

Committee on Natural Toxins and Food Allergens

Phycotoxins

JAMES M. HUNGERFORD

U.S. Food and Drug Administration, Seafood Products Research Center, 22201 23rd Dr SE, Bothell, WA 98021, Tel: +1-425-483-4894, Fax: +1-425-483-4996, E-mail: James.Hungerford@fda.gov

Summary

Over the course of the past year many changes have occurred in the Phycotoxins topic, the first being the appointment of a new General Referee. Starting in 2004, the "Phycotoxins" General Referee topic will be renamed "Marine and Freshwater Toxins." This will allow inclusion of other important seafood toxins, such as the tetrodotoxins and emerging toxins, while retaining the freshwater toxins. Many topic areas require new appointments and the General Referee is actively recruiting these volunteers. The General Referee and AOAC are in the process of establishing an Association "analytical community" to promote, expedite, and seek funding for the validation of methods for marine toxins and freshwater toxins. Although replacement methods based on liquid chromatography, mass selective detection, *in vitro*, and other alternative approaches are being sought and in some cases validated, mouse bioassays are still in wide use. This is particularly true of monitoring programs for detecting the saxitoxins which cause paralytic shellfish poisoning (PSP). Similarly, a mouse bioassay is also still used in many countries for detecting diarrhetic shellfish poisoning (DSP) toxins. Alternative bioassays, based on cultured nerve cells, are being pursued for their high sensitivity and (like the mouse) their ability to respond to multiple toxins.

With the exception of domoic acid (implicated in amnesic shellfish poisoning, or ASP), classical LC-UV detection methods are not sufficiently sensitive or selective to detect the very toxic phycotoxins. LC-fluorescence methods have also been developed but the general trend in LC methods is towards the use of mass selective detection. Over the last several years instrumentation for liquid chromatography-mass spectrometry (LC/MS) has become more affordable, and methods based on LC/MS have now been developed for nearly all of the phycotoxins. Some of these procedures, such as a Cawthron Institute method capable of monitoring 16 different ASP and DSP toxins in a single pass (http://www.cawthron.org.nz/news/news_LCMS_method.htm), are in the process of being adopted into a regular marine biotoxin management program.

Upcoming international symposia will address phycotoxins and other natural toxins. Cosponsored by AOAC, the XI International IUPAC Symposium on Myco-

toxins and Phycotoxins will be held May 17–21, 2004, in Bethesda, Maryland. Information is available at <http://www.aoac.org/meetings1/IUPAC/Main.htm>. The 5th International Conference on Molluscan Shellfish Safety will be held June 14–18 in Galway, Ireland (<http://www.icmss04.com>) and an international symposium, "Marine Toxins: Structure, Toxicology, and Detection," will be held at Pacificchem 2005 December 15–20, 2005, in Honolulu, Hawaii (*see* www.pacificchem.org).

Selected Study Director Topics

Amnesic Shellfish Poisoning Toxins

Study Director Michael A. Quilliam, National Research Council of Canada, Institute for Marine Biosciences, 1411 Oxford St, Halifax, Nova Scotia, B3H3Z1, Canada, Tel: +1-902-426-9736, Fax: +1-902-426-9413, E-mail: michael.quilliam@nrc.ca. The Study Director is seeking funding for the AOAC collaborative study fees.

Bioassays for Phycotoxins

Topic Advisor Donald J.A. Richard, Richard Biologics, 20 Walker St, Moncton, New Brunswick, E1C 4L8, Canada, Tel: +1-506-855-4824, E-mail: donr@nb.sympatico.ca. Previous reports have focused on the scientific, legal, and ethical justifications of proposed amendments to Method **959.08** (Paralytic Shellfish Poison, Biological Method). This report to committee will communicate a proposed action plan for 2003–2005 with the ultimate aim of submitting a revised method that reflects present day expectations of quality control, reduced animal usage, international trade requirements, accuracy, and precision. In summary, previous in-house initiatives have identified the following issues which will be addressed in the upcoming collaborative study: (a) the use of mice larger than the 23 g presently specified; (b) the requirement for established guidelines in regards to analyst competency, accuracy, and precision; (c) smaller tolerances in regards to pH at all stages of analysis; (d) specifying gravimetric measurements as opposed to the present volumetric standards; (e) establishing new guidelines for sample homogenization; (f) new specifications on the number of animals required for both screening and quantification purposes; (g) new guidelines on sample integrity from collection to analysis; (h) redefining the amount and number of animals which constitute a sample; (i) redefining the toxin standards so that future comparisons with instrumental analysis are not affected by the sodium factor; (j) new standardization and quality control guidelines aimed at reducing animal use while maintaining confidence in results; and (k) defining any parameter which may be subject to varying interpretation. It is anticipated that a collaborative protocol will be prepared and submitted in 2004.

A call for reference materials and standards will be issued concurrently and issues regarding international shipments and permits will be addressed by early 2004. Some specific instrumental analysis will be required in order to incorporate certain amendments in regards to sample extraction. These will be scheduled for early 2004 and possibly continue into the summer depending on findings. Because of the high probability of unforeseen problems in logistics, a smaller interlaboratory trial may prove beneficial in identifying shortcomings in planning. Details of the new procedures will be provided to all collaborators in early fall 2004 with allowance for comments and incorporation of any last minute changes with new data. The full collaborative study will be scheduled for early 2005 and collated, analyzed, and reported by late summer.

Capillary Electrophoretic Methods for Marine Toxins

Topic Advisor Ana Gago-Martinez, Universidad de Vigo, Dpto. Química Analítica y Alimentaria, Facultad de Ciencias, Campus Universitario de Vigo 36200-Vigo-E, Spain, Tel: +34-986 812284, Fax: +34-986 812382, E-mail: anagago@uvigo.es. Gago-Martinez reports that capillary electrophoresis (CE) has been applied as an alternative for the analysis of algal toxins (1–9). The separation in this case is based on the different mobilities of these compounds in an electric field. This technique offers high efficiency and resolution capabilities. The main advantages of using CE in comparison with LC include simplicity, rapid method development, fast analysis times, low cost, and a variety of separation modes. CE also requires minimal quantities of reagents and separation media, which represents a significant advantage to avoid the large volumes of organic waste associated with HPLC methods. On the other hand the main disadvantage of using CE is associated with the lack of sensitivity which makes particularly difficult its application for the analysis of compounds that are present in complex matrixes at trace levels. Nevertheless different CE approaches have been and are still in development for the analysis of various algal toxins, particularly for the saxitoxins, domoic acid, and recently for microcystins present in freshwaters. Both ultraviolet and mass selective detection have been used for these applications and intense work is being devoted to overcome the lack of sensitivity associated with this technique, using different preconcentration approaches. Another novel electro-separation approach, capillary electrochromatography (CEC), has also been applied to the analysis of algal toxins. A pioneer application in the field of marine toxins been carried out, particularly for the analysis of domoic acid. This technique is resulting in a promising approach which combines both HPLC and CE features, nevertheless further improvements are still required, especially regarding the lack of adequate stationary phases commercially available and particularly for this application.

