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ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

JOURNAL

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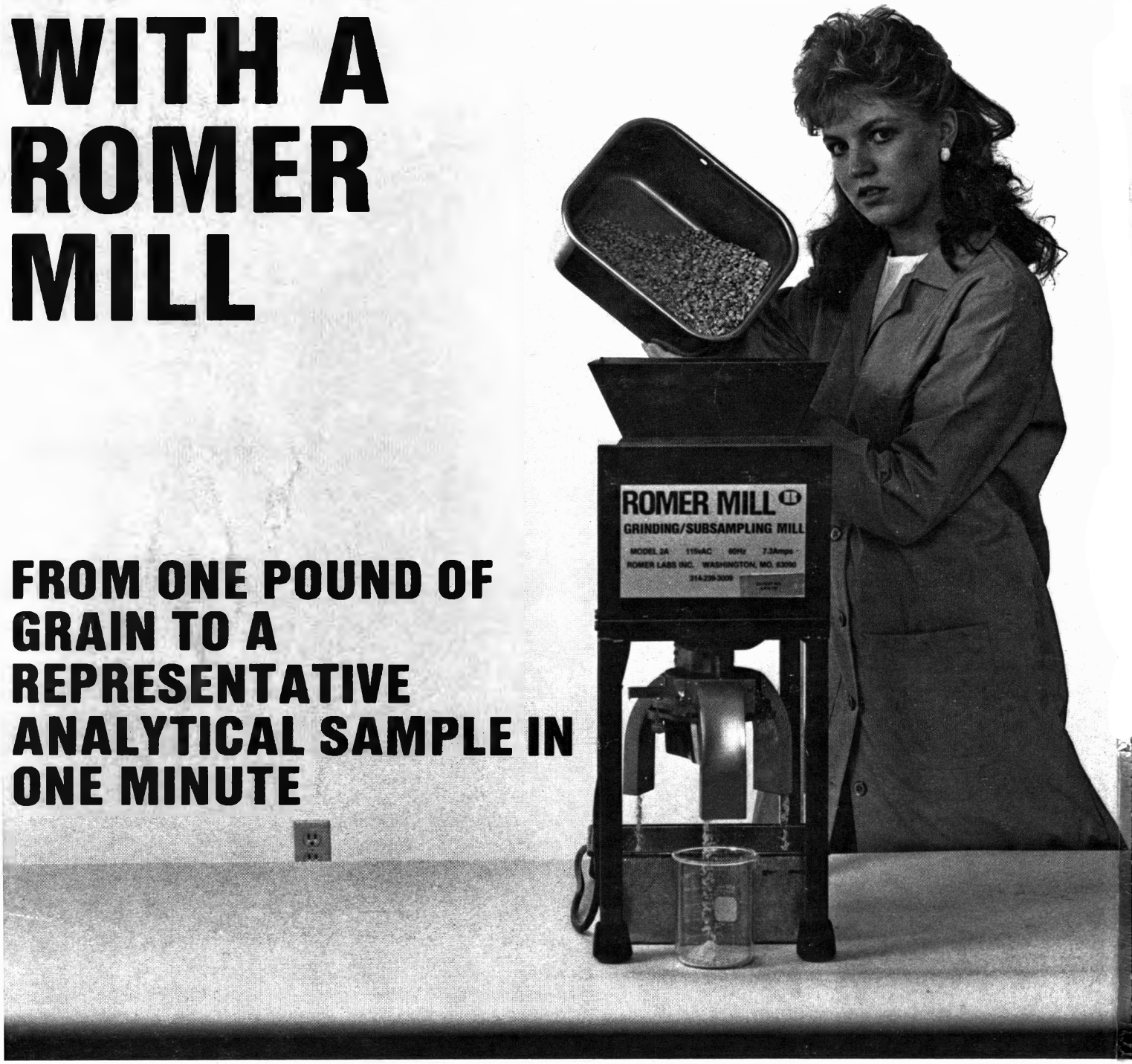
VOL. 72, NO. 1

JANCA 72(1) 1–218 (1989)
ISSN 0004-5756

NO MORE RIFFLING WITH A ROMER MILL



FROM ONE POUND OF GRAIN TO A REPRESENTATIVE ANALYTICAL SAMPLE IN ONE MINUTE

- **EFFICIENT:** Simultaneously Grinds & Subsamples 1 Lb. Per Minute
- **ACCURATE:** Prepares **25 g.** Representative Analytical Sample From **5 Lb.** of Aflatoxin Contaminated Corn
- **VERSATILE:** Can Be Used For Mycotoxins, Pesticides, Fumigants, Antibiotics, Drugs, Vitamins, Mineral & Proximate Analyses

EASY TO CLEAN: Can Be Thoroughly Cleaned in One Minute

FLEXIBLE: Will Grind High Moisture Corn

DUST FREE: Little or No Dust Generated

PORTABLE: 40 Lb. - 26" x 20" x 10"

CAPACITY: 100 g to 15 Lb.

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No other automatic sampler measures up to this all-new Bio-Rad instrument. It's the most complete, most versatile, and most compact refrigerated sampler on the market. Call your Bio-Rad representative or contact us directly.

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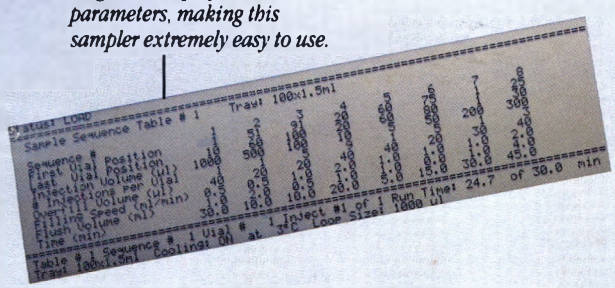

Three interchangeable sample trays. Use 0.5 or 1.5 ml vials or 96 well microtitration plates. No special vials needed.

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MYCOSEP #224 CLEANUP COLUMN PURIFIES FOOD & FEED EXTRACTS IN JUST TEN SECONDS. PURIFIED EXTRACTS ARE SUITABLE FOR IMMEDIATE TLC, HPLC OR GC ANALYSIS

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- Aflatoxin in Corn, Peanut Butter, Nuts and Feeds
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- Aflatoxin, Zearalenone and Deoxynivalenol in Corn
- Sterigmatocystin and Patulin in Corn and Feeds

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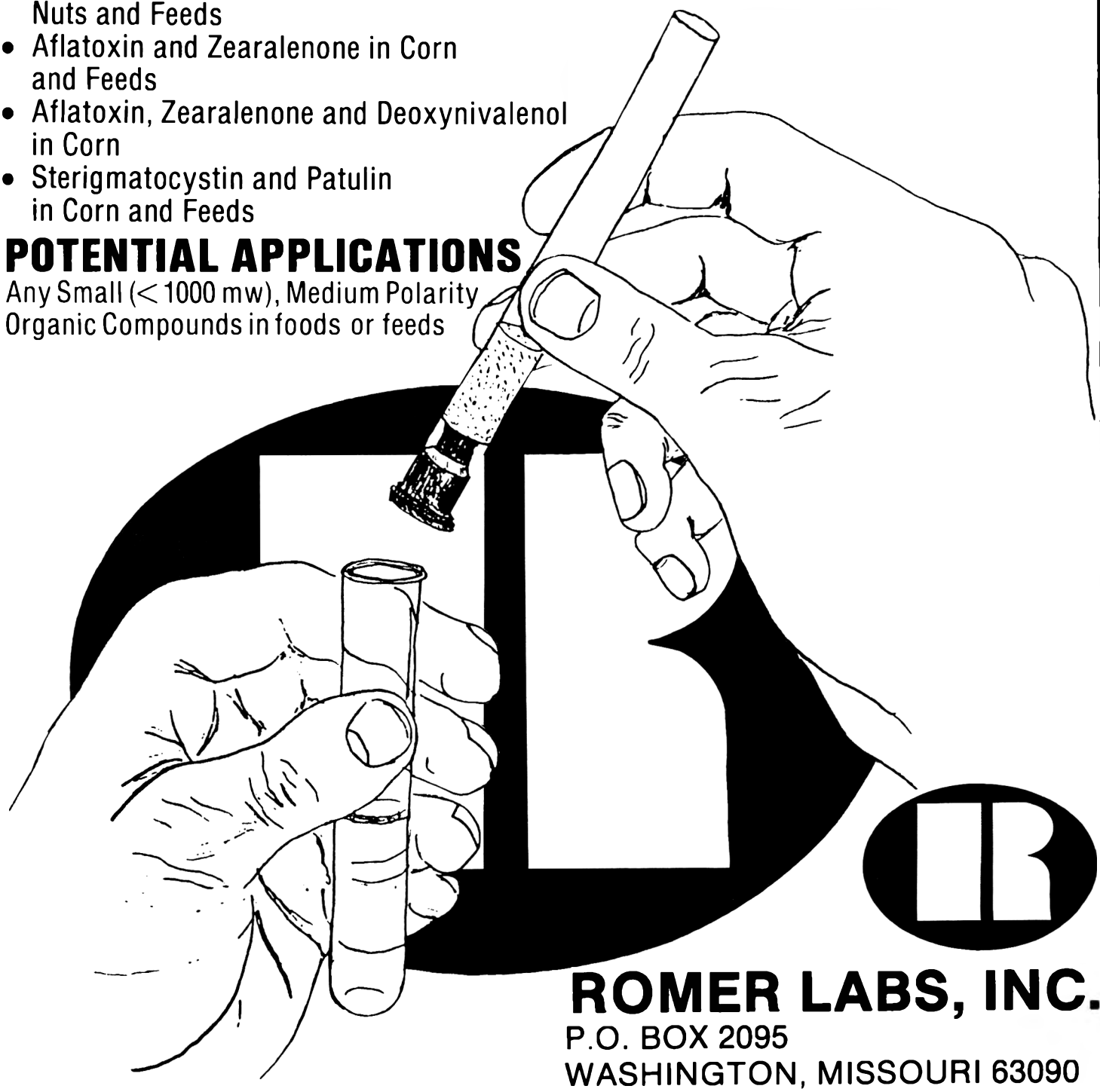

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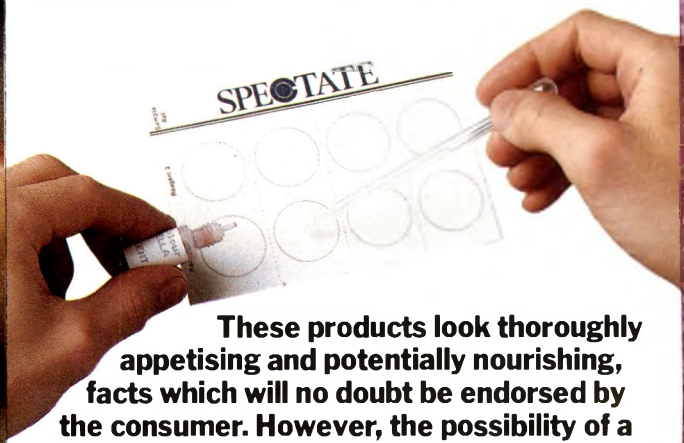
THIS FOOD IS FULL OF CALCIUM, PROTEIN, VITAMINS, AND SALMONELLA?

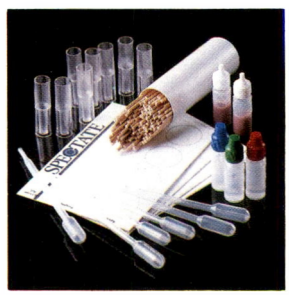
THIS RAPID TEST WILL TELL YOU.



These products look thoroughly appetising and potentially nourishing, facts which will no doubt be endorsed by the consumer. However, the possibility of a salmonella presence could change that appraisal if you could in fact see it.

Salmonella screening has until now been a lengthy and often unreliable procedure as 12,000 cases of poisoning in Britain last year will testify.

Spectate is a revolutionary salmonella testing kit from May & Baker Diagnostics, utilising latex antibody technology, making it simple, reliable and very, very fast.



