Joint Mycotoxin Committee

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AOAC INTERNATIONAL

Mary Trucksess (U.S. Food and Drug Administration [FDA], Washington, DC) reported on AOAC activities and referred to the General Referee report presented at the 114th AOAC Annual Meeting during the Natural Toxins poster session. Trucksess highlighted some of the contents of the General Referee report, by topic area. In 1999, the Codex Commission accepted the recommendation of the Codex Committee on Food Additives and Contaminants and set a guidance of 15 ppb of aflatoxins in peanuts. The General Referee report indicated that a document that will assist member nations to implement the Codex aflatoxin sampling plan is needed. Also, this past year, a collaborative study of an immunoaffinity column (IAC) method with HPLC detection for aflatoxin M_1 (AFM_1) in milk, with a limit of detection of 0.02 ppb, was adopted Official First Action by AOAC INTERNATIONAL. The General Referee report indicated that methods using immunoassay approaches are needed and will be discussed by the Task Force. AFM_1 in milk powder is being proficiency tested by FAPAS and is available to interested participants. Biosensors for aflatoxins appear promising, and continue to be developed. A method using mushroom tyrosinase was developed for the detection of *Alternaria* toxins. Citrinin is a mycotoxin produced by fungi that also produce a red color, and the General Referee report suggested that the association be further investigated. An IAC method was developed for cyclopiazonic acid (CPA). CPA is of interest to FDA, particularly with regard to possible toxic effects together with the aflatoxins. This toxin was also found at levels up to 250 ng/g in an Italian cheese, and was found in both the rind and the core. The CPA may partition into the milk fat. An IAC method was also developed for one of the ergot alkaloids, LSD. The Associate Referee for fumonisins, Larry Rice, resigned and has been replaced by Chris Maragos. A substantial number of studies during 1999 illustrated the worldwide nature of the contamination of maize with the fumonisin mycotoxins. In the past year, a method for the extraction of fumonisins from maize using aqueous buffers was described in the literature and merits further investigation. A number of studies on the stability of fumonisins during processing, in particular extrusion and nixtamalization, were also described in the General Referee report. In Europe, regulators are considering limits for ochratoxin A. Levels of 5 ng/g for "raw" (unfinished) cereal materials and 3 ng/g for finished cereal products, such as flour, are being considered. In Brazil, a survey found very few samples of apple juice contaminated with patulin. The Associate Referee for trichothecenes recommended that methods be developed to detect multiple mycotoxins, such as fumonisins, zearalenones, and the trichothecenes. Trucksess also summarized methods that were recently approved Official First Action by AOAC INTERNATIONAL. Details may be obtained from the General Referee report.

International Dairy Federation Group E501

Jean-Marc Fremy (AFSSA, Maisons-Alfort, France) reported on the activities of the International Dairy Federation (IDF) Group E501 (Organic Contaminants) related to mycotoxins. The structure within the IDF has changed and the oversight of methodology for mycotoxins is now part of a new task force: the Task Force on Organic Compounds, which includes pesticides as well. The IDF is currently involved in validation of an IAC method for AFM_1 in milk, with detection by TLC. The goal of the program is to provide a quantitative method for AFM_1 using low cost equipment. Such methods are particularly needed by developing countries. The IDF is also supporting a risk assessment of the carry-over of fungal contaminants from animal feed into milk. Fremy, who is chair of the AOAC Method Committee on Natural Toxins, invited all those interested to attend the meeting of this committee.

American Oil Chemists' Society

Catherine Chen (Nabisco Foods, Fair Lawn, NJ) presented a summary of the projects with which the American Oil Chemists' Society (AOCS) Technical Committee on Mycotoxins is involved. The committee, chaired by James V. Falk (USDA-AMS, Washington, DC) met on April 27, 2000, in San Diego, CA. Samples prepared from naturally contaminated raw materials are being made available as part of a new AOCS Smalley check sample series for the fumonisins and deoxynivalenol (DON). The fumonisin series will be introduced in October 2000 and the DON series in 2001.

