

## GENERAL REFEREE REPORTS

## Committee on Natural Toxins and Food Allergens

### Mycotoxins

#### MARY W. TRUCKSESS

U.S. Food and Drug Administration, 5100 Paint Branch Pkwy, College Park, MD 20740, Tel: +1-301-436-1957 (office), +1-301-436-1522 (laboratory), Fax: +1-301-426-2644, E-mail: mtruckse@cfsan.fda.gov

Topic Advisors: David Avramson, Joe Dorner, Robert M. Eppley, Winston M. Hagler, Benedicte Hald, Chris Maragos, Myrna Sabino, Michele Solfrizzo, Hans P. van Egmond, George M. Ware, Thomas B. Whitaker, David M. Wilson

#### Summary

It is always a happy occasion to have one of our colleagues being honored and recognized for his/her achievement. Thomas B. Whitaker, our Sampling and Subsampling Topic Advisor, received the Peanut Research and Education award from the American Peanut Council for his contributions in developing an Origin Certification Program with several key European Union (EU) countries which import U.S. peanuts. We offer Tom our congratulations!

Last year various changes in policies were adopted by AOAC. The implementation of new rules and involvement of new people resulted in last year's General Referee report of Mycotoxins not being ready in time for publication in the *J. AOAC Int.* in January. Some AOAC terminology has been redefined. Associate Referees are now called Topic Advisors. Those who conduct interlaboratory studies are called Study Directors.

Since the occurrence of terrorist attacks in the U.S. in September 2001, in New York City and the Pentagon in Washington, DC, as well as the spreading of anthrax through the mails, many different methods of terrorism are being considered, including poisoning the food supply by various methods. Some of the mycotoxins are included on the list of possible toxins. Further information on food safety was presented at a symposium, "Mycotoxins and Food Safety," 220th American Chemical Society National Meeting, Washington, DC. The proceedings have been published in a book (1).

The Codex Alimentarius Commission (CAC) of the United Nations Food and Agricultural Organization (FAO), through the Codex Committee on Food Additives (CCFAC) is focused on harmonization of regulatory limits for mycotoxins and other contaminants that will protect the consumers as well as facilitate fair international trade practices. At the 34th CCFAC (March 2002) it was agreed to forward to CAC the following documents:

(1) The draft maximum level of 5 µg/kg for ochratoxin A in raw wheat, barley, and rye and derived products for final adoption at step 8 by CAC in July 2003.

(2) The draft maximum level of 50 µg/kg for patulin in apple juice and apple juice ingredients for adoption at step 8 by CAC in July 2003.

At the same meeting CCFAC agreed to forward to the Codex Executive Committee (CEC) 2 draft codes of practice: (1) The prevention (reduction) of mycotoxin contamination in cereals, including Annexes on ochratoxin A, zearalenone, fumonisins and trichothecenes. (2) The reduction of patulin contamination in apple juice, apple juice ingredients, and other beverages.

Both documents were adopted by the CEC at step 5 in June 2002. Currently discussion papers on aflatoxins in tree nuts and deoxynivalenol in cereals are being prepared by CCFAC.

Along with the advance of analytical technology comes pressure for laboratory accreditation and more stringent quality assurance programs with short turn around time for analysis. These factors have resulted in rapid acceleration in mycotoxin research, with numerous publications and many interlaboratory collaborative studies. With the increasing cost of disposing of waste solvents there is great effort to develop biotechnology-based methods to minimize the use of organic solvents. It is not unexpected that all of the recent publications and many completed collaborative studies involve biotechnology-based techniques. Most of the adopted First Action Official Methods for mycotoxins utilize antibodies for separation and isolation and some are included in the recently published 17th Ed. of *Official Methods of Analysis of AOAC INTERNATIONAL*. The disposal of large quantities of leftover test samples and the unused extracted test portions is a problem and requires the development of innovative techniques for reliable analysis of smaller samples or of development of safe methods for disposal of leftover products.

During 2001, the following 3 methods were approved as Official First Action:

(1) **2001.01** Determination of Ochratoxin A in Wine by Immunoaffinity Column Cleanup and LC

(2) **2001.04** Determination of Fumonisin B<sub>1</sub> and B<sub>2</sub> in Corn and Cornflakes by LC and Immunoaffinity Cleanup

(3) **2000.06** Veratox for Fumonisin

In 2002, a method was evaluated for possible Official First Action Method adoption: Determination of Aflatoxin in Animal Feed by Immunoaffinity Column Cleanup and LC with Post-Column Derivatization.

In addition 5 other collaborative studies are in progress.

## Topic Advisor Reports

### *Sampling and Subsampling*

Topic Advisor Thomas B. Whitaker, U.S. Department of Agriculture, Agricultural Research Service, PO Box 7625, North Carolina State University, Raleigh, NC 27695-7625, Tel: +1-919-515-6731, Fax: +1-919-515-7760, E-mail: whitaker@eos.ncsu.edu. Reported that preliminary discussions concerning the design of aflatoxin sampling plans for tree nuts were initiated at the March 2002 meeting of the Codex Committee on Food Additives and Contaminants in The Netherlands. It was decided to take no action on this subject until the position paper on aflatoxin contamination in tree nuts is finalized.

The U.S. peanut industry in cooperation with U.S. Department of Agriculture (USDA) has developed an Origin Certification Program with several key European Union (EU) countries that import U.S. peanuts into Europe. These key markets, in a memorandum of understanding, have agreed to recognize the sampling and testing of U.S. peanuts for aflatoxin before being exported to these markets. Documents showing positive lot identification and aflatoxin test results can be used to certify that the peanuts meet EU aflatoxin regulations. As a result, U.S. peanuts are now only subjected to spot surveillance at the ports of entry into the United Kingdom (UK) and The Netherlands. This achievement was recognized by the peanut industry in 2002 when the American Peanut Council (APC) presented Thomas Whitaker and the other developers of the Origin Certification Program with the APC Peanut Research and Education award.

### *Aflatoxin M<sub>1</sub>*

Topic Advisor Hans P. van Egmond, National Institute of Public Health and the Environment, Laboratory for Residue Analysis, Postbok 3, PO Box 1, 3720 BA Bilthoven, The Netherlands, Tel: +31-30-2742440, Fax: +31-30-2744403, E-mail: hp.van.egmond@rivm.nl. Reported that the 56th report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA; 2), which presented the conclusions of a joint expert committee convened to assess the risks associated with the consumption of food contaminated with certain mycotoxins, contains a 102 page chapter about aflatoxin M<sub>1</sub>. This comprehensive and up-to-date review includes sections on biological data, analytical methods, sampling protocols, effects of processing, levels and patterns of contamination of food commodities, food consumption and dietary intake estimates, prevention and control and dose-response relationship and estimation of carcinogenic risk. As for the latter subject, the expert committee concluded that, with worst case assumptions, the additional risks for liver cancer predicted when a limit of 0.05 µg/kg milk would be raised to 0.5 µg/kg milk are very small. For example, in a population with a prevalence of hepatitis B virus infection of 1%, the additional numbers of liver cancer cases associated with contamination of all milk at 0.5 µg/kg versus 0.05 µg/kg would be 29 cancers/1000 million persons per year.

Whether the JECFA assessment has any influence on national and regional regulations for aflatoxin M<sub>1</sub> is unclear as yet. An updated FAO Food and Nutrition Paper on mycotoxin regulations, including regulations on aflatoxin M<sub>1</sub> in milk and milk products, is in preparation, to appear in 2003.

In the EU, where aflatoxin M<sub>1</sub> limits in milk remain stringent, the Institute for Reference Materials and Measurements of the European Commission (Geel, Belgium) is in the process of preparing 3 new batches of reference milk powders, certified for their aflatoxin M<sub>1</sub> content. These milk powder certified reference materials (CRMs) are intended to replace the exhausted supplies of BCR CRMs 282, 283, 284, and 285.