Within minutes you can have a positive detection, saving you time, your reputation for quality – even your customers health.

Protect yourself against the repercussions of salmonella contamination by screening with Spectate.

Contact May & Baker Diagnostics now for further details.

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A Peer-Reviewed Journal Publishing Basic and Applied Research in the Analytical Sciences Related to Foods, Drugs, Agriculture, and the Environment

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

JOURNAL

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 The *Journal of the Association of Official Analytical Chemists* (ISSN 0004-5756) is published bimonthly by the Association of Official Analytical Chemists, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301 USA. **Subscriptions** for Vol. 72 (1989) are: Members, $84.50 in U.S., $125.00 outside U.S.; Nonmembers, $94.50 in U.S., $145.00 outside U.S. Second class postage paid at Arlington, VA, and additional mailing offices. Abstracted or indexed in Analytical Abstracts, Biological Abstracts, Cambridge Scientific Abstracts, Chemical Abstracts, Current Advances in Ecological Sciences, Current Contents, and others. Printed in USA.
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General Referee Reports: Committee on Foods II

General Referee Reports: Committee on Residues

General Referee Reports: Committee on Microbiology and Extraneous Materials

General Referee Reports: Committee on Feeds, Fertilizers, and Related Materials

General Referee Reports: Committee on Hazardous Substances in Water and the Environment

Transactions: Association of Official Analytical Chemists

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Now there's a rapid-method breakthrough that lets you screen for Listeria and Salmonella and get accurate results in just 48 hours.

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NEW PRODUCTS

On-Line Pyro-chemiluminescence® Nitrogen Analyzer

Make the analysis of nitrogen-containing compounds faster and safer than using Kjeldahl. Antek Industrial Instruments Inc. offers a method that is entirely instrumental, and requires no reagents. The system can detect and quantitate the total nitrogen content of liquid or gas samples. Little or no sample preparation is needed for most samples. Liquids and gases can be analyzed in 30–45 s. Gas samples may be analyzed as a continuous flow through or as discrete samples. Liquids are run as discrete samples. The analyzer can detect total chemically bound nitrogen in amounts from 1 ppm to low %. Applications include petroleum, petrochemicals, environmental monitoring (air or water), and pharmaceutical/biological samples.

Circle No. 1 on reader service card.

Chromatography Software

Antek Instruments now offers the DELTA chromatographic software family, providing high quality PC-based data acquisition and analysis at budget prices. Two packages are available. DELTA Junior is designed as a single instrument, dual detector, integration system. It provides automatic calibration, autosampler control, and disk storage of chromatograms and methods for later analysis and manipulation. DELTA, a more sophisticated package, will access and process data from 1 or 2 instruments with 1 or 2 detectors each. It also adds a multitasking feature which will allow the chromatographer to acquire data from 1 or both instruments in the background while simultaneously reviewing and manipulating previously acquired data in the foreground.

Circle No. 2 on reader service card.

Ion Exchange Membranes and Sample Preparation Discs

Bio-Rad Laboratories introduces the new Bio-Rex ion exchange membranes, extremely flexible, strong membranes composed of Analytical Grade ion exchange resin beads permanently enmeshed in a polytetrafluoroethylene (PTFE) membrane. AG ion exchange resins are exhaustively purified and narrowly sized for reproducible performance in sensitive laboratory applications. Three ion exchange resin types have been incorporated into membranes: AG 1-X8 strong anion exchange resin, AG 40W-X8 stron cation exchange resin, and Chelex 100 chelating ion exchange resin. The membranes offer a fast and convenient alternative to column and batch ion exchange resin methods.

Circle No. 3 on reader service card.

96 Well Pipetting

Bio-Rad's complete line of products for 96 well pipetting. The 12-Pette adjustable multi-channel pipet delivers precise volumes in a continuous range from 20 to 200 μL. Disposable polyethylene reagent reservoirs fit all multi-channel pipets. The 96 well flat bottom EIA plates bind more protein more uniformly than any other plate. Type 28 pipet tips which fit the 12-Pette pipet as well as the Octapette pipet, and Titertube 1 mL micro test tubes, are available in bulk boxes, racked for use with 96 well plates, or sterilized.

Circle No. 4 on reader service card.

CHEF-DR II System

Bio-Rad Laboratories CHEF-DR II system (Clamped Homogeneous Electric Fields with Dynamic Regulation), which resolves DNA fragments from 200 to over 10 million pairs. The CHEF-DR II system is unique among pulsed field technologies because it provides a reliable electronic means of achieving uniform fields. Electrodes are actively regulated during the separations to maintain field uniformity. Thus the system provides straight lanes and optimal resolution over a wide size range reproducibly.

Circle No. 5 on reader service card.

High Performance Electrophoresis (Capillary Electrophoresis)

Bio-Rad's new HPE 100 system is a versatile yet simple to operate High Performance Electrophoresis (HPE) System, which provides fast, sensitive methods for identification and quantitation of biopolymers such as peptides, proteins, and DNA fragments. With HPE, extremely high voltage gradients are used in free solution in very thin silica capillary tubes. Functionally, HPE behaves like a typical chromatography system. Results are produced within minutes. Samples are injected with a microliter syringe and the results, an electropherogram rather than a chromatogram, are read out on a strip chart recorder, sent to an integrator, or processed by a computer.

Circle No. 6 on reader service card.

System Gold Chromatography

The System Gold, a liquid chromatography (HPLC) system, is used for separating, isolating, and purifying proteins in body fluid samples. This use of scientific instrumentation to monitor athletes is part of a growing role for science in sport. Through science, or biochemical monitoring, doctors are able to help the athlete avoid overtraining and optimize peak levels of performance.

Circle No. 7 on reader service card.

Dissolution 6/12 Soft-Pac®

Beckman's Dissolution 6/12 Soft-Pac software module for use with the Beckman DU Series 60 spectrophotometers and the Dissoscan II autosampler provides convenient, automatic on-line dissolution analysis from 1 or 2 baths. The software program integrates system operation and control, sample delivery, data acquisition, and hard copy reporting to meet *U.S. Pharmacopeia* and *National Formulary* requirements. The flexible Soft-Pac program allows the user to select the analytical wavelength for productive analysis extending up to 166.6 h determined by the time interval and number of baths. Using 1 or 2 wavelengths, it also allows for automatic background correction of raised or sloping baselines caused, for example, by turbidity in the samples. Volume corrections are not required.

Circle No. 8 on reader service card.

Infrared CO_2 Monitor

The Riken RI-411 from CEA Instruments, Inc. is an accurate, lightweight, rugged CO_2 infrared gas monitor with digital readout and audible alarm. The unit is battery-operated and microprocessor-controlled. It has a standard range of 0–4975 ppm in 25 ppm increments; 0–1%, 0–20%, and 0-50% range units are also available. The readings of CO_2 concentrations can be continuous or averaged over 1, 3, or 15 min. The RI-411 has

Society for **APPLIED SPECTROSCOPY**
will present

THREE SHORT COURSES

at the 40th Annual Pittsburgh Conference
at Atlanta on March 4th and 5th

PLASMA SPECTROCHEMICAL ANALYSIS

Instructors: R.M. Barnes and A.T. Zander

Topics Include:

Introduction to Plasmas and Principles of Atomic Spectroscopy;
Measurement of Spectrochemical Information in Plasma Spectroscopy;
Principles and Applications of DC Plasmas;
Principles and Applications of Microwave Plasmas;
Introduction to Inductively Coupled Plasmas;
Characteristics, Instrumentation, and Applications of ICP Emission, Fluorescence and Mass Spectrometry;
Comparison of Plasma Techniques

FOURIER TRANSFORM INFRARED SPECTROMETRY

Instructors: P.R. Griffiths and J.A. de Haseth

Topics Include:

Multichannel Methods of Spectrometry; Theory of FT-IR Spectrometry (resolution, apodization, phase);
Interferometers for FT-IR (sources, beamsplitters, optics, detectors, etc.);
Conventional Applications (absorption, microsampling, ATR, diffuse reflectance);
Unconventional Applications (emission, low temperature, photoacoustic);
Data Handling (quantitative, subtraction, deconvolution);
Chromatography and FT-IR;
Problem Solving by FT-IR

SCIENTIFIC APPLICATIONS OF PERSONAL COMPUTERS

Instructor: T.C. O'Haver

Topics Include:

Introduction to personal computers (PCs);
Comparison of PCs for scientific applications;
Programming and higher-level languages;
Personal computer networking/communication;
Personal computer interfacing;
Commercial interfacing packages for PCs;
Data transfer between dissimilar PCs;
Scientific word processing with PCs;
Publication quality graphics with PCs;
PC construction with readily available components

The Society for Applied Spectroscopy will sponsor three short courses prior to the 1989 Pittsburgh Conference at Atlanta, GA. Each course covers basic introduction to theory, instrumentation, data handling and applications, as well as the latest state-of-the-art advances. These courses are suitable for analysts new to these fields, experienced analysts who desire further background, and management personnel who need familiarity with these fields. The FT-IR course includes a hardbound text (650 pages) authored by the instructors. The courses will be presented from 8:30 to 5:30 on Saturday and Sunday, March 4 and 5, 1989 at Atlanta, GA. For registration and general course information call JoAnn Brown (301) 694-8122.

REGISTRATION FORM

(Pre-registration is required)

NAME _____ PHONE (___) _____

ADDRESS _____

COURSE _____ REMITTED $ _____

Please make the check payable to: **Society for Applied Spectroscopy**

Early rates through Feb. 10, 1989. After this date add $25.

	Plasma	FT-IR	PCs
SAS Members	160	230	200
Non-SAS Members	200	270	240
Students	60	125	60

Mail Registration form to:
Jo Ann Brown
Society for Applied Spectroscopy
P.O. Box 64008
Baltimore, MD 21264

a solid state detector, illuminated dot-matrix digital display, and recorder output, and can operate on AC using optional power supply.
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With the introduction of CDM-II Conductivity Detector, Dionex offers the most sensitive and specific detection method for inorganic ions and organic ionic compounds. Improvements in both electronics and flow design give the advanced CDM-II rapid correction for eluant temperature, high baseline stability, high resolution, and convenient selection of conductivity range settings.
Circle No. 10 on reader service card.

Simultaneous Multi-Element Zeeman Graphite Furnace AA Spectrophotometer

The Hitachi model Z-9000 is a dedicated graphite furnace atomic absorption spectrophotometer designed for unattended operation and maximum sample throughput. It provides the simultaneous determination of as many as 4 elements in a single atomization, and 8 elements automatically.
Circle No. 11 on reader service card.

Diverse Bench-Top pH Meters

The Horiba Instruments F series bench-top pH meters meet a diverse range of needs, including high technology research, biomedical and pharmaceutical analysis, quality control, clinical laboratory analysis, and educational use. The 6 models in the series are designed to meet specific requirements while enabling users to expand their capabilities.
Circle No. 12 on reader service card.

New Proprietary Closure Developed For Environmental Sample Containers

I-Chem Research announces the development a proprietary formulation of reinforced polypropylene which is now used to produce "I-Chem Blue" open-top closures supplied on I-Chem Research's 40 mL septum vials. Laboratory studies confirmed that the new I-Chem Blue closures are the most temperature-resistant and provide an effective barrier against the loss of volatile organics.
Circle No. 13 on reader service card.

New Cyanopropyl Polysiloxane Phase for GC Users

J&W Scientific has introduced the new DB-23 capillary column for gas chromatography. DB-23 is a recently developed polar stationary phase consisting of 50% cyanopropyl on a proprietary polysiloxane backbone. This proprietary stationary phase exhibits more complete cross-linking and higher thermal stability than conventional cyanopropyl phases. DB-23 is used for the separation of both cis/trans isomers of fatty acid methyl esters and positional isomers.
Circle No. 14 on reader service card.

Buchner-Style Funnel with Replaceable Frit

Deltaware introduces a filter assembly with replaceable frits. Buy one funnel assembly and choose from a selection of interchangeable frits that include fine, medium, coarse, and extra coarse porosities. The glass filled polypropylene threaded clamp makes assembly and disassembly quick and easy. Frits are reusable but may be discarded if the possibility of cross-contamination exists.
Circle No. 15 on reader service card.