Cell Bioassays for Phycotoxins

Topic Advisor Ronald Manger, Fred Hutchinson Cancer Research Center, PO Box 19024, Seattle, WA 98109-1024, Tel: +1-206-667-5838, Fax: +1-206-667-4182, E-mail: rmanger@fhcrc.org. Manger reports that refinements to the

current MTT cell-based assay for voltage gated sodium channel specific neurotoxins are being explored. Our current modification (10) of these methods offers a robust cell-based assay for toxins associated with paralytic shellfish poisoning that can be completed within an average of 6 to 8 h. This shortened assay time is comparable to our previously reported cell-based assays (11, 12) for voltage-gated sodium channel enhancing toxins, brevetoxin, and ciguatera toxin. Over the past year the 2 cell assay formats have been successfully applied by FDA laboratories to several ciguatera outbreaks and also to unusual outbreaks of pufferfish poisoning (13). In the latter, cell assays were performed by T. Hawryluk of FDA's Northeast Regional Laboratory, Jamaica, New York. Saxitoxins (rather than tetrodotoxins) were identified as the causative agents via LC/MS work by M. Quilliam of NRC Canada in Halifax, Nova Scotia (14).

Significant advancements to cell-based detection methods for sodium channel active marine toxins have been made within the past few years; in particular the studies by Louzao et al. (15) utilizing a voltage-sensitive oxonol dye for a specific, sensitive, and reliable fluorimetric detection assay for PSP toxins. The method has been adapted to a microplate format offering the potential for a high throughput in vitro assay for PSP toxins (16). Advantages of this methodology are that assay time can be accomplished within minutes, and it represents another alternative to controversial mouse bioassays. Nicholson et al. (17) have taken a different approach to voltage-sensitive dye methodologies, using rhodamine 6G and mouse brain synaptoneuroosomes (rather than the oxonol dye and neuroblastoma cells) for the detection of saxitoxins. David et al. (18) have extended this approach to the detection of brevetoxins. The latter 2 procedures amount to lethal mouse bioassays since the mouse brain synaptoneuroosomes must be prepared fresh before use. Noting the advantages of the methods developed by Louzao et al. using neuroblastoma cells, we have recently explored the potential of flow cytometry coupled with the rapid response of the oxonol dye fluorescence. The significant gain in signal response by flow cytometry has allowed the detection of toxins that either block or enhance the activity of the voltage-gated sodium channel. We are currently exploring the application of this method to naturally incurred toxins associated with either PSP or ciguatera activity.

Diarrhetic Shellfish Poisoning Toxins, Assay Methods

Topic Advisor J. Marc Fremy, Agence Française de Sécurité Sanitaire des Aliments, Laboratoire d'Etude et de Recherche sur l'Hygiène et la Qualité des Aliments, 10 Rue P. Curie, Maisons Alfort, F-94704, France, Tel: +33-1-4977-2751, Fax: +33-1-4977-2695, E-mail: j.fremy@afssa.fr. A specific ELISA was employed for the analysis of DSP toxins in small sized plankton fractions using a mouse monoclonal anti-okadaic acid antibody which recognizes okadaic acid, DTX-1, and DTX-3. Based on this ELISA, the monitoring of plankton in seawater could detect DSP toxins 2 weeks before the detection of toxins in shellfish (scallops) and 2 weeks after the loss of bivalve toxicity detected by

the mouse bioassay. The authors mentioned the usefulness of such a test for monitoring phytoplankton as a practical tool for predicting DSP toxin contamination in bivalves from shellfish aquaculture (19).

Polyclonal antibodies that bind to yessotoxin (YTX) and its analogs have been produced and used to develop an ELISA as rapid screening test for YTXs in shellfish. Direct and indirect formats for the assays have been optimized and YTXs have been quantified in water samples, shellfish (both whole meat and digestive gland), and algal cell extracts. The assay is able to detect all the YTX analogues and a limit of quantitation (LOQ) was found at 20 pg/mL (20, 21). Regarding the Protein Phosphatase 2A (PP2A) inhibition assay, Sato et al. (22), to solve some problems encountered using enzymes from commercial sources, examined PP2A from mammals, plants, and aquatic animals. The best PP2A production at low cost was found from the marine snail, *Neptuna arthritica*. Based on the use of this enzyme a simple microplate assay was developed and able to quantify Okadaic acid at a lower level than 0.1 mg/kg which is a level below the new allowance level (0.16 mg/kg) decided by the European Union.

Immunological Methods for Seafood Toxins

Topic Advisor Joanne F. Jellett, Jellett Rapid Testing, 4654 Route 3, Chester Basin, NS, B0J 1K0 Canada, Tel: +1-902-275-5104, Fax: +1-902-275-2242, E-mail: jjellett@ns.sympatico.ca, jjellett@jellett.ca, Web site: www.jellett.ca. Jellett Rapid Testing has successfully completed validation work on the Rapid Test for PSP in the United States and other countries. The Rapid Test for ASP is currently being validated in a study led by the National Research Council of Canada and involving several APEC countries. The company is working toward the initial release of their Rapid Test for DSP in the spring of 2004.

The Rapid Test for PSP was recently accepted by the Interstate Shellfish Sanitation Conference (ISSC) for use in screening for PSP. This acceptance is now subject to final review by the FDA, at which time the changes will be reflected in the new NSSP. The method was accepted through the new Laboratory Methods Review (LMR) Committee of the ISSC, a standing committee put in place to review and accept/reject new methods based on immediate and ongoing need that are not AOAC or APHA validated methods. The particulars of the ISSC acceptance can be seen below. The Rapid Test for PSP and ASP were the subject of a study by the UK Food Standards Agency, and a quote from their report can be seen below. Sales information on the company's products can be found on the Web site (www.jellett.ca) and technical information can be seen in the primary literature references below. The LMR Committee of ISSC: 3a. Recommend to Task Force I to adopt as a Type IV method with the following restrictions: (i) Method can be used to determine when to perform a mouse bioassay in a previously closed area; further (ii) a negative result can be substituted for a mouse bioassay to maintain an area in the open status. Also (iii) a positive result can be used for a precautionary closure. *Note:* The technology in the Jellett Rapid Test was marketed formerly as the MIST Alert™ test.

Also the UK Food Standards Agency under Project Code B04006 made the following recommendations for the use of MIST Alert for PSP in the UK: MIST Alert for PSP has proven to be a fast, robust, and accurate screen for PSP toxins in both the field and laboratory conditions. The MIST Alert for PSP has the potential of reducing the numbers of animals required for testing during routine monitoring. Field trial results indicate that MIST Alert for PSP could also be used by shellfish farmers as a shellfish management tool and by processors in end product testing. For additional information on Jellett rapid tests, see refs. 23–32.

