with FB<sub>1</sub> and D-glucose or sucrose and manufactured into extrusion products at various temperatures and mois-

ture levels. An LC/electrospray ionisation-MS method was developed for the determination of NCM-FB<sub>1</sub>. The detection limit achieved with this method was 10 µg/kg. Low concentrations of NCM-FB<sub>1</sub> were detected in all samples spiked with D-glucose and FB<sub>1</sub>, whereas those spiked with FB<sub>1</sub> and sucrose showed only NCM-FB<sub>1</sub> in samples produced at 180°C. Various corn containing food samples from the German market were analysed for the presence of NCM-FB<sub>1</sub>, FB<sub>1</sub> and hydrolysed FB<sub>1</sub>. All samples were contaminated with FB<sub>1</sub> (22–194 µg/kg) and hydrolysed FB<sub>1</sub> (5–247 µg/kg). Six of nine samples contained NCM-FB<sub>1</sub> in low concentrations ranging from 10 to 76 µg/kg.

SCARANO, G., GRASSO, L., ARACE, O., OLIVIERO, G. and SERPE, L. 2001. [Use of semiautomatic equipment for the determination of fumonisin B<sub>1</sub> in milk]. *Industrie Alimentari* **40(401)**: 257–260.

An HPLC method for the determination of fumonisin in milk was modified to include semiautomatic equipment in the purification step by means of immunoaffinity columns, and in the derivatisation and LC injection steps. The modified method provided good repeatability, short process time and the analysis of several samples in the same time. The detector response was linear in the range 0.001–0.010 mg/L and the detection limit was 0.010 mg/L. Recoveries from milk spiked with FB<sub>1</sub> at 0.025, 0.05 and 0.1 mg/L were 83.1, 79.7 and 73.5%, respectively. (In Italian).

RIBAR, S., MESARIC, M. and BAUMAN, M. 2001. **High-performance liquid chromatographic determination of sphinganine and sphingosine in serum and urine of subjects from an endemic nephropathy area in Croatia. Presented at the 6th International Symposium on New Achievements in Chromatography, Plitvice Lakes, October 11–13, 2000.** *Journal of Chromatography B* **754**: 511–519.

A modified method has been used for the determination of the sphinganine (Sa)/sphingosine (So) ratio in human serum and urine of healthy subjects and endemic nephropathy patients from Brodska Posavina, Croatia. Free sphingoid bases, Sa and So were obtained by base hydrolysis. Afterwards, precolumn *o*-phthalaldehyde derivatisation, HPLC separation and quantification by fluorescence detection were performed. The results obtained pointed to a sphingolipid metabolism impairment which may have been induced by fumonisins or fumonisin-like mycotoxins. As statistically significant differences

were recorded in the subjects not yet affected with endemic nephropathy, an impairment in the metabolism of sphingolipids might be considered as an early indicator of endemic nephropathy.

DELONGCHAMP, R.R. and YOUNG, J.F. 2001. **Tissue sphinganine as a biomarker of fumonisin-induced apoptosis.** *Food Additives and Contaminants* **18**: 255–261.

Sa concentrations in the livers of mice and in the livers and kidneys of rats were measured in conjunction with a tumour bioassay. The utility of Sa levels in this role was initially questioned because they were highly variable when compared across time points. However, a conceptual framework and data are presented that support the use of Sa as a biomarker for a dose response of FB<sub>1</sub> on cell death. This framework is reasonably consistent with observed Sa concentrations in the examined tissues, the literature on fumonisin effects on sphingolipid synthesis and an hypothesised mechanism through which FB<sub>1</sub> increases age-specific tumour incidence.

## Fumonisin – Toxicology

QIU, M.F. and LIU, X.M. 2001. **Determination of sphinganine, sphingosine and Sa/So ratio in urine of humans exposed to dietary fumonisin B<sub>1</sub>.** *Food Additives and Contaminants* **18**: 263–269.

An LC method sufficiently sensitive to determine the low concentration of free Sa in male human urine is described. The method involves isolation from human urine of exfoliated cells followed by an extraction of free sphingoid bases and their separation and quantification by HPLC. The detection limits for So and Sa were 0.15 µg/L in female urine and 0.005 µg/L in male urine. Twenty-eight healthy adult volunteers consumed for 1-month a normal diet containing their home-grown corn potentially contaminated with FB<sub>1</sub>. All the home-grown corn samples contained FB<sub>1</sub> ranging from 0.08 to 41.1 mg/kg, and the estimated daily FB<sub>1</sub> intakes ranged from 0.4 to 740 µg/kg body weight/day. The 1-month monitoring results suggest that sphingolipid metabolism of humans could be affected by FB<sub>1</sub> intake, the urinary Sa/So ratio may be useful for evaluating FB<sub>1</sub> exposure when the contamination of FB<sub>1</sub> is high, and that males are more sensitive to FB<sub>1</sub> disruption of sphingolipid metabolism than females.

MERRILL, A.H., SULLARDS, M.C., WANG, E., VOSS, K.A. and RILEY, R.T. 2001. **Sphingolipid metabolism:**

**Roles in signal transduction and disruption by fumonisins.** *Environmental Health Perspectives* **109**: 283–289.

An overview with 59 references. The accumulation of sphingoid bases is a primary cause of the toxicity of fumonisin. Nonetheless, the full effects of fumonisins probably involve many biochemical events. The elevations in sphingoid bases also affect the amounts of other lipids, including the 1-phosphates and N-acetyl derivatives of Sa. Furthermore, the aminopentol backbone of FB<sub>1</sub> (AP<sub>1</sub>) is both an inhibitor and a substrate for ceramide synthase, and the resultant N-palmitoyl-AP<sub>1</sub> is an even more potent inhibitor of ceramide synthase.

JONES, C., CIACCI-ZANELLA, J.R., ZHANG, Y.G., HENDERSON, G. and DICKMAN, M. 2001. **Analysis of fumonisin B<sub>1</sub>-induced apoptosis.** *Environmental Health Perspectives* **109**: 315–320.

Genes have been identified that inhibit FB<sub>1</sub>-induced apoptosis in African green monkey kidney fibroblasts (CV-1) cells and two mouse embryo fibroblasts (MEF). A baculovirus gene, inhibitor of apoptosis (CpIAP), protected these cells from apoptosis. CpIAP blocks apoptosis induced by the tumour necrosis factor (TNF) pathway as well as other mechanisms. Further support for the involvement of the TNF signal transduction pathway in FB<sub>1</sub> induced apoptosis was the cleavage of caspase 8. Inhibition of caspases by the baculovirus gene *p35* also inhibited FB<sub>1</sub> induced apoptosis.

ZHANG, Y., JONES, C. and DICKMAN, M.B. 2001. **Identification of differentially expressed genes following treatment of monkey kidney cells with the mycotoxin fumonisin B<sub>1</sub>.** *Food and Chemical Toxicology* **39**: 45–53.

In order to identify genes that are induced by FB<sub>1</sub>, a PCR based subtraction approach was employed. Eight genes that showed high similarity (>90%) to known mammalian genes were identified. These genes included tumour necrosis factor type 1 receptor associated protein 2 (TRAP2), human leukaemia virus receptor (GLVR1), human Scaffold attachment factor A (SAFA) also called heterogeneous nuclear ribonucleoprotein U (hnRNP-U), human protein kinase C-binding protein (RACK7), human oligosaccharyl transferase STT3 subunit, mouse WW-domain binding protein 2 (WBP2), human fibronectin, and an unknown human clone. The ability of FB<sub>1</sub> to alter gene expression and signal transduction pathways may be necessary for its carcinogenic and toxic effects.

VANDERWESTHUIZEN, L., SHEP-HARD, G.S. and VANSCHALKWYK, D.J. 2001. **The effect of a single gavage dose of fumonisin B<sub>2</sub> on the sphinganine and sphingosine concentrations in vervet monkeys.** Food and Chemical Toxicology **39**: 455–459.

The disruption in Sa and So concentrations in plasma and urine of vervet monkeys (*Cercopithecus aethiops*) was measured following a single gavage dose of FB<sub>2</sub> at either 1 or 10 mg/kg body weight. In the low dose monkeys, none of the parameters measured increased significantly above the control values. In the high dose monkeys the plasma Sa/So ratios were significantly increased above the corresponding control ratios after 3 days and continued to be significantly raised for another 27 days, whereafter the ratios declined to control values after 51 days. The plasma aspartate transaminase activities increased significantly above their control values from day 5 to day 23 and the gamma-glutamyl transferase activities from day 7 until the end of the study period. The urinary Sa/So ratio, plasma creatinine and urea values in both groups of monkeys did not increase above the control values.

VANDERWESTHUIZEN, L., SHEP-HARD, G.S. and VANSCHALKWYK, D.J. 2001. **The effect of repeated gavage doses of fumonisin B<sub>1</sub> on the sphinganine and sphingosine levels in vervet monkeys.** Toxicol **39**: 969–972.

Vervet monkeys (*Cercopithecus aethiops*) were dosed with repeated gavages of FB<sub>1</sub> at 1 mg/kg body weight three times/week continuously over a 51-day period. The plasma Sa/So ratio reached a maximum after 30 days with a 3-fold increase above the ratio of the control monkeys and then declined slowly to double the value in controls after 51 days. The lack of a clear elevation in urinary Sa/So ratios after 51 days of multiple exposure in the dosed monkeys indicates that the plasma ratio is more sensitive than urinary changes in monkeys. This is confirmed by the plasma levels of liver function enzymes of which aspartate transaminase, glutamyl-transferase and lactate dehydrogenase were increased in the dosed monkeys, while the plasma indicators of renal function were not increased above the levels in the control monkeys.

GUMPRECHT, L.A., SMITH, G.W., CONSTABLE, P.C. and HASCHKE, W.M. 2001. **Species and organ specificity of fumonisin-induced endothelial alterations: Potential role in porcine pulmonary edema.** Toxicology **160**: 71–79.

Lung, liver, heart and kidney tissues from fumonisin-exposed pigs, sheep, rabbits, and rats were examined ultrastructurally. Endothelial alterations were present in the pulmonary capillary endothelial cells of pigs, but at doses that did not induce pulmonary oedema. These alterations were present only in pigs and not in other species. In addition, these endothelial alterations were not present in any other organs of pigs.