Methodology for Determination of Alcohol

Labconco Corp. has published a rapid Kjeldahl methodology for the determination of alcohol in a mixture by direct distillation. This methodology details apparatus and reagents used in the process. The determination procedure is given with step-by-step instructions. The methodology is based on data which was tested in Labconco's laboratories with Labconco rapid Kjeldahl apparatus.
Circle No. 16 on reader service card.

Fiberglass Blowers

Labconco Corp. introduces its new fiberglass blowers. The new line is designed for use with Labconco fume hoods and will accommodate a variety of exhaust applications with moderate to highly corrosive vapors, including vapors with high acid content. The blower is equipped with an injection molded polypropylene wheel to make the blower more

chemically resistant and allow more efficient operation.
Circle No. 17 on reader service card.

New Long-Life Reverse-Phase Polymerica LC Column

Researchers using reverse-phase LC separations in pharmaceutical, industrial, and life science applications can create high-resolution chromatograms faster with new PolySpher RP18 polymeric reverse-phase LC columns from EM Science. They can perform in minutes analyses of organic acids, amino acids, peptides, carbohydrates, and metal ions that typically require 3 h or more using conventional polymeric reverse-phase columns. Separation is also improved.
Circle No. 18 on reader service card.

New Microplate Reader Software

Molecular Device's new SOFTmax 2.0 significantly expands the capabilities of the end-user to collect, analyze, and manage data from any of the MAXline family of microplate readers. Major new kinetic features for use with Vmax and UVmax kinetic microplate readers include dual wavelength kinetics and calculation of Vmax (the maximum slope of the kinetic reaction) for each well over a user determined number of points.
Circle No. 19 on reader service card.

AVIR Oxygen Enrichment Systems

A/G Technology Corp. introduces a line of standard oxygen enrichment systems. The AVIR systems use a patented permeable membrane to separate compressed air into an oxygen enriched stream and oxygen depleted stream. The AVIR systems can generate oxygen enriched air concentrations of over 35% oxygen in a single step or over 50% oxygen concentration in a 2-stage unit. The AVIR systems come complete with a solid state oxygen monitor and all necessary instrumentation to adjust product flow and concentration easily.
Circle No. 20 on reader service card.

New Post-Column Interlock System

Pickering Laboratories has introduced a new post-column-eluant flow interlock designed to protect against equipment damage caused by the accidental reverse flow of reagents. The interlock monitors pressure at the outlet of the eluant pump. Should eluant pressure drop below 500 psi, electrical power to the post-column system is shut off, stopping reverse reagent flow that can damage both the analytical column and post-column equipment. Such pressure drops can be caused by pump malfunction, automatic or intentional shut down, or empty reservoirs.
Circle No. 21 on reader service card.

Epifluorescence Microscopy Technique

Poretic's black polycarbonate screen membrane filters are ideally suited for use in the epifluorescence microscopy technique. This technique provides a way to observe and count microorganisms faster and more efficiently than traditional culturing methods. Microorganisms can be observed and counted in 30 min compared to traditional culturing methods that require 18–48 h.
Circle No. 22 on reader service card.

Centrifugal Partition Chromatograph for Analysis and Purification of Chemical Substances

Sanki Laboratories announces the introduction of its new preparative/analytical centrifugal partition chromatography system, Model LLN-8. Centrifugal partition chromatography is a new liquid chromatographic technique which utilizes liquid-liquid partition, counter-current distribution to fractionate complex mixtures of chemical substances. It has been used to separate and purify a broad range of synthetic and naturally occurring chemical species; it offers distinct advantages for the isolation of polar substances and materials of biological origin.
Circle No 23 on reader service card.

Rapid, Complete Solute Recovery for all Preparative Applications

Savant Instruments has introduced 4 SpeedVac systems for concentrating aqueous or organic samples. Whatever the solute reduction/drying application and sample volume, Savant Instruments now offers a complete SpeedVac system that can recover 100% of the solute faster and more efficiently than conventional methods such as rotary evaporation, freeze drying, or nitrogen blowdown. Each SpeedVac system combines vacuum evaporation with centrifugal force to produce an easily redissolved, compact button of dry residue at the bottom of each tube.
Circle No. 24 on reader service card.

FOR YOUR INFORMATION

Meetings

April 8–12, 1989: APhA 136th Annual Meeting and Exhibit, Anaheim, CA. Contact: Linda M. Karson, American Pharmaceutical Association, 2215 Constitution Ave, NW, Washington, DC 20037, telephone 202/628-4410.

April 11, 1989: "Chromexpo-1989," Sheraton Washington Hotel, Washington, DC. Contact: Janet E. Cunningham, Barr Enterprises, PO Box 279, Walkersville, MD 21793, telephone 301/898-3772.

April 24–26, 1989: Chromatographic Separation of Enantiomers, short course, Henry VIII Hotel and Conference Center, St. Louis, MO. Contact: Daniel W. Armstrong or Michael Van De Mark, University of Missouri–Rolla, MO 65401, telephone 314/341-4419.

May 8–9, 1989: "Prep-89, International Symposium on Preparative Chromatography," Sheraton Washington Hotel, Washington, DC. Contact: Janet E. Cunningham, Barr Enterprises, PO Box 279, Walkersville, MD 21793, telephone 301/898-3772.

May 11–12, 1989: National Research Conference, Pesticides in Terrestrial and Aquatic Environments, Richmond, VA. Contact: Tamim M. Younos, Virginia Water Resources Research Center, Virginia Polytechnic Institute and State University, 617 N Main St. Blacksburg, VA 24060, telephone 703/961-5624.

May 15–17, 1989: AOAC Northeast Regional Section Meeting, Novotel North York Hotel, North York (Toronto), Ontario, Canada. Contact: C. G. Halliday, Chipman, PO Box 9910, Stoney Creek, Ontario, L8G 3Z1, Canada, telephone 416/643-4123.

May 22–25, 1989: "3rd Annual Seminar on Analytical Biotechnology," Sheraton Inner Harbor Hotel, Baltimore, MD. Contact: Janet Cunningham, Barr Enterprises, PO Box 279, Walkersville, MD 21793, telephone 301/898-3772.

June 11–13, 1989: AOAC Midwest Regional Section Meeting, Concourse Hotel and Convention Center, Madison, WI. Contact: David Zoromski, Wisconsin Animal Health Laboratory, 6101 Mineral Point Rd, Madison, WI 53705, telephone 608/266-2465.

June 1989: AOAC Pacific Northwest Regional Section Meeting. Contact: Steve Pope, Environmental Protection Agency, PO Box 549, Manchester, WA 98353, telephone 206/442-0370.

June 1989: AOAC Southeast Regional Section Meeting. Contact: Martha Hudak-Roos, National Marine Fisheries Service, 3209 Frederik St, Pascagoula, MS 39568-1207, telephone 601/762-7402.

September 25–28, 1989: 103rd Annual International Meeting and Exposition, The Clarion Hotel, St. Louis, MO. Contact: Margaret Ridgell, AOAC, 1111 N. 19th St, Suite 210, Arlington, VA 22209, telephone 703/522-3032.

October 28–November 2, 1989: 10th Annual Meeting, Society of Environmental Toxicology and Chemistry, Royal York Hotel, Toronto, Ontario, Canada. Contact: Peter Hodston, Canada Centre for Inland Waters, Box 5050, Burlington, Ontario L7R 4A6, Canada, telephone 416/336-4864.

November 5–8, 1989: "9th International Symposium for HPLC Separation of Proteins, Peptides, and Polynucleotides," Wyndham Franklin Plaza Hotel, Philadelphia, PA. Contact: Janet E. Cunningham, Barr Enterprises, PO Box 279, Walkersville, MD 21793, telephone 301/898-3772.

September 10–13, 1990: 104th Annual International Meeting and Exposition, The Clarion Hotel, New Orleans, LA. Contact: Margaret Ridgell, AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209, telephone 703/522-3032.

Short Courses

For the 26th summer, short courses in powder diffraction and x-ray spectrometry will be offered at the State University of New York at Albany. Both courses are tutorial and develop the entire field of chemical analysis. The course in powder diffraction, scheduled June 19-30, 1989, will emphasize quantitative methods of analysis, and the course in x-ray spectrometry, scheduled June 5-25, 1989, will concentrate on mathematical and computer methods to solve the matrix problem and probe into in-depth problems of the advanced spectroscopist. For course description, specific dates, costs, and enrollment information, contact the Department of Physics, State University of New York at Albany, 1400 Washington Ave, Albany, NY 12222, telephone 518/442-4512.

First AOAC Section Outside North America

AOAC members from Europe, Israel, and Egypt will be meeting on March 9, 1989, in Almere, The Netherlands, to organize a new AOAC regional section in the area. All who are interested are urged to attend and participate. The meeting will be held at the Boehringer Mannheim offices in Almere. A scientific program on Immunochemistry Techniques for Foods and Feeds will follow the organizational portion of the meeting. For information, contact the AOAC representative, Margreet Lauwaars, PO Box 153, 6720 AD Bennekom, The Netherlands, telephone +31-8389-18725.

New Sustaining Members

AOAC welcomes the following new private sustaining members: California State Department of Health Services, Division of Laboratories, Berkeley, CA; D.C.A. Food Industries, Inc., New York, NY; Malthus Instruments Ltd, Crawley, West Sussex, UK; Quaker Oats Co., Barrington, IL; Unilever Research Laboratory, Vlaardingen, The Netherlands.

Wiley Award Contributors

Many thanks to the following AOAC members who have recently contributed to the Wiley Awards Fund: Mark Billedeau, Benjamin T. Dunhart, Forrest W. Quackenbush, Paul F. Taylor, Dick H. Kleyn, Louis L. Gershman, Willie L. Hinze, Paul C. Stefan, Gunars J. Zikmanis, Paul G. King, Don C. LaBerge, Canan D'Avela, Bernard F. Taylor, Carl E. Johnson, James W. Fitzgerald, C. R. Todd, Richard P. Moody, Neil J. Butler, and Leo J. Lipinski, Jr.

Important Notice Concerning Use-Dilution Methods for Testing Disinfectants—Analysts Are Advised to Use Great Care

The AOAC official first action use-dilution methods for testing disinfectants against *Salmonella choleraesuis* (sections **4.007**-**4.009**), *Staphylococcus aureus* (section **4.010**), and *Pseudomonas aeruginosa* (section **4.011**) are technique-sensitive and may produce questionable results unless conducted by experienced, trained analysts under strictly controlled conditions. Specific editorial revisions to identify potentially critical control points have been incorporated in the methods.

Users of the AOAC official use-dilution methods are advised to use great care and are notified that they must follow the editorially revised official methods appearing in the 5th supplement to *Official Methods of Analysis,* 14th edi-

tion, as printed in the *Journal of the Association of Official Analytical Chemists,* Vol. **72,** No. 1 (January/February), pages 205–208 (1989).

Analysts are also advised to consult the following reports of recent studies for current scientific data and interpretations: *J. Assoc. Off. Anal. Chem.* **69,** 1003–1005 (1986); **70,** 635–637 (1987); **70,** 903–906 (1987); **71,** 9–11 (1988); **71,** 288–289 (1988); **71,** 868–871 (1988); **71,** 1187–1194 (1988); *Infect. Control* **8,** 501–506 (1987).

