

GENERAL REFEREE REPORTS

Committee on Natural Toxins and Food Allergens

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Summary

Topic Advisor David Abramson will be retiring and has submitted his last advisor's report. The committee thanks him for his past services to AOAC and wishes him all the best in his retirement. The topic advisors have all reported on recent advances in analytical methods. Multitoxin methods combined with liquid chromatography (LC) and the use of antibody methods continue to be the areas of research activity. Two reviews published during 2006 highlighted these approaches. The first dealt with mycotoxin analysis by LC with atmospheric pressure ionization mass spectrometry (MS; 1) and the second reviewed the application of fluorescence polarization to the determination of mycotoxins (2). The economic impact of mycotoxins, an area of importance frequently neglected, has begun to receive more attention with the publication of economic models relating to fumonisin and aflatoxin contamination of conventional and Bt corn (3).

A number of international meetings have been held over the past year. These have included a European Union (EU) Myco-Globe meeting in Monopoli, Italy on "Advances in Genomics, Biodiversity and Rapid Systems for Detection of Toxigenic Fungi and Mycotoxins," which was followed in Bari, Italy, by a Workshop-Training Course on Detection Techniques for Mycotoxins and Toxigenic Fungi in the Food Chain. Other meetings included a special session of the American Chemical Society (San Francisco, CA, September 2006) and the World Mycotoxin Forum (Cincinnati, OH, November 2006). During May 2007, the XII International IUPAC Symposium on Mycotoxins and Phycotoxins was successfully held in Istanbul, Turkey with over 500 participants. Besides parallel sessions for oral presentations, a large number of posters were also presented and a large suppliers' exhibition was organized. Topics included mycotoxins and human health, analytical techniques, animal feed and dairy products, dried fruits, spices and botanicals, nuts, cereals, toxicology, coffee and cocoa, and a final session on risk assessment, regulations and international trade. The Third Turkish National Mycotoxin Symposium was held at the end of the meeting. The next IUPAC Symposium will be organized by Benjamin Suarez in 2010 in Valparaiso, Chile. The Gordon Research Conference on Mycotoxins and Phycotoxins was successfully held in June 2007 at Colby College, Waterville, ME.

Books on mycotoxins continue to be published and include "The Mycotoxin Factbook" containing peer-reviewed papers

of the third World Mycotoxin Forum (4) and "Mycotoxins and Phycotoxins—Advances in Determination, Toxicology and Exposure Management," containing the proceedings of the XI International IUPAC Symposium on Mycotoxins and Phycotoxins held in Bethesda, MD in 2004 (5).

The new International Society for Mycotoxicology held an open meeting during the XII International IUPAC Symposium on Mycotoxins and Phycotoxins at which the future role and activities of the society were discussed. Further information on the society and membership application can be found on the Website <http://www.mycotox-society.org>. During the past year a study protocol for "Determination of aflatoxins and ochratoxin A in ginseng and ginger by multi-toxin immunoaffinity column cleanup and liquid chromatographic quantitation" with Study Director Mary Trucksess (U.S. Food and Drug Administration) was approved.

Codex Activities.—The first Session of the Codex Committee on Contaminants in Foods (CCCF) was held in The Hague, The Netherlands, April 24–28, 2006. The draft maximum level for ochratoxin A in raw wheat, barley, and rye (5 µg/kg) was held at step 7. The proposed draft code of practice for the prevention and reduction of ochratoxin A contamination in wine is at step 4. It was agreed to develop codes of practice for aflatoxin contamination in dried figs and ochratoxin A in coffee, whereas the development of a discussion document for ochratoxin A in cocoa will continue. Further work by Codex is discussed under *Sampling* below.

Study Director Topics

Sampling and Subsampling

Topic Advisor Thomas B. Whitaker, U.S. Department of Agriculture, Agricultural Research Service, North Carolina State University, PO Box 7625, Raleigh, NC 27695-7625, Tel: 919-515-6731, Fax: 919-515-7760, E-mail: tom_whitaker@ncsu.edu. Whitaker reported that improving sampling methods to detect mycotoxins and other quality attributes in food and feed products continues to be a high priority among regulatory agencies, international organizations, and commodity industries worldwide. Examples of several worldwide efforts are discussed below.

After the 38th meeting in 2006 of the Codex Committee on Food Additives and Contaminants (CCFAC) in The Hague, The Netherlands, CCFAC was split into 2 committees: (1) Codex Committee on Food Additives (CCFA) and (2) CCCF. In April 2007, the 1st CCCF met in Beijing, People's Republic of China, and continued discussing the harmonization of aflatoxin limits and sampling plans for the following: treenuts, almonds, hazelnuts, pistachios, and Brazil nuts. CCCF established an Electronic Working Group, led by the EU, to make recommendations to members of CCCF for aflatoxin limits and sampling plans for the 4 treenuts. JECFA

(SPELL OUT JECFA) will also met in June 2007 to estimate the effects of 5 maximum limits (4, 8, 10, 15, and 20 total ng/g aflatoxin) on health risks to the consuming public.

While limits are being discussed, sampling studies have been completed on almonds, hazelnuts, and pistachios. Sampling studies are in the planning stages for Brazil nuts. Within the Electronic Working Group, the United States has taken the lead to prepare sampling plans to detect aflatoxin in almonds, hazelnuts, and pistachios for circulation, consideration, and comments by CCCF member nations.

Sampling studies were completed by the Iranian delegation to CCCF to determine the uncertainty and distribution among sample test results used to quantify aflatoxin in pistachios. The uncertainty and distribution information was used to develop a computer model to predict the performance of aflatoxin sampling plan designs for pistachios. Computer models were used to develop performance curves for almonds, hazelnuts, and pistachios sampling plan designs to assist the Electronic Working Group make sampling plan recommendations to the CCCF membership.

Three manuscripts, 2 describing the uncertainty associated with sampling almonds (6, 7) and 1 associated with sampling hazelnuts for aflatoxin (8) were published. One additional manuscript has been accepted by *J. AOAC Int.* for publication that demonstrates the effect of sample size and maximum limits on the performance of aflatoxin sampling plans for hazelnuts.

Sampling studies by the Brazil Ministry of Agriculture have provided information to develop models to predict the effect of sample size and maximum limits on risks of misclassifying lots when testing coffee beans for ochratoxin A. The model and performance curves for various sampling plan designs are described in a paper published in *J. AOAC Int.* (9).

Aflatoxin M₁

Topic Advisor Hans P. van Egmond, National Institute for Public Health and the Environment, Laboratory for Food and Residue Analysis (ARO), PO Box 1, 3720 BA Bilthoven, The Netherlands, Tel: 31-30-2742440, Fax: 31-30-2744403, E-mail: hp.van.egmond@rivm.nl. van Egmond reported that in 2006/2007 scientific research related to aflatoxin M₁ resulted again in a significant stream of publications. Many of these deal with occurrence issues, but there are also some analytical developments worth mentioning. In addition to publications in the scientific literature, there were a few oral presentations and 10 posters dealing with the subject area at the XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins in Istanbul. This demonstrates the continuous interest for aflatoxin M₁ as a mycotoxin of concern.

A few dozen studies were published on surveys of aflatoxin M₁ in milk and milk products, carried out in Brazil (10, 11), Colombia (12), Iran (13–19), Italy (20–22), Kuwait (23), Mexico (24), Morocco (25), Nigeria (26), Pakistan (27), Trinidad (28), and Turkey (29–34). Aflatoxin M₁ was frequently detected in most studies and the levels found sometimes exceeded legal limits. Contamination levels were comparable with those found in previous years, and

generally not of significant public health concern. The highest levels were reported in Nigeria (4 µg aflatoxin M₁/kg milk).

In a study carried out in Egypt, aflatoxin M₁ was detected in 248 of 443 (56%) samples of human breast milk (35). In a multilevel model of the data, there was a highly significant effect of month of sampling on the frequency of aflatoxin M₁ detection with summer months having the highest frequency (>80%) and winter months the lowest frequency (<20%) of detection. Aflatoxin M₁ was observed most frequently in June. The level of aflatoxin M₁ detection also followed this seasonal pattern with highest mean level in July (64 pg/mL milk, range 6–497 pg/mL) and the lowest mean level in January (8 pg/mL milk, range 4–108 pg/mL). The duration of lactation and peanut consumption also contributed to the model. The authors emphasized that the identification and understanding of factors determining the presence of toxicants in human milk is important and may provide a knowledge driven basis for controlling the transfer of chemicals to infants.

An analysis of the methodologies used in the surveys on aflatoxin M₁ in milk and milk products shows the following techniques and their frequencies of use: enzyme-linked immunosorbent assays (ELISA; 39%), LC (31%), thin-layer chromatography (TLC; 18%), **SPELL OUT RIA** (RIA; 4%), fluorometry (4%), and lateral flow immunoassay (4%). LC/MS, a sophisticated technique increasingly used in mycotoxin methodology, was obviously not (yet) applied. ELISA and LC with fluorescence detection were used in 70% of the surveys on aflatoxin M₁ in milk and milk products. Two articles published comparative studies on the performance characteristics of both techniques (36, 37), and in both papers it was concluded that the techniques performed equally well, with slight overestimations for ELISA at higher aflatoxin M₁ concentrations up to 0.1 µg/L.

An interlaboratory study of the Charm[®] aflatoxin M₁ quantitative lateral flow test for raw bovine milk was published (38). In the test aflatoxin M₁ in a milk test portion competes with the antibody gold beads for binding to 2 test lines. Remaining unbound binder forms on the control line. The test and control lines are compared with a reflectance reader, and the concentration of aflatoxin M₁ is determined with an algorithm. For the study, in which 29 U.S. laboratories took part, milk samples were fortified with aflatoxin M₁ in the range 0.3–0.55 µg/L. The combined false-negative rate was <5% for samples at 0.5 and 0.55 µg/L (the U.S. limit is 0.5 µg/L). False violatives at 0, 300, 350, 400, and 450 µg/L were 0, 0, 21, 14, and 93%, respectively. LC analyses of the study samples by 5 laboratories showed 38% false negatives with the 0.5 and 0.55 µg/L samples, and a 0% false-violative rate with samples containing <500 µg/L.

Several other techniques were recently developed or are in development for the determination of aflatoxin M₁. One of these makes use of a single-well fluorometer (39). The method utilizes a novel “mobile head” configuration immunoaffinity column and requires <40 min to complete. The method is claimed to have a limit of detection (LOD) of 12.5 ng/kg milk. Micro/nanosensors and fluorometric sensors

for detection of aflatoxin M₁ in milk are in development (40, 41). These techniques have potential for preliminary screening at the early stages of the industrial process of producing consumption milk.

The few papers that appeared on carry-over of aflatoxin M₁ into dairy products generally did not provide much novel information. Original work was done on a steady-state model for carry-over of aflatoxins from feed to cow's milk (42). The model used in the study related dairy intake of aflatoxin B₁ in feed of dairy cattle and the cow's lactation status to resulting concentrations of aflatoxin M₁ in milk. The authors concluded that the EU limit of 5 µg aflatoxin B₁/kg concentrate has proved thus far an appropriate level in preventing milk from exceeding the EU limit of 0.05 µg aflatoxin M₁/kg milk.

Aflatoxin Methods

Topic Advisor Gordon S. Shephard, Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council, PO Box 19070, Tygerberg 7505, South Africa, Tel: 27-21-938-0279, Fax: 27-21-938-0260, E-mail: gordon.shephard@mrc.ac.za. Shephard reported that a successful interlaboratory study has been published for aflatoxin B₁ in medicinal herbs at levels of ≥1 µg/kg (43) and a collaborative study indicating the equivalence of a photochemical post-column UV reactor to results obtained by iodine or Kobra cell methods for peanuts (44). The UV system had a slightly high bias for corn compared to the other 2 systems evaluated.