HASCHKE, W.M., GUMPRECHT, L.A., SMITH, G., TUMBLESON, M.E. and CONSTABLE, P.D. 2001. **Fumonisin toxicosis in swine: An overview of porcine pulmonary edema and current perspectives.** Environmental Health Perspectives **109**: 251–257.

An overview with 69 references. Porcine pulmonary oedema has been reproduced experimentally in pigs by feeding of naturally contaminated corn, *F. verticillioides* culture material and by iv administration of FB<sub>1</sub>. Hepatic lesions consisting of apoptosis, necrosis and hepatocyte proliferation also are observed. Fumonisin has been shown to induce an accumulation of membranous material in pulmonary capillary endothelial cells and this change appears specific to this cell type and to swine. In short-term cardiovascular studies, fumonisin decreased left ventricular dP/dt(max) (an index of cardiac contractility), mean systemic arterial pressure, heart rate and cardiac output, and increased mean pulmonary artery pressure and pulmonary artery wedge pressure. These changes are compatible with the inhibition of L-type calcium channels by increased So and/or Sa concentration. Therefore, fumonisin-induced pulmonary oedema in swine appears to result from acute left-sided heart failure mediated by altered sphingolipid biosynthesis.

VOSS, K.A., RILEY, R.T., NORRED, W.P., BACON, C.W., MEREDITH, F.I., HOWARD, P.C., PLATTNER, R.D., COLLINS, T.F.X., HANSEN, D.K. and PORTER, J.K. 2001. **An overview of rodent toxicities: Liver and kidney effects of fumonisins and *Fusarium moniliforme*.** Environmental Health Perspectives **109**: 259–266.

An overview with 124 references. FB<sub>1</sub> is poorly absorbed and rapidly eliminated in faeces of rodents. Minor amounts are retained in liver and kidneys. FB<sub>1</sub> induces apoptosis of hepatocytes and of proximal tubule epithelial cells. More advanced lesions in both organs are characterised by simultaneous cell loss (apoptosis and necrosis) and proliferation (mitosis). Microscopic and other findings suggest that an imbalance between cell loss and replacement develops, a condition favourable for carcinogenesis. On the molecular level,

fumonisins inhibit ceramide synthase and disrupt sphingolipid metabolism and, theoretically, sphingolipid-mediated regulatory processes that influence apoptosis and mitosis. Liver sphingolipid effects and toxicity are correlated, and ceramide synthase inhibition occurs in liver and kidney at doses below their respective no-observed-effect levels.

KODELL, R.L., YOUNG, J.F., DELONGCHAMP, R.R., TURTURRO, A., CHEN, J.J., GAYLOR, D.W., HOWARD, P.C. and ZHENG, Q. 2001. **A mechanistic approach to modelling the risk of liver tumours in mice exposed to fumonisin B<sub>1</sub> in the diet.** Food Additives and Contaminants **18**: 237–253.

Data from the National Toxicology Program's carcinogenesis study of FB<sub>1</sub> in B6C3F<sub>1</sub> mice were used to fit the Moolgavkar-Venzon-Knudson (MVK) two-stage, clonal-expansion model of carcinogenesis. In addition to tumour data from the conventional 2-year bioassay, the study included data on tissue weights, cell proliferation, cell death and sphingolipid metabolism in primary target organs. The model was used to predict 2-year liver tumour rates in mice. The model was able to reproduce reasonably well the observed tumour rates in both female and male mice, predicting substantially increased rates above background only at the highest doses of FB<sub>1</sub> in females.

DRAGAN, Y.P., BIDLACK, W.R., COHEN, S.M., GOLDSWORTHY, T.L., HARD, G.C., HOWARD, P.C., RILEY, R.T. and VOSS, K.A. 2001. **Implications of apoptosis for toxicity, carcinogenicity, and risk assessment: Fumonisin B<sub>1</sub> as an example.** Toxicological Sciences **61**: 6–17.

In a recent National Toxicology Program study in Fischer rats and B6C37F<sub>1</sub> mice, FB<sub>1</sub> caused renal carcinomas in male rats and liver cancer in female mice. In an earlier study in male ED-IX rats, FB<sub>1</sub> caused hepatic toxicity and hepatocellular carcinomas. An early effect of FB<sub>1</sub> exposure in these target organs is apoptosis. However, there is also some evidence of oncotic necrosis following FB<sub>1</sub> administration, especially in the liver. Induction of apoptosis may be a consequence of ceramide synthase inhibition and disruption of sphingolipid metabolism by FB<sub>1</sub>. FB<sub>1</sub> is not genotoxic in bacterial mutagenesis screens or in the rat liver unscheduled DNA-synthesis assay. FB<sub>1</sub> may be the first example of an apparently nongenotoxic agent producing tumours through a mode of action involving apoptotic necrosis, atrophy and consequent regeneration.

HOWARD, P.C., EPPLEY, R.M., STACK, M.E., WARBRITTON, A., VOSS, K.A., LORENTZEN, R.J., KOVACH, R.M. and BUCCI, T.J. 2001. **Fumonisin B<sub>1</sub> carcinogenicity in a two-year feeding study using F344 rats and B6C3F<sub>1</sub> mice.** Environmental Health Perspectives **109**: 277–282.

Female and male F344/N/Nctr BR rats and B6C3F<sub>1</sub>/Nctr BR mice were fed a diet containing FB<sub>1</sub> for 2 years: female rats received 0–100 mg/kg; male rats, 0–150 mg/kg; female mice, 0–80 mg/kg, and; male mice, 0–150 mg/kg. FB<sub>1</sub> was not tumorigenic in female F344 rats up to 100 mg/kg. FB<sub>1</sub> in the diets of male rats induced renal tubule adenomas and carcinomas in 0, 0, 0, 19 and 31% rats at 0, 5, 15, 50 and 150 mg/kg, respectively. FB<sub>1</sub> in the diet of male mice did not affect tumour incidence. Hepatocellular adenomas and carcinomas were induced by FB<sub>1</sub> in the female mice, occurring in 11, 6, 2, 40 and 83% of female mice that consumed diets containing 0, 5, 15, 50 and 80 mg/kg, respectively.

GELDERBLOM, W.C.A., ABEL, S., SMUTS, C.M., MARNEWICK, J., MARASAS, W.F.O., LEMMER, E.R. and RAMLJAK, D. 2001. **Fumonisin-induced hepatocarcinogenesis: Mechanisms related to cancer initiation and promotion.** Environmental Health Perspectives **109**: 291–300.

A review with 79 references of the hepatocarcinogenic effects of fungal cultures of *Fusarium verticillioides* (= *Fusarium moniliforme*) strain MRC 826 in male ED IX rats. Fumonisin intake levels of between 0.8 and 1.6 mg/kg body weight/day over approximately 2 years produce liver cancer in male ED IX rats. Exposure levels of less than 0.8 mg/kg body weight/day fail to induce cancer, although mild toxic and preneoplastic lesions are induced. These studies supported the findings of long-term investigations indicating that a cytotoxic/proliferative response is required for cancer induction and that a no-effect threshold exists for cancer induction. The mechanisms proposed for cancer induction are highlighted and include the possible role of oxidative damage during initiation and the disruption of lipid metabolism, integrity of cellular membranes, and altered growth regulatory responses as important events during promotion.

RILEY, R.T., ENONGENE, E., VOSS, K.A., NORRED, W.P., MEREDITH, F.I., SHARMA, R.P., SPITSBERGEN, J., WILLIAMS, D.E., CARLSON, D.B. and MERRILL, A.H. 2001. **Sphingolipid perturbations as mechanisms for fumonisin carcinogenesis.** Environmental Health Perspectives **109**: 301–308.

An overview with 116 references. The biochemical consequences of fumonisin disruption of sphingolipid metabolism most likely to alter cell regulation are increased free sphingoid bases and their 1-phosphates, alterations in complex sphingolipids, and decreased ceramide biosynthesis. Because free sphingoid bases and ceramide can induce cell death, the fumonisin inhibition of ceramide synthase can inhibit cell death induced by ceramide but promote free sphingoid base-induced cell death. Theoretically, at any time the balance between the intracellular concentration of effectors that protect cells from apoptosis (decreased ceramide, increased sphingosine 1-phosphate) and those that induce apoptosis (increased ceramide, free sphingoid bases, altered fatty acids) will determine the cellular response.

HOWARD, P.C., WARBRITTON, A., VOSS, K.A., LORENTZEN, R.J., THURMAN, J.D., KOVACH, R.M. and BUCCI, T.J. 2001. **Compensatory regeneration as a mechanism for renal tubule carcinogenesis of fumonisin B<sub>1</sub> in the F344/N/Nctr BR rat.** Environmental Health Perspectives **109**: 309–314.

In studies with F344/N/Nctr BR rats consuming diets containing FB<sub>1</sub> at up to 484 mg/kg for 28 days, female rats demonstrated more sensitivity than male rats in the induction of hepatocellular apoptosis and mitosis. Conversely, induction of renal tubule apoptosis and regeneration were more pronounced in male than in female rats. Induction of renal tubule apoptosis and hyperplasia correlated with the incidence of renal tubule carcinomas that developed in the 2-year feeding study with FB<sub>1</sub> in the F344/N/Nctr BR rats. The data are consistent with the hypothesis that the induction of renal tubule carcinomas in male rats could be partly due to the continuous compensatory regeneration of renal tubule epithelial cells in response to the induction of apoptosis by FB<sub>1</sub>.

GELDERBLOM, W.C.A., LEBEPE-MAZUR, S., SNIJMAN, P.W., ABEL, S., SWANEVELDER, S., KRIEK, N.P.J. and MARASAS, W.F.O. 2001. **Toxicological effects in rats chronically fed low dietary levels of fumonisin B<sub>1</sub>.** Toxicology **161**: 39–51.