Interim Methods

The following methods have been approved interim official first action by the respective methods committees and by the chairman of the Official Methods Board: by the Methods Committee on Drugs and Related Topics—Reverse Phase Liquid Chromatographic Determination of Clioquinol in Cream and Ointment Preparations, submitted by E. J. Wojtowicz (Food and Drug Administration, Buffalo, NY); by the Methods Committee on Foods I—Visual and Semiquantitative Spectrophotometric Enzyme-Linked Immunosorbent Screening Assay for Aflatoxin B_1 in Corn and Peanut Products, submitted by D. L. Park, S. Nesheim, M. W. Trucksess, and L. H. Brown (Food and Drug Administration, Washington, DC), and B. M. Miller, A. Vekich, B. Bidigare, and J. L. McVey (Neogen Corp., Lansing, MI); by the Methods Committee on Foods II—Liquid Chromatographic Method for Determination of Vanillin and Related Flavor Compounds in Vanilla Extract, submitted by S. Kahan (Kahansultants Inc., Roslyn Heights, NY); by the Methods Committee on Residues—Gas Chromatographic Determination of Polychlorinated Biphenyls (as Aroclor 1254) in Serum, submitted by V. W. Burse, M. P. Korver, L. L. Needham, C. R. Lapeza, Jr, E. L. Boozer, S. L. Head, J. A. Liddle, and D. D. Bayse (Centers for Disease Control, Center for Environmental Health and Injury Control, Atlanta, GA); and by the Methods Committee on Microbiology and Extraneous Materials—Extraction of Light Filth from Spirulina Powders and Tablets, submitted by M. J. Nakashima (Food and Drug Administration, Division of Microbiology, Washington, DC).

The methods will be submitted for adoption official first action at the 103rd AOAC Annual International Meeting, September 25–28, 1989, at St. Louis, MO. Copies of the methods are available from the AOAC office.

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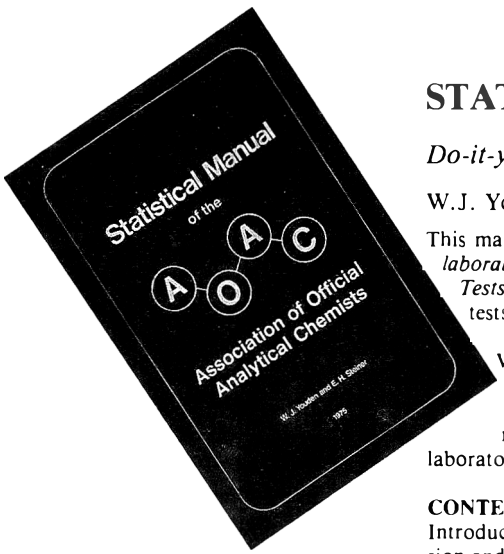

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This manual is a composite, with revisions, of two publications — *Statistical Techniques for Collaborative Tests,* by the late W.J. Youden, and *Planning and Analysis of Results of Collaborative Tests,* by E.H. Steiner — for use in statistical analysis regarding interlaboratory collaborative tests. The manual also presents guidelines for planning collaborative tests.

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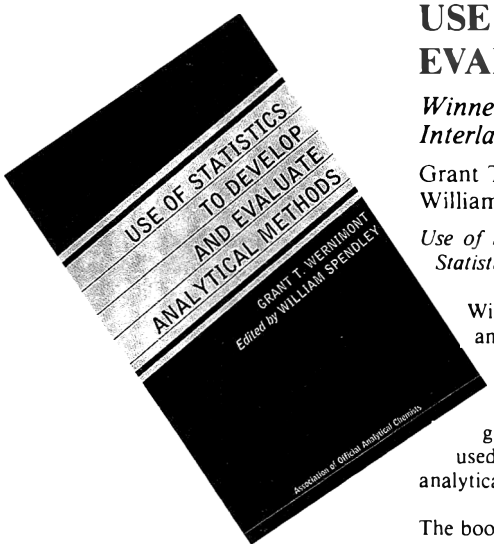

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BOOKS IN BRIEF

Alternative Methods in Toxicology Series, Volume 6, Progress in *In Vitro* Toxicology. Edited by Alan M. Goldberg, Published by Mary Ann Liebert, Inc., 1651 3rd Ave, New York, NY 10128, 1988. 350 pp. Price $85.00 (U.S.); $105.00 (export). ISBN 0-913-113-12-3.

In vitro toxicology—or non-whole-animal testing—is a very important aspect of safety-testing strategies. Not only does it produce a reduction in the number of animals used in testing but more effective methodologies evolve to evaluate safety. *Alternative Methods in Toxicology* is a series on the proceedings of the annual symposia of the Johns Hopkins Center for Alternatives to Animal Testing and reflects its goal: to develop appropriate basic scientific knowledge that can lead to innovative methods, using non-whole-animal systems, for the safety evaluation of commercial and therapeutic products.

Fluorinated Carbohydrates, Chemical and Biochemical Aspects. Edited by N. F. Taylor. Published by the American Chemical Society, 1155 16th St, NW, Washington, DC 20036, 1988. 213 pp. Price $49.95 (U.S. and Canada); $59.95 (export). ISBN 0-8412-1492-1.

Since 1944, chemists and biologists have been intrigued by the natural occurrence, synthesis, and properties of the C-F bond. New synthetic methods have led to explosive growth in the numbers and variety of fluorinated sugars. As a result, these compounds are now readily accessible for chemical and biochemical research. This 11-chapter volume examines recent studies in the synthesis, reactivity, and biochemical aspects of fluorinated carbohydrates. It looks at the potential applications and importance of carbohydrates in biomedical and pharmaceutical research.

Dictionary of Antibiotics and Related Substances. Edited by B. W. Bycroft. Published by Chapman and Hall, 29 W 35th St, New York, NY 10001, 1988. 964 pp. Price $675.00. ISBN 0-412-25450-6.

The intention of this dictionary is to include every well-defined microbial compound showing antibiotic activity known up to the present, together with the most important of those compounds of unknown structure, as well as many other substances, both natural and synthetic, which show antibacterial, antitumor, or related effects. Over 8000 compounds are listed within 4000 entries, each carefully diagrammed and extensively referenced.

Dictionary of Alkaloids. Edited by John Buckingham and Ian Southon. Published by Chapman and Hall, 29 W 35th St, New York, NY 10001, 1988. 1850 pp. in 2 volumes. Price $1295. ISBN 0-412-249103.

The new *Dictionary of Alkaloids* is the only definitive source of essential information on all known available alkaloids. It is the first systematic and critical compilation of data from journals and references, fully tabulating the approximately 10 000 known alkaloids, providing a wide range of data, accessible by 5 different indexes.

Handbook of Hazardous Waste Management for Small Generators. Edited by Russell H. Phifer and William R. McTigue, Jr, Lewis Publishers, Inc., 121 S Main St, PO Drawer 519, Chelsea, MI 48118, 1988. 284 pp. Price $39.95. ISBN 0-87371-102-5.

This new compliance handbook covers in all the necessary detail how to design and operate your waste management program. By experienced authors, this new volume deals thoroughly with CFR 40 and CFR 49 to help you avoid the consequences of noncompliance. Special features include: Practical "how-to" instructions, state/federal regulations—and overview, laboratory waste management, generator checklist, complete coverage, and interpretations of regulations and enforcement.

CRC Handbook of Data on Organic Compounds, 2nd Edition. Edited by Robert C. Weast and Jeanette G. Grasselli, CRC Press, Inc., 200 Corporate Blvd, NW, Boca Raton, FL 33431, 1988. 9000 pp. Price: $2000 (U.S.); $2350.00 (export). ISBN 0-8493-0420-2.

The new 9-volume 2nd edition of the CRC Handbook of Data on Organic Compounds (HODOC II) is a massive revision of the 2-volume 1st edition. With over 30 000 compounds covered, HODOC II not only features the most frequently used physical and chemical data but presents for the first time in a single source, extensive spectral data. Additionally, HODOC II provides 21 exhaustive indexes which allow immediate identification of a compound if one or more spectral, physical, and/or chemical properties are known. Designed to fill the most diverse needs, HODOC II will serve as the definitive, master reference source in organic chemistry.

Chemical Hazards of the Workplace, 2nd Edition. Edited by Nick H. Proctor, James P. Hughes, and Michael L. Fischman. Published by J. B. Lippincott Co., E. Washington Square, Philadelphia, PA 19105, 1988. 589 pp. Price: $54.50. ISBN 0-397-53025-0.

The new edition provides authoritative, documented data on some 438 chemicals most likely to be encountered at work in industry and commerce, including all of the 386 chemicals identified in the NIOSH/OSHA Standards Completion Project. The chief signs and symptoms of overexposure are given, as well as clinical effects in humans as related to exposure levels. Diagnostic features, special tests, and treatment for overexposure, when valid, are presented.

Infrared Microspectroscopy: Theory and Applications. Edited by Robert G. Messerschmidt and Matthew A. Harthcock. Published by Spectra-Tech Inc., 652 Glenbrook Rd, PO Box 2190-G, Stamford, CT 06906, 1988. 280 pp. Price: $85.00. ISBN 0-8247-8003-5.

Containing more than 200 illustrations and tables, *Infrared Microspectroscopy: Theory and Applications* is designed to be a valuable source of information about this analytical technique, which couples infrared spectroscopy and optical microscopy. It describes the technique's applications in such areas as manufacturing and quality control; semiconductors and electronics; industrial problem solving using microvibrational spectroscopy; polymers; pharmaceuticals; paper chemistry; and different aspects of biomedical and biotechnological research. The book also includes information about the development of a high performance infrared microscope and discusses sampling techniques that can be handled on

a micro scale, such as diffuse reflectance.

Automatic Methods of Analysis. Edited by M. Valcarcel and M. D. Luque de Castro. Published by Elsevier Science Publishers, PO Box 211, 1000 AE Amsterdam. The Netherlands, 1988. 560 pp. Price: $131.50/Dfl. 250.00. ISBN 0-444-43005-9.

This new monograph provides a comprehensive overview of the state of the art of the automation of laboratory processes in analytical chemistry. The topics have been chosen according to such criteria as the degree of consolidation, scope of application, and most promising trends. The first part of the book begins with the basic principles behind the automation of laboratory processes, then describes automation systems for sampling and sample treatment. In the second part the principal types of analyzers are discussed: continuous, batch, and robotic. The third part is devoted to the automation of analytical instrumentation: spectroscopic, electroanalytical, and chromatographic techniques, and titrators. The last part presents some examples of the application of automation to clinical chemistry, environmental pollution monitoring, and industrial process control.

Molecular Luminescence Spectroscopy, Methods and Applications: Part 2. Edited by Stephen G. Schulman. Published by John Wiley & Sons, Inc., One Wiley Dr, Somerset, NJ 08873, 1988. 526 pp. Price: $79.95. ISBN 0-471-63684-3.

A list of recent advances in analytical applications of luminescence spectroscopy goes on and on. *Molecular Luminescence Spectroscopy, Part II,* and its companion volume, investigate several applications of fluorescence, phosphorescence and chemiluminescence spectra to the analysis of organic and inorganic compounds. This volume focuses on the various aspects of the analysis of solids and solid solutions. Additionally, it covers: time-resolved and phase-resolved emission spectroscopy; fiber optical fluorosensors in analytical and clinical chemistry; highly resolved molecular luminescence spectroscopy; applications of lanthanide ion luminescence from inorganic solids, plus studies of fast reaction kinetics by fluorescence spectroscopy (proton transfer kinetics of electronically excited acids and bases).

Trace Minerals in Foods. Edited by Kenneth T. Smith. Published by Marcel Dekker, Inc., 270 Madison Ave, New York, NY 10016, 1988. 488 pp. Price: $125.00 (U.S. and Canada); $150.00 (export). ISBN 0-8247-7835-9.

Combining a wealth of information about the relationships of trace elements to nutrition and health, this valuable new reference meets the need for a source integrating these advances into a cohesive framework that food scientists, nutritionists, biochemists, physicians, and pharmacologists can use effectively. Containing over 1600 citations of pertinent literature, *Trace Minerals in Foods* evaluates the chemistry of trace elements in food preparations and their potential bioavailability to the consumer; considers palatability, mineral interactions, and other nutritional factors; discusses trace element biology, chemistry, and pharmacokinetics; details the myriad interrelationships among foods and food customs; focuses on radioisotope and stable-isotope techniques; and defines problems in trace element nutrition from a global perspective.