Cleanup of extracts for aflatoxin determination has received research attention. A simple inexpensive minicolumn packed with Florisil achieved satisfactory results for corn, rice, cottonseed, almonds and various tree nuts (45). Florisil was also used for cleanup in the development of an overpressured layer chromatographic method for aflatoxins in red paprika (46). The chromatoplate was a TLC silica gel plate and a development time of 760 s was achieved using chloroform–acetone (9 + 1) at 50 bar with a flow rate 400 µL/min. Quantification was by densitometer. An immunoaffinity monolithic disk has been developed for the on-line solid-phase extraction (SPE) of aflatoxin B₁ from aqueous solutions (47). This system will subsequently be applied to real samples. Extracts of farm commodities such as chili powder, green beans, and black sesame have been purified using matrix solid-phase dispersion (48). This procedure involves blending the milled sample with neutral alumina in a pestle, transferring the mixture to a column whose base contains 70 mg graphitic carbon black, and eluting the aflatoxins with acetonitrile. Determination of aflatoxins and ochratoxin A in chili samples has also been achieved by the development of a membrane-based immunoassay (49). In the analysis of Chinese medicinal herbs, the problem in certain matrixes of low recovery of aflatoxins during the cleanup of extracts by immunoaffinity columns was addressed by substitution of 0.1 M phosphate buffer (pH 8.0) for the phosphate buffered saline normally used in these columns (50). A confirmatory method for aflatoxins in corn was developed based on LC-ESI-MS/MS in

which the sample extract, obtained by triple extraction of corn with acetonitrile–water (80 + 20), was diluted with water and cleaned up on a Carbograph-4 cartridge (51). Quantification was by matrix-matched standard solutions.

Various methods for determination of multiple toxins, including aflatoxins, have been published. An immunoaffinity column with antibodies specific to both aflatoxin and ochratoxin A was used to purify extracts of ginseng, ginger, kava-kava, and licorice prior to determination by separate LC runs (52). A single cleanup method with separate LC determinations and quantification by fluorescence detection was described for aflatoxin B₁ and ochratoxin A in olive oil (53). After extraction of an olive oil–hexane mixture with methanol–water and back extraction into chloroform, the resulting extract was purified on a silica cartridge, which allowed the separation of the 2 toxins. Aflatoxins and ochratoxin A were extracted from beer on an SPE cartridge containing polymeric sorbent and then separated by ultra-performance LC (UPLC) coupled to tandem MS with run times of 3.2 min (54). Sixteen mycotoxins possibly related to sick building syndrome have been extracted with organic solvents from cellulose filters and determined by LC/MS/MS, using deepoxy-deoxynivalenol as an internal standard (55). Thirteen fusarium and aspergillus mycotoxins, including deoxynivalenol, T-2 toxin, zearalenone, and aflatoxins, have been simultaneously determined by LC-time-of-flight MS (TOFMS) from corn, wheat, cornflakes, and biscuits after cleanup with multifunctional columns (56). A total of 39 mycotoxins possibly occurring in corn and wheat have been determined by LC-ESI-MS/MS (57). Extraction was with acetonitrile–water–acetic acid (79 + 20 + 1) and extracts were diluted prior to injection. The diversity of analytes required the use of 2 chromatographic runs of 21 min each. Ion-suppression effects were negligible for wheat but caused signal suppression for 12 analytes in the maize extracts.

The use of analytical methods other than LC continues to attract considerable attention. A spectrophotometric method based on acetylcholinesterase inhibition was tested on aflatoxin B₁ in barley (58) and a synchronous spectrofluorimetric method for aflatoxin B₁ in pistachio nuts was developed using multivariate analysis (59). Various approaches to the development of immunosensors have been published. These include the use of atmospheric plasma torch for immunosensor preparation (60), the use of an optical waveguide lightmode spectroscopy (OWLS) technique (61), the development of an immune-biosensor for aflatoxin B₁ using bio-electrocatalytic reaction on a micro-comb electrode (62), and a filtration-based tyramide amplification technique (63). This later (**LATER OR LATTER?**) had a detection limit of 12.5 µg/kg. A 96-well screen-printed electrochemical immunosensor has been developed to operate in an indirect competitive ELISA format using intermittent pulse amperometry as measurement signal (64). These new developments have mainly been tested only on a limited number of samples. The development of noninstrumental test devices for cleanup and detection in the same assay column has been highlighted for fumonisins in corn flakes, aflatoxin

B₁ in pig feed, and ochratoxin A in roasted coffee (65). This type of system has been further developed for the simultaneous determination of aflatoxin B₁ and ochratoxin A using one cleanup layer and separate immunolayers with a competitive ELISA format (66). The system was applied to spices with cut-off levels of 5 and 10 µg/kg, respectively.

The majority of analytical reports relates to the determination of aflatoxins in food or feed matrices. However, a few papers have been published on biological systems such as aflatoxin and its metabolites in urine (67) and aflatoxin B₁ in animal liver (68) and human hair (69). Methods are generally adapted from those in use for food matrices. The effects of industrial processing of corn (70) and hulled rice (71) have received attention.

Widespread studies continue to be published concerning the natural occurrence of aflatoxins in a range of foods. Some of those appearing in the past year include the determination of aflatoxin in wheat in India (72); black table olives and cereals in Morocco (73, 74); peanut butter in Japan (75); corn in Uganda (76); brewers grain, sultanas and dried figs in Brazil (77, 78); nuts in South Korea (79); dried figs, helva, red pepper, wheat, and corn in Turkey (80–84); peanuts in Trinidad (28); peanuts and cereals in Ivory Coast (85); maize and rice in China (86); breakfast and infant cereals in Canada (87); cereals in Ethiopia (88); chili in Pakistan (89); pistachio nuts in Iran (90); nuts, spices, and cereal products in Poland (91); and spices, herbs, and medicinal plants in Italy (92).

Alternaria Toxins

Topic Advisor Michele Solfrizzo, Institute of Sciences of Food Production, National Research Council, Via Amendola 122/o, Bari 70126, Italy, Tel: 39-080-5929367, Fax: 39-080-5929374, E-mail: michele.solfrizzo@ispa.cnr.it. Solfrizzo reported that an evaporative light-scattering detector coupled to LC has been used for quantitative measurement of AAL toxin TA (AAL-TA) in fungal culture of *Alternaria alternata* f. sp. *lycopersici*. The culture broth was sequentially filtered through filter paper and 0.2 µm syringe filter and directly analyzed by LC. The response was linear in the range of 400–4000 ng toxin. The method was only applied to fungal culture broth probably because of the high LOD (6000 ng/mL; 93). All tested Japanese strains of the tomato pathotype of *A. alternata* produced AAL-TA, but no fumonisin. The toxigenicity of fungal isolates was checked by LC with fluorometric detection after precolumn derivatization of the toxin with *o*-phthalaldehyde reagent (94). The relationship of AAL toxins (AAL-TA and AAL-TB) and fumonisin contamination in maize silage to seasonal weather conditions and to agronomic and ensiling practices has been studied. AAL toxins were measured by LC/MS method after extraction with a mixture of methanol–water and purification through a strong anion exchange column (95).

The occurrence of *Alternaria* mycotoxins has been investigated in 80 samples of tomato puree processed and sold in Argentina and 35 cereal samples (barley, wheat, oat, and triticale) collected in the southern part of Sweden in 2006, a

season with heavy and frequent rainfall. High incidence (49%) of contaminated samples was found for tomato puree with levels up to 4021 µg/kg tenuazonic acid (TeA), 8756 µg/kg alternariol (AOH), and 1734 µg/kg alternariol monomethyl ether (AME; 96). All tested cereal samples were found contaminated with TeA (up to 4310 µg/kg) with the highest levels being found in oats and triticale. AOH and AME were found in 89 and 66% of the samples at levels up to 335 and 184 µg/kg, respectively (97).

Total synthesis of altenuene and isoaltenuene has been reported for the first time (98). Four new altenuene derivatives called dihydroaltenuenes A and B, and dehydroaltenuenes A and B along with known altenuene, isoaltenuene and 5'-epialtenuene were isolated from cultures of an unidentified freshwater aquatic fungal species belonging to *Tubeufiaceae* family (99).

Citrinin

Topic Advisor Dave Abramson, Agriculture and Agri-Food Canada, Cereal Research Center, 195 Dafoe Rd, Winnipeg MB R3T 2M9, Canada, Tel: 204-984-5536, Fax: 204-983-4604, E-mail: dabramson@agr.gc.ca. Abramson reported that a new review on the qualitative and quantitative analysis of the mycotoxin citrinin has been recently published (100). This summary evaluates advantages and disadvantages of different analytical methods and compares chromatographic properties, sample pretreatment, recovery rates and detection limits. Published literature from 1980 to 2004 was used as the source for this review. A novel LC/MS multitoxin method has been proposed to detect citrinin and 6 other mycotoxins in corn silage (101). Following SPE, citrinin was monitored at positive ions *m/z* 251 and 273 and quantified at *m/z* 251. Recovery of citrinin was 80%, and LODs and limits of quantification (LOQ) were 1.5 and 5 ppb, respectively. Between 4 and 25 ppb citrinin were found in corn silage. A new method was devised to detect citrinin as well monacolin K acid and monacolin K lactone in red mold rice (102). The method uses LC with both ultraviolet and fluorescence detection; recoveries for citrinin at spiking levels of 50, 100, 150, 200, and 250 ppb averaged 86.2%. Up to 11.2 ppm citrinin were found in capsules of powdered red mold rice.

Citrinin and other mycotoxins in grain dusts have become a matter of concern. A multitoxin method (103) for screening 15 mycotoxins included citrinin, giving a 74% recovery at a 40 ppb spiking level [coefficients of variation (CV) = 8.9%] in wheat dust with an LOQ of 1.4 ppb for this toxin. In 14 samples of grain dusts, citrinin ranged from 34 to 958 ppb (median = 186 ppb). In a related paper (104) examining ochratoxin A and citrinin in grain dusts, a similar methodology was used for the 2 toxins, which gave the same method performance for citrinin. The range for citrinin in these wheat dust samples was 137–344 ppb.

Cyclopiazonic Acid

Topic Advisor Joe Dorner, U.S. Department of Agriculture, Agricultural Research Service, National Penut

Research Laboratory, PO Box 509, 1011 Forrester Dr SE, Dawson, GA 31742, Tel: 912-995-7408, Fax: 912-995-7416, E-mail: jdorner@nprl.usda.gov. Dorner reported that there is little work currently being performed on cyclopiazonic acid, other than its use as an inhibitor of the sarcoplasmic reticulum calcium pump (105). Investigations of secondary metabolite production by fungal strains has been aided by the development of multitoxin LC/MS methods. The application of this methodology in which fungal culture extracts were directly injected into the LC/MS system has demonstrated the production of cyclopiazonic acid by the newly described *Aspergillus lentulus* (106). Cyclopiazonic acid has also been shown to be produced by 50% of strains from *Aspergillus* section Flavi, isolated from corn in Italy during investigations of an episode of aflatoxin contamination of corn destined for animal feed (107). Genetic studies have shown that in *A. flavus* a common gene regulates the production of aflatoxins B₁ and B₂, as well as cyclopiazonic acid and aflatrem (108).

Ergot Alkaloids

Topic Advisor George M. Ware, U.S. Food and Drug Administration, 60 8th St, Atlanta, GA 30309, Tel: 404-347-2131, ext. 5215, Fax: 404-347-4225, E-mail: gware@fda.hhs.gov. Ware reported that the only information reported on ergot alkaloids analytical methodology was an LC/MS method for detecting ergot in rye flour (109). This confirmatory method detected 5 ergot alkaloids, ergocristine, ergotamine, ergonovine, ergocornine, and α -ergokryptine, using LC coupled to MS/MS. Detection was monitored by 2 transition reactions per analyte. The method entailed a liquid-solid extraction followed by a cleanup step using C18 SPE. The method was fully validated according to the EU criteria. LODs and LOQs of all analytes were calculated and ranged from 7 to 11 $\mu\text{g}/\text{kg}$ and from 23 to 37 $\mu\text{g}/\text{kg}$, respectively.

Fumonisin

Topic Advisor Chris Maragos, U.S. Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, 1815 N. University St, Peoria, IL 61604, Tel: 309-681-6266, Fax: 309-681-6689, E-mail: maragocm@ncaur.usda.gov. Maragos reported that development of analytical methodology for fumonisins continued to be an active area, in particular with regards to LC methods with either fluorescence or MS detection. Relating to the isolation of fumonisins, the use of immunoaffinity column techniques were reviewed (110). Also relating to isolation of fumonisins, Riley et al. reported on a novel mechanism for conducting international analyses of fumonisin-contaminated maize (111). Namely, by extracting maize at the country of origin, performing C18 SPE, and shipment of the SPE cartridges to the destination country, they were able to avoid the need to obtain permits for importing the maize itself.