Falk continues to investigate supercriticial fluid extraction (SFE) procedures to extract aflatoxins from peanuts and cottonseed using 1,1,1,2-tetrafluoroethane (R-134a). Some of the conditions for this extraction have been developed. Optimal SFE conditions for the aflatoxin extraction using tetrafluoroethane are 110°C, 7500 psi pressure, and a flow rate

of 3.0 mL/min. A 3 g sample size resulted in the most efficient recoveries. Falk is also initiating research at the USDA laboratory in Blakely, GA, to find a replacement for chloroform in AOCS method **Ab 6-68**. The current AOAC Official Method **998.03** for analyzing aflatoxins in peanuts (water slurry method or modified BF method) also involves an initial extraction with various organic solvents, including chloroform. Nine alternative solvents or solvent mixtures were investigated as replacements for chloroform. Falk will also be preparing a summary report of the AOCS Laboratory Proficiency results for aflatoxins.

Arthur E. Waltking and Henry Joshua are organizing a collaborative study to determine the applicability of the quantitation of aflatoxins B_1, B_2, G_1, and G_2 by HPLC with post-column in-line photochemical derivatization (PHRED). Waltking requested that those in attendance who have a PHRED detector and who wish to participate in the study contact him for further information.

American Association of Cereal Chemists (AACC)

Dionisia Trigo-Stockli (Kansas State University, Manhattan, KS), chair of the Technical Committee on Mycotoxins in Cereal Products, described activities of the AACC. In the past year, a major emphasis was placed on revision of the AOAC Official Methods for mycotoxins. These changes are included in the revised (2000) edition of the AACC official methods. Trigo-Stockli also described the mycotoxin check sample service of the AACC. This service is designed to support the analytical accuracy and promote quality control of participating laboratories. The service is available for the aflatoxins (B_1, B_2, G_1, G_2), DON, or the fumonisins (FB_1 and FB_2). Samples of ground whole grain or other food or feed materials with varying levels of the toxins are distributed quarterly to laboratories that subscribe to the service. The subscribing laboratory performs the analyses of the samples and returns its results to AACC. AACC then provides a statistical evaluation of the analytical results wherein the confidentially coded results of the subscribing laboratory are compared with those of other laboratories. This mechanism lets subscribers compare the accuracy of their own laboratory to those of other industrial and government laboratories. An annual fee for the service is $450 for AACC corporate members and $675 for nonmembers. A brochure describing the service was distributed to the attendees.

International Union for Pure and Applied Chemistry

John Gilbert (MAFF CSL, Norwich, UK) reported on the activities of the International Union for Pure and Applied Chemistry (IUPAC), in particular the Food Chemistry Commission. The IUPAC is being reorganized and Gilbert is the final chairman of this committee in the current form. The IUPAC recently sponsored *The X International IUPAC Congress on Mycotoxins and Phycotoxins* in Sao Palo, Brazil, and proceedings from the meeting will be available later this year or early next year. IUPAC and FAO/IAEA are jointly involved in development of analytical methods needed by developing countries, in particular for AFM_1 and fumonisin B_1. The study of AFM_1 in milk by combined IAC cleanup and TLC determination (Project 650/94/97) is being done in collaboration with the IDF (described above in the IDF report). Lastly, a combined IAC cleanup and TLC determination for aflatoxins is being compared to a combined solid phase extraction cleanup and TLC determination (Project 650/95/98-Mycotoxin Methods for Developing Countries). The joint projects for developing countries are described further in the FAO/IAEA section of this report.

Comité Européen de Normalisation (CEN, Technical Committee 275/W.G.5 "Biotoxins")

Hans van Egmond (National Institute of Public Health and the Environment, Bilthoven, The Netherlands) described recent activities of the European Committee for Standardization Working Group 275 on Biotoxins. CEN is the European counterpart of ISO. The convenor for the working group is Hans Jeuring. Most European Union countries and Norway participate in the Working Group. The group is tasked with standardizing analytical methods for mycotoxins and phycotoxins in cooperation with the Standards, Measurements and Testing (SMT) Program of the European Commission (EC). The mycotoxin program, begun in 1992, uses a criteria approach for selection of methods. Methods accepted as standards are described in CEN report CR13505. Mycotoxin methods accepted as standards include those for ochratoxin A (OA) in cereals (2 methods), and aflatoxins in cereals, shell fruits, and derived products.