The toxicity of low dietary levels of FB<sub>1</sub> (1, 10 and 25 mg/kg diet) were monitored in rats over a period of 24 months. Mild toxic effects, including single cell necrosis (apoptosis), proliferation of bile duct epithelial cells, and early signs of fibrosis, bile duct hyperplasia and in one case, adenofibrosis, were noticed in the liver of the rats fed the 25 mg/kg diet. A significant increase in the level of oxidative damage was also noticed

in the liver of the rats in this group. Hepatocyte nodules, staining positively for glutathione-S-transferase placental form, were observed macroscopically in the 25 mg/kg group and to a lesser extent in the 10 mg/kg group. The most prominent toxic lesions caused by FB<sub>1</sub> in the kidneys were restricted to the tubular epithelium manifesting as granular cast, necrosis, apoptosis, calcification and the presence of regenerative foci in the proximal convoluted tubules.

SOLFRIZZO, M., CARRATU, M.R., AVANTAGGIATO, G., GALVANO, F., PIETRI, A. and VISCONTI, A. 2001. **Ineffectiveness of activated carbon in reducing the alteration of sphingolipid metabolism in rats exposed to fumonisin-contaminated diets.** Food and Chemical Toxicology **39**: 507–511.

Rats were fed fumonisins (FB<sub>1</sub> + FB<sub>2</sub>) in the diet at 4 mg/kg with and without the addition of activated charcoal at 20 g/kg diet for 1 week. In rats fed the fumonisin contaminated diet, the Sa concentration and Sa/So ratio increased significantly and reversibly in kidney, while urine and liver did not show a significant increase of Sa/So ratio. The addition of activated charcoal to the fumonisin diet did not alter the change of Sa/So biomarker for fumonisin exposure.

MATHUR, S., CONSTABLE, P.D., EPPLEY, R.M., TUMBLESON, M.E., SMITH, G.W., TRANQUILLI, W.J., MORIN, D.E. and HASCHEK, W.M. 2001. **Fumonisin B<sub>1</sub> increases serum sphinganine concentration but does not alter serum sphingosine concentration or induce cardiovascular changes in milk-fed calves.** Toxicological Sciences **60**: 379–384.

Ten milk-fed male Holstein calves were administered purified FB<sub>1</sub> at 1 mg/kg, iv, daily for 7 days. Calves were euthanised on day 7. In treated calves, serum Sa concentration increased from day 3 onward, whereas, serum So concentration was unchanged. Other parameters including heart rate, cardiac output, stroke volume, mean arterial pressure and mean pulmonary artery pressure were unchanged in treated and control calves. Fumonisin treated calves developed metabolic acidosis but all survived for 7 days.

MATHUR, S., CONSTABLE, P.D., EPPLEY, R.M., WAGGONER, A.L., TUMBLESON, M.E. and HASCHEK, W.M. 2001. **Fumonisin B<sub>1</sub> is hepatotoxic and nephrotoxic in milk-fed calves.** Toxicological Sciences **60**: 385–396.

Ten milk-fed male Holstein calves aged 7 to 14 days were administered FB<sub>1</sub> at 1 mg/kg, iv, daily until euthanised on day 7. FB<sub>1</sub> treated calves were lethargic and had

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decreased appetite from day 4 onward, serum biochemical evidence of severe liver and bile duct injury, and impaired hepatic function. Treated calves also had biochemical evidence of renal injury that functionally involved the proximal convoluted tubules. Sa and So concentrations in liver, kidney, lung, heart and skeletal muscle were increased in treated calves. Sa, but not So, concentration was increased in brains of treated calves. This is the first report of FB<sub>1</sub> induced renal injury and organ sphingolipid alterations in cattle.

HENRY, M.H. and WYATT, R.D. 2001. **The toxicity of fumonisin B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>, individually and in combination, in chicken embryos.** Poultry Science **80**: 401–407.

The toxicities of purified FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>, individually and in combination (3:1:1 ratio), were evaluated with regard to their embryo toxicity by injection of the toxins into the air cell of chicken eggs at 72 hr of incubation. The 50% lethal dose for FB<sub>1</sub> was 18.73 µg/egg. A comparison of the toxicity of FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>, individually and in combination found that FB<sub>1</sub> was the most toxic. Microscopic examination of chicken embryos exposed to fumonisin did not reveal any gross developmental abnormalities, however, severe haemorrhages of the head, neck and thoracic area of the dead embryos were evident.

YU, C.H., LEE, Y.M., YUN, Y.P. and YOO, H.S. 2001. **Differential effects of fumonisin B<sub>1</sub> on cell death in cultured cells: The significance of the elevated sphinganine.** Archives of Pharmacal Research **24**: 136–143.

FB<sub>1</sub> elevated the intracellular free Sa concentrations in both LLC-PK1 and Chinese hamster ovary (CHO) cells. However, CHO cells are resistant to fumonisin cytotoxicity at 50 µM, while LLC-PK1 cells are sensitive at concentrations greater than 35 µM. The intracellular concentration of free Sa in LLC-PK1 cells treated at 50 µM FB<sub>1</sub> for 72 hr was approximately 1450 pmol/mg protein relative to the 37 pmol observed in the control culture. Under the same conditions, the population of apoptotic cells in the 50 µM FB<sub>1</sub> treated culture was approximately 37% of the total compared to 12% in the control. The intracellular concentration of free Sa in CHO cells treated with 50 µM FB<sub>1</sub> for 72 hr was approximately 460 pmol/mg protein, indicating that the mass amount of elevated free Sa in the CHO cells was about 32% of that in LLC-PK1 cells. Adding exogenous Sa to the CHO cells along with 50 µM FB<sub>1</sub> treatment for 72 hr caused both necrosis and apoptosis.

GAUMY, J.L., BAILLY, J.D., BURGAT, V. and GUERRE, P. 2001. **[Zearalenone: properties and experimental toxicity].** Revue de Medecine Veterinaire **152**: 219–234.

A review with 107 references. Zearalenone (ZEA) is resistant to most food processing treatments. When administered orally it is well absorbed and is able to reach intracellular targets. Its metabolism is complex. Urinary and biliary excretion of the mycotoxin and metabolites occur, with a possible entero-hepatic cycle. Milk excretion is also observed. Acute toxicity of ZEA is weak. It provokes reproductive disorders after competitive fixation to the intracellular receptors of oestrogens. Although it is not genotoxic, ZEA is carcinogenic in animals. For lack of epidemiological data, no evaluation of its carcinogenicity in humans has been proposed. (In French).

WALKER, S.L., LEATH, S., HAGLER, W.M. and MURPHY, J.P. 2001. **Variation among isolates of *Fusarium graminearum* associated with *Fusarium* head blight in North Carolina.** Plant Disease **85**: 404–410.

Sixty-six isolates of *Fusarium graminearum* associated with *Fusarium* head blight were collected in North Carolina and tested for *in vitro* growth rate, *in vitro* production of DON and ZEA, and pathogenicity on three cultivars of soft red winter wheat. Significant differences among isolates were found for all three traits. Randomly Amplified Polymorphic DNA (RAPD) analysis revealed high levels of genotypic diversity among isolates. There were no significant differences between levels of DON produced by the five isolates associated with the highest levels of disease.

BAI, G.H., PLATTNER, R., DESJARDINS, A. and KOLB, F. 2001. **Resistance to *Fusarium* head blight and deoxynivalenol accumulation in wheat.** Plant Breeding **120**: 1–6.

An international collection of 116 wheat lines was evaluated for *Fusarium* head blight (FHB) resistance and concentration of DON in grain. Plants were inoculated with mixed isolates of *F. graminearum* in the greenhouse by injecting conidia into a single spikelet of each spike, and in the field by scattering *F. graminearum*-infected wheat kernels on the soil surface. Significant differences in FHB ratings and DON levels were observed among cultivars. Correlation coefficients were significant between FHB symptom ratings, seed quality traits and DON levels. Thus, the percentage of

scabbed spikelets and kernels can be generally used to predict DON levels in harvested wheat grain.

VIGIER, B., REID, L.M., DWYER, L.M., STEWART, D.W., SINHA, R.C., ARNASON, J.T. and BUTLER, G. 2001. **Maize resistance to gibberella ear rot: Symptoms, deoxynivalenol, and yield.** Canadian Journal of Plant Pathology **23**: 99–105.

The effects of different environments on maize resistance to *Gibberella* ear rot, disease symptoms, DON concentration and grain yield were measured in three maize inbred lines and five hybrids at six locations in eastern Canada. All genotypes were inoculated with a three-isolate macroconidial mix of *Fusarium graminearum* using a kernel stab inoculation technique. Results show that year to year variation is more important than variation associated with multiple locations in testing for genotypic resistance to *Gibberella* ear rot, according to disease symptoms and DON content. Regression models indicated that higher ear rot severity and DON concentration were associated with an increase in the total number of days from July to September with relative humidity equal to or greater than 80%.

BROWN, D.W., MCCORMICK, S.P., ALEXANDER, N.J., PROCTOR, R.H. and DESJARDINS, A.E. 2001. **A genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum*.** Fungal Genetics and Biology **32**: 121–133.

T-2 toxin biosynthesis by *Fusarium sporotrichioides* and DON biosynthesis by *F. graminearum* were studied by comparing the nucleotide sequence of the 23-kb core trichothecene gene cluster from each organism. This comparative genetic analysis allowed the prediction of proteins encoded by two trichothecene genes, TRI9 and TRI10, that had not previously been described from either *Fusarium* species. Differences in gene structure also were correlated with differences in the types of trichothecenes that the two species produce. Gene disruption experiments showed that *F. sporotrichioides* TRI7 (FsTRI7) is required for acetylation of the oxygen on C-4 of T-2 toxin. Sequence analysis indicated that *F. graminearum* TRI7 (FgTRI7) is nonfunctional.

EDWARDS, S.G., PIRGOZLIEV, S.R., HARE, M.C. and JENKINSON, P. 2001. **Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against *Fusar-***

**ium head blight of winter wheat.** Applied and Environmental Microbiology **67**: 1575–1580.

A PCR based assay to quantify trichothecene producing *Fusarium* based on primers derived from the trichodiene synthase gene (*Tri5*) is described. The primers were tested against a range of FHB pathogens and found to amplify specifically a 260-bp product from 25 isolates belonging to six trichothecene producing *Fusarium* species. Amounts of the trichothecene-producing *Fusarium* and DON in harvested grain from a field trial designed to test the efficacies of the fungicides metconazole, azoxystrobin and tebuconazole to control FHB were quantified. No correlation was found between FHB severity and DON in harvested grain, but a good correlation existed between the amount of trichothecene-producing *Fusarium* and DON present within grain. Azoxystrobin did not affect levels of trichothecene-producing *Fusarium* compared with controls. Metconazole and tebuconazole significantly reduced the amount of trichothecene-producing *Fusarium* in harvested grain.