Microcystins

Topic Advisor Geoffrey A. Codd, University of Dundee, Department of Biological Sciences, Dundee DD1 4HN, Scotland, UK, Tel: +44-1382-344272, Fax: +44-1382-344275, E-mail: g.a.codd@dundee.ac.uk. Codd reports that research and development continues on the detection and analysis of the hepatotoxic microcystins and nodularins of cyanobacteria. Advances in the use of physicochemical and biochemical methods (33–35) are being complemented by increasing application of immunological methods (36). The need for standardized and validated methods for the analysis of cyanobacterial toxins, including microcystins, is being increasingly recognized by international agencies (e.g., the World Health Organization) and national bodies acknowledge the health hazards presented by the toxins and national guidelines for the protection of human and animal health are introduced. An international intercomparison exercise has been completed on microcystin analysis (37). This was carried out as part of 2 European Union projects and involved 31 laboratories in 13 countries. The exercise included the analysis by all partners of a purified, gravimetric standard solution of microcystin-LR prepared in the project, plus lyophilized cells of a natural cyanobacterial bloom containing microcystins. HPLC, with ultraviolet or photodiode array detection, was used by all laboratories, with some participants additionally using ELISA, protein phosphatase inhibition assays, or HPLC-MS. It was concluded that the microcystin-LR standard was measured with adequate precision by all participants, independently of the method used. Significantly more variability occurred with the lyophilised cells, indicating the need for optimization and better standardization of extraction procedures. Further variability occurred where additional analyses included the use of commercially available microcystin reagents, indicating the need for quantitative analytical standards (37).

A further international project is in progress for the determination of microcystins in raw and treated waters. This International Organization for Standardization (ISO) exercise in the Water Quality Programme aims to develop an ISO standard for microcystin. It began in 2000 and is expected to be completed in 5–6 years.

Paralytic Shellfish Poisoning Toxins, Instrumental Methods

Study Director James F. Lawrence, Health Canada, Food Research Division, Banting Bldg, 2203D, Ottawa, Ontario, K1A0L2, Canada, Tel: +1-613-957-0947, Fax: +1-613-941-4775, E-mail: jim_lawrence@hc-sc.gc.ca. The Study Director has already conducted a collaborative study on the prechromatographic oxidation LC method. A manuscript describing the results of this study has been accepted for publication (38).

Receptor Assays for Phycotoxins

Topic Advisor Frances Van Dolah, NOAA-National Ocean Service, Center for Coastal Environmental Health & Biomolecular Research, 219 Fort Johnson Rd, Charleston, SC 29412, Tel: +1-843-762-8529, Fax: +1-843-762-8700, E-mail: Fran.Vandolah@noaa.gov. Ruberu et al. (39) reported the results of an interlaboratory comparison of PSP in shellfish extracts using the receptor assay formatted for high throughput using microplate scintillation counting. Modifications were made to the original protocol (40) to accommodate differences in counting efficiency of 2 commercial microplate scintillation counters (Wallac TriLux1450 vs Packard TopCount). Shellfish samples tested ($n = 75$) ranged from nondetectable by mouse bioassay (<40 g/100 g) to 137 g/100 g. LOD of the optimized assay was 0.2 g/100 g with a between-assay RSD of 10%. The ratio of STX equiv. concentrations reported by the 2 laboratories averaged 0.9. STX equiv. determinations were somewhat higher than those reported by the mouse bioassay for samples <80 g/100 g, as has been previously observed. It is likely that the shellfish matrix provides a "protective" effect for the mouse, as it is injected undiluted at these low levels. Further work on the receptor assay for PSP toxins is currently impeded by the worldwide unavailability of tritiated STX, previously produced by Amersham. The International Atomic Energy Agency is currently pursuing alternative sources of reagent to employ in planned international collaborative testing of the receptor assay for PSP toxins.

A multilaboratory study was carried out to test the performance of 4 methods as candidate alternatives to the mouse bioassay for determination of brevetoxins in shellfish tissue extracts (41). Methods tested included ELISA, N2A neuroblastoma cytotoxicity assay, HPLC-MS, and 2 formats of the receptor assay (test tube and microplate). In the receptor assay, recovery of PbTx-3 from spiked test samples was 97% (tube format) and 137% (microplate format). Within-laboratory variation of all test samples averaged 27% (tube format) and 16% (microplate format). Between-laboratory variation averaged 39% (tube format) and 23% (microplate format). Of the methods tested, the ELISA and the microplate format receptor assay showed statistically acceptable correlation to the mouse bioassay, with HORRAT values of 0.9 and 1.4, respectively.

The microplate format domoic acid receptor assay using a cloned glutamate receptor (42) requires the removal of com-

peting glutamate (Glu) from sample extracts by incubation in the presence of glutamate decarboxylase (GAD), which irreversibly converts Glu to gamma-aminobutyrate (GABA) such that it does not bind to the glutamate receptor. This step was efficient at removing up to 10mM Glu from samples, well above the levels of Glu found in shellfish extracts. In 2002, commercial suppliers discontinued production of glutamate decarboxylase previously used in the assay, due to difficulty in expressing the enzyme. This led to 2 separate solutions. Baugh et al. (43) designed an alternative enzymatic digestion using glutamate dehydrogenase (GLDH), which reversibly converts Glu to alpha-ketoglutarate. The equilibrium of this reaction favors Glu formation at neutral pH; however, by increasing pH to 9.0 and addition of hydrazine hydrate, a trapping agent, this method was shown to convert up to 700 M Glu, sufficient to remove competing Glu from seawater samples for which the method was designed. Quantification of domoic acid in seawater samples using the GLDH digestion showed excellent correlation ($r^2 = 0.94$) with HPLC using the method of Hatfield et al. (44). The method was not efficient at removing higher Glu concentrations that may be present in shellfish or tissue samples. Doucette et al. (personal communication) resolved the lack of commercial GAD by purification of GAD enzyme from a cloned bacterial GAD gene expressed in an *E. coli* over expression system (manuscript in preparation), which yielded GAD activity sufficient to remove 10mM Glu in spiked samples using the original assay method.

Sample Pretreatment Methods for Marine Toxin Analysis

Topic Advisor Ana Gago-Martinez. Gago-Martinez reports that the complexity of the matrix in which algal toxins are commonly found as well as the low levels at which they can produce toxicity, make sample preparation critical for a reliable determination of these toxins when present in such complex matrixes. A lot of attention has been paid over the last few years to the development of sensitive analytical techniques, which allowed to identify new toxins as well as to determine them with high levels of sensitivity; nevertheless, further improvements in sample preparation protocols are still required.