There are numerous methods at the stage of being draft CEN standards, including fumonisins in maize; patulin in apple juice and puree (AOAC approved 2000); aflatoxins in fig paste, pistachios, peanut butter, and paprika powder (AOAC approved 1999, Method **999.07**); OA in barley and roasted coffee (AOAC approved 2000); aflatoxin B_1 in baby food (AOAC approved 2000); OA in wine and beer (AOAC review stage); fumonisins in maize and maize products (AOAC review stage); and OA in baby food (AOAC review stage). Other items discussed in the CEN working group included a call for proposals for development of a method for DON in grains and development of certified reference materials for zearalenone in maize.

American Phytopathological Society

David M.Wilson (University of Georgia, Tifton, GA), member of the American Phytopathological Society (APS) Mycotoxicology Committee, described events at the APS annual meeting, held August 12, 2000, in New Orleans, LA. The committee has 12 members and was chaired by Frances Trail (Michigan State University). Special sessions at the APS meeting included: (*1*) *Significance of Mycotoxins to Pathogens*, which was held on August 13, 2000, and (*2*) *The Aflatoxin Elimination Program: A Model for Directed Research*, which was held on August 15, 2000. Special sessions for the 2001 APS meeting will include the role of mycotoxins in international food security (Kitty Cardwell is the organizer) and

mycotoxin synthesis and biotechnology. The committee is actively trying to recruit international members as well as graduate students. There is considerable interest within APS on genomics. Wilson noted that an AOAC-sponsored Web page for mycotoxins is needed. The existing Web pages are good, but the Joint MTX committee should consider establishing their own Web page. A list of existing Web pages would also be beneficial. Gary Munkvold (Iowa State University, Ames, IA) will be the chair and Anne Desjardins (USDA, Peoria, Illinois) the vice chair of the 2001 APS Mycotoxicology Committee. At the conclusion of the presentation, Trucksess noted that the 17th Edition of the *Official Methods of Analysis of AOAC INTERNATIONAL* (OMA) is now available. A suggestion has been made to publish the mycotoxin methods separately as their own book, for purchase separately from OMA. Trucksess asked for volunteers to edit such a book.

Japanese Association of Mycotoxicology

Tetsuhisa Goto (National Food Research Institute, Tsukuba, Japan) provided an overview and historical perspective of mycotoxin research in Japan. In Japan, modern mycotoxin research started before Turkey X disease. In the late 1940s, Japan imported rice due to the severe shortage of food after World War II. During that period, some of the imported rice from Indonesia and other southeast Asian countries was found to be contaminated with *Penicillium* mycotoxins. Both university and government scientists worked on this problem and found liver toxicity associated with the rice. They then halted the supply of this contaminated rice to the public. Because of the color of the contaminated rice, the problem was termed the yellow rice (not yellow rain) problem. Then in the1960s, after *A. flavus* and *A. parasiticus* were identified as aflatoxin producing fungi, questions were raised about the safety of traditional Japanese fermented foods such as miso (soy bean paste), shoyu and sake (rice wine), which used *A. oryzae* and/or *A. sojae* as starter cultures. It is now known that the *A. oryzae* and *A. sojae* used in the food fermentation industry did not produce aflatoxins. Also, aflatoxigenic fungi are rarely found in Japanese soil, except in the warmer southwest area such as Okinawa.

With this perspective, the Japanese Association of Mycotoxicology was founded in 1974. H. Kurata, who is the current president of the AOAC Japan Section, was the first president of the this association. Since 1975, 2 regular meetings have been held each year, in the winter and the summer. A journal, *Mycotoxins*, is also published semi-annually. The Japanese Association of Mycotoxicology has also hosted the 7th IUPAC Symposium for Mycotoxins and Phycotoxins (1988, Tokyo) and the International Symposium of Mycotoxicology (1999, Chiba). Proceedings from the Chiba meeting are now available and many of the presentations have been published in a special issue of the journal *Mycotoxins*.