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   (2) Hurn, B. A. L., & Chantler, S. M. (1980) in *Methods in Enzymology,* Vol. 70, H. VanVunakis & J. J. Langone (Eds), Academic Press, New York, NY, pp. 104–142

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   (3) Siegel, S. (1956) *Nonparametric Statistics for the Behavioral Sciences,* McGraw-Hill Book Co., New York, NY

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   (4) *Official Methods of Analysis* (1984) 14th Ed., AOAC, Arlington, VA, secs 29.070–29.072

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SPECIAL REPORTS

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- Review of Mycotoxin Contamination: Worldwide Occurrence of Mycotoxins in Foods and Feeds—An Update—by *Charles F. Jelinek, Albert E. Pohland,* and *Garnett E. Wood*

METALS AND OTHER ELEMENTS

- Methods of Analysis for Toxic Elements in Food Products: 1. Mineralization Methods to Determine Heavy Metals and Arsenic According to the USSR Standard—by *Igor M. Skurikhin*
- Methods of Analysis for Toxic Elements in Food Products: 2. Review of USSR Standards on Determinations of Heavy Metals and Arsenic—by *Igor M. Skurikhin*
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Keynote Address

From Parochial to International

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The customers for analytical measurement services have international requirements; they require data of proven validity that will be accepted internationally. This means that well researched and tested analytical methods must gain better international acceptance. The methods should be capable of producing results to the accuracy required by the customer. They need to be supported by the use of appropriate certified reference materials, and the analyst, in reporting results, should give an estimate of the accuracy obtained. In addition, an independent accreditation is needed of the quality assurance procedures of the laboratory to assure customers that the analytical methods have been used correctly.

I am delighted and honored to be giving the opening address at the AOAC 102nd Annual Meeting. However, I must confess that I am here under slightly false pretenses. I come under the impressive title of The Government Chemist but I am in fact by training a physicist. I am not sure that President Rund was aware of this when he invited me to give the address. However, I can offer something in my defense besides giving you the opportunity of meeting someone who is a candidate for an entry in the *Guinness Book of World Records* as the first physicist to have made it to Government Chemist—I have in fact spent most of my career working as a measurement scientist. The only break from that activity was during a previous appointment when I had certain responsibilities for Research and Technology Policy within the United Kingdom Department of Industry. Although we tried to measure what we were doing, our measurements were far from successful, and in the context of today's gathering, many would claim that our analysis was far from sound.

Coming to analytical chemistry late on at least gives me an opportunity to see what the various measurement fields can teach each other. Certainly we can learn much from the practices in other disciplines and I will draw on my own experiences in what I have to say. On the other hand, analytical chemistry can offer much to those involved with physical measurement, but that can be the subject of another lecture.

When President Rund invited me to speak, while not actually providing me with the text of what I should say, he gave me some very broad hints on what he would wish me to cover. Certainly this is a good opportunity to discuss the future of AOAC. But before we can begin to decide what the future should be we need to be clear about the role of AOAC and its members and how that role is changing and will change.

The Needs of Our Customers

So, what is our role as Official Analytical Chemists? I am fairly clear what that means for my organization—it is meeting the current and future needs of my customers. I was tempted to use as my title, "Are We Meeting the Needs of Our Customers?" We, being the analytical community, the official analytical community, and AOAC.

We have certain evidence that we in all three senses are meeting those needs. AOAC is well supported, as evidenced by today's assembly; someone has paid for us to be here as a result of the services we have provided based on analytical chemistry. One presumes they are satisfied, but assuming one's customers are satisfied and taking them for granted is the road to bankruptcy. One thing is certain: The needs of our customers are international, not parochial.

However, let me make you feel good by reminding you of the community we serve by giving a brief overview of the range of activities with which we are associated.

Industry

Industry has a major requirement for analytical science. Generally, analytical measurements are made to monitor production conditions and the quality and suitability of the raw material and the finished product. Conformity to statutory specifications may need to be checked in many cases. Quality assurance activities are dependent on analytical data and in many cases, such as in the pharmaceutical industry, may constitute a significant part of a production process.

Trade and Commerce

The regulation of trade depends heavily on analytical measurement. Law enforcement frequently depends on recognized analytical methods. Most decisions taken in the areas of national and international trade involve compliance of goods with contractual or statutory requirements and many of these requirements are derived from chemical analysis.

Consumer Protection

Every item available for purchase in the shops must be fit for the use for which it is intended, must comply with the specification issued by the manufacturer, and must not constitute an unreasonable hazard to the consumer. Compliance is frequently tested by analytical measurement.

Environmental Protection and Safety Issues

Many industrial processes could have a harmful effect on the environment. The discharge of noxious fluids from manufacturing plants or power stations is continually monitored by analytical measurement. These measurements allow the regulation of such emissions and provide the means of enforcement.

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The safety of the working environment and the exposure of workers to chemical, biological, or radiochemical hazards can only be assessed by analytical measurement. Hazards may arise from the use of new materials or substances used in their production.

Law Enforcement

Forensic analysis encompasses many areas of analytical measurement. Such results are primarily of use to the police and customs and tax officials.

Health

Clinical analyses account for a significant proportion of the nonindustrial analytical measurements performed. Routine body fluid analyses are performed in hospital laboratories, and clinical trials evaluate the effectiveness of new drugs and identify undesirable side effects. Many types of diagnoses and treatment would not be possible without information obtained from analytical measurement.

Food

The public health is protected through the analysis of food and drink. Foodstuffs and beverages are examined for microbiological or chemical contaminants, and the nutritional value of food is assessed from the measurement of a number of components.

Agriculture

The use of feedingstuffs, animal antibiotics and other medicines, pesticides, and fertilizers are all dependent to some degree on analytical data. These may provide information on the correct dose, possible metabolites and breakdown products associated with drugs, and the most economic use of fertilizers and feedingstuffs.

Water

The provision of a clean drinking water supply and monitoring the pollution of waterways and oceans is a routine and long-established application of chemical analysis.

This is an impressive list of activities which no doubt you can add to. So, how well do we meet the needs of our customers in these areas? The common theme through all the activities I have listed is that our customers are looking for "cost-effective, sound advice based on authenticated analytical data, the validity of which will be accepted internationally."

This statement about the international acceptability of the data is one of vital importance to the analytical community and this Association. If we look at the history of regulation to control trade and protect consumers, then we see the gradual evolvement from a system that was particular to an individual town, city, or region. In many market places within Europe, you can see embedded in the wall of a building the standard length of measure for use in that city's market. In other areas we have seen the development from the need for town and city regulation and measurement to needs at state and federal levels. Most of our customers no longer take a parochial view, because most of the issues they are addressing have international implications.

Let us then examine how we set about meeting that need. We recognize (or do we just assume) that good analytical data require the adoption of a "validated" analytical method. By validated I of course mean a method that has been checked by some means, for example, by a properly conducted ring test. My physicist colleagues would throw up their hands in horror at the suggestion that one should use an approved or validated method; however, the difference is more apparent than real. In practice, physicists will use the same method of measuring the volt or the meter but will occasionally challenge and test the method by applying some new technique much the same way as the analytical chemist does. This is the normal circumstance of progress.

Methods

The preeminence of the method leads to the major activity of this Association, that of method development and verification. In this, it has an outstanding record. But AOAC is an international association. How well is it serving the international community? How often are the AOAC methods accepted by the official bodies in other countries? I am afraid that as far as Europe is concerned, the answer is "Not very."

If we look at Figure 1 we can see why. We have a very complex international network, with much overlapping of functions. To further confound things, this is duplicated at a national level; Figure 2 shows the structure for just one area. Within each country in Europe, we have separate organizations developing and verifying analytical methods—not a very cost-effective activity. And this cost must be borne by our customers. Aside from cost considerations, how well do these methods agree, based on solid evidential data? We would be hard-pressed to say, although we can have a certain amount of confidence because the same members of the analytical community are likely to be involved in the development and testing of methods for both national and international bodies. In certain cases, pesticides, for example, an agreement exists between the Collaborative International Pesticide Analytical Council (CIPAC) and AOAC for jointly approving methods, but, in general, a multiplicity of organizations is operating in different ways in each country.

Compare this with the situation for physical measurement (Figure 3). At the center is an organization, Bureau International des Poids et Mesures, of which the governments of all industrial countries are members. The definition and realization of physical quantities is carried out on an agreed international basis. While in some respects this comparison is not entirely fair (there is little similarity between the measurement of the meter and a typical analytical measurement), the differences disappear somewhat when you consider the physical measurement of color, for example. However, we believe the situation in analytical measurements is more complex, and the range of measurements and number of methods are far greater than on the physical or engineering side. Are we correct in this belief? Consider the range of measurements that are dependent on the standard of length; the field is enormous, from the fine structure of lines on microcircuits to large structures such as bridges and buildings, from the dimension of threads to the shape of complex surfaces such as aircraft turbine blades.

The one difference of some significance between the organizations for cooperation for physical and chemical sciences is the fact that the governments or their agents are members of the international organization dealing with physical measurement; a clearly defined hierarchy assures that the methods and standards developed by the international organizations will be acceptable to the individual governments. Perhaps chemical sciences would benefit from a more formal international structure.

The benefits of a hierarchical structure have of course been recognized by the analytical community. Within AOAC, the methods committees deal with broad subject areas and these are mirrored by the committees of various organizations in

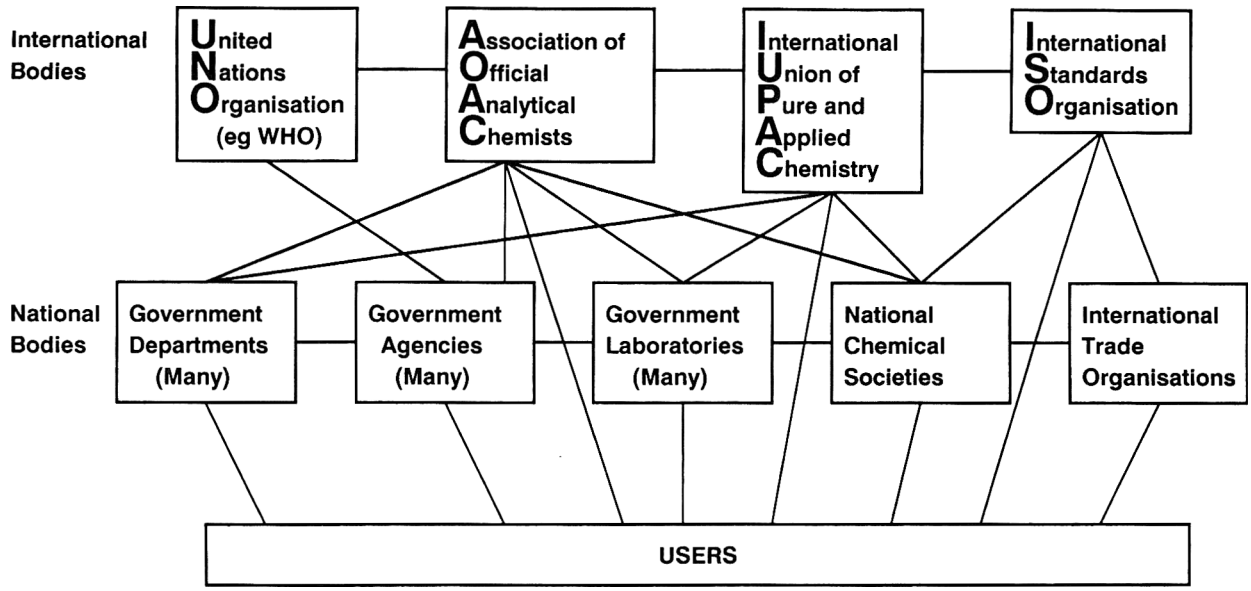

Figure 1. Interactions between international and national bodies on methods development.

most major countries. While, as I have said, there are strong informal links among these various committees, these arise mainly from the individuals involved.