The most common methods used for sample extraction and cleanup involve conventional solid-phase extraction (SPE). The main drawbacks encountered with this approach relate to selectivity, specificity, and variability due to the packing materials used for SPE. This variability is seen as lack of reproducibility, repeatability, and sometimes as low efficiency. An example of this was observed for the sample preparation of ASP toxins and cyanobacterial toxins such as microcystins (MCs). Different extraction and cleanup procedures have been proposed in the literature for the analysis of these toxins (45–52). The efficiency of the extraction and cleanup procedures for ASP toxins are presently being evaluated in the author's laboratory as is laboratory sample pretreatment of cyanobacterial toxins such as microcystins. The results obtained show significant variability which could

compromise the reliability of the analysis. Thus the evaluation of these steps is strictly required. Generally, the development of more efficient sample preparation protocols including faster and efficient cleanup procedures is still a priority in this field.

Immunoaffinity chromatography (IAC) is a powerful tool for improved selectivity and specificity. A few examples of the results obtained using IAC for the cleanup of samples contaminated with MCs is shown in refs. 53–55. Modern alternatives for extraction using microwaves, solid-phase microextraction, accelerated solvent extraction, etc. are being developed in the author's laboratory for algal toxins. It is therefore recommended that alternative sample preparation protocols be sought to ensure the reliability of the analytical data especially when confirmatory techniques using mass selective detection are not available.

Recommendations

Amnesic Shellfish Poisoning Toxins: Study Director Michael A. Quilliam. Continue study.

Bioassays for Phycotoxins: Topic Advisor Donald J.A. Richard. Continue study.

Capillary Electrophoretic Methods for Marine Toxins: Topic Advisor Ana Gago-Martinez. Continue study.

Cell Bioassays for Phycotoxins: Topic Advisor Ronald Manger. Continue study.

Diarrhetic Shellfish Poisoning Toxins, Assay Methods: Topic Advisor J. Marc Fremy. Continue study.

Immunological Methods for Seafood Toxins: Topic Advisor Joanne Jellett. (Note: This topic was broadened from the previously termed *Paralytic Shellfish Poisoning Toxins, Immunological Methods.*) Continue study.

Microcystins: Topic Advisor Geoffrey A. Codd. Continue study.

Paralytic Shellfish Poisoning Toxins, Instrumental Methods: Topic Advisor James F. Lawrence. Continue topic.

Receptor Assays for Phycotoxins: Topic Advisor Frances Van Dolah. Continue study.

Sample Pretreatment Methods for Marine Toxin Analysis: Topic Advisor Ana Gago-Martinez. Continue study.

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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: +1-301-436-2644, E-mail: mtruckse@cfsan.fda.gov

Topic Advisors

David Abramson, 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, and David M. Wilson

Thomas Whitaker, Sampling and Subsampling Topic Advisor, received the 2003 AOAC Wiley Award for his contribution to cross-cutting sampling technology. The Wiley Award Symposium covered Whitaker's work on peanut sampling for aflatoxins, peanut origin certification, biotechnology derived grains, dwarf bunt spores (TCK) in wheat, and food allergens.

Herbs are produced in many different countries in some of which quality control may be inadequate. The materials may be moldy from exposure to adverse weather conditions, insect

damage, improper harvesting procedures, damage during transportation, inadequate drying, and improper storage facilities. The growing mold may have produced mycotoxins. In the United States about 50% of botanical supplements on the market have been treated with low-dose radiation (1–10 kGy) for microbial decontamination (1). Radiation-processed herbs may contain more mycotoxins than nonirradiated herbs. The effects of radiation used for preservation and sterilization of mold and metabolites have been studied. Radiation reduced mold flora that produced aflatoxins and ochratoxin A but stimulated the remaining viable molds' ability to produce toxins. A series of papers on toxin producing mold reported an increased production of aflatoxins by *Aspergillus flavus* following gamma irradiation in dose ranges of 0.25 to 6.0 kGy (2). The increased aflatoxin production by *A. flavus* and *A. parasiticus* was attributed to formation of mold mutants (3). Enhancement of ochratoxin A production by *A. ochraceus* after exposure to gamma radiation has also been reported (4). Aflatoxins are stable to radiation. AFB₁ exposed to up to 20 kGy was not destroyed (5).

Many analytical methods have been developed for aflatoxins in grains and nuts. Collections of herbal products for analysis of mycotoxins were limited and the methods cited or described were deficient in confirmation of identity of the toxins (6–8). There is great need to develop reliable, validated, quantitative methods for mycotoxins in dietary supplements.

During 2003, the following method was approved as Official First Action: **2003.02** Immunoaffinity Column Cleanup with Liquid Chromatography Using Post-Column Bromination for the Determination of Aflatoxin B₁ in Cattle Feed. In addition 5 other collaborative studies are in progress.

Codex Activities

Codex Committee on Food Additives and Contaminants (CCFAC): The 35th CCFAC meeting was held in Arusha, Tanzania, March 17–21, 2003.

The following documents (1 and 2 below) were discussed and forwarded by CCFAC to the 26th Session of the Codex Alimentarius Commission (CAC) for final adoption at Step 8: (1) Draft Code of Practice for the Prevention and Reduction of Patulin Contamination in Apple Juice and Apple Juice Ingredients in Other Beverages; (2) Draft Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals, Including Annexes on Ochratoxin A, Zearalenone, Fumonisin, and Trichothecenes.

The following document (3 below) was discussed and forwarded by CCFAC to the 26th Session of the CAC for preliminary adoption at Step 5. (3) Proposed Draft Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Peanut.

Other issues: The Committee discussed and agreed to request the Drafting Group (led by China) to revise the Proposed Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Tree Nuts for further consideration at the next CCFAC meeting (March 2004).

The Committee discussed and agreed to request the Drafting Group (led by Iran) to revise a Discussion paper on Aflatoxins in Tree Nuts to include the elaboration of maximum levels for aflatoxins in almonds, hazelnuts, and pistachio nuts for further consideration at the next CCFAC meeting.

The Committee discussed and agreed to request the Drafting Group (led by Belgium) to commence work on the elaboration of maximum levels for deoxynivalenol in cereals for consideration at the next CCFAC meeting.

The 26th Session of the CAC was held in Rome, Italy June 30–July 7, 2003.

Documents 1 and 2 (above) were adopted by the CAC at Step 8 as Final Texts. The document Maximum Levels for Patulin in Apple Juice and Apple Juice Ingredients in Other Beverages was also adopted by the CAC at Step 8 as Final Texts. Document 3 (above) was adopted by the CAC at Step 5.

Topic Advisor Reports

Sampling and Subsampling

Topic Advisor Thomas B. Whitaker, U.S. Department of Agriculture, North Carolina State University Agricultural Research Service, PO Box 7625, Raleigh, NC 27695-7625, Tel: +1-919-515-6731, Fax: +1-919-515-7760, E-mail: whitaker@eos.ncsu.edu. Whitaker reported that the Codex Commission approved as new work in June/July 2003, the establishment of aflatoxin limits in almonds, hazelnuts, and pistachios. Appropriate sampling plans will be part of the discussion as the CCFAC begins their consideration of aflatoxin limits.