Research on Mycotoxins in Latin America

Myrna Sabino (Instituto Adolfo Lutz, São Paulo, Brazil) discussed ongoing research on mycotoxins in South America. Research papers published within the last 5 years (February 1995 to January 2000) were used as the basis for developing an insight into mycotoxin research in Latin America. Of 112 papers, 37% surveyed the occurrence of mycotoxins in foods and feeds. Aflatoxins (B and G) continued to receive the most attention, followed by zearalenone, trichothecenes, OA, and the B series of fumonisins. The major commodity analyzed was corn, followed by peanut and peanut products and wheat and wheat products. The B and G series of aflatoxins were generally determined by TLC, while fumonisins and patulin were generally determined by HPLC. AFM_1 was determined by HPLC and ELISA, the trichothecenes by GC and TLC, and the *Alternaria* mycotoxins by TLC and HPLC.

Zearalenone and OA were assayed in various papers together with aflatoxins and sterigmatocystin with a multi-toxin TLC method. Data from Argentina, Brazil, Costa Rica, and Uruguay all showed widespread, high level contamination of corn and corn-based products with fumonisins. In Argentina, DON in wheat and wheat products, zearalenone in corn, and *Alternaria* mycotoxins in sunflower seeds also appeared to pose serious risks. In Brazil, aflatoxins in peanut and peanut products continued to be an alarming problem. DON in wheat, barley, and feed, ergot alkaloids in wheat and feed, and AFM_1 in milk were of concern in Uruguay. Relative to that of Brazil, aflatoxin contamination of feeedstuffs appeared to be greater in Colombia and Uruguay. Aflatoxin occurrence in corn was apparently more serious in Mexico than in Argentina, Brazil, and Uruguay.

Work on analytical methods continued and mycological examination notably increased. Attempts to find means of preventing or controlling fungal growth and mycotoxin production constituted 31% of the papers. Included were investigations on influencing factors (e.g. state of maturity, genotype resistance, water content or activity, temperature, presence of metals, type of soil, mite infestation, sieving) and the antagonistic potential of other micro-organisms against mycotoxin-producing fungi. Effects of plant extracts, essentials oils, flavonoids, fungicides and other chemicals, storage bag materials, adsorbents, cooking and processing of food were also studied. Most of the mycotoxin papers originating from Latin America are being conducted by investigators in Brazil, Argentina, and Uruguay.

The IAEA Projects in Developing Countries

Gordon Shephard (Medical Research Council, Tygerberg, South Africa) described the activities of the joint Food and Agriculture Organisation (FAO)/International Atomic Energy Agency (IAEA) Training and Reference Centre for Food and Pesticide Control. The center is currently undertaking a coordinated research program entitled “Evaluation of methods of analysis for determining mycotoxin contamination of food and feed” under the coordination of Maya Pineiro (Laboratorio Technologico del Uruguay, Montevideo, Uruguay). The program is designed to strengthen the analytical capabilities of laboratories undertaking mycotoxin analysis in developing countries and to assist national food control laboratories in identifying and validating time and cost efficient

methods for mycotoxin analysis of agricultural commodities. The specific modus operandi of the program has been to select mycotoxin and commodity combinations of high risk and evaluate analytical methods that can be used by food control laboratories. Recommendations specific to analytical methods are compiled for developing countries. The first research coordination meeting was held in Kuala Lumpur, Malaysia, in April 1999 and the following work groups were established within the program: aflatoxins in corn and peanut butter, fumonisin B_1 in corn, AFM_1 in milk, OA in green coffee, and trichothecenes in wheat and corn. In addition, a working group is investigating development of ELISA methods for aflatoxin B_1 and fumonisin B_1 and another group is investigating the effects of processing on fumonisins. In general, methods using technology available in laboratories in developing countries are being evaluated, with an emphasis on TLC methods. About 20 countries are participating, including both developed and under-developed countries. A TLC method for fumonisin B_1 in maize is currently being evaluated by 14 laboratories. The maize is extracted with methanol–water, and the fumonisins are isolated with strong anion exchange columns and then derivatized with fluorescamine. The derivative is then separated by reversed-phase TLC. Shephard also distributed brochures describing the Medical Research Council and the Program on Mycotoxins and Experimental Carcinogenesis (PROMEC). PROMEC has a wide variety of analytical services available, and has analytical standards for the fumonisins as well. These are described on the web site www.mrc.ac.za/promec/contents.htm.