LEGUEVEL, R. and PAKDEL, F. 2001. **Assessment of oestrogenic potency of chemicals used as growth promoter by in vitro methods.** Human Reproduction **16**: 1030–1036.

Three *in vitro* bioassays were used to compare the oestrogenic potency of chemicals used as growth promoters in beef cattle or found as food contaminant such as the mycotoxin ZEA and some of their metabolites (17 alpha-oestradiol, oestrone, 17 alpha-epitestosterone, 19-nortestosterone, androstendione, zearalanone, alpha-zearalanol, beta-zearalanol, alpha-zearalenol, beta-zearalenol). The first bioassay was based on the activation of a reporter gene by oestrogens in recombinant yeast expressing human or rainbow trout oestrogen receptor. In the second bioassay, the vitellogenin gene induction of rainbow trout hepatocyte cultures was used as a biomarker for the exposure to oestrogens. The third bioassay was based on the alkaline phosphatase gene induction by oestrogens in the human endometrial Ishikawa cell line. The strong oestrogenicity of ZEA and its metabolites and particularly alpha-zearalenol which was as potent as ethinyl oestradiol and diethylstilboestrol in the human endometrial Ishikawa cell line was clearly demonstrated.

NAMIKOSHI, M., AKANO, K., MEGURO, S., KASUGA, I., MINE, Y., TAKAHASHI, T. and KOBAYASHI, H. 2001. **A new macrocyclic trichothecene, 12,13-deoxyroridin E, produced by the**

**marine-derived fungus *Myrothecium roridum* collected in Palau.** Journal of Natural Products **64**: 396–398.

A new macrocyclic trichothecene, 12,13-deoxyroridin E, and three known compounds, roridin E, verrucarins A and verrucarins J, were obtained as cytotoxic components from the marine-derived fungus *Myrothecium roridum*, isolated in Palau.

KANG, Z.S., HUANG, L.L., KRIEG, U., MAULER-MACHNIK, A. and BUCHENAUER, H. 2001. **Effects of tebuconazole on morphology, structure, cell wall components and trichothecene production of *Fusarium culmorum* in vitro.** Pest Management Science **57**: 491–500.

The effects of tebuconazole, a systemic fungicide, on the morphology, structure, cell wall components and toxin production of *Fusarium culmorum* were investigated *in vitro*. Mycelial growth was strongly inhibited by fungicide treatment. Immunogold labelling with antiserum against DON revealed that DON was localised in the cell walls, cytoplasm, mitochondria and vacuoles of the hyphae and that tebuconazole dramatically reduced DON production by the fungus.

## Trichothecenes – Methodology

KRSKA, R. and JOSEPHS, R. 2001. **The state-of-the-art in the analysis of oestrogenic mycotoxins in cereals.** Fresenius Journal of Analytical Chemistry **369**: 469–476.

A review with 54 references discussing the state-of-the-art in the analysis of oestrogenic mycotoxins in cereals, with special emphasis on ZEA as its most relevant representative. Immunoaffinity columns followed by HPLC with fluorescence detection and immunoassays are currently the most frequently used methods for the determination of ZEA and its metabolites, and these techniques are discussed in more detail. The great potential of HPLC-MS(MS) for the simultaneous detection and identification of several oestrogenic mycotoxins is discussed. International intercomparison studies have revealed the need for better means of external quality assurance measures, especially the availability of certified reference materials and certified standards.

JOSEPHS, R.D., SCHUHMACHER, R. and KRSKA, R. 2001. **International interlaboratory study for the determination of the *Fusarium* mycotoxins zearalenone and deoxynivalenol in agricultural commodities.** Food Additives and Contaminants **18**: 417–430.

Twenty-eight laboratories from 12 different countries participated in an interlaboratory study for the determination of ZEA in maize and DON in maize and wheat employing their usual in-house methods. For the final separation and quantification either GC, HPLC, TLC or ELISA were employed. Coefficients of variation (CV) between laboratory mean results ranged from 28 to 41% for ZEA and from 32 to 38% for DON. A good trueness was obtained for the wheat samples spiked with DON at 475 µg/kg. However, a significant deviation from the respective target value was observed for the maize samples spiked with ZEA at 102 µg/kg. The high CVs can be traced back to problems occurring by determination of the concentration of the participants' own calibrant solutions. Additionally, the variability of the results is strongly influenced by the use of different final separation and quantification procedures.

OSTRY, V. and SKARKOVA, J. 2000. **Development of an HPTLC method for the determination of deoxynivalenol in cereal products.** JPC – Journal of Planar Chromatography – Modern TLC **13**: 443–446.

A method is described for the quantification of DON in cereals and cereal products (wheat flour and malt). The toxin was extracted with water and polyethylene glycol and the extracts were purified on immunoaffinity columns, then analysed by instrumental HPTLC on silica gel layers with chloroform–acetone–2-propanol (8 + 1 + 1) as mobile phase. The chromatogram was sprayed with a solution of aluminium chloride in ethanol to reveal the spots of DON and scanned in fluorescence mode. Recovery was 84% in the range 0.2–0.67 mg/kg cereal products. The average RSD<sub>r</sub> was 6.9% and the limit of quantification was 0.2 mg/kg.

SCHOTHORST, R.C. and JEKEL, A.A. 2001. **Determination of trichothecenes in wheat by capillary gas chromatography with flame ionisation detection.** Food Chemistry **73**: 111–117.

A method for the determination of DON, NIV, 3-acetylDON, fusarenon X, T-2 toxin, HT-2 toxin, DAS and neosolaniol in wheat, based on capillary GC with flame ionisation detection (FID) has been developed and validated. The trichothecenes were extracted from the sample by acetonitrile/water (84/16, v/v). Two different Mycosep(R) cleanup columns were used to purify the extract. The extract was evaporated to dryness and the trichothecenes were derivatised to trimethylsilyl ethers. The residue was dissolved in iso-octane and washed with water. The final extract was analysed for trichothecenes by GC with FID. The average recoveries

ranged from 79% for NIV to 116% for DAS. The limit of quantification was 75 µg/kg for each of the individual trichothecenes. A temporary tolerance limit of 500 µg/kg is in effect in the Netherlands for DON in cleaned wheat. A survey was carried out in the Netherlands in 1999 and 7/22 imported wheat samples exceeded this limit. Thirteen of the 22 wheat samples exceeded a proposed DON tolerance limit of 120 µg/kg for cleaned wheat.

MATEO, J.J., LLORENS, A., MATEO, R. and JIMENEZ, M. 2001. **Critical study of and improvements in chromatographic methods for the analysis of type B trichothecenes.** *Journal of Chromatography A* **918**: 99–112.

Various analytical methods used in the analysis of type B trichothecenes (DON, NIV, 3- and 15-acetylDON) in cereals were compared and optimised. The extraction solvent of choice was a mixture of acetonitrile–water (84:16, v/v). The MycoSep 225 column was chosen as the best alternative for cleanup of grain samples. For GC-electron capture detection analysis, derivatisation of analytes with heptafluorobutyric anhydride prior the final determination was chosen as the most suitable procedure. HPLC-photodiode array (at 221 nm) analysis was more suitable than HPLC of the fluorescent coumarin-3-carbonyl derivatives. Recoveries obtained in spiked corn, rice and wheat are reported.

## Trichothecenes – Toxicoses

FROQUET, R., SIBIRIL, Y. and PAR-ENT-MASSIN, D. 2001. **Trichothecene toxicity on human megakaryocyte progenitors (CFU-MK).** *Human & Experimental Toxicology* **20**: 84–89.

Four trichothecenes, T-2 toxin, HT-2 toxin, DAS and DON, have been tested on human platelet progenitors (CFU-MK) using a culture model of CFU-MK optimised for toxicological studies. At low concentrations, trichothecenes cause cytotoxic effects in megakaryocyte progenitors, which could induce thrombocytopenia. Sensitivity of human CFU-MK is compared to respective sensitivities of human red blood cell progenitors (BFU-E) and white blood cell progenitors (CF-U-GM) that were described in previous works.

GAUMY, J.L., BAILLY, J.D., BERNARD, G. and GUERRE, P. 2001. **[Zearalenone: Origin and effects on farm animals].** *Revue de Medecine Veterinaire* **152**: 123–136.

A review with 89 references. The ingestion of feed contaminated with ZEA leads to reproductive disorders in a great number of

species. Pigs are the most sensitive, especially young females. Poultry are considered to be resistant to ZEA. Ruminants are only very rarely affected, but excrete the mycotoxin and its metabolites in milk. (In French).

EL-MAKAWY, A., HASSANANE, M.S. and ABDALLA, E.S.A.M. 2001. **Genotoxic evaluation for the estrogenic mycotoxin zearalenone.** *Reproduction Nutrition Development* **41**: 79–89.

ZEA was administered to Albino male and pregnant female mice at 5 or 10 µg/kg and genotoxic effects were evaluated. Chromosome aberrations in bone marrow and spermatocytes of adult male mice and chromosome analysis and teratological effects of mouse embryos were assessed. ZEA was found to reduce the mitotic activity in treated males and embryos. In treated males and females, ZEA induced some chromosome abnormalities but with no significant increase over controls.

JUHASZ, J., NAGY, P., KULCSAR, M., SZIGETI, G., REICZIGEL, J. and HUSZENICZA, G. 2001. **Effect of low-dose zearalenone exposure on luteal function, follicular activity and uterine oedema in cycling mares.** *Acta Veterinaria Hungarica* **49**: 211–222.