Codex Committee on Methods of Analysis and Sampling (CCMAS) is developing 2 documents, Sampling Guidelines and Measurement Uncertainty. These documents are currently at Step 6 of the Codex process.

Three studies are currently underway to determine the uncertainty associated with measuring mycotoxins and other analytes in foods and food products: (1) FDA and USDA scientists are collaborating to measure the variability associated with 4 analytical kits to measure peanut protein in 4 food matrices. (2) Scientists with the Brazil Ministry of Agriculture are studying the variability associated with testing green coffee beans for ochratoxin A. (3) FDA and USDA scientists are collaborating to measure the variability associated with testing grains for genetically modified seed.

Aflatoxin M₁

Topic Advisor Hans P. van Egmond, National Institute of Public Health and the Environment, Laboratory for Residue Analysis, Postbox 3, 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 an updated FAO Food and Nutrition Paper on mycotoxin regulations, prepared by the National Institute for Public Health & the Environment under contract with the Food and Agriculture Organization of the United Nations approaches the stage of finalization (9). The document is based upon an extensive worldwide enquiry carried out in 2002/2003. The paper will

include updated information about specific regulations on aflatoxin M₁ (AF M₁) in milk and milk products. A first impression from the draft (Figure 1) shows the following: Regulations for AF M₁ are now seen in 60 countries, a more than 3-fold increase as compared to 1995. It is again in the EU, EFTA (European Free Trade Association), and candidate EU countries where 0.05 g/kg limits for AF M₁ in milk are applied, but some countries in Africa, Asia, and Latin America also apply this limit. Another often occurring legal limit is at 0.5 g/kg. This higher regulatory level is applied in the United States, several Asian, and some European countries, and it occurs most frequently in Latin America, where it is established as a harmonized MERCOSUR (South American Free Trade Association) limit. Apart from these sub- g/kg regulatory levels, a few countries indicated in the 2002 inquiry that they regulate AF M₁ in milk at levels of 5 and 15 g/kg. These levels are not realistic and probably these are mistakes that will need to be adjusted during the correction stages of the draft document.

Because of the high acceptance of certified reference materials (CRMs) for AF M₁ in milk powder the Institute for Reference Materials and Measurements (IRMM) of the European Commission's Directorate General Joint Research Centre (JRC) has certified 3 new batches of aflatoxin M₁ in milk powder (BCR-282R, -283R, and -284R). The provisionally certified AF M₁ mass fractions of <0.02, 0.11, and 0.44 g/kg, respectively, conform with requirements as laid down by several EU legal instruments. The materials will be on sale not later than beginning of 2004 (10).

There have not been many publications on analytical developments in the area of AF M₁. Methods applied in surveillance and monitoring programs are usually the immunoaffinity column (IAC)/liquid chromatography (LC) methods, and ELISAs are also used.

Recently published surveys on AF M₁ (and incidentally aflatoxin M₂) involved countries such as Brazil, Greece, Spain, and Turkey. The survey in Brazil of 60 ultra high temperature milk samples and 79 pasteurized milk samples showed 29 samples positive for AF M₁ in the range 5 to 240 ng/kg (11). No aflatoxin M₂ was found. The authors concluded that the levels were not a serious health problem according to MERCOSUR Technical regulations (*see also* above), but they recognized that the 21% incidence of positives would not fulfil EU regulations. In Greece a total of 297 samples of raw (cow, sheep, and goat) and market milk were investigated for AF M₁ (12). Only in a few cases the EU limit of 0.05 g/kg was exceeded. The authors concluded that the current regulatory status in Greece is effective. Raw cow's milk collected from dairy farms in Spain was examined for AF M₁ (13). In only 3.3% of samples could the presence of AF M₁ be confirmed, but concentrations were below the EU limit. A survey of aflatoxins in retail products in Turkey (14) revealed that 15% of the investigated 130 cheese samples (fatty Turkish white cheeses, fresh kashars, old kashars, Gravyer cheeses, and cream cheeses) exceeded the Turkish tolerance limit of 250 ng/kg.

Egyptian researchers investigated human exposure to mycotoxins by using aflatoxin M₁ in human milk as a



Figure 1. Frequency distribution of specific regulatory limits for aflatoxin M₁ in milk in 60 countries, in 2002.

biomarker for aflatoxin B₁ (15). In 120 human milk samples aflatoxin M₁ could be found at a mean level of 0.3 0.5 ng/mL. Aflatoxin M₁ was also found in 25% of blood samples at a measured level of 1.2 ng/mL. If correct, these results point to substantial exposure of lactating mothers to aflatoxin B₁.

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@tifton.cpes.peachnet.edu. Wilson reported that he and Study Director Arthur Walking conducted a study using AOAC *Official Methods* for extraction, purification, and separation for aflatoxins. The test solutions were divided into 3 portions and 3 different post-column derivatization techniques were used to enhance the fluorescence of aflatoxins B₁ and G₁: the post-column photochemical reactor (PHRED cell), post-column iodine, and the Kobra cell. The data was comparable. The Study Director is planning to submit data of the study to AOAC requesting the addition of the use of the PHRED cell as method modification of the existing methods in which the cell was used.

Several interesting papers relating to aflatoxin assays were published. Advances in analytical methods and novel detection systems were reviewed from 1995 onwards (16). An immunochemical biosensor assay was used to detect 4 mycotoxins, AFB₁, fumonisin B₁ (FB₁), zearalenone, and deoxynivalenol (DON), in a single measurement, following extraction, sample cleanup, and incubation with a mixture of appropriate anti-mycotoxin antibodies. The assay time was 25 min and detection limits were <1 ng/g for most of the analyte except FB₁ was 50 ng/g (17). A method for screening the aflatoxins production by *A. flavus* was reported (18). The strains were grown on yeast extract agar. Methylated -cyclodextrin derivative and sodium deoxycholate were added. After 3 days at 28 C aflatoxins was detectable by the appearance of a beige ring surrounding the colonies. The ring exhibited blue fluorescence under longwave UV light. An ELISA method was used to detect AFB₁ in chicken liver extracts that were with or without prior purification with IAC (19). Results were similar. A radioimmunoassay procedure for AFB₁ was developed for rice, wheat, soybeans, and

ground nuts in India (20). The sample analysis was compared with a commercially available ELISA method. Results showed good correlation.