We are sufficiently concerned in the United Kingdom about the lack of an effective structure within the European Community that we are taking an initiative that we have called EUROCHEM which is aimed at strengthening the formal links between the organizations concerned with analytical measurement within Europe. This is no easy task; unlike the physical measurement area, in general, no central national laboratory covers the whole range of analytical measurement. But most of the work falls into the five categories of (*1*) customs and excise; (*2*) consumer protection; (*3*) food and agriculture; (*4*) public health; (*5*) environment.

To provide an appropriate structure, it will be necessary to identify the lead organization and laboratory responsible for giving advice on the basis of analytical measurement in each of the above areas. We would then look to these lead organizations, first, to ensure agreement among themselves on analyses in their own areas. They would also have to take on the responsibility of helping to ensure the validity of measurements made by other analytical laboratories within their sector in their own country. Quite a responsibility, but I will come to how this might be fulfilled later.

What, then, about the effectiveness of the methods themselves. They are certainly well researched and tested. The thoroughness of this work sets an example that other areas of scientific measurement would do well to follow. They

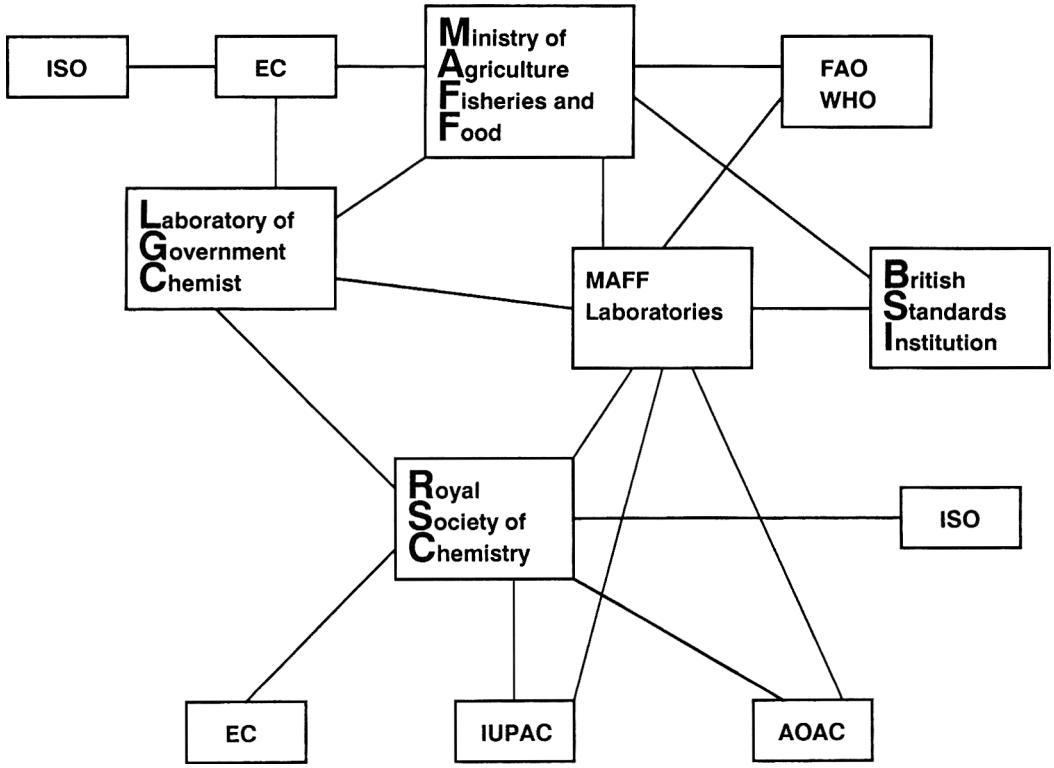

Figure 2. Interactions at national level on methods development in just one area.

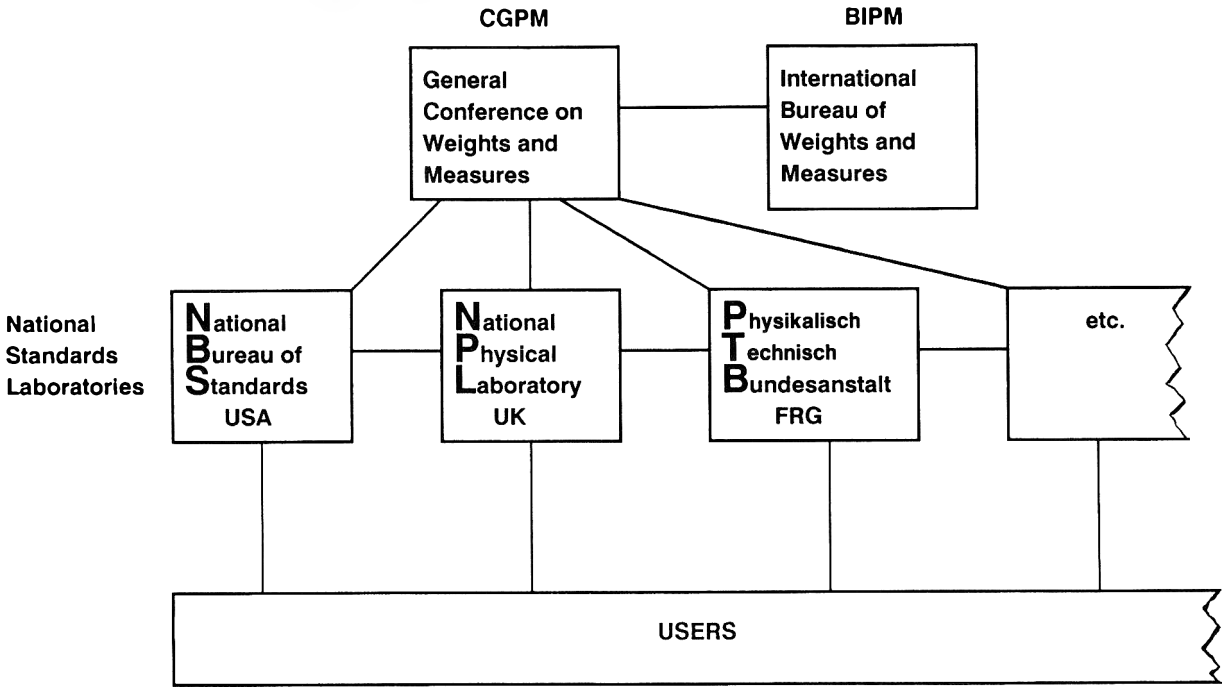

Figure 3. International organization for standards in physical measurement.

would certainly benefit from reading the excellent manual by Youden and the other manuals on collaborative studies published by the Association, which are well known to all of you. But are the methods sufficiently accurate and do we know whether in practice they produce results that enable us to give the sound advice our customers are requesting? Is enough attention being paid to determining and, if necessary, improving the accuracy of the method? There is always the opportunity for doing more, if the extra expense can be justified.

Certainly more could be done to investigate the accuracy of the various stages of the analysis. Each laboratory should prepare its own "error budget" and, ideally, verify that error budget, using, for example, the ruggedness test suggested by Youden. A comparison of the components of the error budget of the participating laboratories would reveal where the method required attention. A comparison between the error budget and the spread of results among laboratories would enable a judgment to be made on whether the major sources of variability on the method had been identified. One may not be able to eliminate the sources of uncertainty but one must be aware of them and have reliable estimates of their size. It is also of course necessary to have quality control procedures built into the method which will check the consistency of the results obtained when the method is used routinely and which will ideally show the part of the measurement that is moving out of control. Reference materials are an essential part of these quality control procedures and more needs to be done to ensure their supply and encourage their use.

My second point is that, when appropriate, the evaluation of the method could be taken further. Consider for example some typical results shown on the traditional two-dimensional plot (Figure 4). A grouping with an elliptical/linear scatter of the type shown is considered to be satisfactory. It is evidence that the method can be used reproducibly but with (acceptable) systematic differences among laboratories. However, in general, the reasons for these systematic differences are not investigated (that is, unless they are unreasonably large); since their causes are not known, we have no evidence of their likely variation in practice. Are they, for example, comparable with the measurement uncertainties estimated in each laboratory's error budget? Since we do not have error budgets, we do not know. It is clear from the results that the precision is sufficient to investigate the differences and possibly to reduce them. Certainly, the precision is sufficient to examine the likely causes of these systematic differences and to estimate their likely variation.

A third point on methods is that when results of analysis are reported, an estimate of the accuracy of the result should always be given; this is a practice that the Association should be encouraging. One can argue that a statement of a result that does not give a measure of the accuracy is of very little

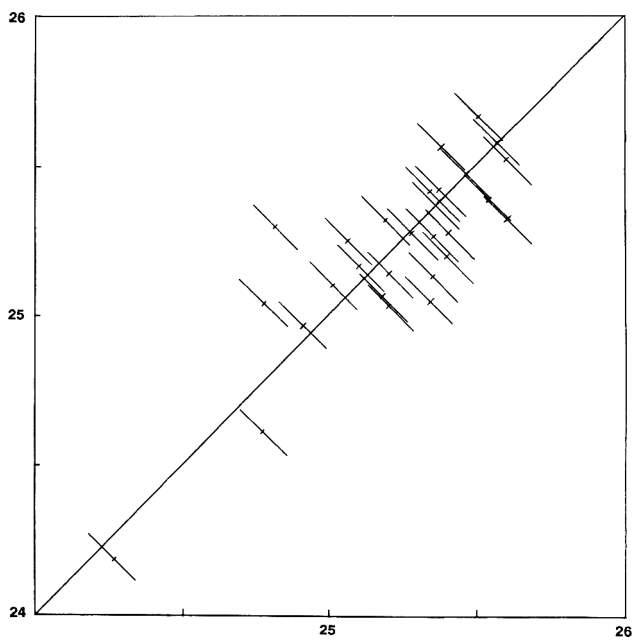

Figure 4. Typical Youden plot of results of a collaborative study (S_o = 0.11, S_x = 0.37).

value. Much of the information required to estimate the accuracy has been obtained during the collaborative assessment of the method, particularly if error budgets and the results of the ruggedness tests are available. I am pleased that a summary of the precision of the method is now included for methods in *Official Methods of Analysis,* but is it sufficient to enable analysts to estimate the accuracy of their results?

Mention of *Official Methods of Analysis* reminds me of two developments that I think would be of value. The book is already produced from electronic keystrokes; it would be very helpful if that electronic text were made available in a computer-readable format, structured to enable appropriate searching. Looking further ahead with the increasing use of automation, one can envisage the time when at least some of the information in the method could be used to control its use automatically. I think it would be worthwhile giving some thought on how the book could be produced as a data base structured for searching and with the data on, for example, equipment settings, and set out in a standard way in a computer readable format that would enable automatic control of the equipment.

Therefore, in looking at how we might better serve our customers in the future, I have pointed out how we could improve the efficiency of method development and the manner of its publication. I have also indicated how we could improve our confidence in and possibly the accuracy of the method, both important parameters affecting the advice we can give based upon the results of the analysis. In addition, if we had a more hierarchical structure for methods development of the type that is in existence in the physical area, then we could have some expectation of the international compatibility of the results.

Accreditation

However, this would not be sufficient. I have already said that, in many cases, results have to be acceptable across national boundaries. That we are using methods of proven validity, whether or not the methods are identical, does not show that the methods were competently executed. Within our own organizations, we require, and I would expect most of you already have, quality assurance procedures for the control of our analytical measurement. However the procedures have to be made visible and be acceptable to our customers and third parties who will be basing their actions on the advice they obtain from us. You will be aware that some of these actions will be of significant importance: whether to ban certain products, to enact new legislation, to license some pharmaceutical, or to enter into some course of possibly quite severe treatment for a patient. Those making these decisions will wish to be convinced about the effectiveness of our quality assurance procedures. Particularly since in many cases they will have to gain international acceptance of their decision.

This was a situation that arose nearly a decade ago in the United Kingdom, in other areas of testing and measurement. When we examined this in detail we found that laboratories were incurring significant costs in demonstrating separately to each of their major customers the effectiveness of their quality assurance procedures. They were in fact being assessed several times over and it was clearly much more economic to have one assessment or accreditation system which was acceptable to all of their customers. We introduced a national accreditation scheme run by the government but financed out of accreditation fees. The scheme, now called NAMAS (National Measurement Accreditation Service) has proved to be very satisfactory; there are nearly 700 approved laboratories and the annual growth rate is 25%.