Derivatization of fumonisins followed by LC and fluorometric detection continues to be extensively used for fumonisin detection. General reviews of LC methods for

fumonisins include those by Kushiro et al. (112), Biselli (113), and Takatori et al. (114). In particular, the review by Kushiro et al. contains a summary of 5 reagents previously reported for the precolumn fluorescence derivatization of fumonisins, including fluorescamine, *o*-phthalaldehyde (OPA), naphthalene-2,3-dicarboxaldehyde (NDA), 4-fluoro-7-nitro-benz-2-oxa-1,3-diazole (NBD-F), and 4-(*N,N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazol (DBD-F). The OPA derivative, for which there is an AOAC validated method, continues to be the most widely reported. However, other fluorophores continue to be explored and recently a comparison of OPA, 4-chloro-7-nitrobenzofurazan (NBD-Cl), and dansylchloride (DC) was reported (115). Under the conditions used, the NBD-Cl gave the lowest fluorescence response, while OPA in combination with 2-mercaptoethanol gave the largest response. The dansylated derivative was more stable than the OPA derivative but also was less fluorescent.

Fumonisin analysis by LC/MS continues to be a very active area of research, with applications ranging from multitoxin assays in commodities to the analysis of complex matrixes, to the discovery of novel fumonisin analogs and congeners. The application of LC/MS to a variety of mycotoxins, including fumonisins, was recently reviewed by Zöllner and Mayer-Helm (1). There is a very extensive literature on the application of LC/MS and LC/MS/MS to fumonisin analysis, and this review has done an excellent job of summarizing the salient aspects of many of the methods including the matrix tested, form of sample preparation, form of chromatography, and type of MS. Applications of LC/MS and LC/MS/MS to fumonisin analysis in a variety of matrixes were also summarized by Kushiro et al. (112). Recently a method was validated for the measurement of 39 mycotoxins in wheat and maize using LC with electrospray injection MS/MS (ESI-MS/MS; 57). The method involved a single extraction using acetonitrile-water-acetic acid (79 + 20 + 1, v/v/v). The extract was diluted and analyzed without cleanup. Two consecutive chromatographic runs (21 min each) were conducted, with switching of the instrument between positive and negative ion modes in order to capture all of the mycotoxins, which have different ionization characteristics. Recoveries for many of the mycotoxins were quite good, however extraction of the fumonisins was reportedly incomplete. While the observed ion-suppression effects were minor for wheat, with maize there were significant ion-suppression effects for 12 of the mycotoxins.

In addition to routine monitoring, MS methods are useful in detecting new toxin analogs or congeners. Fumonisin and fumonisin-like compounds were produced by *Fusarium verticillioides* in rice culture and examined by LC-ESI with ion trap MS/MS (116). A variety of known and previously unreported fumonisin analogs were detected, including iso-FA₁, iso-FB₂, partially hydrolyzed (PH) FB₂ and FB₄, FB₅, iso-FB₅, FBK₁ 2TCA, FBK₄ 2TCA, FC₂, iso-FC₂, PHFC₄, FD, and FBX. The FD series contained a backbone shorter than the C20 backbone of the fumonisins. The FBX series consisted of 12 analogs that contained other acids (acids other than tricarballic acid) esterified to the backbone, for

example aconitic acid, oxalylsuccinic acid, or oxalylfumaric acid. Among the reported analogs several were suggested as possible precursors of fumonisins. Using ^1H and ^{13}C NMR spectroscopy Gelderblom et al. identified 2 stereoisomers of fumonisins B₃ and B₄ (117). The 2,3-anti stereoisomers (2S,3R absolute configuration), or 3-epi series, represented 10 to 40% of fumonisin in samples of standards. The 3-epi-FB₃ was also found to occur naturally in maize at low levels (<20% of the level of FB₃).

The fumonisins are found worldwide and in a variety of commodities, in particular cereal grains susceptible to the producing fungi such as *Fusarium verticillioides* and *F. proliferatum*. Space does not permit a comprehensive review of the occurrence of fumonisins in maize during the past year. However, fumonisins were reported in less commonly surveyed matrixes, such as wild plants, silage, and sorghum. Sewram et al. (118 (**CORRECT AS CHANGED?**)) tested wild plants, 19 dietary and 30 medicinal, used by residents of Eastern Cape Province of South Africa (118). Four of the dietary plants and four of the medicinal plants were found to contain FB₁, at levels ranging from 8 to 1553 $\mu\text{g}/\text{kg}$. Detection methods for FB₁ and FB₂, as well as their aminopolyol hydrolysis products (HFB₁, HFB₂, or AP₁, AP₂), were also developed and applied to sorghum grown in Tlayca, Mexico (119, 120). The mean concentrations of FB₁ and FB₂ were found to be 0.53 and 0.47 $\mu\text{g}/\text{g}$, respectively. FB₁ and FB₂ as well as AAL toxins were detected in silage collected in 2001 and 2002 from 30 to 40 dairies throughout Pennsylvania (95). The average concentrations found were 2.02 μg FB₁/g (range 0.20 to 10.10), and 0.98 μg FB₂/g (range 0.20 to 20.30). Prevalence of the fumonisins was also high, with 92 and 55% of the samples having detectable FB₁ or FB₂ respectively. Interestingly, ensiling did not affect the toxin concentrations.

A number of immunoassays have been developed for the fumonisins, and several manufacturers offer commercial test kits based upon various platforms, such as ELISAs or immunochromatographic (lateral flow) devices. Two recent reports review the various immunoassay formats currently in use (65, 121). In addition to the commercially available test formats, the review by De Saeger et al. also covers the recent approach of using a cleanup tandem column assay. The review by Zheng also covers the traditional formats but in addition covers emerging technologies such as surface plasmon resonance, fiber optic immunosensors, molecularly imprinted polymers, microarrays, and microspheres. Improving ELISAs is also possible, and the use chemiluminescence, rather than color development as an endpoint in microplate ELISAs was shown to enhance the sensitivity of the assay for FB₁ by roughly 10-fold (122). The same group also reported on a lateral flow assay using colloidal gold (123). The membrane-based colloidal gold assay had a visual LOD of 1.0 μg FB₁/L. A rather unique bioassay that does not rely upon antibodies was reported (124). The assay was based upon the inhibition of dextranase activity, as measured fluorometrically. While the assay did not respond to up to

2 μg FB₁/mL, responses were observed with some other mycotoxins, including aflatoxins B₁ and G₁, and T-2 toxin.

Mycotoxins in Botanicals

Topic Advisor Bruce Malone, Trilogy Analytical Laboratory, 111 West 4th St, Washington, MO 63090, Tel: 636 239-1521, Fax: 636 239-1531, E-mail: bruce@trilogylab.com. Methods for the determination of aflatoxins B₁, B₂, G₁ and G₂ in ginseng and other botanical roots including echinacea, ginger, kava-kava, and licorice have been developed which involve immunoaffinity column cleanup and LC determination using precolumn trifluoroacetic acid derivatization or postcolumn bromination or ultraviolet irradiation (52). Recoveries of about 80% were achieved. Extension of the method to include simultaneous isolation of ochratoxin A using immunoaffinity columns containing antibodies for both toxins achieved recoveries of about 60–70%, whereas ochratoxin A recoveries for ginseng, ginger, and kava-kava were about 55% at a spiking level of 4 ng/g. Supercritical fluid extraction has been used to develop an LC/MS/MS method for the determination of aflatoxins in *Zizyphi fructus* (fruits of *Zizyphus jujube*), a traditional Chinese medicine (125). A small survey of 8 random samples from different geographical areas of Taiwan yielded only 1 sample with a low level of aflatoxin contamination. The problem of low recoveries of aflatoxins during analysis of Chinese medicinal herbs has been overcome by substituting 0.1 M phosphate buffer (pH 8.0) for the buffered saline normally used (50). The analysis of 48 medicinal plants and herbal infusions in Italy failed to find any aflatoxin contamination (LOD of 0.5 $\mu\text{g}/\text{kg}$ for aflatoxin B₁; 92).

Ochratoxins

Topic Advisor Mary Trucksess, U.S. Food and Drug Administration, 5100 Paint Branch Pkwy, College Park, MD 20740, Tel: 301-436-1957, Fax: 301-436-2665, E-mail: mary.trucksess@fda.hhs.gov. Trucksess drew attention to 2 recent reports of immunosensor-based methods for multitoxins. One method applied OWLS technique for deoxynivalenol (DON) and ochratoxin A (OTA; 61). Antibodies or mycotoxin conjugate were immobilized on the sensor chip for direct or indirect measurement, respectively, and were used in a flow-injection analyzer (FIA) system. The sensitive detection range was between 0.5 and 10 ng/mL. Good correlation with ELISA was obtained when the method was applied to barley and wheat flour. The other method employed evanescent-wave fluorescence excitation to probe binding events of OTA and DON occurring on the surface of a waveguide (126). After sample analysis was completed, the surface was regenerated with 6 M guanidine hydrochloride in 50 mM glycine, pH 2.0. The LODs in cornmeal determined with the automated array biosensor were 15 and 150 ng/g for OTA and DON, respectively.

There were several interesting methods published. A solid-phase microextraction (polydimethylsiloxane/divinylbenzene; PDMS/DVB) interfaced with an LC-fluorescence method was used to analyze OTA in human urine (127). The urine samples

were diluted, partitioned against chloroform and the aqueous phase extracted by the PDMS/DVB fiber, desorbed through an interface into an LC system. The LOD and LOQ were 0.01 and 0.05 ng/mL, respectively. A method using zinc acetate and silica cartridges for OTA in beer was published (128). The LOD and LOQ of the method were 0.0008 and 0.0025 ng/mL, respectively. The recoveries were 80.6–87.6% for beer spiked at 0.025–0.40 ng/mL. Sixty-nine beer samples purchased in Spanish markets were analyzed and were found to be contaminated with OTA from 0.008–0.498 ng/mL. A method using immunoaffinity chromatography (IAC) cleanup and MS for detection of OTA in cereals was validated in-house (129). Ochratoxin B was used as the internal standard. Recoveries of OTA in rice were about 100% at spiking levels of 0.05–0.15 ng/g. Screening methods for OTA in cocoa powder (130), and simultaneously detecting aflatoxin B₁ and ochratoxin A in virgin olive oil (53) and chili (49) were reported. A multitoxin method for aflatoxins and OTA in ginger and ginseng was developed (52). Recoveries of the added toxins were about 80% for aflatoxins in the range 2–16 ng/g and OTA in the range 1–8 ng/g.

Occurrence of OTA in human plasma in agricultural zones of Chile was investigated (131). Fifty-four percent of the samples collected in March and July 2004 in Colbun and 91% of samples collected in October in San Vicente de Tagua-Tagua were positive for OTA in the range of 0.07–2.75 ng/mL and 0.22–2.12 ng/mL, respectively. The OTA levels in serum did not show a good correlation with normal dietary consumption. The same relationship was found in many of the European studies. There were 3 studies comparing the occurrence of OTA in organic and conventional products (132–134). OTA occurred in organic wholemeal wheat flours collected in Belgium during 2002–2003 more frequently than conventional samples (132). Organic beers collected in Belgium during 2003–2004 were more frequently OTA contaminated (95%, $n = 40$) than the conventional beer (50%, $n = 40$), and at higher mean concentration (133). However, a study in The Netherlands found that the concentration of OTA in organically produced wines was not significantly different from that in conventional products (134). OTA is commonly found in licorice roots and licorice products. Thirty samples of licorice root, licorice confectionery, licorice block, and licorice extract were analyzed for OTA by LC and confirmed by methyl-ester formation (135). All samples contained OTA. The 15 dry root samples had an average of OTA 63.6 µg/kg (1.4–252.8 µg/kg), the 8 fresh roots averaged 9.2 µg/kg (3.3–14.7 µg/kg), and the 4 sweets averaged 3.8 µg/kg (0.5–8.2 µg/kg). OTA was also present in the liquid licorice extract and solid licorice block (16 and 40 µg/kg, respectively).