Alternaria Toxins

Topic Director Michele Solfrizzo, Institute of Sciences of Food Production, National Research Council, Viale L. Einaudi 51, Bari 70125, Italy, Tel: +39-080-5912838, Fax: +39-080-5486063, E-mail: michele.solfrizzo@ispa.cnr.it. Solfrizzo reported that an LC/mass spectrometry (MS) and LC/MS/MS method for quantitative analysis of alternariol (AOH) and alternariol methyl ether (AME) in fruit beverages has been developed. Positive and negative ion mass spectra of AOH and AME under electrospray and atmospheric pressure chemical ionization conditions were obtained. Electrospray LC/MS/MS with negative ion detection and multiple reaction monitoring mode showed higher sensitivity and specificity allowing the detection of 4 pg of both mycotoxins. The method, which includes a sample cleanup on C18 and aminopropyl solid-phase extraction columns, was applied to the confirmation and determination of ng/mL and sub-ng/mL levels of AOH and AME in apple juice, grape juice, cranberry nectar, raspberry juice, prune nectar, and red wine. AOH at levels of 0.84–5.6 ng/mL was found in 5 out of 9 fruit beverages whereas AME at levels of 0.23–1.4 ng/mL was found in 3 out of the 9 tested samples (21). The mutagenicity of the major *Alternaria* metabolites (AOH, AME, tenuazonic acid, altenuene, altertoxin I, tentoxin, and radicinin) and *A. alternata* culture broth was examined using the Ames Salmonella test. Nitrosylation increased the mutagenicity of altertoxin I, and to a lesser extent that of several other *Alternaria* toxins. The mutagenic response of *A. alternata* culture broth was different from that found with the purified toxins suggesting the presence of other mutagenic compounds in the culture broth (22).

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@agr.gc.ca. Abramson reported that several publications have appeared regarding citrinin production by *Penicillium expansum* and other species in fruit and culture media. In Portuguese apples, levels of citrinin between 0.32 and 0.92 mg/kg have been found, along with 3.06–5.37 mg/kg patulin (23). In fruit juices marketed in Germany, citrinin was found at levels between 0.10 and 0.20 ng/mL, although this may be an underestimation due to low recoveries with the immunochemical procedure (24). In another study, *P. expansum* was isolated from retail-level apples in Croatia containing 0.05–0.24 mg/kg citrinin, and cultured in yeast extract-sucrose (YES) liquid medium. In culture, 11/29 isolates produced citrinin at 0.07–9.0 mg/kg (25). In a study of this fungus on various agar media, production of citrinin on YES agar proved quite consistent, while production of patulin varied with the strain and culture medium used (26).

In other foods and food additives, French workers found that *P. expansum* and *P. citrinum* could produce citrinin in cheese at 20 °C. Levels up to 600 mg/kg after 10 days were found in the cheeses studied, namely goat's milk, Saint Marcellin, and Soignon. Surface trimming left behind about 33% of the citrinin (27). In a study on *Monascus* culture material, which is used as a natural red food colorant, a GC/MS method was devised to detect citrinin in this matrix down to about 1 µg/kg. In a small sampling of retail-level *Monascus* colorant in Taiwan, citrinin levels between 4.2 and 25.1 mg/kg were found (28).

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. Dorner reported that in a study of the mutagenicity of cyclopiazonic acid (CPA) and AFB₁ using the Ames test, it was found that CPA was not mutagenic (29). The mutagenicity of AFB₁ was significantly inhibited in the presence of CPA in a dose-dependent relationship. This is an important finding given the relatively common co-occurrence of aflatoxins and CPA in various commodities.

The production of CPA by *Penicillium commune*, a frequent contaminant of dry-cured meat products, was evaluated over a range of temperatures and water activities (30). CPA in extracts was quantified by LC on a Supelcosil LC18 column and a mobile phase gradient consisting of water and various percentages of 5% trifluoroacetic acid in acetonitrile. The optimal condition for production of CPA was a temperature of 30 °C at a water activity of 0.96.

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. Ware reported on a survey for mycotoxins in infant cereal foods from the Canadian retail market (31). Three hundred and sixty-three samples of cereal-based infant foods were collected from the Canadian retail marketplace over 3 years. The samples included oat, barley, soy, and rice-based infant cereals, mixed-grain infant cereals, teething biscuits, creamed corn, and soy-based formulas. Forty-one out of 161 samples contained detectable levels of ergot alkaloids. Barley-based cereals had the highest incidence of ergot alkaloid contamination (31/55). The calculated LOQ for each of the individual ergot alkaloids was 4 ng/g.

The separation of 39 ergot alkaloids using thin-layer chromatography (TLC) and LC were reported (32). Chromatographic separation of alkaloids on silica plates was reported using different solvents: (a) chloroform, methanol, and 25% NaOH (90:10:1, 90:10:0.1, or 80:20:0.2); (b) chloroform and acetone (9:1); and (c) ethyl acetate, methanol, and 25% NH₄OH (85:15:10). LC gradient elution conditions for separation of clavine alkaloids on ODS2 and Supelcosil LC-18

columns were optimized. The retention values of 22 alkaloids were compared to those of agroclavine and roquefortine.

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. Maragos reported that much interesting research was published concerning fumonisins in the past year. Previous studies have indicated that fumonisins occur worldwide and the majority of the recent publications deal with prevalence. Fumonisin was found in maize or maize products in Argentina (33), Taiwan (34), Iran (35); in marketed foods in Italy (36); in corn flakes in Canada (37), and in barley and corn-based foods from Korea (38). Mycotoxins, including the fumonisins were found in retail samples of cereal-based infant foods in Canada (31), and in a corn/cottonseed-based nutritional supplement (Incaparina) from Guatemala (39). Fumonisin was also found in cowpea seed samples in South Africa at levels up to 0.61 g/g (40). These studies reinforce that fumonisins are found worldwide and in a wide variety of products. FB₁ decreased the cardiovascular function of horses, which, combined with increased serum sphinganine and sphingosine, may contribute to the development of leukoencephalomalacia in that species (41). A 28-day feeding study of FB₁ and 6 related compounds in B6C3F mice indicated that, of these compounds, FB₁ was the only one that was hepatotoxic to the mice (42).

The widespread occurrence of fumonisins derives from the worldwide distribution of the fumonisin producing fungi of the genus *Fusarium*. The production of fumonisins and their analogs by 17 *Fusarium* species was reviewed (43). Visible and near-infrared spectroscopy were used to detect maize contaminated with *F. verticillioides* (44). Single kernels contaminated with more than 100 g/g or less than 10 g/g FB₁ could be identified with the technique, suggesting that a near-infrared device might be useful for the nondestructive screening of maize for fumonisins.

The factors affecting the occurrence of fumonisins in commodities and processed foods was also an active area of study. Maize that contained up to 10⁵ CFU/g fungi, predominantly *Fusarium* and *Penicillium* species, was dried to 11% moisture and stored for 12 months. No significant change in fumonisin levels from the initial level of 9.9–6.0 g/g was reported (45). In some South African communities maize is used to prepare a traditionally cooked stiff porridge. Fumonisin levels in naturally contaminated maize were reduced by an average of 23% by the process, which involves addition of maize meal to boiling water with salt and simmering for 20 min (46). The fumonisins can undergo alkaline hydrolysis, and can be substantially reduced by nixtamalization. The reaction of the primary amine of FB₁ with glucose is also known to occur under the appropriate conditions. A method was developed to isolate the fumonisin-glucose reaction products (47). Control of fumonisin production by limitation of *F. verticillioides* growth in culture using plant phenolic compounds was tested (48).