We had all the arguments about the need and the effectiveness of such an organization during the period leading to its launch: One, it would be too expensive. Well, the laboratories are not going to agree that it is not expensive, but there is a good deal of evidence that it is less expensive than the multiple assessment procedures that were in place previously. Two, it will be too bureaucratic and there will be excessive paperwork. This need not be the case and I think we can claim that it has not been the case. Quality control requires well documented quality control procedures; accreditation requires no more than the procedures that should be in place in any case. Three, who will have the expertise to assess us? Which was really a substitute for "Are you challenging our professional competence?" This did not turn out to be an issue. We had very little difficulty in putting together assessment teams with the requisite expertise. There have been problems but none has been insuperable. The benefits have been very significant. Customers are assured of the quality of the work, and new customers are attracted by the enhanced status of the laboratories. International acceptance of the results of these accredited laboratories has grown as we have negotiated mutual acceptability with other accreditation schemes overseas.

These developments are of importance in support of open and free trade, in particular, within Europe with the plans for the completion of a single market by 1992. The developments form an integral part of the EUROCHEM proposal I referred to earlier. The lead laboratory will be able to use the accreditation service to ensure the validity of the measurements being carried out by accredited laboratories within its sector, by helping the accreditation services to define the accreditation criteria, by participating in the assessment, by carrying out proficiency tests, and by supporting the development and use of certified reference materials.

Some significant changes are required to meet the needs of our customers as their demands become less parochial and more international. We need to move toward a more hierarchical structure that encourages the development of methods on an international basis. With this concentration of effort, we can then afford to put more effort into evaluating the ruggedness and accuracy of the method. We need also to develop accreditation schemes to assure and demonstrate to our customers that the required accuracy is obtained in practice.

In all of these areas, AOAC has a major contribution to make.

Wiley Award Address

Theory and Practice of Microbiological Assaying for Antibiotics

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I was surprised and pleased to receive the AOAC Wiley Award for 1988. It was given in recognition of my contributions to the theory and practice of microbiological assaying for antibiotics. This address is about one person's efforts to provide a scientific basis for the assays. To do my job of developing media for new strains of antibiotic producers, I needed accurate assays. None were available in 1953. I had no choice: I had to develop new assays or improve existing assays. However, I had no authority; I could only suggest changes. The effort became a part-time, off and on activity for the next 35 years. During that time, I was an inventor of instruments, designer of assays, trainer of personnel, teacher, author, editor, and a participator in AOAC programs. Many of these activities were done on my own time, and most were done at my own initiative.

Microbiological assays are used when chemical ones are inappropriate, insensitive, nonspecific, too expensive, or nonexistent. Microbiological and chemical assays measure different things and should be expected to disagree except on pure, single-component materials. However, excessive disagreement signals a problem to be investigated.

Complete dependence on nonspecific chemical assays when assaying for microbiologically active compounds can lead to gross error, as illustrated by the following example: The puzzled quality control official (a bacteriologist) held a bottle of Tincture of Merthiolate (thimerosal, a mercury antiseptic). The chemist reported no loss of thimerosal, but large globules of mercury were evident in the bottom of the bottle. Chemists can either assay for mercury or determine mercury by a polarographic method; this chemist had used the latter. Whatever he had measured, it was not thimerosal. Obviously, he had not carefully examined the contents of the bottle. I developed an assay for thimerosal which can be used for other antiseptics and formaldehyde (1).

A collaborative study is a collection of assays of a few samples done by numerous analysts following a written procedure. Results are greatly influenced by the competence of the analysts because it is possible that important details are absent from the official method, and the study is no better than the assays; hence, what follows in my address will emphasize assaying. I shall discuss, briefly, assaying as I found it and as I left it 20 years later, the importance of the people who do the work, the problems of supervision, and a suggestion that AOAC try to increase the supply of competent analysts.

Analytical chemists, believing microbiological methods to be some sort of black magic practiced by bacteriologists using "bugs," avoided the field. This was a wise decision because microbiological methods have at least 26 potential sources of error, not all of which are under the control of the analyst. Few chemical methods are as complex. Bacteriologists were assigned the assays because of the belief that only they could handle bacteria. All assumptions and beliefs were wrong.

For the benefit of those who do not use microbiological assays, I shall give a brief description of the diffusion (also called plate) and turbidimetric methods. They differ radically in theory, design, mechanics, and calculation of antibiotic potencies.

The plate method is one in which a zone of inhibition of bacterial growth forms around the point of application of the antibiotic. Movement of the antibiotic through the thin bacteria-containing agar layer is governed by laws of diffusion. The bacteria are both the reagent reacting with the diffusing front of the antibiotic and the indicator of position of the front at a critical time and at a critical concentration of antibiotic. The method is analogous to a permanganate titration. Analytical quality assays require control of, or compensation for, 11 variables.

The turbidimetric assay consists of a large number of reaction vessels—test tubes of inoculated broth—to which antibiotic is added. The tubes are incubated in a water bath for a fixed time, and turbidity is measured. The antibiotic reduces the growth rate of the test organism to give an inverse relation between turbidity and concentration of antibiotic. Analytical quality work requires careful control of 6 variables.

Essential details of plate and turbidimetric methods were worked out by bacteriologists by 1944. They did it without any guidance from theory because there was none. Plate methods have undergone little change in design since 1945. The big improvement in turbidimetric assaying came in 1970 with introduction of the Autoturb® system.

By 1958, I had in mind the factors from dishwashing to selecting and training personnel that are essential for analytical quality turbidimetric assays. I wrote a report (2) that described the assays as then done and possible improvements which were later incorporated in the Autoturb system. I also discussed improvements in plate assays.

Academic Press asked me to edit a book on microbiological assaying. I began by recruiting K. E. Cooper of Bristol University to write about the theory of zone size in diffusion (plate) assays for antibiotics. His manuscript is Chapter 1 of *Analytical Microbiology* (3). I wrote the corresponding chapter on turbidimetric assaying for the excellent reason that I could not find anyone else to do it. Volume 2 (1) brought the information on assaying up to 1970. It is a continuation

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of, and is not independent of, the 1963 volume. The 2 books should be used together. For those who assay growth-promoting substances, I wish to call attention to the extraordinary chapter in volume 2 by Arne Bolinder on assaying for amino acids. He gives principles, practices, and the numerous pitfalls in such difficult assays. Certain procedures in microbiological assays for amino acids and vitamins in "Methods of Analysis" are vintage Snell and Strong and are 40 years out of date.

I tried to keep plate and turbidimetric methods up-to-date by means of journal publications after 1971 (4–9). The articles contain much raw data, an unusual feature these days, for others to use to check ideas.

Autoturb System

The Autoturb system revolutionized turbidimetric assaying by automating sample-tube filling, incubation, and turbidity measurement (10, 11). Uncoupling these 3 elements of an assay gave the system the flexibility required for microbiological assaying. The large number of test organisms, incubation times, and temperatures that can be used are easily managed by people but poorly managed by machines. The special water bath is as essential as the diluter and reader. The system can also be applied to chemical and microbiological procedures in which color, fluorescence, pH, or turbidity is the response to be measured. The Autoturb system improves precision and can reduce the unit cost of assays for amino acids, antibiotics, B-vitamins, and other growth-promoting or growth-inhibiting substances.

A brief description of operations will explain why the 1953 turbidimetric method was grossly flawed and had to be drastically changed. The assays had 10 closely spaced concentrations of standards in quadruplicate in a rack, and 3–5 racks of 24 samples each, at one concentration in triplicate. Standards were diluted in water; samples were diluted in buffer. One mL volumes of standards or samples were added from 5 mL serological pipets. Tubes were incubated in a war surplus unstirred serological water bath. Growth was stopped by steaming stacked racks for 10 or more min. Turbidity was measured by the McMahan procedure in which the entire span of turbidities from zero tube to highest standard was represented by about 80 divisions (63 mm) of a galvanometer scale. The deflection position of the galvanometer was estimated while it was still moving! The tubes were shaken vigorously, and their contents were poured immediately into the photometer cuvet. Turbidity of air bubbles and bacteria was measured. From 3 to 5 people worked on a test, depending on work load. Penicillin V was assayed in terms of penicillin G standard!

One very important purpose of the assays was to identify new strains of a culture that were at least 5% more productive than the control. Chances of finding such a strain with the 1953 assay were so small that strains could have been selected for further testing by flipping a coin. This would have been at least as productive and much more cost-effective. But, such a practice was not scientific. By working slowly so that I did not disturb anyone, I had made all the improvements possible with the facilities available to me by 1963. I had made an analytical instrument from the photometer; standards and samples could be measured accurately with the same special pipet; each rack of 40 tubes was a complete assay of 15 samples done by one person. Now, a 5% change in potency could be detected. Strain selection and media improvement became productive.

About this time, I discovered Norbert Kuzel and Henry Coffey measuring turbidities of bacterial suspension in flow cells in a spectrophotometer. All of their flow cells were unsatisfactory for measuring rod-shaped bacteria. They had observed the influence of flow birefringence on absorbance and were puzzled by it. An A. H. Thomas Co. flow cell was recommended; it was used later in all Autoturb readers.

Kuzel and Coffey had been assigned the project of automating the measurement of turbidity. They were appropriate people to receive the assignment because Coffey was a genius at designing mechanisms and Kuzel was an expert at AutoAnalyzer assays. I joined the team as a volunteer. What they were doing was straightforward and easy. The difficult operation would be automatic preparation of assay tubes.

I taught Kuzel about the principles of turbidimetric assaying. He used this information and devised a way to measure small volumes of samples accurately and wash them into assay tubes with inoculated broth. That idea completed the design of the Autoturb system. I was testing the prototype in 6 months; it was not a crash program. The commercial version was available in 1970. The diluter had a unique system for preparing assay tubes. It was the same in the commercial instrument as in the prototype. The first commercial system has operated for at least a half-million times during the last 18 years with minimum servicing.

Design of the Autoturb system was a true collaboration; none of us could have developed it alone. We were not involved with the design of the Autoturb II.

Purchasers of the system were urged to send 2 people to Indianapolis to learn how to operate and service the system. A majority were bacteriologists with degrees ranging from B.S. to Ph.D. Most were supervisors and would not be operating the equipment. A certain number quite obviously did not understand what they were being told by the instructor, through no fault of the instructor. The following experience illustrates the reason for the training program.

The young woman stormed across the 15 ft separating her laboratory from my desk to say, "Your Autoturb is no good." Her evidence was that the value of the check sample in the second carrier of her test was only 70% of its true value. We went over details of operations and found at least 9 easily identified sources of error. She had 23 samples, but a test maximum for the Autoturb system was 15. She, therefore, had added a half-carrier of tubes to receive the additional samples, producing one test and part of a quite different second one—one without standards. Obviously, the half-carrier of tubes had incubated for a significantly shorter time than the full carrier. She did not understand the principles of turbidimetric assaying and proper use of the Autoturb system. I arranged a meeting with everyone doing assays in that department and gave a quick lesson on assaying and proper operation of the Autoturb system. Most of the 9 errors were removed from their operations during the next 2 years. All could have been removed the next day.

Diffusion Assays

Diffusion methods of assaying for antibiotic were derived from the one brought from Oxford by Heatley in 1941. He worked at NRRL in Peoria, teaching all he knew about assaying for penicillin and its production. A slight modification of his method was published by Grove and Randall (12). It had 10 closely spaced concentrations of standards ranging from 0.6 to 1.5 U/mL. Zone sizes were measured by projecting the image of a zone and of a millimeter scale onto a screen. Standard and sample zone sizes were corrected to the observed mean reference diameter. Potency was interpolated

from the standard curve drawn through the 10 points. Later, the standard curve was assumed to be a straight line and was located by calculation, not by eye. The number of standards was reduced to 5 and given a logarithmic spacing to facilitate hand calculation of the line through the points (13).