Immunoaffinity cleanup in combination with LC and fluorescence detection remains a major technique in aflatoxin M<sub>1</sub> determination. A method description, based on these principles has been included in the book "Mycotoxin Protocols" (3). Other techniques applied in practice involve ELISA and, incidentally, TLC. Recently published surveys for aflatoxin M<sub>1</sub> in milk and milk products were from Austria (4), Brazil (5), Egypt (6), Italy (7-10), India (11), Kuwait (12), Turkey (13) and the United Kingdom (14). With the exception of Egypt and India the levels found were low, usually fulfilled legal requirements, and were not of health concern. In contrast, in Egypt and India, levels of aflatoxin M<sub>1</sub> in milk were reported as high as 6.3 and 48 µg/kg. For the survey in India, CRMs were used to verify the reliability of the analytical procedure. If correct, these analysis results point to a potentially high exposure of humans to aflatoxin M<sub>1</sub>, as well as to very high levels of aflatoxin B<sub>1</sub> in the cow's feedstuffs.

### *Aflatoxin Methods*

Topic Advisor David M. Wilson, University of Georgia, Department of Plant Pathology, Coastal Plain Section, Tifton, GA 31793, Tel: +1-912-386-3368, Fax: +1-912-386-7285, E-mail: dwilson@tifon.cpes.peachnet.edu. Reported that several interesting developments relating to aflatoxin assays appeared in late 2000 and 2001. There were 2 reports on aflatoxin-producing fungi in culture. The first report by Fente et al. (15) was on the addition of cyclodextrin to culture media used for testing aflatoxin production. The incorporation of the cyclodextrin resulted in an enhanced fluorescence around cultures that produced aflatoxin. The other culture related paper reported that an ELISA technique had been developed for the aflatoxin producing fungi (16). The ELISA could be used to detect the aflatoxin producing fungi before any aflatoxin was detectable. A modified chick embryotoxicity screening test was developed for testing the effects of aflatoxin using a bioassay (17). Transmittance (500 to 900 nm) and reflectance spectroscopy (550 to 1700 nm) were used to detect aflatoxin in single corn kernels (18). More than 95% of the kernels were correctly classified as having high (>100 µg/kg) or low (<10 µg/kg) contamination based on correlations with analytical data. This technology may be valuable in designing rapid screening detection methods in corn. Younis and Malik (19) compared the efficiency of extraction procedures and LC versus TLC for aflatoxin estimation in peanut and peanut products. LC gave better precision than TLC in these tests. A

rapid multifunctional column method for aflatoxins in spices was developed, the recovery of the aflatoxins was 80–85%, and the minimum detectable concentration was 0.5 µg/kg for each aflatoxin (20). Chiavaro et al. (21) developed a method to detect aflatoxins by adding cyclodextrins to the LC eluent. Succinyl-β-cyclodextrin gave the greatest fluorescence enhancement for B<sub>1</sub> and M<sub>1</sub> while dimethyl-β-cyclodextrin showed better results for G<sub>1</sub>. Detection limits lower than 0.3 µg/kg were achieved for the 4 major aflatoxins, while M<sub>1</sub> could be detected at much lower concentrations.

New aflatoxin assay approaches include a fluorescence polarization assay for the determination of aflatoxins in grain (22), a novel capillary electrokinetic chromatography method using multiphoton-excited fluorescence (23), a method for determining aflatoxins in groundnut and groundnut cake using Fourier transform infrared spectroscopy (24), and a method for multiple mycotoxin analysis using LC separation of extracts followed by electrospray ionization/mass spectrometry/mass spectrometry (ESI-MS-MS) detection (25). These new methodologies will most likely lead to further analytical advances for aflatoxin determination. A novel immunoassay was developed for B<sub>1</sub> using a rapid colorimetric sequential injection immunoassay with a jet flow cell packed with polymethylacrylate beads (26). The 15 min assay could detect aflatoxin B<sub>1</sub> at levels as low as 0.2 µg/L in spiked test sample extract, which is comparable to microtitre plate ELISA methods. Ho and Wauchope (27) reported a strip liposome immunoassay for aflatoxin B<sub>1</sub> (27). The technique could be used to detect as little as 20 ng B<sub>1</sub> and could serve as a useful visual or densitometric screening method. One collaborative study on the determination of aflatoxin B<sub>1</sub> using immunoaffinity column cleanup followed by LC with post-column bromination to enhance the fluorescence of B<sub>1</sub> was reported in 2001 (28). Samples of infant formula were spiked with 0.1 and 0.2 µg/kg and recoveries ranged from 92 to 101%. The method had acceptable within- and between-laboratory precision at the target levels.

### *Alternaria Toxins*

Topic Advisor Michele Solfrizzo, Institute of Science of Food Production, National Research Council, Viale L. Einaudi 51, Bari 70125, Italy, Tel: +39-080-5912838, Fax: +39-080-5486063, E-mail: m.solfrizzo@area.ba.cnr.it. Reported that alternariol (AOH), alternariol methyl ether (AME), and altertoxin I (ATX-I) added to fruit juices and white wine were found to be sufficiently stable at room temperature for a period of 8–27 day periods (29). Analytical methods and natural occurrence of mycotoxins produced by *Alternaria alternata* have been reviewed recently (30). Most of the reviewed methods use solvent partition for the cleanup of crude extracts especially for the analysis of tenuazonic acid (TeA). Solid-phase extraction columns are used for the analysis of AOH and AME and also for ATX-I in fruit juices, tomato paste, and wine. Reversed-phase liquid chromatography with UV, fluorescence, electrochemical, and recently MS detection is mainly used for separation and determination of these mycotoxins.

High levels of AOH, up to 12 700 µg/kg, and AME, up to 5390 µg/kg, were found in 4 out of 100 feed samples collected in Poland (31). Natural occurrence of TeA, up to 111 µg/kg, has been reported in 7 out of 80 samples of tomato products collected in Brazil; 3 samples contaminated with TeA also contained cyclopiazonic acid, up to 134 µg/kg (32). TeA was found in 3 out of 49 samples of moldy kokonte, a cassava product from Ghana, at levels of 20–340 µg/kg, although no relevant toxigenic fungi were isolated from contaminated samples (33).

Production of AOH and AME by toxigenic strains of *A. alternata* grown on surface disinfected grapes and apples stored at various temperatures (4–21°C) for 3 weeks has been demonstrated. The production of TeA and ATX-I was not investigated (34).

### *Citrinin*

Topic Advisor David Abramson, Agriculture and Agri-Food Canada, Cereal Research Center, 195 Dafoe Rd, Winnipeg MB R3T 2M9, Canada, Tel: +1-204-984-5536, Fax: +1-204-983-4604, E-mail: dabramson@em.agr.ca. Reported that citrinin has been detected in foods from Bulgarian villages having a high incidence of endemic nephropathy. Some cereals collected from these villages contained substantial amounts of citrinin (up to 420 µg/kg), and 6 samples were co-contaminated with ochratoxin A (35). In a related study, composite weekly diet samples were assayed. Citrinin levels between 1 and 6 µg/kg were found in the diet of 11–19 healthy volunteers. Three of the volunteers had a total weekly citrinin intake between 9 and 40 µg, and one had a maximum intake close to 100 ng/kg BW/day (36).

In studies on citrinin production in Spanish cheeses by *P. citrinum*, eugenol and thymol were evaluated for their mycotoxin inhibition properties (37). In Arzua-Ulloa cheese, no citrinin was detected at a concentration of 150 µg/L of eugenol, but citrinin was detected after 5 days in the case of thymol at the same concentration. In Cebreiro cheese, neither eugenol nor thymol at similar concentrations prevented the production of citrinin.