Chlorophorin, iroko, benzoic acid, caffeic acid, ferulic acid, and vanillic acid reduced FB₁ levels by greater than 90% in this system.

Fumonisin is very water soluble and is often isolated from foods using reverse-phase or ion-exchange solid-phase extraction (SPE) columns. The migration of FB₁ in columns filled with soils containing various sand contents were investigated to provide insights into the movement of fumonisins through soils (49). Sand retarded the migration slightly, while Cecil sandy loam retained much of the applied FB₁. Of the FB₁ bound to the Cecil sandy loam approximately 75% was eluted with formic acid or formic acid/acetonitrile.

Instrumental methods for measuring fumonisins continued to be investigated, particularly those using LC. LC with fluorescence detection of the *o*-phthalaldehyde (OPA)/*w*-mercaptoethanol derivative remains a popular method. Limits of detection for FB₁ and FB₂ were lower using an OPA derivatization procedure than using an NBD-F (4-fluoro-7-nitrobenzofurazan) derivatization procedure (50). Thirty-seven alternatives to 2-mercaptoethanol in the OPA derivatization of fumonisins were examined (51). Several of these, including 3-mercaptopropionic acid, *N*-acetyl-cysteine, and thioglycerol were considered superior to 2-mercaptoethanol, particularly when immunoaffinity columns were used to prepare the samples. Less decay of signal attributable to FB₁ was observed with these 3 reagents over 2.5 h relative to when 2-mercaptoethanol was used. The structure of the FB₁ derivative obtained with *N*-acetyl-cysteine was verified by LC/MS. Naphthalene-2,3-dicarboxaldehyde, which has been used previously for fumonisin derivatization, was compared to OPA and found to give more stable derivatives of sphingoid bases (52). Aside from the derivatization, many laboratories have begun to use LC/MS for fumonisin detection. One of the LC/MS equipment manufacturers has described a method for FB₁ in corn, which is published as an application note on their Web site (www.thermoquest.com).

Ochratoxins

Topic Advisor Benedicte Hald, Royal Veterinary and Agricultural University, Department of Veterinary Microbiology, Stigbøjlen 4, Friedriksberg C, Copenhagen 1870-DK, Denmark, Tel: +45-3528-2762, Fax: +45-3528-2757, E-mail:vetmi@kvf.dk. Hald reported recent publications on ochratoxin A (OTA). Zollner et al. (53) emphasized the use of LC/MS/MS as an analytical tool for determining trace amounts of OTA in a variety of food matrixes and biological fluids. Possibilities and limitations of the technique were outlined with special attention to the impact of sample preparation. Performance characteristics of an LC method for determination of OTA in cacao powder such as within- and between-day repeatability, reproducibility, and accuracy were described (54), showing reliable results adequately matching the criteria suggested by the European Committee for Standardization (CEN) for the analysis of OTA. The advantages of using this method was saving analytical time and the possibility of detecting OTA at ng/kg level. Recently food safety issues received increasing attention. Both monitoring programs

and researches have been performed, aimed at focusing the status of contamination worldwide and hazard analysis critical control points (HACCP) in the wine making process. A study to carry out an automated method analysis for the determination of OA in wine samples was published (55), in order to process a high number of samples for HACCP purposes.

Belli et al. (56) gave a general overview of methods of analysis and occurrence of OTA in wines. The results of more than 2000 samples summarized from the literature have been taken into account to quite extensively describe the present situation of OTA in wine. LC/fluorescence detection preceded by extraction of OTA using commercially available immunoaffinity columns is currently the method most applied for OTA determination in wines.

A total of 408 Brazilian coffee samples were examined during the 1999–2000 coffee harvest seasons for the presence of OTA and fungi with the potential to produce it (57). OTA was found in some samples, but occurrence was sporadic. Only 7% of 135 samples had OTA levels higher than 5 g/kg and in all cases this was found to involve local environmental conditions and poor harvesting, drying, and storage practices. During the past year there were reports of occurrence of OTA in Australian wines (58), South African wines (59), Greek wines (60), fungi in grapes grown in Italy (61), Greek domestic wines and dried vine fruits (62), cereals from Puglia, Italy (63) and Danish wheat and rye, 1992–1999 (64).

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. Sabino reported that studies were conducted to evaluate the influence of different culture preservation techniques on the production of patulin and citrinin (26). Variability in the profiles of the mycotoxins tested seems to be more strain-specific than dependent on the preservation techniques used. Influence of gamma-radiation on mycotoxin producing molds and mycotoxins in fruits were studied (65). Analysis of fruits revealed the occurrence of penicillic acid, patulin, CPA, citrinin, OTA, and aflatoxin B₁. Irradiation of fruits at doses of 1.5 and 3.5 kGy decreased significantly the total fungal counts compared with nonirradiated controls. Mycotoxins production in fruits decreased with increasing irradiation dose and were not detected at 5.0 kGy.

The biocontrol capacity of *Candida sake* and *Pantoea agglomerans* on growth of blue and gray mold on apples and pears was investigated (66). This study demonstrated that the combination of *C. sake* and *P. agglomerans* improves the control of *P. expansum* and *B. cinerea* in some fruits and allows the required concentration to achieve reduction of the pathogens to half, with a subsequent reduction in the cost of treatment.

In order to find an alternative to postharvest fungicides currently used in the prevention and control of the damage by *P. expansum* in apples, the antimicrobial activity of several substances against *P. expansum* were evaluated in vitro using different end point methods: agar diffusion assay, volatility method, and agar dilution and broth dilution MIC assays (67).

Essential oils, such as thymol, eugenol, citral, and cineole, vanillin, sodium hypochloride, acetic acid, potassium sorbate, and hydrogen peroxide, were the substances evaluated. Thymol and citral were the essential oil components that showed the greatest inhibitory effects. The use of hydrogen peroxide for postharvest treatment of Golden Delicious apples gave the best result.

The effects of *P. expansum* isolate, apple cultivar, storage temperature, duration, and pH on the production of patulin were investigated (68). The highest patulin levels were by those isolates displaying aggressive growth (characterized by rapidly increasing acidity) accompanied by profuse mycelial development. Extensive fungal growth and higher patulin levels in apple ciders were associated with incubation at room temperatures (23 °C), although potentially toxic patulin levels were also found in refrigerated ciders inoculated with *P. expansum*. The effect of vitamins such as thiamine hydrochloride, pyridoxine hydrochloride, and calcium d-pantothenate on the patulin content of apple juice concentrate was also studied (69). These vitamins were added to apple juice concentrate at various doses in order to reduce the patulin content. Apple juices concentrates containing high levels of patulin were stored at 22° and 4 °C for 6 months after vitamins were added. Patulin was fully degraded at the end of a 6 month period in samples stored at 22 °C, on the other hand, other quality parameters diminished significantly.