The effects of growth stage on OTA contamination of grapes were reported in several publications. Musts ($n = 24$) obtained from grapes collected in Tunisian vineyards at different sampling times were analyzed for their OTA content (136). Up to 37% of the musts contained OTA at levels varying between 0.59 and 2.57 µg/L. The amounts of OTA in musts increased as grapes matured. A Portuguese study found OTA production differed significantly with the composition of the grape berries

at different maturation stages, with a mean value of OTA production at pea berry, early veraison, and ripe berry of 3402, 1530, and 22 µg/kg, respectively (137). The production of OTA by *Aspergillus carbonarius* was correlated positively and negatively with the total acidity of grapes and the reducing sugar content, respectively. An Italian study reported significant reduction of OTA in must at the end of alcoholic fermentation (138). However, depending on the yeast strain involved in the fermentation, there was a difference in the content of OTA in the wines. The percentage of OTA removal during the fermentation was between 46.8 and 52.1% in white wine and between 53.2 and 70.1% in red wine.

Patulin

Topic Advisor Myrna Sabino, Instituto Adolfo Lutz, Av. Dr Arnaldo 355–01246-902, São Paulo, Brazil, Tel: 55-11-3068-2921, Fax: 55-11-3085-3505, E-mail: mysabino@ial.sp.gov.br. Sabino reported that the use of a solid-phase multifunctional column (Mycosep 228) and LC for determination of patulin in apple and hawthorn beverages was investigated (139). Comparable tests revealed that the results obtained by both the new Mycosep-based method and the Official AOAC Method did not differ significantly. The performance of 4 purification methods for the analysis of patulin in apple juice was evaluated by LC (140). Samples were spiked with patulin, extracted by 1 of 4 methods (3 SPE and one liquid–liquid extraction), and then analyzed by LC-UV under the same isocratic conditions. Recoveries were all >70% for the spiking range 10–150 ppb. The relative standard deviation for repeatability was found to meet EU Directive requirements. In addition, all the methods showed baseline separation from hydroxymethylfurfural. The synthesis of 2 new derivatives of patulin was described, along with their conjugation to bovine serum albumin for the production of polyclonal antibodies. A fluorescence competitive immunoassay was developed for the on-line detection of patulin (141).

The role of apple variety (Golden or Fuji), degree of ripeness and size of lesions in the extent of patulin contamination in apples artificially inoculated with *Penicillium expansum* and stored at room temperature for short periods of time was reported (142). Higher accumulation of patulin occurred in Golden apples, with less ripened apples showing higher concentrations. Patulin accumulation occurred rapidly at room temperature, which indicates that standing time for apples prior to processing should be minimized. The development and production of patulin in apples by *Penicillium expansum* during cold storage and in the steps prior to processing of apple products has been investigated (143). Cold storage periods of 6 weeks did not lead to patulin accumulation. Shortening preprocessing times at warm temperatures would result in a reduction in patulin content of fruit entering the processing plant. Deck storage subsequent to cold storage can lead to enhanced patulin accumulation (144).

The occurrence of patulin in a large group of organic, conventional, and handcrafted apple juices marketed in

Belgium was investigated (145). The patulin content was higher than the European legal limit of 50 µg/L in 2 samples of organic apple juice. Although, the incidence of patulin in organic (12%), conventional (13%), and handcrafted (10%) apple juices was not significantly different, the mean concentration of patulin in contaminated samples was significantly higher in organic (43.1 µg/L) than in conventional (10.2 µg/L) and handcrafted (10.5 µg/L) apple juice. Patulin contamination in 20 apple juice samples sold in retail outlets in Italy and South Africa was investigated (146). Eleven of 12 Italian samples had no detectable levels of patulin, whereas one was just slightly above the lowest regulatory limit of 10.0 µg/L. On the other hand, 5 of the 8 South African samples had patulin levels exceeding 10 µg/L, with one showing a concentration of 75.0 µg/L, well above the highest regulated limit of 50.0 µg/L.

The aflatoxin, patulin, and ergosterol contamination of 3 different categories of dried figs (palatable, fluorescent, and cull) were investigated (147). Samples were obtained from 4 fig processing plants located in a major fig producing area in the Aegean Region of Turkey. Palatable figs were contaminated with trace amounts of aflatoxins, patulin, and ergosterol. Fluorescent figs were contaminated with high aflatoxin levels and cull figs with high patulin and ergosterol levels.

A bacterium isolated from patulin-contaminated apples was found to be capable of degrading patulin to a less toxic compound, ascladiol (148). The bacterium was identified as *Gluconobacter oxydans* by 16S rRNA gene sequencing. Ascladiol was identified by LC MS/MS and proton and carbon nuclear magnetic resonance. Degradation of up to 96% of patulin was observed in apple juices containing up to 800 µg/mL of patulin and incubated with *G. oxydans*. The potential of 19 different strains of *Byssochlamys nivea* and *B. fulva* to produce patulin in relation to the presence of 2 genes involved in the patulin biosynthetic pathway in the genome of these fungal strains was reported (149). All of the 8 *B. nivea* strains tested produced patulin. By contrast, none of the 11 strains of *B. fulva* produced this toxin. The absence of 6-methylsalicylic acid and isoeoxydon dehydrogenase genes can explain the inability of *B. fulva* to produce patulin.

The patulin exposure of children consuming organic, handcrafted, or conventional apple juice was investigated using a probabilistic approach to evaluate the effectiveness of several risk management options aiming at reducing the risk of patulin exposure in children (150). Reduction of the apple juice consumption is less effective than a reduction of the patulin concentration in apple juice and is only useful when the patulin concentration of apple juice is <25 µg/kg.

Trichothecenes

Topic Advisor Gary A. Lombaert, Health Canada, 510 Lagimodiere Blvd, Winnipeg, MB, R2J 3Y1, Canada, Tel: 204-984-2088, Fax: 204-983-5547, E-mail: gary_lombaert@hc-sc.gc.ca. Lombaert reported that the potential health risks associated with occurrence of toxic trichothecene mycotoxins in foods, feeds, and the environment continue to be a global concern. This concern

drives a need for improved analytical methods, which is being addressed by many techniques, ranging from rapid immunoassays to the most sensitive and specific MS techniques.

Accurate quantitative methods depend upon reliable reference standards. The certification of solutions of type B trichothecenes by multilaboratory analysis was found unsuitable due to the high variability of results between laboratories (151). Instead, the authors recommended certification based upon gravimetric preparation.

Immuno-based systems continue to provide rapid alternatives to more traditional chromatographic techniques for the analysis of trichothecenes. A fluorescence polarization immunoassay for DON was optimized for the quantitative analysis of durum wheat kernels, semolina, and pasta (152). After subtraction of matrix-dependant background signals and naturally incurred DON, accurate quantitation was possible, with a LOD of about 100 ng/g. At spiking levels between 250 and 1750 ng/g, recoveries averaged 98, 102, and 101 for wheat, semolina, and pasta, respectively. Comparison to a method employing IAC cleanup and LC showed good correlation. A multiplexed competitive assay for the simultaneous determination of DON (and ochratoxin A), employing evanescent-wave fluorescence excitation, has been described (126). Both a manual and automated version of the system was evaluated. The LODs (for DON) by the manual version were reported to be 1, 180, and 65 ng/g in cornmeal, wheat, and barley, respectively; the LOD by the automated system was reported to be 150 ng/g in cornmeal.

For the first time, this reviewer found no significant advances over the past year in the use of gas chromatography for the determination of trichothecenes. This likely reflects the increasing application of LC as the separation technique of choice. Also in the past year, the UV detector appears to have regained favor. Fourteen participants contributed to an interlaboratory study examining an LC-UV method of analysis for DON in baby food and animal feed (153). For baby food, mean recoveries ranged from 85% (at 120 ng/g) to 89% (at 240 ng/g), and the relative standard deviation for reproducibility (RSD_R) ranged from 9.4 to 19.5%. For animal feed, mean recoveries (at 200 and 400 ng/g) ranged from 93 to 100%, and the RSD_R ranged from 10.5 to 25.2%. The method demonstrated acceptable precision for each matrix as required by European legislation. Another collaborative study for the determination of DON in wheat employed a multifunctional column prior to LC-UV detection, with an LOD of 100 ng/g (154). The mean recovery (at 1100 ng/g) was 100%. The relative standard deviation for repeatability (RSD_r) for the analysis of naturally contaminated wheat ranged from 6 to 11%, while the RSD_R ranged from 12 to 21%. A multimycotoxin method of analysis for wheat included DON, 3-acetyl-DON (3-ADON), and 15-acetyl-DON (15-ADON; 132). Extracts were cleaned up on a strong ion exchange (SAX) column followed by a MycoSep cartridge, and the trichothecenes were detected by LC-UV. The LOD and average recovery of DON were reported to be 50 ng/g and 92%, respectively. The development and application of DON-specific sol-gel IACs has

been reported (155). An advantage of these columns is their reusability for up to 20 determinations without any loss of binding efficiency or DON carryover in the extracts. Furthermore, the authors report that regeneration (after 25 uses) can be accomplished by long-term storage in phosphate-buffered saline. The columns were applied to a method for the analysis of maize, wheat, and spaghetti. The LODs were reported as 240, 200, and 207 ng/g for maize, wheat, and spaghetti, respectively; recoveries (at 400–1200 ng/g) ranged from 83–97%. An LC-fluorescence detection (FLD) method for the quantitative determination of T-2 toxin (T-2) and HT-2 toxin (HT-2) in eggs has been reported (156). Trichothecenes from spiked eggs were isolated on a charcoal-alumina-florisil column, followed by IAC cleanup. Extracts were derivatized with 1-anthrolylnitrile prior to injection into the LC. LODs were reported as 1 ng/mL whole (shelled) egg for each trichothecene. Mean recoveries from eggs spiked with 5 to 50 ng/mL were 89 and 100% for T-2 and HT-2 respectively, with CV of 3.5 and 8.2%.

LC with MS or MS/MS provides the advantages of selective, multitoxin, quantitative data from a single instrumental analysis. Trichothecenes were included in a review of analytical methods for mycotoxins by LC/MS employing atmospheric pressure ionization (1). An LC/MS/MS method for the detection of 39 mycotoxins, including both A- and B-trichothecenes and DON-3-glucoside, in wheat and maize was reported (57). Samples were extracted with acetonitrile–water–acetic acid (79 + 20 + 1; which was described as the best compromise for multitoxin extraction for wheat and maize) and injected without any cleanup. The method employed electrospray ionization (ESI) and multiple-reaction monitoring (MRM); sequential chromatographic runs used positive and negative ionization modes for the optimal detection of trichothecenes. The trueness of the method for DON was confirmed through the analysis of certified reference materials. Another LC/MS/MS method reported the determination of 17 mycotoxins, including DON, 3- and 15-ADON, NIV, fusarenon-X (F-X), T-2, and HT-2 (157). Samples were extracted with an aqueous acetonitrile solution containing zearalanone (ZAN) as an internal standard. Extracts were cleaned up with a MycoSep multifunctional cartridge, and injected onto a UPLC column. This MS/MS was also operated with ESI, MRM, and both polarities. Mean recoveries, at 3 levels, from a peanut butter matrix ranged from 76 (HT-2) to 93% (T-2). LC/MS/MS was linked to a fully automated sample preparation and SPE routine for the analysis of beer and wine for DON, T-2, and HT-2 (158). The method also used ZAN as an internal standard, and reported LODs of 0.4, 0.4, and 2.0 ng/mL for T-2, HT-2, and DON, respectively. Recoveries ranged from 61 to 113% at spike levels of 35 and 350 ng/mL. The application of stable isotopic ¹³C-DON as an internal standard for LC/MS determination of DON was reported to correct for fluctuations during extraction, cleanup, and ionization (159). Recoveries from maize improved from 76 to 101% using the internal standard method. Another method reported excellent sensitivity and good precision using ¹³C-labeled acetyl trichothecene

derivatives as internal standards prior to LC/MS/MS (160). The method was applied to the quantitative analysis of the B-trichothecenes DON, 3- and 15-ADON, and F-X in cereals and cereal products, after sample cleanup on multifunctional columns.