In studies involving German silage-type feeds, *Monascus ruber* was found in 43/233 samples; of these *Monascus*-positive samples, citrinin was found in 14 at levels up to 62.5 µg/kg (38). In other studies on toxigenic fungi, isolates of *Penicillium expansum* from Portuguese grapes were screened for citrinin production. All isolates produced citrinin in yeast extract–sucrose medium, while one produced this toxin in a grape juice medium (39).

### *Cyclopiazonic Acid*

Topic Advisor Joe Dorner, USDA, ARS, National Peanut Research Laboratory, PO Box 509, 1011 Forrester Dr, SE, Dawson, GA 31742, Tel: +1-912-995-7408, Fax: +1-912-995-7416, E-mail: jdorner@nprl.usda.gov. Reported that there continues to be interest in cyclopiazonic acid (CPA). Co-occurrence of CPA and aflatoxins was found in 2 of 50 samples of Argentine peanuts analyzed (40). Samples were

extracted with methanol–2% sodium hydrogen carbonate (7 + 3), defatted with hexane, and partially purified by liquid–liquid partition. TLC was used to quantify CPA and aflatoxins. In the contaminated samples, CPA concentrations were 493 and 4300  $\mu\text{g}/\text{kg}$ , respectively, and the totals of aflatoxins B<sub>1</sub> and G<sub>1</sub> were 518 and 1250  $\mu\text{g}/\text{kg}$ , respectively.

Monaci et al. (41) reported a solid-phase microextraction (SPME) technique for determination of CPA in cheese samples. Cheese was extracted with methanol by sonication, evaporated to dryness, dissolved in phosphate buffer (pH 2.8), and subjected to SPME interfaced with LC and diode array detection. Recoveries from cheese samples spiked at 100 and 1000  $\mu\text{g}/\text{kg}$  were 82 and 86%, respectively, with a detection limit of 7  $\mu\text{g}/\text{kg}$ . Analysis of white surface cheese samples showed concentrations of CPA ranging from 20 to 80  $\mu\text{g}/\text{kg}$ .

A new LC method was reported allowing a very low detection limit for CPA of 25 pg (42). The method utilizes amino-bonded silica as the stationary phase, a mobile phase of acetonitrile–50mM ammonium acetate (80 + 20; pH 5), and UV diode array detection. The method was applied to analysis of fungal cultures, but was not applied to the analysis of food or commodities. This LC method in combination with electrospray ionization tandem mass spectrometry was used to quantify CPA in milk samples (43). Milk was extracted with methanol–2% sodium hydrogen carbonate (7 + 3), defatted with hexane, partitioned after acidification into chloroform, and evaporated to dryness. Residues were dissolved in methanol and subjected to LC analysis. The selective reaction monitoring acquisition mode was used for MS detection of CPA. Recoveries of CPA from spiked milk samples were consistent at 95–96% over a range of 5–80  $\mu\text{g}/\text{L}$ . Three of 20 milk samples were found to be naturally contaminated with CPA, but concentrations were not reported.

Natural contamination of tomato products with CPA has been reported for the first time. Eighty samples of tomato products were analyzed by LC with diode array detection, and CPA was found in 6 samples of pulp (64–178  $\mu\text{g}/\text{kg}$ ) and 2 samples of puree (36–117  $\mu\text{g}/\text{kg}$ ). Co-occurrence of CPA with tenuazonic acid was found in 2 samples of puree and one of pulp (32).

### **Ergot Alkaloids**

Topic Advisor George M. Ware, U.S. Food and Drug Administration, 60 8th St, Atlanta, GA 30309, Tel: +1-404-347-2131 ext. 5215, Fax: +1-404-347-4225, E-mail: gware@ora.fda.gov. Reported that 2 papers were published for the determination of ergot alkaloids. A semi-quantitative competitive enzyme-linked immunosorbent assay (ELISA) was used to determine ergot alkaloids in seed and straw samples (44). Straw samples had an average coefficient of variation (CV) of 10.2% within-runs and 18.4% between-runs and the seed samples had an average CV of 13.3% within-runs and 24.5% between-runs. An LC method for the determination of ergot alkaloids in cereal grains was reported in a recent publication (45).

### **Fumonisin**

Topic Advisor Chris Maragos, USDA, ARS National Center for Agricultural Utilization Research, 1815 N. University St, Peoria, IL 61604, Tel: +1-309-684-6266, Fax: +1-309-681-6689, E-mail: maragocm@mail.ncaur.usda.gov. Reported that fumonisins were a very active field of study in the past year, particularly in the areas of toxicology and occurrence. Previous studies have indicated that fumonisins occur worldwide, especially in maize. Fumonisin was found in maize, or maize products, in Taiwan (46), Turkey (47), Guangxi, China (48), Brazil (49, 50), Korea (51, 52), Argentina (53), and Zimbabwe (54). Co-occurrence of fumonisins and aflatoxins was reported in several of the studies. A method for evaluating the performance of sampling plans for fumonisins in maize was developed by Whitaker et al. (55).

Fumonisin was also found in matrices other than maize, such as black tea and medicinal plants (56). Using an isolated-perfused bovine udder fumonisin B<sub>1</sub> (FB<sub>1</sub>) was found to carry over from feed into milk at very low levels (57). LC–ESI–MS was used to detect fumonisins in asparagus spears and garlic bulbs infected with *Fusarium proliferatum* (58). Sewram et al. (59) developed an LC–ESI–MS method for fumonisins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and their hydrolysis products in the hair of vervet monkeys and rats. FB<sub>1</sub> was confirmed to be present in hair of monkeys and rats exposed to fumonisins through contaminated feed. Therefore samples of hair may be useful as indicators of long term exposure to dietary fumonisins. The use of LC–MS for detection of fumonisins in cereal grains was recently reviewed by Musser et al. (60).

The fumonisins are relatively stable compounds and the extent to which they can be affected by food processing has a direct bearing on potential exposure of humans to contaminated food. Fumonisin was found in dry-milled fractions of maize in Argentina (61). FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub> were found in flour. Concentrations in the germ and bran were roughly 3-fold higher than in the whole kernel corn. Bullerman et al. (62) described the effect of processing temperature on fumonisin stability. Losses of fumonisins occurred at  $\leq 125^\circ\text{C}$  but were greatest at temperatures  $> 175^\circ\text{C}$ . Addition of glucose to corn muffins and extrusion mixes also increased losses of fumonisins during baking or extrusion. De Girolamo et al. (63) observed significant losses (60 to 70%) in fumonisin content of maize as it was extruded and roasted to produce corn flakes. A reaction product of FB<sub>1</sub> with D-glucose, N-(1-deoxy-D-fructos-1-yl) FB<sub>1</sub>, is believed to be the first stable product formed after the Amadori rearrangement of the Schiff base (64). Fumonisin levels were also reduced significantly during the commercial process used to produce fried tortilla chips (65). The nixtamalization and rinsing steps of the process accounted for much of the loss. The effects of processing on fumonisin content were reviewed by Saunders et al. (66).

Analysis of commodities and foods for fumonisins encompasses a variety of methods. Most of the articles describing the occurrence of fumonisins or surveys for fumonisins use instrumental methods such as LC with fluorescence detection

(generally of the *o*-phthalaldehyde/mercaptoethanol derivative) or enzyme linked immunosorbent assay (ELISA). The Methods Committee on Natural Toxins and Food Allergens of AOAC INTERNATIONAL recommended First Action approval of an ELISA method for fumonisins in corn. A collaborative study of the method, which uses a commercially available test kit (Veratox for fumonisin, Neogen Corp., Lansing, MI) was recently published (67). Established LC methods use one or more solid-phase extraction steps to isolate the fumonisins before determination. An automated method for cleanup and analysis of corn and corn-based feed using C18 columns was also described (68). An immunoaffinity column (IAC) method for the cleanup of corn and corn flakes was collaboratively studied (69) and recommended for First Action approval by the Methods Committee on Natural Toxins and Food Allergens.