ZEA administration starting 10 days after ovulation was studied in 6 cycling trotter mares. After an entire oestrous cycle (Cycle 1), mares were given 7 mg purified ZEA per os daily beginning on day 10 of Cycle 2. Toxin exposure was continued until the subsequent ovulation. The toxin had no effect on the length of the interovulatory intervals, luteal and follicular phases. It did not influence significantly the plasma progesterone profiles, follicular activity and uterine oedema.

ALEXOPOULOS, C. 2001. **Association of *Fusarium* mycotoxicosis with failure in applying an induction of parturition program with PGF2alpha and oxytocin in sows.** *Theriogenology* **55**: 1745–1757.

In a farrow-to-finish pig unit, an induction-of-parturition program was applied in gilts and sows with PGF2alpha 113 days post service, followed by oxytocin 24 hr later. This program resulted in a high proportion of animals farrowing within the working hours of the day. When splay-legs and oedematous swelling and reddening of the vulva started to be observed in newborn piglets, a concurrent decline or parameters related to a parturition also was noticed. Mycotoxicological analysis of the feeds revealed a co-occurring contamination with DON and ZEA. For a 4-week period, sows were divided into two groups: an induction-of parturition group and a non-induction-of parturition group. Signifi-

cant differences were found between the two groups relating to prevalence of dystocia and pregnancy duration. Moreover, it was found that prevalence of splay-legs and swelling of the vulva were highly correlated with reduction of percentage of sows farrowing within the working day and increase of pre-weaning mortality.

WONG, S.S., SCHWARTZ, R.C. and PESTKA, J.J. 2001. **Superinduction of TNF-alpha and IL-6 in macrophages by vomitoxin (deoxynivalenol) modulated by mRNA stabilization.** *Toxicology* **161**: 139–149.

Post-transcriptional effects of DON on TNF-alpha and IL-6 gene expression were studied in lipopolysaccharide (LPS)-stimulated macrophage RAW 264.7 cells. DON was found to enhance both TNF-alpha and IL-6 protein secretion in the presence of LPS. Upon addition of the transcriptional inhibitor, 5,6-dichloro-1-beta-D-ribofuranosyl benzimidazole (DRB), secretion of both cytokines was inhibited. Using Northern analysis, the mRNA stabilities of TNF-alpha and IL-6 were studied in DRB-treated cells exposed to DON and LPS in both asynchronous and delayed synchronous modes. TNF-alpha and IL-6 mRNA were rapidly stabilised by DON in both models. These results suggest that post-transcriptional control via enhancement of mRNA stability is likely to contribute to proinflammatory cytokine superinduction in macrophages by DON and other trichothecenes.

ISHIGAMI, N., SHINOZUKA, J., KATAYAMA, K., NAKAYAMA, H. and DOI, K. 2001. **Apoptosis in mouse fetuses from dams exposed to T-2 toxin at different days of gestation.** *Experimental and Toxicologic Pathology* **52**: 493–501.

T-2 toxin at 2 mg/kg body weight was orally inoculated to pregnant mice at different days of gestation and the foetuses were examined 24 hr later. The number and region of pyknotic or karyorrhectic cells varied according to inoculation date. In the gestation day 13.5 subgroup, a moderate to high number of pyknotic or karyorrhectic neuronal cells were observed in the central nervous system, peri-ventricular zone to subventricular zone, and pyknosis or karyorrhexis were also observed in a small number of chondroblasts and chondrocytes. In the gestation day 16.5 subgroup, a moderate to high number of pyknotic or karyorrhectic cells were observed in the thymus and renal subcapsular parenchyma. T-2 toxin is known to readily cross the rat placenta and it seems that these effects might be a direct effect of T-2 toxin on foetuses.

ALBARENQUE, S.M., SUZUKI, K., SHINOZUKA, J., NAKAYAMA, H. and DOI, K. 2001. **Kinetics of apoptosis-related genes mRNA expression in the dorsal skin of hypotrichotic WBN/ILAHt rats after topical application of T-2 toxin.** *Experimental and Toxicologic Pathology* **52**: 553–556.

The expression of apoptosis-related genes mRNAs was examined in the dorsal skin of hypotrichotic WBN/ILAHt rats topically applied with T-2 toxin. The total mRNA was obtained from skin biopsy samples at 3, 6, 12 and 24 hr after T-2 toxin treatment, and RT-PCR was carried out with pairs of oligonucleotide primers corresponding to the cDNA sequences of rat p53, bcl-2, c-ki-ras, c-fos and c-jun oncogenes. The expression of c-fos mRNA markedly increased at 3 hr, peaked at 6 hr, and greatly decreased at 12 hr. However it maintained a higher level, compared with the control level, even at 24 hr. Although not prominent, the expression of c-jun mRNA also showed significant elevation from 3 to 12 hr. The induction of c-fos and perhaps of c-jun mRNAs might be associated with T-2 toxin induced epidermal cell apoptosis.

## Aflatoxins – General

GOVARIS, A., ROUSSI, V., KOIDIS, P.A. and BOTSOGLU, N.A. 2001. **Distribution and stability of aflatoxin M<sub>1</sub> during processing, ripening and storage of Telemes cheese.** *Food Additives and Contaminants* **18**: 437–443.

Telemes cheeses were produced using milk that was artificially contaminated with AFM<sub>1</sub> at 0.050 or 0.100 µg/L. The cheeses produced were allowed to ripen for 2 months and stored for an additional 4 months to simulate commercial production of Telemes cheese. Concentrations of AFM<sub>1</sub> in whey, curd, brine and the produced cheeses were determined at intervals by LC and fluorometric detection coupled with immunoaffinity column extraction. Concentrations of AFM<sub>1</sub> in the produced curds were 3.9 and 4.4 times higher than those in milk, whereas concentrations in whey were lower than those in curd and milk. AFM<sub>1</sub> was present in cheese at higher concentrations at the beginning than at the end of the ripening/storage period. Concentrations of AFM<sub>1</sub> in brine started low and increased by the end of the ripening/storage period but only a portion of the amounts of AFM<sub>1</sub> lost from cheese was found in the brine.

MIDIO, A.F., CAMPOS, R.R. and SABINO, M. 2001. **Occurrence of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in cooked food components of whole meals marketed in**

**fast food outlets of the city of Sao Paulo, SP, Brazil.** *Food Additives and Contaminants* **18**: 445–448.

Samples of cooked components of regular meals served at fast food outlets of the city of Sao Paulo, Brazil were analysed for AFB<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. The 322 samples were composed of prepared traditional Brazilian and ethnic foods in which aflatoxins might be present. Aflatoxins were detected in 30 samples in the range 2.80–1323 µg/kg. AFB<sub>1</sub> was detected in all contaminated samples. The contamination levels and frequency of AFB<sub>1</sub> and G<sub>1</sub> in positive samples above 20 µg/kg were high.

MARTINS, M.L., MARTINS, H.M. and BERNARDO, F. 2001. **Aflatoxins in spices marketed in Portugal.** *Food Additives and Contaminants* **18**: 315–319.

Seventy-nine prepackaged samples of 12 different types of spice powders were selected from supermarkets and ethnic shops in Lisbon, Portugal, and analysed for aflatoxins. AFB<sub>1</sub> was detected in 34 samples. All of the cayenne pepper samples were contaminated with AFB<sub>1</sub> at levels ranging from 2 to 32 µg/kg. Eight of 10 nutmeg samples contained AFB<sub>1</sub> at levels in the range 1–58 µg/kg. Paprika contained AFB<sub>1</sub> at levels ranging from 1 to 20 µg/kg. Chilli, cumin, curry powder, saffron and white pepper samples had levels ranging from 1 to 5 µg/kg. Aflatoxins were not detected in cardamom, cloves, ginger and mustard. None of the samples analysed contained AFB<sub>2</sub>, G<sub>1</sub> or G<sub>2</sub>.

BROWN, R.L., CHEN, Z.Y., MENKIR, A., CLEVELAND, T.E., CARDWELL, K., KLING, J. and WHITE, D.G. 2001. **Resistance to aflatoxin accumulation in kernels of maize inbreds selected for ear rot resistance in West and Central Africa.** *Journal of Food Protection* **64**: 396–400.

Thirty-six maize inbred lines selected in West and Central Africa for moderate to high resistance to maize ear rot under conditions of severe natural infection were screened for resistance to aflatoxin contamination using the previously established kernel screening assay. Results showed that more than half the inbreds accumulated aflatoxins at levels as low as or lower than the resistant U.S. Lines GT-MAS:GK or M182. In 10 selected aflatoxin-resistant or aflatoxin-susceptible inbreds, *Aspergillus flavus* growth was, in general, positively related to aflatoxin accumulation. Confirmation of resistance in promising African lines in held trials may significantly broaden the resistant germplasm base available for managing aflatoxin contamination through breeding approaches.

TUBAJIKA, K.M. and DAMANN, K.E. 2001. **Sources of resistance to aflatoxin production in maize.** *Journal of Agricultural and Food Chemistry* **49**: 2652–2656.

Drought tolerant maize genotypes (Huffman, Z08-004, Tuxpan, PH 9, NRC 5348, Chunco, Saint Croix, and Arizona) were compared in the field and laboratory to toxin-resistant GT-MAS:GK and Yellow Creole. Kernel screening assays showed that AFB<sub>1</sub> levels in inoculated drought-tolerant genotypes differed significantly from those in GT-MAS:GK and Yellow Creole. AFB<sub>1</sub> levels in the inoculated genotypes differed significantly from those of GT-MAS:GK or Yellow Creole when grown under drought stress conditions. Pearson correlation coefficients were significant between *Aspergillus* ear rot and aflatoxin levels. On the basis of the parameters studied, there are indications that these genotypes were potential sources of *A. flavus* resistance.

OBERHEU, D.G. and DABBERT, C.B. 2001. **Aflatoxin contamination in supplemental and wild foods of northern bobwhite.** *Ecotoxicology* **10**: 125–129.

Oesophagi were removed from northern bobwhite (*Colinus virginianus*) that were killed by hunters in Wheeler County, Texas, and Roger Mills County, Oklahoma. Oesophagi were segregated into three categories based upon their contents: all wild seeds, all supplemental foods and mixed foods. Contents of oesophagi were then analysed for aflatoxin concentration. Wild seeds had higher aflatoxin concentrations (mean 2.44 µg/kg) than either the supplemental or mixed categories, although these levels are below those found to cause damage to northern bobwhite.