At the end of the presentation, Trucksess concurred with the need for low cost methods for mycotoxins, in particular those that require minimal instrumentation, such as TLC, and further described the need for methods that reduce solvent use and facilitate the disposal of large volumes of material potentially contaminated with mycotoxins.

Society for Mycotoxin Research

Ewald Usleber (Institute of Veterinary Food Science, Justus-Liebig University, Giessen, Germany) summarized the activities of the Society for Mycotoxin Research. The Society was founded in 1997 primarily to support the traditional annual conference "Mykotoxin Workshop" held in Germany since 1979. The 22nd Mykotoxin Workshop was held June 5–7, 2000, in Bonn, Germany. Although the predominant language of the Society is German, and its members are mainly from Austria, Germany, and Switzerland, the Society is truly international in nature, and mycotoxin researchers from all countries are encouraged to join the activities. Visit the Society's homepage at http:/www.mycotoxin.de to find information about current mycotoxin research. A collection of international mycotoxin legislation, tables of contents of past proceedings of Mykotoxin-Workshops, as well as chemical structures and physicochemical data of almost 100 mycotoxins, are available to download. The page is available in both German and English, and participants were encouraged to check both versions because the German version may be slightly more current.

During the last year, the society principally worked to support the organizer of the 22nd Mykotoxin-Workshop, Holger Hindorf from the University of Bonn. This meeting had a record attendance of more than 200 scientists, and its interdisciplinary character was reflected in the many disciplines represented by the participants: human and veterinary medicine, biology, chemistry, agricultural sciences, food sciences, industry as well as academia and public/animal health.

The scientific program included 45 oral and 23 poster presentations, covering all aspects of mycotoxin research, in particular ecology of toxin production, toxicology, detoxification, effects of fungicides, occurrence in foods, feeds, and the environment, and, of course, analytical methods. DON was a major topic, because the contamination rates and DON levels in cereals contaminated with this toxin have recently been very high in many parts of middle Europe.Other major topics were *Fusarium* toxins, OA, aflatoxins, and stachybotrys toxins. However, virtually all groups of mycotoxins were covered by scientific presentations. The proceedings from the 22nd Workshop will be published as a supplement to the journal *Mycotoxin Research*.

During the workshop, the first Brigitte Gedek Award for Mycotoxin Research was presented to Michael Gekle, University of Würzburg, Germany, for his outstanding research on the toxicology of OA. Presentation of this award was a major event and will be made biennially through sponsorship of Brigitte Gedek (Ismaning, Germany) to a scientist for creative and outstanding research in the area of mycotoxins. The award is intended to be truly international in nature and is further intended as a means of encouraging younger researchers. The award is in the amount of 10 000 EURO, and is given to promote scientific research in mycotoxinology. The next award will be given in 2002, and information on how to apply will be available in the future. Eligible for consideration are individual scientific contributions (master thesis, PhD thesis, or equivalent) as well as publications in renowned scientific journals. The next Mykotoxin Workshop will be held in Vienna, Austria, May 28–30, 2001.

Lastly, Usleber summarized the results from a large project conducted in Germany on OA. Levels of OA in foods were determined, and exposure based on various diets were predicted. Levels of OA in the blood were also measured. Almost everyone in Germany is exposed to OA, with cereal grains as the major source. There is no indication of an unknown source of OA. Mean daily intake of this toxin was estimated at about 0.3 ng/g BW, with the worst case intake predicted to be 5–10 fold higher. The average level of OA in the serum was about 0.2 ng/mL. Details of this study will soon be posted on the Website. The results from the project will be put into the "SCOOP" report. It is possible that Germany may set lower tolerances for OA than the current EU levels.