### *Ochratoxins*

Topic Advisor Benedicte Hald, Royal Veterinary and Agricultural University, Department of Veterinary Microbiology, 13 Bulowsvej, Friedriksberg C, Copenhagen 1870-DK, Denmark, Tel: +45-3528-2760, Fax: +45-3528-2757, E-mail: vetmi@kvf.dk. Reported that there were several interesting studies in 2001–2002 that are relevant to ochratoxin A (OA) analysis. The review of methodology for OA detection by P.M. Scott addressed reversed-phase liquid chromatography (LC) with detection by fluorescence (excitation 330–340 nm, emission 460–470 nm) detection methods or, more recently, tandem mass spectrometry, but ELISA methods are also available (70). Different analytical methods for determination of OA in wine have been compared (71); sample cleanup was based on solid-phase extraction with immunoaffinity or RP-18 sorbent materials applying different experimental protocols. The accuracy, repeatability, and reproducibility characteristics of an LC method for the determination of OA in wine and beer were established in a collaborative study involving 18 laboratories in 10 countries, using immunoaffinity column cleanup and fluorometric detection (72). A study to validate novel field immunoassay for rapid mycotoxin detection was published (73). Some of the new approaches to OA include work on a C-18 cartridge to achieve a 100-fold sample concentration followed by LC on a C-18 column with gradient elution and quantitation at 333 nm by means of a photodiode array detector and determination of OA using gas chromatography with mass selective detection monitoring 8 specific ions (74). The last method is not suitable for routine quantitation but is potentially useful as a confirmatory tool for samples with OA greater than 0.1 µg/L. Molecular imprinted polymers displaying selective binding properties for OA in polar/protic media were prepared (75). The possibility of solvent-dependent tuning of substrate selective/affinity and the high binding capacity recommend the developed molecularly imprinted polymers as promising solid-phase extraction adsorbents for cleanup and preconcentration of OA from various biologically relevant matrixes.

A thin-layer chromatographic screening method was developed for detection of OA in green coffee at a control level

of 10 µg/kg (76) and validation by comparing results measured by a quantitative immunoaffinity/LC method. This screening method is very rapid, simple, robust, and inexpensive. A simple method for determination of OA in pig blood serum was presented (77). It fulfills the criteria for a routine method and could be a suitable tool for surveying OA in pig herds and in slaughtered pigs.

During the past year there was report of occurrence of OA in Brazilian coffee (78), Finnish cereal samples in 1998 (79), Romanian slaughtered pigs (80), pig liver-derived patés (81), cereal-based baby foods (82), grapes and musts from France (83), and airborne dust from fungal conidia (84).

### *Patulin*

Topic Advisor Myrna Sabino, Instituto Adolfo Lutz, Dr Arnaldo 355, 355-CEP, São Paulo 01246-902, Brazil, Tel: +55-11-3068-2921, Fax: +55-11-853-3505, E-mail: mysabino@ial.sp.gov.br. Reported recent publications on patulin. A survey of patulin was conducted, from 1996 to 1998 on 60 locally produced commercial apple products purchased from retail outlets in South Africa (85). The incidence of patulin found was low indicating that the fruit quality was high and the manufacturing practices good. The AOAC Method to determine patulin in apple juice was validated for apple juice and fruit puree by LC, C-18 column, and UV detection (86). For juice, mean recoveries of 82.5% and CV of 3.33% were obtained at a level of 50 µg/L with limits of detection and quantitation of 1.72 and 5.2 µg/L, respectively. Purees were diluted and centrifuged prior to analysis. For mangoes an additional purification column was proposed.

Various activated carbons were tested for removal of patulin from apple juice of different Brix levels and at different temperature (87). At a carbon dosage level of 1 g/L, 70 and 80% of patulin was removed by 2 commercial steam activated carbons in a 12 Brix juice at 12 and 55°C, respectively. At higher Brix, more carbon was required for the same removal efficiency. In a second study (88) activated charcoal efficiently reduced patulin in apple juice at 62.3 µg/L. No patulin was found in apple juice aroma after distillation (89). In clarification processes for apple juice the patulin is bound to the solid substrate, filter cake, pellet or sediment, which if used for animal feed might be harmful (90).

A survey of patulin in Turkish apple juice was conducted by Yurdun et al. (91). Levels above the regulatory limit of 50 µg/L were found in 44% of 45 samples tested. Levels of 19.1 to 733 µg/L were found. In another study of 351 apple samples of 7 varieties with small rotten areas, 20% were found on TLC analysis to be contaminated with both patulin and citrinin, while 69% were contaminated with only patulin and 4% with only citrinin (92). The contamination levels of patulin and citrinin were 120–130 and 15–20 µg/kg, respectively. Of a total of >1000 apple juice samples analyzed in the United States (93), half were contaminated with patulin but only 10% showed levels >50 µg/L. Identity of patulin in some test samples was confirmed by GC–MS.

The use of modified atmospheres and packing on the growth of *Penicillium expansum* was studied. Packing in

polypropylene (PE) did not inhibit fungal growth (94). PE inhibited patulin production in atmospheric gas and 58% CO<sub>2</sub>/42% N<sub>2</sub>. Patulin production in PE-packaged apples was almost completely inhibited by the 2 gas combinations. The fate of patulin in the presence of the yeast *Saccharomyces cerevisiae* was investigated (95). Patulin was degraded but not aerobically. Polar patulin degradation products were formed which remained in the clarified fermented cider. Patulin did not appear to bind to yeast cells or apple juice sediment.

### *Trichothecenes*

Topic Advisor Robert M. Eppley, U.S. Food and Drug Administration, 5100 Paint Branch Pkwy, College Park, MD 20740, Tel: +1-301-436-1951, Fax: +1-301-436-2644, E-mail: reppley@cfsan.fda.gov. Reported that the interest in methods for the determination of trichothecenes in grains continues to dominate the literature. Several reports present updated procedures (96–100) based on gas–liquid chromatography (GLC). Three of these publications investigated the use of mass spectrometry detection. In a review of these methods, Lombaert (96) preferred to use GLC–negative ion chemical ionization/mass spectrometry (GLC–NICI/MS) to determine 8 of the more significant trichothecenes in a variety of commodities. Another report (97) compares GC–MS and an electronic nose. Analyses of off-odors using the electronic nose were used to determine if the off-odors detected in grains could be used to indicate the presence of mycotoxins. Deoxynivalenol was one of the mycotoxins evaluated. GC–tandem MS (98) was used for rapid screening of trichothecenes in fungal cultures. Two other GC methods were noted, one used flame ionization detection (FID) of the trimethylsilyl derivative (99) of both type A and B trichothecenes and the other group preferred the heptafluorobutyrate derivative with electron capture detection (ECD) for the determination of type B trichothecenes (100).

A rapid immunoassay kit for T-2 toxin was evaluated by collaborative study (73). The immunoassay procedure uses a flow-through device and the analysis can be performed in less than 15 min. Another rapid immunoassay uses the principle of fluorescence polarization immunoassay (101, 102) for determination of deoxynivalenol in wheat.

Surveys for the presence of various feed and food commodities were noted (103–105). In 2 of the reports (103, 104), DON was the most frequently found trichothecene, although both type A and B trichothecenes were detected. A fairly high incidence of T-2 toxin, 23–31%, was found in cereals and pulses from Turkey (105). The maximum level detected was 1.9 mg/kg; however, TLC was the method of confirmation.

### *Zearalenone*

Topic Advisor Winston M. Hagler, Jr, North Carolina State University, College of Agriculture and Life Sciences, Department of Poultry Science, Mycotoxin Laboratory, PO Box 7608, Raleigh, NC 27695-7608, Tel: +1-919-515-3228, Fax: +1-919-515-2625, E-mail: winston\_hagler@ncsu.edu. Summarizes some highlights appearing in recent literature.