A rapid multidetection TLC method was used to detect patulin and citrinin simultaneously in 351 samples of 7 different varieties of apples with small rotten areas (23). The minimum detectable concentration of patulin and citrinin were 120–130 and 15–20 g/kg, respectively. The percentage contamination with patulin only was higher (68.6%) than that with citrinin only (3.9%). Patulin and citrinin (19.6%) were also detected simultaneously and all apples showed low level of citrinin contamination ranging from 0.32 to 0.92 mg/kg.

The cellular mechanisms associated with the intestinal toxicity of patulin were reported (70). Two human epithelial intestinal cell lines (HT-29-D4 and Caco-2-14) were exposed to the mycotoxin. Inactivation of an active site of protein tyrosine phosphatase was found.

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: reppey@cfsan.fda.gov. Eppley reported that DON, of all the known trichothecenes, continues to draw the most research interest as indicated by the number of publications (71–79) during the last year. Four of these publications explored new analytical techniques (71–74). One uses 2 commercial cleanup columns for the analysis of wheat products (71). Recoveries ranged from 70 to nearly 100% with a limit of detection of 50 ng/g. Another reports improvements in the rapid fluorescence polarization immunoassay for DON in wheat (72). The authors concluded that the procedure was useful for the screening of wheat for DON, but not corn. The application of near infrared spectroscopy for the determina-

tion of DON in wheat was investigated (73). The results indicated that it might be possible to develop a calibration model, which can be used to rapidly screen for DON in wheat. The development of molecularly imprinted polymers for the analytical purification of DON and zearalenone was investigated (74). The results were promising. Currently available methods for DON determination were used in several surveys. Wheat and rye on the Danish market were surveyed (75). Over a 4 year survey period, DON was found in 78% of the samples. A survey of processed cereals and pulses from Turkey (76) reported DON in 6 of 68 cereal samples. In the analysis for trichothecenes in beer from The Netherlands (77), only DON was found in low quantities. Cereal-based infant foods from the Canadian retail market were surveyed for several mycotoxins (31). DON was detected in 63% of the samples.

The distribution of DON in naturally contaminated wheat during processing in an industrial mill was studied (78). The DON was found in both the flour and gluten with the majority going to the bran.

Some other publications on DON were noteworthy. A urinary biomarker of human exposure to DON was developed using an IAC/LC method (79). An improved method for the large-scale production of DON was the subject of another publication (80).

Two reports on the determination of the type A-trichothecenes were noted. In one case, the authors use LC-atmospheric pressure chemical ionization MS to analyze T-2 toxin, HT-2 toxin, acetyl T-2 toxin, diacetoxyscirpenol, monoacetoxyscirpenol, and neosolaniol in oats, maize, barley, and wheat samples (81). In the other report, LC with fluorescence detection, after cleanup by IAC, was used to determine T-2 toxin in various cereals (82).

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. Hagler summarized selected information appearing in the last year's scientific literature.

A very detailed examination of the metabolic profiles of zearalenone and zeranone in heifers was reported (83). The discussion of the report relative to work in other species is particularly interesting. One conclusion is that definitive detection of the use of zeranone as a growth promoter, in countries in which it is not approved, is not a simple matter.

The European Commission sponsored preparation and definition of CRM for zearalenone determination. One very useful result was the determination of the molar absorptivity of zearalenone in acetonitrile at 274 nm was determined (84).

The fungus, *Clonostachys* sp., converted zearalenone to a nonestrogenic metabolite. The structure of the transformation product, inactive in bioassays, was 1-(3,5-dihydroxyphenyl)-10-hydroxy-1 E-undecene-6-one (85). Golinski et al. reported zearalenone in winter pasture in Poland. The highest

concentration found was 48 ng/g and incidence was as high as 66% (86).

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. The collaborative study report has been submitted to the AOAC Methods Committee.

(2) *Immunoaffinity Column Cleanup with Liquid Chromatography Using Post-Column Bromination for the Determination of Aflatoxin B₁ 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. The study has been approved as First Action Official Method **2003.02**.

Recommendations

The General Referee recommends:

(1) Approve all First Action Official Methods as Official Methods.

(2) Approve the following Methods: **2000.02**, **2000.03**, **2000.08**, **2000.09**, and **2000.16** as Final Action Official Methods.

(3) Terminate the appointment of the Study Directors after their studies have been approved as Final Action Official Methods.

(4) Develop analytical methods for mycotoxins in dietary supplements.

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 M₁*.—Topic Advisor Hans P. van Egmond, National Institute of Public Health and the Environment, Laboratory for Residue Analysis, Postbox 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 Director Michele Solfrizzo, Institute of Sciences of Food Production, National Research Council, Viale L. Inaudi 51, Bari 70125, Italy, Tel:

+39-080-5912838, Fax: +39-080-5486063, E-mail: michele.solfrizzo@ispa.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) *Ochratoxin*s.—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. Discontinue study.

(14) *Determination of Ochratoxin A in Green Coffee by Immunoaffinity Column Cleanup and HPLC*.—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 B₁ in Baby Food by Immunoaffinity Column and HPLC*.—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. Recommended for Final Action. Continue study.

(16) **2001.04** *Determination of Fumonisin B₁ and B₂ in Maize Flour and Cornflakes by HPLC*.—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. Continue study.

(17) *Determination of Aflatoxin B₁ 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. Approved as Official First Action **2003.02**. Continue study.

(18) **2001.06** *Veratox for Fumonisin*s.—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 HPLC*.—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). Study has been completed and report has been submitted to AOAC for review. Continue study.

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

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

(23) *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.

(24) *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. Study Director will submit single laboratory validation data. This study will be evaluated as a matrix extension (modification) of an *Official Method*.

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

(26) **2000.02** *Determination of Patulin in Clear and Cloudy Apple Juices and Apple Puree by HPLC*.—Suzan McDonald, MAFF - UK/Food Safety Directorate, United Kingdom. Approved as First Action in 2000. Recommended for Final Action. Continue study.

(27) **2000.03** *Determination of Ochratoxin in Barley by HPLC*.—Catherine Entwisle, Leatherhead Food RA, United Kingdom. Approved as First Action in 2000. Recommended for Final Action. Continue study.

(28) **2000.08** *Aflatoxin M₁ in Liquid Milk by Immunoaffinity Column Cleanup and HPLC with Fluorescence Detection*.—Sylviane Dragacci, AFSSA, Maisson-Alfort, France. Approved as First Action in 2000. Recommended for Final Action. Continue study.

(29) **2000.09** *Determination of Ochratoxin in a Roasted Coffee by HPLC*.—Catherine Entwisle, Leatherhead Foods RA, UK. Approved as First Action in 2000. Recommended for Final Action. Continue study.

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