ITO, Y., PETERSON, S.W., WICKLOW, D.T. and GOTO, T. 2001. ***Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section *Flavi*.** *Mycological Research* **105**: 233–239.

A recent report of an aflatoxin producing isolate of *Aspergillus tamarii* prompted a taxonomic re-examination of aflatoxigenic and non-aflatoxigenic isolates identified as *A. tamarii* as well as the closely related *A. caelatus*. Because of genetic, morphological and mycotoxin differences, the aflatoxin producing isolates of *A. tamarii* are given species rank as *Aspergillus pseudotamarii* sp. nov.

KLICH, M., MENDOZA, C., MULLANEY, E., KELLER, N. and BENNETT, J.W. 2001. **A new sterigmatocystin-producing *Emericella* variant from agricultural desert soils.** *Systematic and Applied Microbiology* **24**: 131–138.

An unusual, sterigmatocystin-producing taxon with characteristics of both *Emericella nidulans* and *Emericella rugulosa* was isolated repeatedly during a mycofloral survey of desert cotton field soils where aflatoxin is a chronic problem. Members of this taxon had ascospores with smooth convex wails like *E. nidulans* but grew slowly like *E. rugulosa*. Traditional morphological characters, secondary metabolite profiles of mycelial extracts and Southern blot analysis of genomic DNA indicate that these isolates constitute a new non-rugulose variant of *E. rugulosa*.

LIU, D.L., YAO, D.S., LIANG, Y.Q., ZHOU, T.H., SONG, Y.P., ZHAO, L. and MA, L. 2001. **Production, purification, and characterization of an intracellular aflatoxin-detoxifzyme from *Armillariella tabescens* (E-20)**. Food and Chemical Toxicology **39**: 461–466.

Some *Armillariella tabescens* (E-20) multienzymes have previously been reported to present detoxifying activities against aflatoxins. The isolation and purification of an intracellular enzyme, named aflatoxin-detoxifzyme, which exhibited detoxification activity on AFB<sub>1</sub> is described. The enzyme exhibited a specific activity of 7.09 nmol min/mg at pH 6.0 and 28°C. The activity of the purified enzyme was confirmed by Ames test.

HUA, S.S.T. 2001. **Inhibitory effect of acetosyringone on two aflatoxin biosynthetic genes**. Letters in Applied Microbiology **32**: 278–281.

The concentration of acetosyringone has been shown to increase about 10-fold when certain metabolically active plant tissues are wounded. Two GUS (beta-glucuronidase) reporter constructs, nor1::GUS (pGAP12) and ver1::GUS (pGAP13), were used to study the effect of acetosyringone on expression of aflatoxin biosynthetic genes, *nor1* and *ver1*. GUS activities of these two reporter constructs were inhibited by 80% in the presence of acetosyringone at 2 mmol/L. Aflatoxin production in a toxigenic strain was also shown to be inhibited by acetosyringone to the same level.

HODGES, R.L., KELKAR, H.S., XUEI, X., SKATRUD, P.L., KELLER, N.P., ADAMS, T.H., KAISER, R.E., VINCI, V.A. and MCGILVRAY, D. 2000. **Characterization of an echinocandin B-producing strain blocked for sterigmatocystin biosynthesis reveals a translocation in the *steW* gene of the aflatoxin biosynthetic pathway**. Journal of Industrial Microbiology & Biotechnology **25**: 333–341.

Echinocandin B (ECB), a lipopolypeptide used as a starting material for chemical manufacture of the anti-Candida agent LY303366, is produced by fermentation using a strain of *Aspergillus nidulans*. In addition to ECB, the wild-type strain also produces a significant level of sterigmatocystin (STG). Characterization of a mutant strain, which is blocked in STG biosynthesis, was the result of a chromosomal translocation. The chromosomal regions containing the breakpoints of the translocation were isolated and DNA sequencing and PCR analysis of the chromosomal breakpoints demonstrated the translocation occurred within the *steW* gene of the STG biosynthetic pathway, resulting in disruption of the open reading frame for this gene.

MATSUSHIMA, K., CHANG, P.K., YU, J., ABE, K., BHATNAGAR, D. and CLEVELAND, T.E. 2001. **Pre-termination in *aflR* of *Aspergillus sojae* inhibits aflatoxin biosynthesis**. Applied Microbiology and Biotechnology **55**: 585–589.

The *aflR* gene product is the main transcriptional regulator of aflatoxin biosynthesis in *Aspergillus parasiticus* and *A. flavus*. Although *A. sojae* strains do not produce aflatoxins, they do have an *aflR* homologue. When compared with the *aflR* of *A. parasiticus*, the *A. sojae* gene contains two mutations: an HAH motif and a premature stop codon. To investigate the functionality of the *A. sojae aflR* gene product, a GAL4 one-hybrid system in yeast was used. Results indicate that the premature stop codon of the *A. sojae aflR* is the key to its functionality and leads to prevention of aflatoxin biosynthesis through loss of the transcription of aflatoxin biosynthesis-related genes.

## Aflatoxins – Methodology

THIRUMALA-DEVI, K., MILLER, J.S., REDDY, G., REDDY, D.V.R. and MAYO, M.A. 2001. **Phage-displayed peptides that mimic aflatoxin B<sub>1</sub> in serological reactivity**. Journal of Applied Microbiology **90**: 330–336.

Phage-displayed random peptide libraries were tested as sources of peptides that mimic the binding of AFB<sub>1</sub> to monoclonal antibodies (Mabs) raised against the toxin. For two of the three Mabs tested, clones were obtained by panning, producing phage that bound specifically to MAb 13D1-1D9 (MAb 24; specific for AFB<sub>1</sub> and G<sub>1</sub>) and MAb 6E12-1E9 (MAb 13; specific for AFB<sub>1</sub>, G<sub>1</sub> and B<sub>2</sub>) in ELISA. The amino acid sequences of the binding peptides varied. Those binding to MAb 24 contained the sequence of ‘...YMD...’, and those that bound to MAb 13 contained the dipeptide ‘PW’. Mimotope phage was used in a com-

petition ELISA format for assaying aflatoxin concentrations. The results show that mimotope preparations are effective substitutes for pure toxin in these ELISA procedures.

WANG, J.S., ABUBAKER, S., HE, X., SUN, G.J., STRICKLAND, P.T. and GROOPMAN, J.D. 2001. **Development of aflatoxin B<sub>1</sub>-lysine adduct monoclonal antibody for human exposure studies**. Applied and Environmental Microbiology **67**: 2712–2717.

Mouse Mabs were developed against a synthetic AFB<sub>1</sub>-lysine-cationised bovine serum albumin conjugate. The isotype of one of these antibodies, IIA4B3, has been classified as immunoglobulin G1(λ). The affinity and specificity of IIA4B3 were further characterised by a competitive radioimmunoassay. IIA4B3 had about a 10-fold higher affinity for binding to AFB<sub>1</sub>-lysine adduct than to AFB<sub>1</sub> when [<sup>3</sup>H]AFB<sub>1</sub>-lysine was used as the tracer. The concentration for 50% inhibition for AFB<sub>1</sub>-lysine was 0.610 pmol; that for AFB<sub>1</sub> was 6.85 pmol. An analytical method based on a competitive radioimmunoassay with IIA4B3 and [<sup>3</sup>H]AFB<sub>1</sub>-lysine was validated with a limit of detection of 10 fmol of AFB<sub>1</sub>-lysine adduct. The method has been successfully applied to the measurement of AFB<sub>1</sub>-albumin adduct levels in human serum samples collected from the residents of areas at high risk for liver cancer.

YONG, R.K. and COUSIN, M.A. 2001. **Detection of moulds producing aflatoxins in maize and peanuts by an immunoassay**. International Journal of Food Microbiology **65**: 27–38.

An ELISA was developed to detect moulds producing aflatoxins in maize and peanuts by an antibody produced to extracellular antigen from *Aspergillus parasiticus*. This antibody recognised species with phenotypic similarities to *A. parasiticus*, *A. flavus* and the domesticated species *A. sojae* and *A. oryzae*. Maize and peanuts inoculated with spores of *A. parasiticus* and incubated at 15°C for 18 days or 21°C for 7 days were analysed for mould antigens and aflatoxin levels. At 15°C, mould antigens were detected by day 4 in maize when 0.16 µg/kg of aflatoxin was detected by ELISA but not by TLC. Antigens were detected in peanuts by day 4 before aflatoxin was found. Likewise, at 21°C, antigens were detected by day 4 in maize when less than 1 µg/kg of aflatoxin was detected by ELISA but not by TLC, but by day 2 in peanuts when no aflatoxin was detected. *A. parasiticus* could be detected before it could produce aflatoxins.

PAPP, E., FARKAS, A., OTTA, K.H. and MINCSOVICS, E. 2000. **Validation and robustness testing of an OPLC method for the determination of afla-**

**toxins in wheat.** JPC – Journal of Planar Chromatography – Modern TLC **13**: 328–332.

The validation of an OPLC procedure developed for the determination of AFB<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in wheat is described. Samples were extracted with acetonitrile–water (9:1 v/v) and the extracts were filtered and evaporated to dryness. OPLC was performed with chloroform–toluene–tetrahydrofuran (15 + 15 + 1 v/v) as mobile phase. Before the separation an OPLC prewashing step was necessary; this eliminated the time-consuming and costly cleanup steps of other chromatographic methods. Recovery was greater than 84% and the relative standard deviation was less than 10%. The limit of detection for AFB<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> was 0.14, 0.15, 0.100 and 0.018 ng, respectively. The robustness of the procedure was also determined and found to be critically dependent on the type of plate used (TLC or HPTLC).

DRAGACCI, S., GROSSO, F. and GILBERT, J. 2001. **Immunoaffinity column cleanup with liquid chromatography for determination of aflatoxin M<sub>1</sub> in liquid milk: Collaborative study.** Journal of AOAC International **84**: 437–443.