Several reviews on zearalenone were published. A general review as part of the Paul E. Nelson Memorial Symposium (106), and a review of processing effects on zearalenone concentrations in various grain milling fractions, which included a very relevant section on chemical detoxification (107), were presented. The latest methodology for analysis of estrogenic mycotoxins in cereals was recently reviewed (108).

No deleterious effect was found when crystalline zearalenone was administered 7 mg/day in ethanol (7 mL) per os mares beginning 10 days after ovulation until the next ovulation (109). Periods between ovulations, luteal and follicular development (size, growth, number), uterine edema measurements, and plasma profiles of progesterone were unaffected by zearalenone under their experimental conditions. However, Bernhoft et al. (110) demonstrated transplacental transfer of zearalenone and  $\alpha$ -zearalenone in rats which, of course, has implications for the offspring.

Interestingly, zearalenone and  $\alpha$ -zearalenone, and traces of zearalenone and taleranol were identified in muscle tissue of swine fed contaminated oats (111). It was also found that zearalenone and its metabolites were highly conjugated with glucuronic acid in urine and liver, but apparently not in muscle. In another important study, Schneweis et al. (112) identified significant quantities of zearalenone-4- $\beta$ -D-glucopyranoside co-occurring with zearalenone in naturally contaminated Bavarian wheat. At least three observations are possible here: (1) it appears that more zearalenone occurs more often in wheat in Europe than in the United States; (2) if the use of zearalenone implants for growth promotion has implications in human health, the occurrence of zearalenone and metabolites in edible muscle of animals consuming dietary zearalenone is also a significant matter; and (3) despite a number of other reports over the years of contamination of several commodities containing conjugates of zearalenone, the glycoside conjugates are still not well researched. Advances in analytical methodology should facilitate more research in this important area.

Lemke et al. (113) in investigating the effects of modified montmorillonite clays on mouse uterine weights in mice fed dietary zearalenone found that some clays had an adverse effect on body weight gain at 0.5%, but not 0.25%, in the diet and that the ratio of uterus to body weight increased in animals fed the clay plus zearalenone. The clays appeared to potentiate zearalenone's activity. Therefore, the authors concluded that thorough evaluation of potential mycotoxin binding agents was essential before use for that purpose.

### **Selected Study Director Topics**

(1) *Determination of Ochratoxin A in Green Coffee by Immunoaffinity Column Cleanup and LC*: Study Director Eugenia Vargas, Ministry of Agriculture, Laboratory for Quality Control and Food Safety, 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. Reported that the study was participated in by 20 laboratories from Brazil, United Kingdom, Italy,

Switzerland, Canada, Japan, Colombia, and Singapore. Results of the study were subjected to statistical analysis. HORRAT values of <1.0 were obtained for the naturally contaminated test materials as well for spiked test samples. A collaborative study report was submitted to AOAC Natural Toxin Committee. It was recommended that the study director should follow the AOAC format and rewrite the report.

(2) *Immunoaffinity Column Cleanup with Liquid Chromatography Using Post-Column Bromination for the Determination of Aflatoxin B<sub>1</sub> in Cattle Feed*: Study Director Joerg Stroka, European Commission DG Joint Research Center, Institute for Health and Consumer Protection Food Products Unit, TP 260, I-21020 Ispra, Italy, Tel: +0039-0332-785170, Fax: +0039-0332-785707, E-mail: joerg.stroka@jrc.it. Reported that the study was completed and the manuscript of the study was revised to address all the comments of the General Referee and the Natural Toxin Method Committee members. The report has been resubmitted to AOAC.

## Recommendations

The General Referee recommends:

(1) Approve Method **999.07** as a Final Action Official Method.

(2) Approve the "Immunoaffinity Column Cleanup with Liquid Chromatography Using Post-Column Bromination for the Determination of Aflatoxin B<sub>1</sub> in Cattle Feed" method as an Official First Action Method.

(3) Continue study of thin-layer chromatography methods and rapid methods for mycotoxins.

The Topic Advisors and Study Directors recommend:

(1) *Sampling and Subsampling for Mycotoxins*: Topic Advisor Thomas B. Whitaker, U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), PO Box 7625, North Carolina State University, Raleigh, NC 27695-7625, Tel: +1-919-515-6731, Fax: +1-919-515-7760, E-mail: thomas\_whitaker@ncsu.edu. Continue study.

(2) *Aflatoxin M*: Topic Advisor Hans P. van Egmond, National Institute of Public Health and the Environment, Laboratory for Residue Analysis, Postboks 3, PO Box 1, 3720 BA Bilthoven, The Netherlands, Tel: +31-30-2742440, Fax: +31-30-2744403, E-mail: hp.van.egmond@rivm.nl. Continue study.

(3) *Aflatoxin*: Topic Advisor David M. Wilson, University of Georgia, Department of Plant Pathology, Coastal Plain Section, Tifton, GA 31793, Tel: +1-912-386-3368, Fax: +1-912-386-7285, E-mail: dwilson@tifton.cpes.peachnet.edu. Continue study.

(4) *Alternaria Toxins*: Topic Advisor Michele Solfrizzo, Institute of Science of Food Production, National Research Council, Viale L. Einaudi 51, Bari 70125, Italy, Tel: +39-080-5912838, Fax: +39-080-5486063, E-mail: m.solfrizzo@area.ba.cnr.it. Continue study.

(5) *Citrinin*: Topic Advisor David Abramson, Agriculture and Agri-Food Canada, Cereal Research Center, 195 Dafeo Rd, Winnipeg MB R3T 2M9, Canada, Tel: +1-204-984-5536,

Fax: +1-204-983-4604, E-mail: dabramson@em.agr.ca. Continue study.

(6) *Cyclopiazonic Acid*: Topic Advisor Joe W. Dorner, USDA, ARS, National Peanut Research Laboratory, PO Box 509, 1011 Forrester Dr, SE, Dawson, GA 31742, Tel: +1-912-995-7408, Fax: +1-912-995-7416, E-mail: jdorner@nprl.usda.gov. Continue study.

(7) *Ergot Alkaloids*: Topic Advisor George M. Ware, U.S. Food and Drug Administration (FDA), 60 8th St, Atlanta, GA 30309, Tel: +1-404-347-2131 ext. 5215, Fax: +1-404-347-4225, E-mail: gware@ora.fda.gov. Continue study.

(8) *Fumonisin*s: Topic Advisor Chris Maragos, USDA, ARS, National Centre for Agricultural Utilization and Research, 1815 N. University St, Peoria, IL 61604, Tel: +1-309-684-6266, Fax: +1-309-681-6267, E-mail: maragocm@mail.ncaur.usda.gov. Continue study.

(9) *Ochratoxins*: Topic Advisor Benedicte Hald, Royal Veterinary and Agricultural University, Department of Veterinary Microbiology, 13 Bulowsvej, Friedriksberg C, Copenhagen 1870-DK, Denmark, Tel: +45-3528-2760, Fax: +45-3528-2757, E-mail: vetmi@kvl.dk. Continue study.

(10) *Patulin*: Topic Advisor Myrna Sabino, Instituto Adolfo Lutz, Dr Arnaldo 355, 355-CEP, São Paulo 01246-902, Brazil, Tel: +55-11-3068-2921, Fax: +55-11-853-3505, E-mail: mysabino@ial.sp.gov.br. Continue study.

(11) *Trichothecenes*: Topic Advisor Robert M. Eppley, U.S. Food and Drug Administration, 5100 Paint Branch Pkwy, College Park, MD 20740, Tel: +1-301-436-1951, Fax: +1-301-436-2644, E-mail: Reppley@cfsan.fda.gov. Continue study.