The knowledge base of the occurrence of trichothecene mycotoxins in foods and feeds continues to grow. The occurrence of DON and nivalenol (NIV) in *Fusarium*-infected cereal crops from Ethiopia was reported (88). DON was detected in barley, sorghum, and wheat at 40 to 2340 ng/g, while NIV was detected at 40 ng/g in a wheat sample and at 50 to 490 ng/g in sorghum. In a study of South African barley and malt, a high proportion of 82 samples were positive for DON (161). The mean level in maltster's barley was 832 ng/g, while the mean level in retail barley was 150 ng/g. A study of barley grown in Uruguay from 1996 to 2002 also detected frequent contamination with DON (162). Significant seasonal variation was noted with incidence up to 100%, and DON levels as high as 6349 ng/g. A positive correlation between precipitation and DON levels was noted. Other investigations into barley and malt found that electron-beam irradiation (6 to 10 kiloGray) of DON contaminated barley resulted in significant reduction (60–100%) in the levels in the resulting malt (163). A comparison of the DON levels in conventionally and organically grown wheat and flour in Belgium was reported (132). DON was reported to be higher in conventionally grown wheat, but lower in conventionally produced flour purchased from retail shops. Eleven of 45 soy-based food samples from the German market were found to be positive for 1 or more trichothecenes (164). DON, scirpentriol (SCIRP), 15-monoacetoxyscirpenol, 4,15-diacetoxyscirpenol, T-2 tetraol, HT-2, 3- and 15-ADON were each detected in at least 1 sample. DON and SCIRP were found at levels up to 260 and 108 ng/g, respectively. DON levels in soft wheat flour milled by a traditional stone mill were reported to be 40–50% lower than flour produced from a modern rolling mill (165). Fifty commercial beer samples from Turkey were analyzed for the presence of DON (166). Samples were extracted with acetonitrile–water and cleaned up on an alumina-celite-charcoal column followed by a C-18 SPE column. Employing LC-UV detection (LOD–125 µg/L), no DON was detected in any of the samples. Wheat flour samples (*n* = 151) were collected from Danish mills and the retail market over a 5 year period (1998–2003) and were analyzed for DON (167). Contamination levels varied significantly from year-to-year, but no sample exceeded the European standard of 750 ng/g. A study of the carry-over of DON from naturally contaminated wheat to pigs found that most DON (and its metabolite de-epoxy-DON) was expressed in the bile, and the least occurred in muscle and fatty tissue (168). The authors concluded that the exposure risk to humans from consumption of edible tissue is negligible, compared to that from consumption of cereal grain-based foods.

The potential health significance of environmental trichothecenes is still unclear. Whereas much is known about trichothecenes in foods, greater knowledge, including analytical methods, is needed to assess the indoor air question.

GC/MS techniques were investigated for the optimum detection of the macrocyclic trichothecenes, verrucarol and trichodermol, in building material and house dust (169). Negative-ion chemical was the preferred ionization technique due to improved LODs, reduced background noise, and improved specificity. Heptafluorobutyrate derivatives presented greater sensitivity compared to trimethylsilyl ethers. MS/MS was found to reduce background chromatographic noise considerably, in comparison to MS. Settled dust samples from grain storage facilities were analyzed for DON [and ochratoxin A and zearalenone (ZON)]. All were found to contain DON; the mean level was 416 ng/g (170). Additionally, airborne DON concentrations were measured and reported as 2 ng/m³.

Zearalenone

Topic Advisor Rudolf Krska, University of Natural Resources and Applied Life Sciences, Department for Agrobiotechnology-IFA-Tulln, Center for Analytical Chemistry, Christian Doppler Laboratory for Mycotoxin Research, Konrad Lorenz Str. 20, A-3430 Tulln, Austria, Tel: 43 2272 66280 401, Fax: 43 2272 66280 403, E-mail: rudolf.krska@boku.ac.at. Krska and colleague Franz Berthiller summarized selected information on ZON appearing in the last year's scientific literature. The majority of the newly developed methods for ZON analysis continue to rely on LC/MS. In this context, an excellent review dealing with mycotoxin analysis in complex biological and food matrixes by LC/MS has been published (1). An overview on the application of LC-atmospheric pressure-MS in the analysis of frequently occurring mycotoxins, including a separate chapter on ZON, is given. Zöllner and Mayer-Helm compare more than 30 different LC/MS methods for the determination of ZON and its metabolites. Besides the end determination, special focus is also directed towards sample preparation and method validation. The incidences of matrix effects are discussed in particular.

Two LC/MS methods were introduced that allow the determination of multiple classes of mycotoxins, including ZON. The method of Ren et al. (157) utilizes UPLC to separate a total of 17 mycotoxins from the matrix and a triple quadrupole tandem mass spectrometer for their detection. The samples were extracted with acetonitrile–water (84 + 16, v/v), spiked with ZAN as internal standard for ZON analysis and purified with Mycosep multifunctional SPE columns. Two separate 9 min (including re-equilibration) LC runs, one for each electrospray ionization polarity, were used for each sample. The solvent system consisted of 10 mM aqueous ammonium acetate–methanol in positive mode and of aqueous ammonia–methanol in negative mode. LOQ <1 µg/kg were reached.

The method of Sulyok et al. (57) for the determination of 39 mycotoxins in cereals uses a single extraction step with acetonitrile–water–acetic acid (79 + 20 + 1, v/v/v) followed by LC with electrospray ionization triple quadrupole MS without the need for any sample cleanup. The ZON derivatives ZON-4-sulfate, ZON-4-glucoside, α -zearalenol

(α -ZOL), and β -zearalenol (β -ZOL) are also covered by this method. With 2 consecutive 21 min LC runs for each polarity and minimal sample preparation time, this method is also extremely fast for that high number of analytes. Matrix effects were intensively studied and were easily overcome by dilution of the samples before injection as the sensitivity of the used instrumentation is very high. Apparent recoveries for ZON of 108% in wheat and 101% in maize with precisions of about 1% and LOD <1 µg/kg were obtained. Reference materials confirmed the trueness of the method for ZON. The method will be continuously expanded by the authors to cover more matrixes and even more mycotoxins.

Another LC/MS/MS method was developed to quantify ZON, α -ZOL, β -ZOL, ZAN, α -zearalanol (α -ZAL) and β -zearalanol (β -ZAL) in aqueous environmental samples using deuterated internal standards (171). Sample enrichment was performed by C18-SPE cartridges. The recoveries for all analytes were in the range from 70 to 109%, precisions ranged from 2–16%, and LODs were 0.4–12.4 ng/L in drainage water, river water, and treated wastewater (which showed the highest variations). ZON was detected in drainage water from a *Fusarium graminearum* infested crop field up to 30 ng/L.

The same analytes were determined in urine, plasma, and faeces of horses by LC-atmospheric pressure chemical ionization-MS (172). For plasma samples immunoaffinity cleanup was sufficient, while for urine and faeces samples a previous SPE cleanup step had to be included. Dideuterated ZON was used as internal standard. Recovery rates between 56 and slightly above 100% were achieved with LODs <1 µg/kg or 1 µg/L, respectively. LC/MS/MS has also been used as a screening tool to determine phase II metabolites of ZON in the model plant *Arabidopsis thaliana* (173). After treatment of plant seedlings with 50 µM ZON, 17 different metabolites of ZON, most prominently glucosides, malonylglucosides, di-hexose- and hexose-pentose disaccharides of ZON, α -ZOL and β -ZOL, were detected in the plant extracts. Whereas *Arabidopsis* is in no way an agricultural crop, the work still shows the huge capability of plants to metabolize mycotoxins. It has been shown that some of these conjugates can be cleaved easily by the gut microflora of mammals, releasing the precursor toxin. While often only the parent mycotoxin is measured in routine analysis of feed and food, the total available amount of mycotoxins might be currently underestimated.

Besides using LC/MS, methods for ZON determination have also been published featuring LC-UV (174), capillary electrophoresis (CE) with cyclodextrin-enhanced fluorescence (175), an immunoassay (176), and a yeast estrogen bioassay (177). Last year a short review about fluorescence polarization of mycotoxins, including ZON, was released as well (2). The LC-UV method was optimized from existing methods to determine the ZON contamination in corn used for human consumption in Mexico. An average recovery of 90% and an LOD was 0.7 µg/kg was achieved. Seventy percent of the monitored samples were contaminated with ZON with levels ranging from 3 to 83 µg/kg. For the CE method, 22 cyclodextrins were screened for their ability to

enhance the native fluorescence of ZON. Laser-induced fluorescence (LIF) detection was performed after separation. Heptakis(2,6-di-*O*-methyl)- β -cyclodextrin gave the greatest enhancement and was applied to the development of a CE-LIF method for detection of ZON in maize. The method resulted in an average recovery of 103%, a precision of 8.5% and a lower LOQ of 5 $\mu\text{g}/\text{kg}$. The immunoassay was designed in a noncompetitive open sandwich ELISA format for ZON determination. This assay exploits re-association of the weak antibody variable region V_H - V_L complex by a bridging antigen. With the use of immobilized V_L and enzyme-tagged V_H fragments, less ZON can be measured in a shorter time period than using a conventional sandwich assay. A relatively wide working range from about 0.1 to $>100 \mu\text{g}/\text{L}$ was achieved with the optimized assay. A yeast bioassay, expressing both the human estrogen receptor α and yeast-enhanced green fluorescent protein in response to estrogens, was validated and applied to screen for general estrogenic activity in animal feed. Whereas ZON showed a relatively high cut-off value of 1250 $\mu\text{g}/\text{kg}$ and a recovery of about 45%, more potent estrogenic substances had lower LODs. One fish feed sample, found suspect in the bioassay, turned out to contain 17 β -estradiol after confirmatory analysis with LC-TOFMS.

Molecularly imprinted polymers (MIPs) continue to be an attractive target for ZON cleanup (and analysis) with 3 publications in that area by a Spanish group. ZON-binding MIPs have been tailor-made using the ZON analog cyclododecyl 2,4-dihydroxybenzoate as template instead of the natural toxin (178). 1-Allylpiperazine served as the functional monomer, trimethyltrimethacrylate as crosslinker, and acetonitrile as porogen. These MIPs have been applied to the cleanup and preconcentration of ZON and α -ZOL from cereals after pressurized liquid extraction (179). Subsequently, the purified wheat, corn, barley, rye, rice, and swine feed sample extracts were analyzed using LC with fluorescence detection. Recoveries between 85 and 97% and precisions of 2.1–6.7% were achieved for both analytes.

An automated flow through assay for ZON was developed, exploiting the displacement of fluorescent tracers from MIPs by ZON (180). 2,4-Dihydroxybenzoic acid 2-[(pyrene-1-carbonyl)amino] ethyl ester has been finally selected to optimize the ZON displacement fluorosensor, that shows an LOD of 25 μM in acetonitrile. More work is required to increase the sensitivity of this assay, which is currently at approximately 8000 $\mu\text{g}/\text{L}$.

Ten papers investigating the occurrence of ZON (along with other mycotoxins) in various foods and feeds all over the world were identified in last year's literature. In a Portuguese study (181), ZON was detected in 16 out of 52 mixed-feeds for poultry above the detection limit of 5 $\mu\text{g}/\text{kg}$. Contamination levels ranged from 7 to 61 $\mu\text{g}/\text{kg}$, with a mean level of 27 $\mu\text{g}/\text{kg}$. No ZON above 50 $\mu\text{g}/\text{kg}$ was found in Polish food such as cereal products, nuts, dried fruits, coffee, and culinary spices (182). The presence of ZON was further monitored in Belgian conventionally and organically produced wheat grain and in whole meal wheat flours (132).

The mean ZON concentrations were 75 $\mu\text{g}/\text{kg}$ in conventional samples against 19 $\mu\text{g}/\text{kg}$ in organically produced wheat from 2002. Four hundred and eighty poultry feed samples from Brazil were analyzed for ZON using thin layer chromatography, yielding levels from 100 to 7000 $\mu\text{g}/\text{kg}$ (183). Similarly, 865 samples of poultry broiler and layer rations from Pakistan were measured for ZON (184). About half the samples were positive for ZON with mean value of 210 $\mu\text{g}/\text{kg}$. In Kuwait, ZON was found from 46 to 68 $\mu\text{g}/\text{kg}$ in poultry feed (185). Low frequencies of ZON occurred in Ethiopian sorghum samples with concentrations reaching 32 $\mu\text{g}/\text{kg}$ (88). The Bavarian Health and Food Safety Authority examined cereals and cereal products of 2004 (186). From the 77 samples analyzed, only 1 rye sample was highly contaminated with 5070 $\mu\text{g}/\text{kg}$ ZON. However, further investigations showed that this sample was an unprocessed product, which had been taken directly from the field during harvest and was not intended for direct human consumption. In baby foods only 1 sample was contaminated with 4 $\mu\text{g}/\text{kg}$ ZON. In soy food including whole beans, roasted soy nuts, flour and flakes, textured soy protein, tofu, protein-isolate, and soy sauce marketed in Germany, ZON was found up to 214 $\mu\text{g}/\text{kg}$ (187). Finally, ZON was found in 93 of 196 rice samples in Nigeria at concentrations between 24 and 1170 $\mu\text{g}/\text{kg}$ (188).