Mycotoxin Trends in the United States, 1995–2000

Bruce Malone (Trilogy Analytical Laboratory, Beaufort, MO) provided an overview on trends in mycotoxin prevalence in the United States over the past 5 years. During this time frame, there has been increased testing using both test kits and

analytical reference methodology. In particular, there have been significant increases in DON testing and increases in fumonisin testing due to the predicted establishment of FDA guidelines for this group of toxins. Other trends include the testing of more unique sample types, and testing for a wider variety of mycotoxins. With improved analytical capabilities, detection limits have lowered and more sensitive methodologies have been developed. The trend toward lower detection limits is expected to continue.

Aflatoxins, deoxynivalenol, and fumonisins are the toxins of greatest concern in the United States. The geographic "hot spots" for prevalence of these mycotoxins and the associated commodities were presented: aflatoxins affect corn, peanuts, and occasionally cottonseed in the southeastern United States; corn, milo, and cottonseed in Texas and the southern United States; and cottonseed and tree nuts in California and the southwest United States. Aflatoxin occurrence in the Midwest tends to be more sporadic. The regions and commodities affected by DON are different than for aflatoxin. In the Midwest, wheat, barley, and corn have been affected, particularly in recent years. In 2000, DON contamination of barley appears worse than in previous years, while DON contamination of wheat appears less significant than last year. Historically, the prevalence of DON in wheat is fairly high, with levels above at or above 100 ppb common. Levels in corn are generally lower than those in wheat.

Fumonisins are widely distributed in the United States, and in most regions, corn has been the commodity most predominantly affected. Thus far in 2000, fumonisins do not appear to be a major problem in the Midwest, while in the southern United States, much of the corn crop has been lost due to climatic conditions. Other toxins of interest include the ochratoxins, which have been found in wheat and barley, raisins, and milo. The prevalence of zearalenone has been low but sporadic in milo, corn, and wheat. The occurrence of T-2 toxin has been mostly associated with severely damaged crops (corn, cottonseed) or those that have been improperly stored. The fact that DON can not be determined at less than 100 ppb may be an issue, according to the audience. A general discussion ensued about whether improved analytical capabilities lead inexorably to reduced tolerances for mycotoxins in foods. Trucksess commented that FDA still has an advisory level of 1 ppm DON in processed grain for human consumption.

Ochratoxin A in Raisins in the United States

Michael Hurley of the Dry Food Association of California (Fresno, CA) stated that the DFA is an inspection agency for dried fruit and tree nuts. Hurley provided an historical perspective of OA in raisins, and summarized data from multiple sources. A1998 UK retail survey of dried vine fruit found a high percentage of samples positive for OA at 0.2 ppb or greater. A DFA study found 67% of analyzed samples positive for OA at low levels. A study by Trucksess also found that most of the samples tested were positive at greater than 0.5 ppb. If the use of crop protection products such as pesticides decreases, there may be an impact on the prevalence and levels of mycotoxins. A general discussion ensued related to the levels of OA in raisins relative to grapes. In part, the lower levels found in grapes may be due to their much higher water content. A comment was made that 2 major OA producing fungi have been found in California. A collaborative study of OA in dried fruits has been completed and will be submitted to AOAC INTERNATIONAL. Healthy looking grapes, when dried and tested, may have part per trillion levels of OA. When C14-OA was added to soil, the toxin was absorbed by the plant and distributed to the grapes, raising the possibility that OA may also be translocated from the soil.

Caffeine as a Disruptive Element in OA Analysis

Priska Koch of the Swiss Quality Testing Services described recent research on the effects of caffeine on the analysis of OA in coffee. Coffee (25 g), either green or roasted, was extracted with 200 mL of a mixture of methanol and aqueous sodium bicarbonate. After cleanup by IAC using commercially available affinity columns, the OA was eluted with 4 mL methanol and evaporated to dryness before dissolution in solvent for injection (HPLC). Although recovery of OA from rye was adequate, recovery from coffee was initially poor. To test the effects of caffeine, it was added to a blank sample and to caffeine-free coffee spiked with OA. The caffeine content was increased while the OA recovery was decreased. The coffee extraction method was then modified to selectively separate the caffeine from the OA after the extraction and before the IAC cleanup. Dichloromethane and chloroform were the most effective at removing the caffeine, and recovery of OA was best when dichloromethane was used. Therefore, the cleanup was modified by further extracting the methanol–bicarbonate extract twice with 10 mL dichloromethane. Using this treatment, the amount of sample that can be loaded onto the IAC is 3-fold greater, and, therefore, a lower limit of detection was possible and the recovery of OA was dramatically enhanced.