(12) *Zearalenone*: Topic Advisor Winston M. Hagler, Jr, North Carolina State University, College of Agriculture and Life Sciences, Department of Poultry Science, Mycotoxin Laboratory, Box 7608, Raleigh, NC 27695-7608, Tel: +1-919-515-3228, Fax: +1-919-515-2625, E-mail: winston\_hagler@ncsu.edu. Continue study.

(13) *Determination of Veratox for Aflatoxins in Corn and Whole Cottonseed*: Study Director Mark Mozola, Neogen Corp., 620 Leshner Pl, Lansing MI 48912-1595, Tel: +1-517-372-9200, Fax: +1-517-372-0108, E-mail: mmozola@neogen.com. Study on hold.

(14) *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.

(15) **2000.16** *Aflatoxin B<sub>1</sub> in Baby Food by Immunoaffinity Column and LC*: Study Director Elke Anklam, Joint Research Centre of the European Commission, 21020, Ispra, Italy, Tel: +39-332-78-5390, Fax: +39-332-78-5930, E-mail: elke.anklam@jrc.it. Approved as First Action in 2000. Continue study.

(16) **2001.04** *Determination of Fumonisin B<sub>1</sub> and B<sub>2</sub> 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. Approved as First Action in 2001. Continue study.

(17) *Determination of Aflatoxin B<sub>1</sub> in Cattle Feed by Immunoaffinity Column Cleanup and LC with Post-Column Derivatization*: Study Director Joerg Stroka, European Commission DG Joint Research Center, Institute for Health and Consumer Protection Food Products Unit, TP 260,I-21020 Ispra, Italy, Tel: +0039-0332-785170, Fax: +0039-0332-785707, E-mail: joerg.stroka@jrc.it. Continue study.

(18) **2001.06** *Veratox for Fumonisin*: Study Director Mark Mozola (see 13). Approved as First Action in 2001. Continue study.

(19) **2001.01** *Determination of Ochratoxin A in Wine and Beer by Immunoaffinity Column Cleanup and LC*: Study Director Angelo Visconti (see 16). Approved as First Action in 2001. Continue study.

(20) *Determination of Deoxynivalenol in Corn, Wheat, Barley, and Malted Barley, ELISA*: Study Director Mark Mozola (see 13). Continue study.

(21) *Total Aflatoxins in Corn, Poultry Feed, and Pet Food*: Study Director Mark Mozola (see 13). On hold.

(22) *Total Aflatoxins in Almonds and Raw and Roasted Peanuts*: Study Director Mark Mozola (see 13). On hold.

(23) **999.07** *Determination of Aflatoxin in Peanut Butter, Pistachios, Figs, and Paprika*: Study Director Elke Anklam (see 15). Approved as First Action in 1999.

(24) *Aflatoxin of Spices by Aflatest Kit*: Study Director Mike Jackson, Pert Labs, PO Box 267, 145 Peanut Dr, Edenton, NC 27932, Tel: +1-252-482-4456, Fax: +1-252-482-5370, E-mail: mike.jackson@pert\_labs.com. Continue study.

(25) *Analysis of Aflatoxin by an LC with Activation Using a Photochemical Reactor for Enhanced Detection (PHRED)*: Study Director Arthur Waltking, Waltking Associates, 482 Rock Rd, Glen Rock, NJ 07452. Continue study.

(26) *Mycotoxins in Botanicals*: Topic Advisor Bruce Malone, Trilogy Analytical Lab, 111 W. 4th St, Washington, MO 63090, Tel: +1-636-239-1521, Fax: +1-636-239-1531, E-mail: bruce@trilogylab.com. Continue topic.

(27) **2000.02** *Determination of Patulin in Clear and Cloudy Apple Juices and Apple Puree by LC*: Suzan McDonald, MAFF-UK/Food Safety Directorate, United Kingdom. Approved as First Action in 2000. Continue study.

(28) **2000.03** *Determination of Ochratoxin in Barley by LC*: Catherine Entwisle, Leatherhead Food RA, United Kingdom. Approved as First Action in 2000. Continue study.

(29) **2000.08** *Aflatoxin M<sub>1</sub> in Liquid Milk by Immunoaffinity Column Cleanup and LC with Fluorescence Detection*: Sylviane Dragacci, AFSSA, Maison-Alfort, France. Approved as First Action in 2000. Continue study.

(30) **2000.09** *Determination of Ochratoxin in a Roasted Coffee by LC*: Catherine Entwisle (see 28). Approved as First Action in 2000. Continue study.

## References

- (1) DeVries, J.W., Trucksess, M.W., & Jackson, L.S. (Eds) (2002) *Mycotoxins and Food Safety, Advances in Experimental Medicine and Biology*, **504**, Kluwer Academic/Plenum Publishers, New York, NY
- (2) World Health Organization (2001) Safety Evaluation of Certain Mycotoxins in Food, WHO Food Additives Series 47, World Health Organization, Geneva, Switzerland, 1–102
- (3) van Egmond, H.P., & Dragacci, S. (2000) in *Mycotoxin Protocols, Methods in Molecular Biology*, **157**, M.W. Trucksess & A.E. Pohland (Eds.), Humana Press, Totowa, New Jersey, NJ, 59–69
- (4) Pilsbacher, L., & Pfeleger, R. (2000) *Ernahrung* **25**, 348–353
- (5) Taveira, J.A., & Midio, A.F. (2001) *Italian J. Food Sci.* **13**, 443–447
- (6) El-Sayed, A.M., Abd-Alla, Neamat-Allah, A.A., & Soherly, E.A. (2000) *Mycotoxin Res.* **16**, 91–100
- (7) Galvano, F., Galofaro, V., Ritieni, A., Bognanno, M., De Angenis, A., & Galvano, G. (2001) *Food Addit. Contam.* **18**, 644–646
- (8) Grasso, L., Scarano, G., & Serpe, L. (2001) *Rivista Sci. dell'Alimentazione* **30**, 29–34
- (9) Minervini, F., Visconti, A., Bottalico, A., & Montagna, M.T. (2001) *Ind. Aliment.* **40**, 513–516
- (10) Palermo, D., Palermo, C., & Rotunno, T. (2001) *J. Food Sci.* **13**, 435–442
- (11) Thirumala Devi, K., Mayo, M.A., & Hall, A.J. (2002) *J. Agric. Food Chem.* **50**, 933–937
- (12) Srivastava, V.P., Bu-Abbas, A., Alaa-Basuny, Al-Johar, W., Al-Mufti, S., & Siddiqui, M.K.J. (2001) *Food Addit. Contam.* **18**, 993–997
- (13) Oruc, H.H., & Sonal, S. (2001) *Vet. Hum. Toxicol.* **43**, 292–293
- (14) Food Standards Agency (2001) Food Survey Information Sheet 17/1, Document 20013125795, Food Standards Agency, London, UK, 19 pp
- (15) Fente, C.A., Jaminez-Ordaz, J., Vazquez, B.I., Franco, C.M., & Cepeda, A. (2001) *Appl. Environ. Microbiol.* **67**, 4858–4862
- (16) Yong, R.K., & Cousin, M.A. (2001) *Int. J. Food Microbiol.* **65**, 27–38
- (17) Celik, I., Oguz, H., Demet, O., Boydak, M., Donmez, H.H., Sur, E., & Nizamliogu, F. (2000) *Brit. Poultry Sci.* **41**, 401–409
- (18) Pearson, T.C., Wicklow, D.T., Maghirang, E.B., Xie, F., & Dowell, F.E. (2001) *Trans. ASAE* **44**, 1247–1254
- (19) Younis, Y.M., & Malik, K.M. (2001) *J. Saudi Chem. Soc.* **5**, 19–28
- (20) Akiyama, H., Goda, Y., Tanaka, T., & Toyoda, M. (2001) *J. Chromatogr. A* **932**, 153–157
- (21) Chiavaro, E., Dall'Asta, C., Galaverna, G., Biancardi, A., & Gambarelli, E. (2001) *J. Chromatogr. A* **937**, 31–40
- (22) Nasir, M.S., & Jolley, M.E. (2002) *J. Agric. Food Chem.* **50**, 3116–3121