Four noteworthy reviews about ZON (and other mycotoxins) have been published recently. Leung et al. summarizes the worldwide prevalence and preventative strategies for mycotoxins in pet food (189), while Kabak et al. review general strategies to prevent mycotoxin contamination of food and animal feed (190). The toxicity, occurrence, metabolism, detoxification, regulations, and intake of ZON can be found in the article from Zinedine et al. (191). Tiemann and Danicke describe in vivo and in vitro effects of ZON (and deoxynivalenol) on different nonreproductive and reproductive organs in female pigs (192).

Recommendations

General Referee Recommendations

- (1) Appoint a new topic advisor for citrinin.
- (2) Continue to investigate and develop methods for mycotoxins in all commodities relevant to human health.

Topic Advisors' and Study Directors' Recommendations.

- (1) *Sampling and Subsampling for Mycotoxins*: Topic Advisor Thomas B. Whitaker. Continue study.
- (2) *Aflatoxin M₁*: Topic Advisor Hans P. van Egmond. Continue study.
- (3) *Aflatoxin methods*: Topic Advisor Gordon Shephard. Continue study.
- (4) *Alternaria Toxins*: Topic Director Michele Solfrizzo. Continue study.
- (5) *Citrinin*: Topic Advisor David Abramson. Appoint new advisor and continue study.

(6) *Cyclopiazonic Acid*: Topic Advisor Joe W. Dorner. Continue study.

(7) *Ergot Alkaloids*: Topic Advisor George M. Ware. Continue study.

(8) *Fumonisin*s: Topic Advisor Chris Maragos. Continue study.

(9) *Mycotoxins in Botanicals*: Topic Advisor Bruce Malone. Continue study.

(10) *Ochratoxins*: Topic Advisor Mary Trucksess. Continue study.

(11) *Patulin*: Topic Advisor Myrna Sabino. Continue study.

(12) *Trichothecenes*: Topic Advisor Gary Lombaert. Continue study.

(13) *Zearalenone*: Topic Advisor Rudolf Krska. Continue study.

(14) **2001.04** *Determination of Fumonisin B₁ and B₂ in Maize Flour and Cornflakes by LC*: Study Director Angelo Visconti, National Research Council, Institute of Toxins and Mycotoxins, Viale Einaudi 51, Bari 70125, Italy, Tel: 39-080-548-6013, Fax: 39-080-548-6063, E-mail: visconti@area.ba.cnr.it. Remove cornflake from matrix and submit for Final Action.

(15) **2004.10** *Determination of Ochratoxin A in Green Coffee by Immunoaffinity Column Cleanup and LC*: Study Director Eugenia Vargas, Laboratory for Mycotoxin Analysis, Avenida Raja Gabaglia, 245 Cidade Jardim, Belo Horizonte 30380-090, Brazil, Tel: 55-31-250-0398, Fax: 55-31-250-0399, E-mail: gena@cdlnet.com.br. Continue study.

(16) **2005.08** *Liquid Chromatographic Analysis of Aflatoxin Using Post-Column Derivatization (Modification of 991.31, 999.07, and 970.45)*: Study Director Arthur Walkling, Walkling Associates, 482 Rock Rd, Glen Rock, NJ 07452. Continue study.

(17) *Determination of Deoxynivalenol in Corn, Wheat, Barley, and Malted Barley, ELISA*: Study Director Mark Mozola, Neogen Corp., 620 Leshner Pl, Lansing MI 48912-1595, Tel: 517-372-9200, Fax: 517-372-0108, E-mail: mmozola@neogen.com. Study has been discontinued.

(18) *Determination of aflatoxins and Ochratoxin A on Ginseng and Ginger by Multi-Toxin Immunoaffinity Column Cleanup and Liquid Chromatographic Quantitation*: Study Director Mary Trucksess. Study in progress.

References

- (1) Zöllner, P., & Mayer-Helm, B. (2006) *J. Chromatogr. A* **1136**, 123–169
- (2) Maragos, C.M. (2006) *Mycotoxin Res.* **22**, 96–99
- (3) Wu, F. (2006) *Transgenic Res.* **15**, 277–289
- (4) Barug, D., Bhatnagar, D., van Egmond, H.P., van der Kamp, J.W., van Osenbruggen, W.A., & Visconti, A. (Eds) (2006) *The Mycotoxin Factbook*, Wageningen Academic, The Netherlands
- (5) Njapau, H., Trujillo, S., van Egmond, H.P. & Park, D.L. (Eds) (2006) *Mycotoxins and Phycotoxins Advances in Determination, Toxicology and Exposure, Management* Wageningen Academic, The Netherlands
- (6) Whitaker, T.B., Slate, A.B., Jacobs, M., Hurley, J.M., Adams, J.G., & Giesbrecht, F.G. (2006) *J. AOAC Int.* **89**, 1027–1034
- (7) Whitaker, T.B., Slate, A.B., Hurley, J.M., & Giesbrecht, F.G. (2007) *J. AOAC Int.* **90**, 778–785
- (8) Ozay, G., Seyhan, F., Yilmaz, A., Whitaker, T.B., & Slate, A.B. (2006) *J. AOAC Int.* **89**, 1004–1011
- (9) Vargas, E.A., Whitaker, T.B., Santos, E.A., Slate, A.B., Lima, F.B., & Franca, R.C.A. (2006) *J. AOAC Int.* **89**, 1021–1026
- (10) Oliveira, C.A.F., & Ferraz, J.C.O. (2007) *Food Control* **18**, 375–378
- (11) Shundo, L., & Sabino, M. (2006) *Brazil. J. Microbiol.* **37**, 164–167
- (12) Diaz, G.J., & Espitia, E. (2006) *Food Addit. Contam.* **23**, 811–815
- (13) Azizi, I.G., Khoushnevis, S.H., Norozi M., & Poya, N.D. (2007) Abstract 1005, XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey
- (14) Azizi, I.G., Khosravi, A., Hashemi, S.J., Sefidgar, S.A., Azimy, H., & Poya, N.D. (2007) Abstract 1004, XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey
- (15) Ghiasian, S.A., Maghsood, A.H., Neyestani, T.R., & Mirhendi, S.H. (2007) *J. Food Safety* **27**, 188–198
- (16) Kamkar, A. (2006) *Food Control* **17**, 768–775
- (17) Mohsenzadeh, M. (2007) Abstract 1399, XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey
- (18) Oveisi, M.R., Jannat, B., Sadeghi, N., Hajimahmoodi, M., & Nikzad, A. (2007) *Food Control* **18**, 1216–1218
- (19) Tajkarimi, M., Shojaee Aliabadi, F., Salah Nejad, M., Pursoltani, H., Motallebi, A.A., & Mahdavi, H. (2007) *Int. J. Food Microbiol.* **116**, 346–349
- (20) Decasteli, L., Lai, J., & Gramaglia, M. (2007) *Food Control* **18**, 1263–1266
- (21) Gallo, P., Salzillo, A., Rossini, C., Urbani, V., & Serpe, L. (2006) *Ital. J. Food Sci.* **18**, 251–259
- (22) Nachtmann, C., Gallina, S., Rastelli, M., Ferro, G.L., & Decastelli, L. (2007) *Food Control* **18**, 623–629
- (23) Al-Hamili, S., Dashti, B., Al-Omirah, H., Al-Sanki, S., & Sawaya, W. (2007) Abstract 1093, XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey
- (24) Cordova-Izquierdo, A., Saltijeral Oaxaca, J., & Ruiz Lang, G. (2007) *J. Animal Vet. Adv.* **6**, 194–197
- (25) Zinedine, A., González-Osnaya, L., Soriano, J.M., Moltó, J.C., Idrissi, L., & Mañes J. (2007) Abstract 1047, XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey
- (26) Atanda, O., Oguntubo, A., Adejumo, O., Ikeorah, J., & Akpan, I. (2007) *Chemosphere* **68**, 1455–1458
- (27) Raza, R. (2006) *J. Chem. Soc. Pakistan* **28**, 155–157
- (28) Offiah, N., & Adesiyun, A. (2007) *J. Food Prot.* **70**, 771–775
- (29) Ayar, A., Sert, D., & Con, A.H. (2007) *J. Food Safety* **27**, 199–207