Fluorescence Polarization Assay for Fumonisins

Mohammad Nasir (Diachemix Corporation, Grayslake, IL) described a fluorescence polarization (FP) assay for fumonisins in maize, developed by his company in collaboration with the USDA National Center for Agricultural Utilization Research (Peoria, IL). The assay is a rapid and inexpensive method for quantitation of fumonisin in the field. Polarization is independent of intensity and concentration, and the assays can be conducted with colored or cloudy solutions. Previously, FP has been used for the homogeneous analysis of molecular interactions of small molecules with proteins, antigen–antibody interactions, hormone–receptor interactions, and monitoring therapeutic drug levels and substances of abuse. FP measurements are based on the rate of rotation of a molecule in solution. Smaller molecules rotate faster and give lower polarization values than bigger molecules. A fumonisin tracer that bound to a monoclonal antibody specific for fumonisin was developed by linking fumonisin with a fluorophore. Binding of the antibody to the tracer formed an immuno-complex that rotated slower and gave rise to higher

polarization values. Depending upon the quantity of unlabeled fumonisin in the sample, a competition between unlabeled and fluorescently labeled fumonisin occurred for the antibody and the polarization changed accordingly. The observed polarization was, thereby, related to the free fumonisin concentration in samples. The FP assay was rapid and did not require additional separation, washing, or purification steps.

Because of the aqueous solubility of fumonisin B_1, maize samples were extracted with an aqueous buffer, rather than solvent–water mixtures prior to analysis by FP. Aqueous extraction reduced the appearance of colored products in maize extracts. Two independent laboratories analzyed 50 corn samples, and their results correlated well with each other and with an established HPLC method using the OPA derivative for measurement of fumonisins B_1, B_2, and B_3. An extraction method currently under evaluation can be completed in 5 min. Following extraction, the assay was very fast (1 min) and easy to perform. The robustness, speed, and cost effectiveness of the assay suggests that the technique may be applied more widely. At the conclusion of the presentation, the audience asked about the cost effectiveness of the FP approach. The instruments currently are available for about $7 000, and the costs of the reagents (antibody and tracer) are expected to be very low. Discussion ensued about the cost and applicability of the method under the conditions of anticipated use.

FDA Draft Regulations for Fumonisins and Patulin

Bob Eppley (U.S. Food and Drug Administration, Washington, DC) briefly described the FDA guidelines for fumonsins and patulin. For patulin, the CCFAC has forwarded to the Committee on Additives and Contaminants (CAC) for adoption a maximum level of 50 μg/kg patulin in apple juice and apple juice ingredients. FDA has proposed an action level of 50 ppb on June 15, 2000, and CCFAC is developing a Code of Practice for this mycotoxin. The recently released guidance levels for the fumonisins were also discussed. The proposed guidance levels are available in detail at the FDA Website. Limits in de-germed dry mill corn products are 2 ppm, and other levels are suggested for other human foods and commodities incorporated into animal feed rations. The levels were established by FDA after evaluation of toxicology data, the prevalence of the fumonisins in corn, and the levels that may be practically achieved by food processors. The guidance levels are not final, although the public comment period ended August 7, 2000. Final levels have not been released. Although the National Toxicology Program evaluation of fumonisins as carcinogens in rodents is complete, the risk assessment is still in progress.

Randall Lovell (FDA, Center for Veterinary Medicine, Rockville, MD) reviewed the recommended levels for total fumonisins (FB_1 + FB_2 + FB_3) in corn, corn by-products, and the total ration of various animals. Lovell encouraged attendees to visit a CVM Website (www.fda.gov/cvm/fda/mappgs/fumonisin.htm) where a copy of the Draft Guidance for Industry 112 (fumonisin levels in human food and animal feeds) and the CVM background paper in support of fumonisin levels in animal feed are posted.