- (23) Wei, J., Okerberg, E., Dunlap, J., Ly, C., & Shear, J.B. (2000) *Anal. Chem.* **72**, 1360–1363
- (24) Mirghani, M.E.S., Man, Y.B.C., Jinap, S., Baharin, B.S., & Bakar, J. (2001) *J. Amer. Oil Chem. Soc.* **78**, 985–992
- (25) Tuomi, T., Johnsson, T., Hintikka, E.L., & Reijula, K. (2001) *Analyst* **126**, 1545–1550
- (26) Garden, S.R., & Strachan, N.J.C. (2001) *Anal. Chim. Acta* **444**, 187–191
- (27) Ho, J.A.A., & Wauchope, R.D. (2002) *Anal. Chem.* **74**, 1493–1496
- (28) Stroka, J., Anklam, E., Joerissen, U., & Gilbert, J. (2001) *J. AOAC Int.* **84**, 1116–1123
- (29) Scott, P.M., & Kanhere, S.R. (2001) *Mycotoxin Res.* **17**, 9–14
- (30) Scott, P.M. (2001) *J. AOAC Int.* **84**, 1809–1817
- (31) Giryn, H., & Szeke, B. (2001) *Zakl. Anal. Zywnosci.* **56**, 151–162
- (32) da Motta, S., & Valente Soares, L.M. (2001) *Food Addit. Contam.* **18**, 630–634
- (33) Wareing, P.W., Westby, A., Gibbs, J.A., Allotey, L.T., & Halm, M. (2001) *Int. J. Food Sci. Technol.* **36**, 1–10
- (34) Tournas V.H., & Stack M.E. (2001) *J. Food Prot.* **64**, 528–532
- (35) Vrabcheva, T., Usleber, E., Dietrich, R., & Märtilbauer, E. (2000) *J. Agric. Food Chem.* **48**, 2483–2488
- (36) Vrabcheva, T., Usleber, E., Petkova-Bocharova, T., Nikolov, I., Chernozemsky, I., Dietrich, R., & Märtilbauer E. (2000) *Mycotoxin Res.* **16A**, 150–153
- (37) Vazquez, B.I., Fente, C., Franco, C.M., Vazquez, M.J., & Cepeda, A. (2001) *Int. J. Food Microbiol.* **67**, 157–163
- (38) Schneweis, I., Meyer, K., Hoermansdorfer, S., & Bauer, J. (2001) *J. Anim. Physiol. Anim. Nutr.* **85**, 38–44
- (39) Abrunhosa, L., Paterson, R.R.M., Kozakiewicz, Z., Lima, N., & Venancio, A. (2001) *Lett. Appl. Microbiol.* **32**, 240–242
- (40) Losito, I., Monaci, L., Aresta, A., & Zambonin, C.G. (2002) *Analyst* **127**, 499–502
- (41) Monaci, L., Aresta, A., Palmisano, F., Visconti, A., & Zambonin, C.G. (2002) *J. Chromatogr. A* **955**, 79–86
- (42) Pinto, V.F., Patriarca, A., Locani, O., & Vaamonde, G. (2001) *Food Addit. Contam.* **18**, 1017–1020
- (43) Zambonin, C.G., Monaci, L., & Aresta, A. (2001) *Food Chem.* **75**, 249–254
- (44) Schnitzius, J.M., Hill, N.S., Thompson, C.S., & Craig A.M.J. (2001) *Vet. Diagn. Invest.* **13**, 230–237
- (45) Lombaert, G.A. (2000) in *Mycotoxin Protocols Methods in Molecular Biology*, **157**, M.W. Trucksess & A.E. Pohland (Eds), Humana Press, Totowa, NJ, pp 215–224
- (46) Tseng, T.C., & Liu, C.Y. (2001) *Intl. J. Food Microbiol.* **65**, 23–26
- (47) Omurtag, G.Z. (2001) *J. Food Prot.* **64**, 1072–1075
- (48) Li, F.Q., Yoshizawa, T., Kawamura, O., Luo, X.Y., & Li, Y.W. (2001) *J. Agric. Food Chem.* **49**, 4122–4126
- (49) Ono, E.Y., Ono, M.A., Funo, F.Y., Medinal, A.E., Oliveira, T.C., Kawamura, O., Ueno, Y., & Hirooka, E.Y. (2001) *Food Addit. Contam.* **18**, 719–729
- (50) Vargas, E.A., Preis, R.A., Castro, L., & Silva, C.M. (2001) *Food Addit. Contam.* **18**, 981–986
- (51) Kim, E.K., Scott, P.M., Lau, B.P.Y., & Lewis, D.A. (2002) *J. Agric. Food Chem.* **50**, 3614–3620
- (52) Kim, E.K., Shon, D.H., Chung, S.H., & Kim, Y.B. (2002) *Food Addit. Contam.* **19**, 459–464
- (53) Torres, A.M., Reynoso, M.M., Rojo, F.G., Ramirez, M.L., & Chulze, S.N. (2001) *Food Addit. Contam.* **18**, 836–843
- (54) Gamanya, R., & Sibanda, L. (2001) *Int. J. Food Microbiol.* **71**, 145–149
- (55) Whitaker, T.B., Hagler, W.M., Jr, Johansson, A.S., Giesbrecht, F.G., & Trucksess, M.W. (2001) *J. AOAC Int.* **84**, 770–776
- (56) Martins, M.L., Martins, H.M., & Bernardo, F. (2001) *J. Food Prot.* **64**, 1268–1270
- (57) Spotti, M., Caloni, F., Fracchiolla, L., Pompa, G., Vigo, D., & Maffeo, G. (2001) *Vet. Human Toxicol.* **43**, 109–111
- (58) Seefelder, W., Gossmann, M., & Humpf, H.U. (2002) *J. Agric. Food Chem.* **50**, 2778–2781
- (59) Sewram, V., Nair, J.J., Nieuwoudt, T.W., Gelderblom, W.C., Marasas, W.F., & Shephard, G.S. (2001) *J. Anal. Toxicol.* **25**, 450–455
- (60) Musser, S.M., Eppley, R.M., & Trucksess, M.W. (2002) in *Mycotoxins and Food Safety, Advances in Experimental Medicine and Biology*, **504**, J.W. DeVries, M.W. Trucksess, & L. Jackson (Eds), Kluwer Academic/Plenum Publishers, New York, NY, pp 95–105
- (61) Broggi, E.E. (2002) *Toxicol. Lett.* **127**, 19–28
- (62) Bullerman, L.D., Ryu, D., & Jackson, L.S. (2002) in *Mycotoxins and Food Safety, Advances in Experimental Medicine and Biology*, **504**, J.W. DeVries, M.W. Trucksess, & L. Jackson (Eds), Kluwer Academic/Plenum Publishers, New York, NY, pp 195–204
- (63) De Girolamo, A., Solfrizzo, M., & Visconti, A. (2001) *J. Food Prot.* **64**, 701–705
- (64) Poling, S.M., Plattner, R.D., & Weisleder, D. (2002) *J. Agric. Food Chem.* **50**, 1318–1324
- (65) Voss, K.A., Poling, S.M., Meredith, F.I., Bacon, C.W., & Saunders, D.S. (2001) *J. Agric. Food Chem.* **49**, 3120–3126
- (66) Saunders, D.S., Meredith, F.I., & Voss, K.A. (2001) *Environ. Health Persp.* **109** (suppl 2), 333–336
- (67) Bird, C.B., Malone, B., Rice, L.G., Ross, P.F., Eppley, R., & Abouzied, M.M. (2002) *J. AOAC Int.* **85**, 404–410
- (68) Dilkin, P., Mallmann, C.A., de Almeida, C.A., & Corrêa, B. (2001) *J. Chromatogr. A* **925**, 151–157
- (69) Visconti, A., Solfrizzo, M., & De Girolamo, A. (2001) *J. AOAC Int.* **84**, 1828–1837
- (70) Scott, P.M. (2002) in *Mycotoxins and Food Safety, Advances in Experimental Medicine and Biology*, **504**, J.W. DeVries, M.W. Trucksess, & L. Jackson (Eds), Kluwer Academic/Plenum Publishers, New York, NY, pp 117–134
- (71) Leitner, A., Zöllner, P., Paolilla, A., Stroka, J., Papadopoulou-Bouraoui, A., Jaborek, S., Anklam, E., & Lindner, W. (2002) *Anal. Chim. Acta* **453**, 33–41
- (72) Visconti, A., Pascale, M., & Centonze, G. (2001) *J. AOAC Int.* **84**, 1818–1827
- (73) De Saeger, S., Sibanda, L., Desmet, A., & Van Petegham, C. (2002) *Int. J. Food Microbiol.* **75**, 135–142
- (74) Soleas, G.J., Yan, J., & Goldberg, D.M. (2001) *J. Agric. Food Chem.* **49**, 2733–2740
- (75) Jodlbauer, J., Maier, N.M., & Lindner, W. (2002) *J. Chromatogr. A* **945**, 45–63
- (76) Pittet, A., & Royer, D. (2002) *J. Agric. Food Chem.* **50**, 243–247
- (77) Cuitui, V.G., & Gareis, M. (2001) *Food Addit. Contam.* **18**, 635–643