- (30) Baskaya, R., Aydin, A., Yildiz, A., & Bostan, K. (2006) *Med. Weter.* **62**, 778–780
- (31) Baydar, T., Erkekoglu, P., Sipahi, H., & Sahin, G. (2007) *J. Food Drug Anal.* **15**, 89–92
- (32) Gurbay, A., Engin, A.B., Caglayan, A., & Sahin, G. (2006) *Ecol. Food Nutr.* **45**, 449–459
- (33) Tokuşoğlu, Ö., Akşit, S., Tansuğ, N., Dinç, G., & Özcan, C. (2007) Abstract 1462, XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey
- (34) Unusan, N. (2006) *Food Chem. Toxicol.* **44**, 1897–1900
- (35) Polychronaki, N., West, R.M., Turner, P.C., Amra, H., Abdel-Wahhab, M., Mykkänen, H., & El-Nezami, H. (2007) Abstract 1122, XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey
- (36) Colak, H., Hampikyan, H., Ulusoy, B., & Ergun, O. (2006) *Eur. Food Res. Technol.* **223**, 719–723
- (37) Rosi, P., Borsari, A., & Lasi, G. (2007) *Int. Dairy J.* **17**, 429–435
- (38) Salter, R., Douglas, D., Tess, M., Markovsky, B., & Saul, S.J. (2006) *J. AOAC Int.* **89**, 1327–1334
- (39) Liu, J., & Powers, S.P. (2006) Abstracts of papers, 232nd ACS National Meeting, San Francisco, CA, September 2006, p. AGFD–208
- (40) Tothill, I.E., Parker, C., & Heurich M. (2007) Abstract 1077, XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey
- (41) Cucci, C., Mignani, A.G., & Dall'Asta, C. (2006) Proceedings of SPIE-The International Society for Optical Engineering 6189, No. Optical Sensing II, pp 61892D/1–61892D/2
- (42) Van Eijkeren, J.C., Bakker, M.I., & Zeilmaier, M.J. (2006) *Food Addit. Contam.* **23**, 833–838
- (43) Arranz, I., Sizoo, E., van Egmond, H., Kroeger, K., Legarda, T.M., Burdaspal, P., Reif, K., & Stroka, J. (2006) *J. AOAC Int.* **89**, 595–605
- (44) Walting, A.E., & Wilson, D. (2006) *J. AOAC Int.* **89**, 678–692
- (45) Sobolev, V.S. (2007) *J. Agric. Food Chem.* **55**, 2136–2141
- (46) Móricz, A.M., Fatér, Z., Otta, K.H., Tyihák, E., & Mincsovics, E. (2007) *Microchem. J.* **85**, 140–144
- (47) Calleri, E., Marrubini, G., Brusotti, G., Massolini, G., & Caccialanza, G. (2007) *J. Pharmaceut. Biomed. Anal.* **44**, 396–403
- (48) Hu, Y.-Y., Zheng, P., Zhang, Z.-X., & He, Y.-Z. (2006) *J. Agric. Food Chem.* **54**, 4126–4130
- (49) Saha, D., Acharya, D., Roy, D., Shrestha, D., & Dhar, T.K. (2007) *Anal. Chim. Acta* **584**, 343–349
- (50) Ip, S.-P., & Che, C.-T. (2006) *J. Chromatogr. A* **1135**, 241–244
- (51) Cavaliere, C., Foglia, P., Guarino, C., Nazzari, M., Samperi, R., & Laganà, A. (2007) *Rapid Commun. Mass Spectrom.* **21**, 550–556
- (52) Trucksess, M., Weaver, C., Oles, C., D'Ovidio, K., & Rader, J. (2006) *J. AOAC Int.* **89**, 624–630
- (53) Ferracane, R., Tafuri, A., Logrieco, A., Galvano, F., Balzano, D., & Ritieni, A. (2007) *Food Addit. Contam.* **24**, 173–180
- (54) Ventura, M., Guillen, D., Anaya, I., Broto-Puig, F., Lliberia, J.L., Agut, M., & Comellas, L. (2006) *Rapid Commun. Mass Spectrom.* **20**, 3199–3204
- (55) Delmulle, B., De Saeger, S., Adams, A., De Kimpe, N., & Van Peteghem, C. (2006) *Rapid Commun. Mass Spectrom.* **20**, 771–776
- (56) Tanaka, H., Takino, M., Sugita-Konishi, Y., & Tanaka, T. (2006) *Rapid Commun. Mass Spectrom.* **20**, 1422–1428
- (57) Sulyok, M., Berthiller, F., Krska, R., & Schuhmacher, R. (2006) *Rapid Commun. Mass Spectrom.* **20**, 2649–2659
- (58) Arduini, F., Errico, I., Amine, A., Micheli, L., Palleschi, G., & Moscone, D. (2007) *Anal. Chem.* **79**, 3409–3415
- (59) Aghamohammadi, M., Hashemi, J., Kram, G.A., & Alizadeh, N. (2007) *Anal. Chim. Acta* **582**, 288–294
- (60) Akdoğan, E., Çökeller, D., Marcinauskas, L., Valatkevicius, P., Valincius, V., & Mutlu, M. (2006) *Surface Coatings Technol.* **201**, 2540–2546
- (61) Adányi, N., Levkovets, I.A., Rodriguez-Gil, S., Ronald, A., Váradi, M., & Szendrői, I. (2007) *Biosensors Bioelectronics* **22**, 797–802
- (62) Liu, Y., Qin, Z., Wu, X., & Jiang, H. (2006) *Biochem. Eng. J.* **32**, 211–217
- (63) Saha, D., Acharya, D., Roy, D., & Dhar, T.K. (2007) *Anal. Bioanal. Chem.* **387**, 1121–1130
- (64) Piermarini, S., Micheli, L., Ammida, N.H.S., Palleschi, G., & Moscone, D. (2007) *Biosensors Bioelectronics* **22**, 1434–1440
- (65) De Saeger, S., Sibanda, L., Paepens, C., Lobeau, M., Delmulle, B., Barna-Vetro, I., & Van Peteghem, C. (2006) *Mycotoxin Res.* **22**, 100–104
- (66) Goryacheva, I.Y., De Saeger, S., Delmulle, B., Lobeau, M., Eremin, S.A., Barna-Vetro, I., & Van Peteghem, C. (2007) *Anal. Chim. Acta* **590**, 118–124
- (67) Everley, R.A., Ciner, F.L., Zhan, D., Scholl, P.F., Groopman, J.D., & Croley, T.R. (2007) *J. Anal. Toxicol.* **31**, 150–156
- (68) Tavèar-Kalcher, G., Vrtaè, K., Pestevšek, U., & Vengut, A. (2007) *Food Control* **18**, 333–337
- (69) Sabzevari, O., Emrarian, I., Abdi, K.H., Abouhossein, G., & Amirahmadi, M. (2006) *Toxicol. Lett.* **164S**, S233
- (70) Brera, C., Catano, C., De Santis, B., Debegnach, F., De Giacomo, M., Pannunzi, E., & Miraglia, M. (2006) *J. Agric. Food Chem.* **54**, 5014–5019
- (71) Castells, M., Ramos, A.J., Sanchis, V., & Marín, S. (2007) *J. Agric. Food Chem.* **55**, 2760–2764
- (72) Toteja, G.S., Mukherjee, A., Diwakar, S., Singh, P., Saxena, B.N., Sinha, K.K., Sinha, A.K., Kumar, N., Nagaraja, K.V., Bai, G., Prasad, C.A., Vanchinathan, S., Roy, R., & Parkar, S. (2006) *J. Food Prot.* **69**, 1463–1467
- (73) El Adlouni, C., Tozlovanu, M., Naman, F., Faid, M., & Pfohl-Leszkowicz, A. (2006) *Mol. Nutr. Food Res.* **50**, 507–512
- (74) Zinedine, A., Juan, C., Soriano, J.M., Moltó, J.C., Idrissi, L., & Manes, J. (2007) *Int. J. Food Microbiol.* **115**, 124–127
- (75) Sugita-Konishi, Y., Nakajima, M., Tabata, S., Ishikuro, E., Tanaka, T., Norizuki, H., Itoh, Y., Aoyama, K., Fujita, K., Kai, S., & Kumagai, S. (2006) *J. Food Prot.* **69**, 1365–1370
- (76) Kaaya, A.N., & Kyamuhangire, W. (2006) *Int. J. Food Microbiol.* **110**, 217–223

- (77) Simas, M.M.S., Botura, M.B., Correa, B., Sabino, M., Mallmann, C.A., Bitencourt, T.C.B.S.C., & Batatinha, M.J.M. (2007) *Food Control* **18**, 404–408
- (78) Iamanaka, B.T., De Menezes, H.C., Vicente, E., Leite, R.S.F., & Taniwaki, M.H. (2007) *Food Control* **18**, 454–457
- (79) Chun, H.S., Kim, H.J., Ok, H.E., Hwang, J.-B., & Chung, D.-H. (2007) *Food Chem.* **102**, 385–391
- (80) Senyuva, H.Z., Gilbert, J., & Ulken, U. (2007) *J. Food Prot.* **70**, 1029–1032
- (81) Var, I., Kabak, B., & Gök, F. (2007) *Food Control* **18**, 59–62
- (82) Aydin, A., Erkan, M.E., Başkaya, R., & Ciftcioglu, G. (2007) *Food Control* **18**, 1015–1018
- (83) Giray, B., Girgin, G., Engin, A.B., Aydin, S., & Sahin, G. (2007) *Food Control* **18**, 23–29
- (84) Oruc, H.H., Cengiz, M., & Kalkanli, O. (2006) *Animal Feed Sci. Technol.* **128**, 337–341
- (85) Sangare-Tigori, B., Moukha, S., Kouadio, H.J., Betbeder, A.-M., Dano, D.S., & Creppy, E.E. (2006) *Food Addit. Contam.* (2006) **23**, 1000–1007
- (86) Liu, Z., Gao, J., & Yu, J. (2006) *J. Stored Prod. Res.* **42**, 468–479
- (87) Tam, J., Mankotia, M., Mably, M., Pantazopoulos, P., Neil, R.J., Calway, P., & Scott, P.M. (2006) *Food Addit. Contam.* **23**, 693–699
- (88) Ayalew, A., Fehrmann, H., Lepschy, J., Beck, R., & Abate, D. (2006) *Mycopathologia* **162**, 57–63
- (89) Paterson, R.R.M. (2007) *Food Control* **18**, 817–820
- (90) Cheraghali, A.M., Yazdanpanah, H., Doraki, N., Abouhossain, G., Hassibi, M., Ali-Abadi, S., Aliakbarpoor, M., Amirahmadi, M., Askarian, A., Fallah, N., Hashemi, T., Jalali, M., Kalantari, N., Khodadadi, E., Maddah, B., Mohit, R., Mohseny, M., Phaghithy, Z., Rahmani, A., Setoodeh, L., Soleimany, E., & Zamanian, F. (2007) *Food Chem. Toxicol.* **45**, 812–816
- (91) Czerwiecki, L., Wilczyńska, G., & Kwiecień, A. (2006) *Mycotoxin Res.* **22**, 159–162
- (92) Romagnoli, B., Menna, V., Gruppioni, N., & Bergamini, C. (2007) *Food Control* **18**, 697–701
- (93) Xu, L., & Du, L. (2006) *J. Microbiol. Meth.* **64**, 398–405
- (94) Yamagishi, D., Akamatsu, H., Otani, H., & Kodama, M. (2006) *J. Gen. Plant Pathol.* **72**, 323–327
- (95) Mansfield, M.A., Archibald, D.D., Jones, A.D., & Kulda, G.A. (2007) *Phytopathology* **97**, 504–511
- (96) Terminiello, L., Patriarca, A., Pose, G., & Pinto, V. F. (2006) *Mycotoxin Res.* **22**, 236–240
- (97) Solyakov, A., Stepinska, A., & Häggblom, P. (2007) Abstract 1225, XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey
- (98) Altemoeller, M., Podlech, J., & Fenske, D. (2006) *Eur. J. Org. Chem.* **7**, 1678–1684
- (99) Jiao, P., Gloer, J.B., Campbell, J., & Shearer C.A. (2006) *J. Nat. Prod.* **69**, 612–615
- (100) Xu, B.-J., Jia, X.-Q., Gu, L.-J., & Sung, C.-K. (2006) *Food Control* **17**, 271–285
- (101) Garon, D., Richard, E., Sage, L., Bouchart, V., Pottier, D., & Lebailly, P. (2006) *J. Agric. Food Chem.* **54**, 3479–3484
- (102) Lee, C.-L., Wang, J.-J., & Pan, T.-M. (2006) *J. AOAC Int.* **89**, 669–677
- (103) Tangni, E.K., & Pussemier, L. (2007) *J. Sci. Food Agric.* **87**, 1263–1270
- (104) Tangni, E.K., & Pussemier, L. (2006) *Food Addit. Contam.* **23**, 181–189
- (105) Moncoq, K., Trieber, C.A., & Young, H.S. (2007) *J. Biol. Chem.* **282**, 9748–9757
- (106) Larsen, T.O., Smedsgaard, J., Nielsen, K.F., Hansen, M.A.E., Samson, R.A., & Frisvad, J.C. (2007) *Med. Mycol.* **45**, 225–232
- (107) Giorni, P., Magan, N., Pietri, A., Bertuzzi, T., & Battilani, P. (2007) *Int. J. Food Microbiol.* **113**, 330–338
- (108) Duran, R.M., Cary, J.W., & Calvo, A.M. (2007) *Appl. Microbiol. Biotechnol.* **73**, 1158–1168
- (109) Mohamed, M., Gremaud, E., Richoz-Payot, J., Tabet, J., & Guy, P. (2006) *J. Chromatogr. A* **1114**, 62–72
- (110) Castegnaro, M., Tozlovanu, M., Wild, C., Molinié, A., Syla, A., & Pfohl-Leszkowicz, A. (2006) *Mol. Nutr. Food Res.* **50**, 480–487
- (111) Riley, R.T., Torres, O.A., & Palencia, E. (2006) *Food Addit. Contam.* **23**, 826–832
- (112) Kushiro, M., Tanaka, K., Miyazaki, S., & Nagata, T. (2006) *Curr. Pharm. Anal.* **2**, 289–297
- (113) Biselli, S. (2006) *J. Verbr. Lebens.* **1**, 106–114
- (114) Takatori, K., Aihara, M., & Sugita-Konishi, Y. (2006) *Bull. Natl. Inst. Health Sci. (Japan)* **124**, 21–29
- (115) Daško, L., Rauová, D., & Belajová, E. (2006) *J. Food Nutr. Res.* **45**, 127–133
- (116) Bartók, T., Szécsi, A., Szekeres, A., Mesterházy, A., & Bartók, M. (2006) *Rapid Commun. Mass Spectrom.* **20**, 2447–2462
- (117) Gelderblom, W.C.A., Sewram, V., Shephard, G.S., Snijman, P.W., Tenza, K., Van der Westhuizen, L., & Vleggaar, R. (2007) *J. Agric. Food Chem.* **55**, 4388–4394
- (118) Sewram, V., Shephard, G.S., Van der Merwe, L., & Jacobs, T.V. (2006) *J. Agric. Food Chem.* **54**, 5688–5693
- (119) Sharma, M. (2007) *Asian J. Chem.* **19**, 499–504
- (120) Sharma, M., Montes, R., Maroues, C., Bravo, L.L., & Flores, M.H.E. (2007) *Asian J. Chem.* **19**, 396–400
- (121) Zheng, M.Z., Richard, J.L., & Binder, J. (2006) *Mycopathologia* **161**, 261–273
- (122) Quan, Y., Zhang, Y., Wang, S., Lee, N., & Kennedy, I.R. (2006) *Anal. Chim. Acta* **580**, 1–8
- (123) Wang, S., Quan, Y., Lee, N., & Kennedy, I.R. (2006) *J. Agric. Food Chem.* **54**, 2491–2495
- (124) Li, X., Millson, S.H., Coker, R.D., & Evans, I.H. (2006) *Biotechnol. Lett.* **28**, 1955–1964
- (125) Liao, B.C., Jong, T.T., Lee, M.R., & Chang, C.M. (2007) *Rapid Commun. Mass Spectrom.* **21**, 667–673
- (126) Ngundi, M.M., Shriver-Lake, L.C., Moore, M.H., Ligler, F.S., & Taitt, C.R. (2006) *J. Food Prot.* **69**, 3047–3051
- (127) Vatinno, R., Aresta, A., Zambonin, C.G., & Palmisano, F. (2007) *J. Pharm. Biomed. Anal.* (in press, doi:10.1016/j.jpba.2007.04.008 **WHAT IS THIS?**)
- (128) Medina, A., Valle-Algarra, F.M., Gimeno-Adelantado, J.V., Mateo, R., Mateo, F., & Jiménez, M. (2006) *J. Chromatogr. A* **1121**, 178–183
- (129) Chung, S.W., & Kwong, K.P. (2007) *J. AOAC Int.* **90**, 773–777