A collaborative study was conducted to evaluate the effectiveness of an immunoaffinity column cleanup LC method for the determination of AFM<sub>1</sub> in milk at proposed European regulatory limits. Liquid milk was centrifuged, filtered and applied to an immunoaffinity column. The column was washed with water and aflatoxin was eluted with pure acetonitrile. AFM<sub>1</sub> was separated by reversed phase LC with fluorescence detection. Liquid milk samples were sent to 12 collaborators in 12 different European countries. Test portions of samples were spiked with AFM<sub>1</sub> at 0.05 µg/L. The mean recovery of AFM<sub>1</sub> was 74%. The RSD<sub>r</sub> ranged from 8 to 18% RSD<sub>R</sub> ranged from 21 to 31%. The method showed acceptable within- and between-laboratory precision data for liquid milk, as evidenced by HORRAT values at the low level of AFM<sub>1</sub> contamination.

DRAGACCI, S., GROSSO, F., PFAUWATHEL-MARCHOND, N., FREMY, J.M., VENANT, A. and LOMBARD, B. 2001. **Proficiency testing for the evaluation of the ability of European Union-National Reference laboratories to determine aflatoxin M<sub>1</sub> in milk at levels corresponding to the new European Union legislation.** Food Additives and Contaminants **18**: 405–415.

In 1992, the European Union set up a network of National Reference Laboratories and charged the Community Reference Laboratory with the responsibility to design a proficiency testing scheme for assessing the

analytical ability of laboratories involved in the official control of AFM<sub>1</sub> in milk. Since 1996, two exercises of proficiency testing have been performed on samples of milk powder and liquid milk. The trials were conducted according to ISO Guide 43. The interlaboratory RSD<sub>R</sub> obtained for both 1996 and 1998 exercises were in the range 15.7–30.3%. Compared with other published studies, this indicates a very good precision for the performance of this laboratory network in the analysis of traces of AFM<sub>1</sub> in milk.

GATHUMBI, J.K., USLEBER, E. and MARTLBAUER, E. 2001. **Production of ultrasensitive antibodies against aflatoxin B<sub>1</sub>.** Letters in Applied Microbiology **32**: 349–351.

Rabbits were immunised with AFB<sub>1</sub>-bovine serum albumin conjugate. High titres of antibodies with very high affinity for AFB<sub>1</sub> were obtained 15 and 4 weeks after the initial immunisation and the first booster immunisation, respectively. The antibodies were employed in enzyme immunoassay (EIA) and immunoaffinity chromatography (IAC) methods for aflatoxins. With a detection limit of 15.8 ng/L for AFB<sub>1</sub>, the EIA employing these antibodies is the most sensitive test for AFB<sub>1</sub> described so far. In IAC columns, these antibodies provided high binding capacity for all major aflatoxins, including AFB<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. These results indicate that polyclonal antibody based EIA and IAC methods for aflatoxin analysis offer a suitable alternative to the more expensive monoclonal antibody based methods.

### Aflatoxicoses

SMELA, M.E., CURRIER, S.S., BAILEY, E.A. and ESSIGMANN, J.M. 2001. **The chemistry and biology of aflatoxin B<sub>1</sub>: From mutational spectrometry to carcinogenesis.** Carcinogenesis **22**: 535–545.

In this review with 123 references, the chemical reaction of an electrophilic derivative of aflatoxin with specific DNA sequences is examined, along with the types of mutations caused by AFB<sub>1</sub> and the sequence context dependence of those mutations. An attempt is made to assign the source of these mutations to specific chemical forms of AFB<sub>1</sub>-DNA damage. In addition, epidemiological and experimental data are examined regarding the synergistic effects of AFB<sub>1</sub> and hepatitis B virus on hepatocellular carcinoma formation and the predominance of one hotspot GC to TA transversion in the p53 gene of affected individuals.

HOOGENBOOM, L.A.P., POLMAN, T.H.G., NEAL, G.E., VERMA, A., GUY-OMARD, C., TULLIEZ, J., GAUTIER, J.P., COKER, R.D., NAGLER, M.J., HEIDENREICH, E. and DELORT-LAVAL, J. 2001. **Genotoxicity testing of extracts from aflatoxin-contaminated peanut meal, following chemical decontamination.** Food Additives and Contaminants **18**: 329–341.

A new method based on the use of florisil and C<sub>18</sub> solid phase extraction columns has been developed for the preparation of extracts from aflatoxin-contaminated peanut meal which has been decontaminated by a small-scale version of an industrial decontamination process based on ammoniation. The method allowed testing with *in vitro* genotoxicity assays without interference of the residual AFB<sub>1</sub>. Recovery of degradation products in the extracts was evaluated by the use of [<sup>14</sup>C]AFB<sub>1</sub> added to naturally-contaminated peanut meal. Following decontamination, more than 90% of the label could not be extracted from the meal indicating a total AFB<sub>1</sub> reduction of more than 99%. The extraction procedure resulted in AFB<sub>1</sub>-rich and AFB<sub>1</sub>-poor fractions, the latter containing half of the extractable decontamination products but less than 1% of the residual AFB<sub>1</sub>. Testing in the *Salmonella*/microsome mutagenicity assay of the original crude extracts and AFB<sub>1</sub>-rich fractions prepared from non-treated and decontaminated meal, showed the positive results expected from the AFB<sub>1</sub> contents as determined by HPLC analysis. Analysis and testing of the AFB<sub>1</sub>-poor fractions showed that the various decontamination processes not only resulted in a successful degradation of AFB<sub>1</sub> but also did not produce other potent mutagenic compounds.

CAVIN, C., MACE, K., OFFORD, E.A. and SCHILTER, B. 2001. **Protective effects of coffee diterpenes against aflatoxin B<sub>1</sub>-induced genotoxicity: Mechanisms in rat and human cells.** Food and Chemical Toxicology **39**: 549–556.

The coffee-specific diterpenes cafestol and kahweol have been reported to be anti-carcinogenic in several animal models. Cafestol and kahweol added to rat primary hepatocytes reduced the expression of cytochrome P450 CYP 2C11 and CYP 3A2, the key enzymes responsible for AFB<sub>1</sub> activation to AFB<sub>1</sub>-8,9 epoxide. In addition, these diterpenes induced significantly GST Yc2, the most efficient rat GST subunit involved in AFB<sub>1</sub>-8,9 epoxide detoxification. These effects of cafestol and kahweol resulted in a marked dose dependent inhibition of AFB<sub>1</sub>-DNA binding in this rat *in vitro* culture system. In human liver epithelial cell lines (THLE) stably transfected to express AFB<sub>1</sub> metabolising cytochrome P450s, cafestol

and kahweol also produced a significant inhibition of AFB<sub>1</sub>-DNA adducts formation linked with an induction of the human glutathione S-transferase GST-mu.

VERMA, R.J., SHUKLA, R.S. and MEHTA, D.N. 2001. **Amelioration of cytotoxic effects of aflatoxin by vitamin A: An *in vitro* study on erythrocytes.** *Toxicology In Vitro* **15**: 39–42.

Aflatoxin-induced haemolysis in erythrocytes was found to be significantly reduced by the addition of vitamin A at 125–1250 IU/ml to the incubation medium. The decrease in haemolysis was almost dose dependent.

BARRAUD, L., DOUKI, T., GUERRET, S., CHEVALLIER, M., JAMARD, C., TREPO, C., WILD, C.P., CADET, J. and COVA, L. 2001. **The role of duck hepatitis B virus and aflatoxin B<sub>1</sub> in the induction of oxidative stress in the liver.** *Cancer Detection and Prevention* **25**: 192–201.

AFB<sub>1</sub> exposure of duck hepatitis B virus (DHBV) infected Pekin ducks induced a significant increase in viral replication associated with an intense biliary ductular cells proliferation. Extremely high levels of AFB<sub>1</sub>-DNA adducts and AFB<sub>1</sub>-albumin adducts were detected in duck liver and serum respectively, as compared to other animal species exposed to a similar AFB<sub>1</sub> dose. DHBV infection was found to induce a non-significant increase in AFB<sub>1</sub>-albumin adduct levels in duck serum. During the treatment duration there was no effect on formation of oxidative base damage within DNA and no effect on oxidative lipid peroxidation following either viral infection or AFB<sub>1</sub> exposure.

OBASI, S.C. 2001. **Effects of scopoletin and aflatoxin B<sub>1</sub> on bovine hepatic mitochondrial respiratory complexes, 2: a-ketoglutarate cytochrome c and succinate cytochrome c reductases.** *Zeitschrift für Naturforschung C – A Journal of Biosciences* **56**: 278–282.

The *in vitro* effects of AFB<sub>1</sub> on bovine (*Bos indicus*) hepatic mitochondrial respiratory complex III enzymes, succinate cytochrome c and a-ketoglutarate cytochrome c reductases, were examined. Although the observed inhibitory and stimulatory effects of AFB<sub>1</sub> were consistent with the changes in the kinetic parameters (K-m and V-max values), these parameters were not consistent with the observed effects of the toxin at certain concentrations. These observations are discussed in terms of the relative locations of the enzymes in the mitochondria, and the previously reported inhibitory and uncoupling effects of the toxin on cow liver mitochondrial respiration.

MOON, E.Y., RHEE, D.K. and PYO, S. 2000. **Alteration of kinase-mediated signalings in murine peritoneal macrophages by aflatoxin B<sub>1</sub>.** *Cancer Letters* **155**: 9–17.

Murine peritoneal macrophages stimulated with lipopolysaccharide (LPS) after AFB<sub>1</sub> pretreatment, show a decreased production of nitric oxide (NO). The percentage of NO production in AFB<sub>1</sub>-pretreated macrophages was inversely increased by the addition of cholera toxin, phorbol 12-myristate 13-acetate and ionomycin. This suggests that AFB<sub>1</sub> affects the function of signalling constituents, including guanine nucleotide binding protein, protein kinase C (PKC) and the calcium ion. AFB<sub>1</sub> pretreatment significantly decreased PKC activity and tyrosine phosphorylation after LPS stimulation. Taken together, these data propose that in murine peritoneal macrophages the inhibition of LPS stimulated NO production by AFB<sub>1</sub> is related to the suppression of kinase-mediated intracellular signal transduction.