- (78) Urbano, G.R., Taniwaki, M.H., Leitao, M.F.D., & Vicentini, M.C. (2001) *J. Food Prot.* **64**, 1226–1230
- (79) Eskola, M., Parikka, P., & Rizzo, A. (2001) *Food Addit. Contam.* **18**, 707–718
- (80) Curtui, V.G., Gareis, M., Usleber, E., & Märtlbauer, E. (2001) *Food Addit. Contam.* **18**, 730–738
- (81) Jimenez, A.M., de Cerain, A.L., Gonzales-Penas, E., & Bello, J. (2001) *Food Addit. Contam.* **18**, 559–563
- (82) Beretta, B., de Domenico, R., Gaiaschi, A., Ballabio, C., Galli, C.L., Gigliotti, C., & Restani, P. (2002) *Food Addit. Contam.* **19**, 70–75
- (83) Sage, L., Krivobok, S., Delbos, E., Seigle-Murandi, F., & Creppy, E.E. (2002) *J. Agric. Food Chem.* **50**, 1306–1311
- (84) Skaug, M.A., Eduard, W., & Størmer, F.C. (2001) *Mycopathologia* **151**, 93–98
- (85) Leggott, N.L., Shephard, G.S., Stockenstrom, S., Staal, E., & van Schalkwyk, D.J. (2001) *Food Addit. Contam.* **18**, 825–829
- (86) Fernández-Trejejo, E.O., Verdes, J.A.A., & Espinosa, R.S. (2001) *Rev. Cubana Aliment Nutr.* **15**, 20–25
- (87) Leggott, N.L., & Shephard, G.S. (2001) *Food Control* **12**, 73–76
- (88) Kadakal, C., & Nas, S. (2002) *Nahrung* **46**, 31–33
- (89) Kryger, N.A. (2001) *J. Agric Food Chem.* **49**, 4141–4143
- (90) Bissessur, J., Permaul, K., & Odhav, B. (2001) *J. Food Prot.* **64**, 1216–1219
- (91) Yurdun, T., Omurtag, G.Z., & Ersoy, O. (2001) *J. Food Prot.* **64**, 1851–1853
- (92) Martins, M.L., Gimeno, A., Martins, H.M., & Bernardo, F. (2002) *Food Addit Contam.* **19**, 568–574
- (93) Roach, J.A., Brause, A.R., Eisele, T.A., & Rupp, H.S. (2002) in *Mycotoxins and Food Safety, Advances in Experimental Medicine and Biology*, **504**, J.W. DeVries, M.W. Trucksess, & L. Jackson (Eds), Kluwer Academic/Plenum Publishers, New York, NY, pp 135–140
- (94) Moodley, R.S., Govinden, R., & Odhav, B. (2002) *J. Food Prot.* **65**, 867–871
- (95) Moss, M.O., & Long, M.T. (2002) *Food Addit. Contam.* **19**, 387–399
- (96) Lombaert, G.A. (2002) in *Mycotoxins and Food Safety, Advances in Experimental Medicine and Biology*, **504**, J.W. DeVries, M.W. Trucksess, & L. Jackson (Eds), Kluwer Academic/Plenum Publishers, New York, NY, pp 141–152
- (97) Olsson, J., Borjesson, T., Lundsted, T., & Schurer, J. (2002) *International. J. Food Microbiol.* **72**, 203–214
- (98) Nielson, H.F., & Thrane, U. (2001) *J. Chromatogr. A* **929**, 75–87
- (99) Schothorst, R.C., & Jerkel, A.A. (2001) *Food Chemistry* **73**, 111–117
- (100) Mateo, J.J., Llorens, A., Mateo, R., & Jimenez, M. (2001) *J. Chromatogr. A* **918**, 99–112
- (101) Maragos, C.M., Jolley, M.E., & Nasir, M.S. (2002) *Food Addit. Contam.* **19**, 400–407
- (102) Maragos, C.M., & Plattner, R.D. (2002) *J. Agric Food Chem.* **50**, 1827–1832
- (103) Schollenberget, M., Jara, H.T., Suchy, S., Drochner, W., & Muller, H.M. (2002) *Int. J. Food Microbiol.* **72**, 85–89
- (104) Al-Julaifi, M.Z., & Al-Falih, A.M. (2001) *J. Food Prot.* **64**, 1603–1606
- (105) Omurtag, G.Z., & Yazicioglu, D. (2001) *Food Addit. Contam.* **18**, 844–849
- (106) Hagler, W.M., Jr, Towers, N.R., Mirocha, C.J., Eppley, R.M., & Bryden, W.L. (2001) in *Fusarium*, Paul E. Nelson Memorial Symposium, B.A. Summerell, J.F. Leslie, D. Backhouse, W.L. Bryden, & L.W. Burgess (Eds), APS Press, St. Paul, MN, pp 321–331
- (107) Ryu, D., Jackson, L.S., & Bullerman, L.B. (2002) *Adv. Exp. Med. Biol.* **504**, 205–216
- (108) Krska, R., & Josephs, R. (2001) *Fresenius J. Anal. Chem.* **369**, 469–476
- (109) Juhasz, J., Nagy, J.J., Kulesar, M., Szigeti, G., Reiczgel, J., & Huszenica, G. (2001) *Acta Vet. Hung.* **49**, 211–217
- (110) Bernhoft, A., Behrens, G.H., Ingebrigsten, K., Langseth, W., Berndt, S., Haugen, T.B., & Grotmol, T. (2001) *Reprod. Toxicol.* **15**, 545–550
- (111) Zollner, P., Jodlbauer, J., Kleinova, M., Kahlbacher, H., Kuhn, T., Hochsteiner, W., & Lindner, W. (2002) *J. Agric. Food Chem.* **50**, 2494–2501
- (112) Schneweis, I., Meyer, K., Ingelhardt, G., & Bauer, J. (2002) *J. Agric. Food Chem.* **50**, 1736–1738
- (113) Lemke, S.L., Mayura, K., Reeves, W.R., Wang, N., Fickey, C., & Phillips, T.D. (2001) *J. Toxicol. Environ. Health A* **62**, 243–258