- (130) Lobeau, M., De Saeger, S., Sibanda, L., Barna-Vetró, I., & Van Peteghem, C. (2007) *Food Addit. Contam.* **24**, 398–405
- (131) Muñoz, K., Vega, M., Rios, G., Muñoz, S., & Madariaga, R. (2006) *Food Chem. Toxicol.* **44**, 1884–1889
- (132) Pussemier, L., Piérard, J.Y., Anselme, M., Tangni, E.K., Motte, J.C., & Larondelle, Y. (2006) *Food Addit. Contam.* **23**, 1208–1218
- (133) Anselme, M., Tangni, E.K., Pussemier, L., Motte, J.C., Van Hove, F., Schneider, Y.J., Van Peteghem, C., & Larondelle, Y. (2006) *Food Addit. Contam.* **23**, 910–918
- (134) Chiodini, A.M., Scherpenisse, P., & Bergwerff, A.A. (2006) *J. Agric. Food Chem.* **54**, 7399–7404
- (135) Arino, A., Herrera, M., Estopan, G., & Juan, T. (2007) *Int. J. Food Microbiol.* **114**, 366–369
- (136) Lasram, S., Belli, N., Chebil, S., Nahla, Z., Ahmed, M., Sanchis, V., & Ghorbel, A. (2007) *Int. J. Food Microbiol.* **114**, 376–379
- (137) Serra, R., Mendonça, C., & Venâncio, A. (2006) *Int. J. Food Microbiol.* **111 Suppl 1**, S35–S39
- (138) Cecchini, F., Morassut, M., Garcia Moruno, E., & Di Stefano, R. (2006) *Food Microbiol.* **23**, 411–417
- (139) Li, F., Zhao, S., Chin, L., Li, Y., Wu, D., Zhao, X., Han, C., Zhang, H., & Ji, R. (2007) *J. AOAC Int.* **90**, 167–172
- (140) Katerere, D.R., Stockenström, S., Balducci, G., & Shephard G.S. (2007) *J. AOAC Int.* **90**, 162–166
- (141) De Champdoré, M., Bazzicalupo, P., De Napoli, L., Montesarchio, D., Di Fabio, G., Cocozza, I., Parracino, A., Rossi, M., & D'Auria, S. (2007) *Anal. Chem.* **79**, 751–757
- (142) Marin, S., Morales, H., Hasan, H.A., Ramos, A.J., & Sanchis, V. (2006) *Food Addit. Contam.* **23**, 1316–1322
- (143) Morales, H., Marín, S., Rovira, A., Ramos, A.J., & Sanchis, V. (2007) *Let. Appl. Microbiol.* **44**, 30–35
- (144) Morales, H., Marín, S., Centelles, X., Ramos, A.J., & Sanchis, V. (2007) *Int. J. Food Microbiol.* **116**, 260–265
- (145) Baert, K., De Meulenaer, B., Kamala, A., Kasase, C., & Devlieghere, F. (2006) *J. Food Prot.* **69**, 1371–1378
- (146) Katerere, D.R., Stockenström, S., Thembo, K.M., Balducci, G., & Shephard, G.S. (2007) *Food Addit. Contam.* **24**, 630–634
- (147) Karaca, H., & Nas, S. (2006) *Food Addit. Contam.* **23**, 502–508
- (148) Ricelli, A., Baruzzi, F., Solfrizzo, M., Morea, M., & Fanizzi, F.P. (2007) *Appl. Environ. Microbiol.* **73**, 785–792
- (149) Puel, O., Tadriss, S., Delaforge, M., Oswald, I.P., & Lebrihi, A. (2007) *Int. J. Food Microbiol.* **115**, 131–139
- (150) Baert, K., De Meulenaer, B., Verdonck, F., Huybrechts, I., De Henauw, S., Vanrolleghem, P.A., Debevere, J., & Devlieghere, F. (2007) *Food Chem. Toxicol.* (in press, doi:10.1016/j.fct.2007.03.008 **WHAT IS THIS?**)
- (151) Krska, R., Welzig, E., Drs, E., Josephs, R.D., Schothorst, R.C., van Egmond, H.P., Pettersson, H., Chan, D., & MacDonald, S. (2006) *J. AOAC Int.* **89**, 1573–1580
- (152) Lippolis, V., Pascale, M., & Visconti, A. (2006) *J. Food Prot.* **69**, 2712–2719
- (153) Stroka, J., Derbyshire, M., Mischke, C., Ambrosio, M., Kroeger, K., Arranz, I., Sizoo, E., & van Egmond, H. (2006) *J. AOAC Int.* **89**, 1012–1020
- (154) Sugita-Konsihi, Y., Tanaka, T., Tabata, S., Nakajima, M., Nouno, M., Nakaie, Y., Chonan, T., Aoyagi, M., Kibune, N., Mizuno, K., Ishikuro, E., Kanamaru, N., Minamisawa, M., Aita, N., Kushiro, M., Tanaka, K., & Takatori, K. (2006) *Mycopathologia* **162**, 239–243
- (155) Brenn-Struckhofova, Z., Cichna-Markl, M., Bohm, C., & Razzazi-Fazeli, E. (2007) *Anal. Chem.* **79**, 710–717
- (156) Maragos, C.M. (2006) *J. Food Prot.* **69**, 2773–2776
- (157) Ren, Y., Zhang, Y., Shao, S., Cai, Z., Feng, L., Pan, H., & Wang, Z. (2007) *J. Chromatogr. A* **1143**, 48–64
- (158) Rudrabhatla, M., & Wood, J.S. (2007) *Am. Lab.* **39**, 22–25
- (159) Haubl, G., Berthiller, F., Rechthaler, J., Jaunecker, G., Binder, E.M., Krska, R., & Schuhmacher, R. (2006) *Food Addit. Contam.* **23**, 1187–1193
- (160) Asam, S., & Rychlik, M. (2007) *Eur. Food Res. Technol.* **224**, 769–783
- (161) Maenetje, P.W., & Dutton, M.F. (2007) *Environ. Sci. Health, Part B* **42**, 229–236
- (162) Pan, D., Bonsignore, F., Rivas, F., Perera, G., & Bettucci, L. (2007) *Int. J. Food Microbiol.* **114**, 149–152
- (163) Kottapalli, B., Wolf-Hall, C., & Schwarz, P. (2006) *Int. J. Food Microbiol.* **110**, 224–231
- (164) Schollenberger, M., Muller, H.M., Rufle, M., Terry-Jara, H., Suchy, S., Plank, S., & Drochner, W. (2007) *Int. J. Food Microbiol.* **113**, 142–146
- (165) Palpacelli, V., Beco, L., & Ciani, M. (2007) *J. Food Prot.* **70**, 509–513
- (166) Omurtag, G.Z., & Beyoglu, D. (2007) *Food Control* **18**, 163–166
- (167) Rasmussen, P.H., Petersen, A., & Ghorbani, F. (2007) *Food Addit. Contam.* **24**, 315–325
- (168) Goyarts, T., Danicke, S., Valenta, H., & Ueberschar, K.H. (2007) *Food Addit. Contam.* **24**, 369–380
- (169) Bloom, E., Bal, K., Nyman, E., & Larsson, L. (2007) *J. Environ. Monitor.* **9**, 151–156
- (170) Mayer, S., Gareis, M., Degen, G.H., Blaszkewicz, M., Curtui, V., & Usleber, E. (2007) *Gefahrstoffe-Reinhalt. Luft* **67**, 119–125
- (171) Hartmann, N., Erbs, M., Wettstein, F.E., Schwarzenbach, R.P., & Bucheli, T.D. (2007) *J. Chromatogr. A* **1138**, 132–140
- (172) Songsermsakul, P., Sontag, G., Cichna-Markl, M., Zentek, J., & Razzazi-Fazeli, E. (2006) *J. Chromatogr. B* **843**, 252–261
- (173) Berthiller, F., Werner, U., Sulyok, M., Krska, R., Hauser, M.T., & Schuhmacher, R. (2006) *Food Addit. Contam.* **23**, 1194–1200
- (174) Briones-Reyes, D., Gomez-Martinez, L., & Cueva-Rolon, R. (2007) *Food Chem.* **100**, 693–698
- (175) Maragos, C.M., & Appell, M. (2007) *J. Chromatogr. A* **1143**, 252–257
- (176) Suzuki, T., Munakata, Y., Morita, K., Shinoda, T., & Ueda, H. (2007) *Anal. Sci.* **23**, 65–70
- (177) Bovee, T.F.H., Bor, G., Heskamp, H.H., Hoogenboom, R.L.A.P., & Nielen, M.W.F. (2006) *Food Addit. Contam.* **23**, 556–568
- (178) Urraca, J.L., Marazuela, M.D., Merino, E.R., Orellana, G., & Moreno-Bondi, M.C. (2006) *J. Chromatogr. A* **1116**, 127–134
- (179) Urraca, J.L., Marazuela, M.D., & Moreno-Bondi, M.C. (2006) *Anal. Bioanal. Chem.* **385**, 1155–1161

- (180) Navarro-Villoslada, F., Urraca, J.L., Moreno-Bondi, M.C., & Orellana, G. (2007) *Sens. Actuators B Chem.* **121**, 67–73
- (181) Martins, H.M., Guerra, M.M., & Bernardo, F. (2006) *Mycotoxin Res.* **22**, 206–210
- (182) Czerwiecki, L., Wilczynska, G., & Kwiecien, A. (2006) *Mycotoxin Res.* **22**, 159–162
- (183) Oliveira, G.R., Ribeiro, J.M., Fraga, M.E., Cavaglieri, L.R., Direito, G.M., Keller, K.M., Dalcero, A.M., & Rosa, C.A. (2006) *Mycopathologia* **162**, 355–362
- (184) Hanif, N.Q., Naseem, M., Khatoon, S., & Malik, N. (2006) *Pak. J. Sci. Ind. Res.* **49**, 120–124
- (185) Beg, M.U., Al-Mutairi, M., Beg, K.R., Al-Mazeedi, H.M., Ali, L.N., & Saeed, T. (2006) *Arch. Environ. Contam. Toxicol.* **50**, 594–602
- (186) Engelhardt, G., Barthel, J., & Sparrer, D. (2006) *Mol. Nutr. Food Res.* **50**, 401–405
- (187) Schollenberger, M., Mueller, H.M., Ruefle, M., Terry-Jara, H., Suchy, S., Plank, S., & Drochner, W. (2007) *Int. J. Food Microbiol.* **113**, 142–146
- (188) Hussaini, A.M., Timothy, A.G., Olufunmilayo, H.A., Ezekiel, A.S., & Godwin, H.O. (2007) *Afr. J. Biotechnol.* **6**, 99–108
- (189) Leung, M.C.K., Diaz-Llano, G., & Smith, T.K. (2006) *J. Agric. Food Chem.* **54**, 9623–9635
- (190) Kabak, B., Dobson, A.D.W., & Var, I. (2006) *Crit. Rev. Food Sci. Nutr.* **46**, 593–619
- (191) Zinedine, A., Soriano, J.M., Molto, J.C., & Manes, J. (2007) *Food Chem. Toxicol.* **45**, 1–18
- (192) Tiemann, U., & Danicke, S. (2007) *Food Addit. Contam.* **24**, 306–14 (PLEASE CHECK ENDING PAGE NUMBER)