BARTON, C.C., BARTON, E.X., GANEY, P.E., KUNKEL, S.L. and ROTH, R.A. 2001. **Bacterial lipopolysaccharide enhances aflatoxin B<sub>1</sub> hepatotoxicity in rats by a mechanism that depends on tumor necrosis factor alpha.** *Hepatology* **33**: 66–73.

The role of TNF alpha in the enhancement of hepatotoxicity of AFB<sub>1</sub> by lipopolysaccharide (LPS) was investigated in rats. Male Sprague-Dawley rats were treated ip with AFB<sub>1</sub> at 1 mg/kg and 4 hr later with *Escherichia coli* LPS. LPS administration resulted in a marked rise in TNF alpha levels at 6 hr, which preceded the onset of liver injury. When the increase in TNF alpha was attenuated by administration of either pentoxifylline or anti-TNF alpha serum, liver injury was prevented. LPS treatment resulted in the upregulation of gene transcription for cyclooxygenase-2 (COX-2). However, administration of the selective COX-2 inhibitor NS-398 did not decrease injury. TNF alpha and COX-2 inhibitors did not affect hepatic sequestration of neutrophils. Furthermore, it did not appear that TNF-alpha contributed to injury through inhibition of tissue repair. These data support the hypothesis that LPS induced expression of TNF alpha underlies the potentiation of AFB<sub>1</sub> induced hepatotoxicity.

YANG, C.F., LIU, J., WASSER, S., SHEN, H.M., TAN, C.E.L. and ONG, C.N. 2000. **Inhibition of ebselen on aflatoxin B<sub>1</sub>-induced hepatocarcinogenesis in Fischer 344 rats.** *Carcinogenesis* **21**: 2237–2243.

Ebselen, an organic selenium compound, protects against the cytotoxicity of AFB<sub>1</sub> through its antioxidant capability. Fischer

344 rats were first treated with ebselen at 5 mg/kg, 5 days/week via gavage for 4 weeks, then given AFB<sub>1</sub> at 0.4 mg/kg, via gavage once a week, or AFB<sub>1</sub> plus ebselen for another 24 weeks. The results showed that the hepatocarcinogenicity of AFB<sub>1</sub> in rats was significantly reduced by ebselen treatment. Ebselen treatment significantly reduced the formation of hepatic AFB<sub>1</sub>-DNA adducts and 8-hydroxydeoxyguanosine caused by AFB<sub>1</sub> exposure.

CHOU, M.W., MIKHAILOVA, M.V., NICHOLS, J., POIRIER, L.A., WARBRITTON, A. and BELAND, F.A. 2000. **Interactive effects of methyl-deficiency and dietary restriction on liver cell proliferation and telomerase activity in Fischer 344 rats pretreated with aflatoxin B<sub>1</sub>.** *Cancer Letters* **152**: 53–61.

The effects of methyl-deficiency and dietary restriction (DR) on hepatic cell proliferation and telomerase activity was studied in male Fischer 344 rats pretreated with AFB<sub>1</sub>. Rats were gavaged 5 days per week for 3 weeks with AFB<sub>1</sub> at 25 µg/rat/day. Rats were then fed a methyl-sufficient (MS) diet or a methyl-deficient (MD) diet with or without DR. DR reduced hepatic cell proliferation, while the MD diet and AFB<sub>1</sub> pretreatment increased cell proliferation. Telomerase activity was decreased by DR and increased by the MD diet and AFB<sub>1</sub> pretreatment. The same trend was observed with GST-positive foci: in AFB<sub>1</sub>-pretreated rats, methyl deficiency increased the number of foci, while DR decreased the number. These results are consistent with a role of telomerase in hepatocarcinogenesis.

RASTOGI, R., SRIVASTAVA, A.K. and RASTOGI, A.K. 2001. **Biochemical changes induced in liver and serum of aflatoxin B<sub>1</sub>-treated male Wistar rats: Preventive effect of picroliv.** *Pharmacology & Toxicology* **88**: 53–58.

The administration of AFB<sub>1</sub> to rats at 2 mg/kg ip caused significant increase in the activities of gamma-glutamyl transpeptidase, 5'-nucleotidase, acid phosphatase, acid ribonuclease as well as content of lipid peroxides in liver after six weeks. However, the activities of succinate dehydrogenase, glucose-6-phosphatase, catalase, superoxide dismutase, glutathione S-transferase, glutathione peroxidase and glutathione reductase in liver were decreased. The levels of glycogen and reduced glutathione were also decreased. There were significant elevations in the levels of serum transaminases, phosphatases, dehydrogenases and bilirubin following AFB<sub>1</sub> administration. Picroliv at 25 mg/kg/day orally for six weeks significantly prevented the biochemical changes induced by AFB<sub>1</sub>.

RASTOGI, R., SRIVASTAVA, A.K., SRIVASTAVA, M. and RASTOGI, A.K. 2000. **Hepatocurative effect of picroliv and silymarin against aflatoxin B<sub>1</sub> induced hepatotoxicity in rats.** *Planta Medica* **66**: 709–713.

Single doses of AFB<sub>1</sub> at 2 mg/kg, ip caused significant biochemical changes in liver and serum of rats. Oral administration of picroliv at 25 mg/kg/day for 15 days, 6 weeks after AFB<sub>1</sub> treatment, significantly prevented the biochemical changes induced in liver and serum of AFB<sub>1</sub> treated rats. The hepatocurative effect of picroliv and silymarin, a plant based standard hepatoprotective, are comparable.

LI, Y., SU, J.J., QIN, L.L., EGNER, P.A., WANG, J.S., GROOPMAN, J.D., KENSLER, T.W. and ROEBUCK, B.D. 2000. **Reduction of aflatoxin B<sub>1</sub> adduct biomarkers by oltipraz in the tree shrew (*Tupaia belangeri chinensis*).** *Cancer Letters* **154**: 79–83.

The tree shrew (*Tupaia belangeri chinensis*) is a unique species that can be infected with human HBV, is susceptible to AFB<sub>1</sub> induced liver cancer, and shows a synergistic interaction between HBV and AFB<sub>1</sub> for liver cancer. Two groups of tree shrews were fed AFB<sub>1</sub> at 400 µg/kg body weight for 4 weeks. One week prior to AFB<sub>1</sub> administration, one group also received oltipraz at 0.5 mmol/kg, po daily for 5 weeks. Aflatoxin-albumin adducts were determined in serum and urine. Adducts increased rapidly in 2 weeks to plateau then they diminished after cessation of AFB<sub>1</sub> exposure. Oltipraz significantly attenuated the overall burden of aflatoxin-albumin adducts throughout the exposure period with a median reduction of 80%. In a single cross-sectional analysis at the end of AFB<sub>1</sub> dosing, oltipraz treatment decreased urinary aflatoxin-N-7-guanine by 93%.

ROSA, C.A.R., MIAZZO, R., MAGNOLI, C., SALVANO, M., CHIACCHIERA, S.M., FERRERO, S., SAENZ, M., CARVALHO, E.C.Q. and DALCERO, A. 2001. **Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers.** *Poultry Science* **80**: 139–144.

Sodium bentonite from southern Argentina was evaluated for its ability to reduce the effects of total aflatoxins (AFB<sub>1</sub> 5 mg/kg) in the diet of growing broiler chickens. Body weight gains were significantly lower for broilers fed diets containing aflatoxins alone. No differences were found between the body weight gains of chickens fed diets without aflatoxins and those of chickens fed aflatoxins plus sodium bentonite at 0.3% of diet. Alterations in the levels of serum total

protein, albumin and globulins were observed for aflatoxin diets, and moderate protection was provided by the sorbent. However, the histopathological findings in liver sections of broilers fed diets with aflatoxins plus sodium bentonite indicated a non-protective effect of this adsorbent, because a moderate hepatic steatosis was observed.

IVETA, T., REVAJOVA, V., SVICKY, E., MAKOOVA, Z. and SKALICKA, M. 2000. **The number of CD 3 cells in broiler intestines after the administration of aflatoxin and zeolites.** *Acta Veterinaria – Beograd* **50**: 339–344.

The density of CD3+ cells was evaluated in the lamina propria of the intestines of chickens following application of aflatoxin and two kinds of zeolites for 30 days. Analysis of CD3+ cells showed a significantly increased number of T-lymphocytes after the application of both sorbents. There was no increase in the number of examined cells after the application of AFB<sub>1</sub> alone. The possible role of damage to the bacterial biofilm by sorbents is discussed.

CHENG, Y.H., SHEN, T.F., PANG, V.F. and CHEN, B.J. 2001. **Effects of aflatoxin and carotenoids on growth performance and immune response in mule ducklings.** *Comparative Biochemistry and Physiology C – Toxicology & Pharmacology* **128**: 19–26.

Mule ducklings were fed diets containing AFB<sub>1</sub> at 200 µg/kg with or without the addition of beta-carotene (BC) at 200 or 400 mg/kg, or astaxanthin (AS) at 200 mg/kg. AFB<sub>1</sub> alone or with the addition of BC or AS resulted in a significantly lower daily feed intake than for the control group. There were no significant differences in relative organ weights among treatment groups. Blood biochemical parameters and antibody titres were also evaluated. AFB<sub>1</sub> treatment had the highest activities of aspartate aminotransferase and alanine aminotransferase (ALT) in the serum. The addition of BC at 400 mg/kg significantly reduced ALT activity as compared with AFB<sub>1</sub> alone.

MESBAH, L.A., VANDERWEERDEN, G.M., NIJKAMP, H.J.J. and HILLE, J. 2000. **Sensitivity among species of Solanaceae to AAL toxins produced by *Alternaria alternata* f.sp. *lycopersici*.** *Plant Pathology* **49**: 734–741.

Two hundred species of Solanaceae were tested for their sensitivity to AAL toxins T-A and T-B. Twenty-five species were found to be sensitive to AAL toxins at a concentration of 0.2 µM used for distinguishing sensitive and insensitive tomato plants. Three species were as sensitive as the sensitive tomato line, indicating that AAL toxins effectively act on a broader range of plant species within the Solanaceae.

## AUSTRALIAN MYCOTOXIN NEWSLETTER

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

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



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