Since the most efficient way to locate a straight line is to put one-half of the effort into each end, use of 5 points indicates a non-mathematical reason for so many standards. The reason, common in plate assaying, was that an error in one standard of 5 would have little influence on location and slope of the line and would not invalidate the assays. The same error in one end of a 2-point line might invalidate the assay without leaving evidence. Use of superfluous standards indicates that the analyst has little control over the quality of the operators and their work. In other words, the 5 standards are used for operational, not analytical reasons. The most common AOAC standard line is 16-fold with 5 points.

AOAC plate methods have 9 sources of inherent variation to be controlled by the analyst (14) and other potential variations due to manipulation. Obviously, AOAC and FDA methods needed improving. One improvement was use of the BBL nonelectric automatic pipet to fill cylinders with accurately measured volumes of solutions (1963). The Fisher-Lilly antibiotic zone reader was given the capability of recording zone sizes in IBM punch cards for further processing (1960) by computer. The digital voltmeter and system for recording voltage on printed tape used with the prototype of the Autoturb reader was applied to the zone reader to increase resolution and accuracy of zone diameters. This was done by September 1967. Output of the zone reader was sent directly to the computer by 1972. A report of the assay now could be obtained a few minutes after the last zone was read instead of in several days when punched cards were used to record data (assays had low priority). Examples with data were published (8).

Collaborative Studies

The basic assumption concerning collaborative studies is that if 6–15 laboratories obtain results with an acceptable spread of values, then the method can be approved. Another assumption is that those doing the work are experienced analysts who use the best analytical technique, pay attention to details, and have available the equipment required to obtain high quality answers. Generally, these assumptions are false, as shown by collaborative studies of plate methods. Defective analytical procedures and inappropriate computations are sources of many of the errors; the microbiological part is the most reliable. Designs other than current AOAC ones may greatly improve results by reducing the amount of work and by eliminating specified analytical and computational sources of errors (14, 15).

Those performing official microbiological assays must use complex, inefficient, inaccurate, and biased methods. They may use relatively simple, efficient, and accurate methods for routine assays. Chemists do not have this absurd situation of using essentially 1945 methods in 1988 when better ones exist. If Dr. Wiley were to review our indifference to correct analytical procedures, he would consider our proper station in life to be behind a one-horse walking plow.

When he established the concept of collaborative studies in 1884, he assumed that competent analysts given the important details would produce acceptable answers. The emphasis was on the *correct* answer. That point of view changed, once methods became part of the legal system. Now, the methods were developed so that 2 groups using an official method would get *the same* answer on a product. Agreement, not accuracy, became paramount. But, it is accuracy that is important to a purchaser of the product.

There is ample evidence that the whole process of collaborative studies needs examination. Results from chemical and microbiological studies show that it is operations and not the chemistry or microbiology that is being evaluated. A collaborative study is a very inefficient way to measure overall quality of a method. The 2 parts, method and laboratory, should be tested separately: methods by laboratories known to do high quality work and laboratories by means of accurate standards first, and then by accurately prepared typical samples.

Statistical Treatment of Data

Some statisticians evaluating assay results do not quite understand the consequences of their actions. Statisticians are most useful in massaging a data set to reduce its bulk to a manageable amount of information. They are useful consultants for the analyst.

Although an analyst's lot is not always a happy one, statisticians could make it happier if they would first learn the principles of the assay before sitting down at the keyboards of their computers. If their queries forced the analyst to learn the principles, both would benefit. Someday I hope to read an article by a statistician who has been given all the information needed and who then states clearly all the assumptions made in producing the statistical analysis.

Too much statistical treatment of scientific data is mindless number crunching. An example is illustrated by the frustrated statistician who was trying to reconcile data of a drug study. Part of the data came from laboratories using serial dilution methods and part came from laboratories using paper disc diffusion methods. (These are notoriously inaccurate methods of assaying for antibiotics.) No one had told the statistician there were 2 sets of data obtained by very different methods. He left soon after for a less ulcer-generating job.

Statisticians have had a long love affair with the straight dose-response line because its statistics are relatively simple, well worked out, and generally understood. The usable part of the calibration line for turbidimetric assays is slightly sigmoid. Fitting a straight line to the points degrades accuracy. An Autoturb system had to be misused in a particular country to meet the demands of a biostatistician because only straight "dose response lines" were valid to that statistician. Only then could the Autoturb system be used for official assays.

Statisticians should like the Autoturb system because once the diluter starts, the analyst has very little inadvertent effect on the result. The analyst transports the carrier to the incubation bath, the killing bath, the cooling bath, and the reader.

An assay report is a very important part of an analysis. It should be accurate, credible, short, clear, and as simple as the assay design permits. A proper report has all the information needed to obtain the potencies printed in it. The analyst, as the source of information, should take the initiative to design it because statisticians are likely to produce one that is legally indefensible. An AOAC plate assay for chlortetracycline was an example of such a report. It was 6 pages of computer printout—a confusing mass of unidentified or poorly identified numbers. Essential information either was not printed or was not labeled. Should such a report be entered as evidence to a court, the opposing counsel could use it to destroy the credibility of the analyst. The analyst, not the statistician, is the responsible investigator.

A report for a turbidimetric assay is much simpler than

one for an AOAC plate assay. An Autoturb system assay for 14 samples fits on one letter-size sheet of paper.

If one analyst does all of the work on assay, then a control chart on the analyst and method has meaning. Control charts are supplementary evidence of an analyst's competence and have legal significance because they document a pattern of behavior. Data must be entered in ink on the day obtained. Such charts show that certain analysts are more accurate and consistent than others. Control charts show that 2 people working together on a manual assay produce more variable results than the least competent one working alone.

Personnel

The people who do the work are the most important element in any assay. This is true even for highly automated methods such as those of the AutoAnalyzer and Autoturb system. An inexperienced operator and automated methods can produce large quantities of inadequate data at apparent small unit cost but with a large true but hidden cost (16).

Experience with microbiological assay groups in 9 drug companies, 3 government departments, several academic laboratories, and a molecular genetics company revealed general lack of understanding of techniques of analytical chemistry, theories of the methods, and correct laboratory practices. Many of those in charge of the work lacked aptitude for quantitative analysis of any kind, were poorly educated in chemistry, mathematics, and physics and had had no education in analytical chemistry. These groups, generally, did acceptable work because they worked within the wide limits of the 1940s. But only narrow limits should be acceptable in 1988 unless wide ones are appropriate.

Quality of work can depend on the local dominant person who sets the tone for the area. This person, quite often, is not the supervisor. The current trend is to select a supervisor with training in management and who is, too often, inexperienced in the technical details of what is to be managed. However, most of the manager's problems will be technical.

Official FDA and USP methods are essentially those of 1945 with minor changes. FDA required efficient screening methods for identifying samples varying $\pm 20\%$ different from label. The drug companies adopted the *screening* method as their *analytical* method and refused to change because the decision makers did not understand analytical work or the attitude at FDA. The AOAC plate method is the FDA screening method with an extended calibration line.

One of the most important causes of low quality microbiological assaying is a psychological attitude: The assay is regarded as a biological method analogous to the rat assay for vitamin D and, consequently, is considered inherently inaccurate. This attitude causes management, supervisors, and technicians to believe that careful operations are a waste of time. Management is reluctant to spend money for the facilities and equipment required to produce high quality assays. They have no such reluctance in providing very expensive instruments for their analytical chemists.

Another cause of poor work is the kind of people who hire and supervise analysts. I have observed or know of analytical work being done or influenced by an algoldologist, analysts, a virologist, numerous bacteriologists (B.S. to Ph.D.), chemical engineers, failed analytical chemists, a lawyer, pharmacists, physicians, a physical chemist, an organic chemist, a waste disposal environmentalist, and an X-ray crystallographer. Several of these "analysts" did a respectable job. Generally, the best work is done in the few laboratories directed by competent, experienced analysts who are the only people AOAC has insisted for a century should participate in collaborative studies. Obviously, as shown by the results, the problem of competency still exists. At least, we recognize the problems and know the solutions.

Methods can be improved only after theories are developed to guide changes. The theory of the turbidimetric method was published in 1969 (17) and used to guide the design of the Autoturb system. The theory of the diffusion method was developed by Cooper (18) in 1946 and applied to petri dish methods in 1974 (5, 8). The requirements for producing microbiological assays that are equal to or better than certain AOAC chemical ones are now known. With the proper people and equipment, high quality assays take less time, effort, or supplies than official ones.

The demand for analysts greatly exceeds the supply of competent ones. Government agencies mandate ever more analyses but do nothing to increase the analyst supply. One response to inadequate supply is the disastrous one of inadequately trained people operating machines in too many laboratories.

High quality assays, chemical and microbiological, are done by people with aptitude for quantitative work, understanding of analytical procedures, and experience. Such people are rare. Our educational system is not producing the number of competent analysts that society needs to function properly. To obtain an adequate number will require drastic changes in chemical education and of those to be educated. Certain teachers of chemistry recognize the problem and want to do something about it.

Much time, expense, and effort would be saved if people with an aptitude for quantitative chemistry could be identified and urged to take the requisite courses. They are people Garrett Hardin called numerate (19). To start with the numerate would help produce scientists of all kinds. Such people can be identified by means of psychological tests that have been available for many years. I took one in 1963. Aptitude is the determining factor; with it, the person can learn to be an analyst. College degrees cannot substitute for talent (aptitude).

Many chemical companies seem to be unaware of the cost of inadequate chemical assays. Since the cost does not show as a separate line in the annual report, it does not exist for them. They believe that chemists perceived to be unsuited for more prestigious activities are suited for the analytical laboratory as workers and supervisors.

AOAC, as an organization with a large proportion of analysts in its membership, has a great interest in high quality analytical work. Perhaps AOAC could take the initiative and help chemistry departments learn what their students need to know to function as chemists in the nonacademic world. The 2 groups of chemists have been separated for too long. They need to discuss mutual problems, decide on corrective action, and take it. Time's a wastin'.

AOAC as the guardian of official methods needs to establish minimum standards for laboratories and individuals who perform official assays. There is no reason to continue to do assays with 1950 attitudes and facilities. First, establish minimum acceptable precision and accuracy standards and how they are to be measured. Then, require the organizations to have proper equipment and facilities. The third requirement is that official assays should be done by analysts of demonstrated competence (P.A., Professional Analyst). The fourth element can be obtained quickly by updating the *Official Methods of Analysis*. The knowledge has been available for many years.

AOAC has a choice. Either it or the federal bureaucracy can take action. The bureaucracy will be only too happy to

write and impose on analysts rules analogous to that bureaucratic horror, "Title 21 of the Code of Federal Regulations," that was imposed on the pharmaceutical industry. It is a version of the Laws of Medes and Persians, eternal and unchangeable.

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President's Address

Musings, and More

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This is the 104th year of this Association. I am the 101st President (three served two years each) and the fifth to serve as President while an employee of Purdue University. Dr. Harvey Washington Wiley, the first Indiana State Chemist and first professor of chemistry at Purdue, barely escapes this characterization inasmuch as he left the University less than three years before becoming the second AOAC President. I have been privileged to know three of my predecessors from Purdue, both personally and professionally, as well as many of the others who have spoken from a similar platform in the past in the same role as I. The greatness and esteem which I have held for these persons and this office humble me mightily. Some of these persons are here today and, I'm sure, they are now reflecting back on this moment in their lives.

I find it difficult to believe that my chances at attaining this high office were any better than most of my colleagues and yet, here I am, somewhat apologetic for receiving that chance. I ask as a matter of personal privilege that you allow me to reflect upon my own career for a moment. Perhaps, by this process, some of you, as well as myself, may better understand why I find myself in this position.

I was hired directly out of college by a nationally known company to join their laboratory staff as a quality control chemist in their "plant food" manufacturing facility near Chicago. I was 23 years old, a small town youth, and though I had three years of active military service behind me I was still somewhat naive about a lot of things. For instance, I did not recognize the term "plant food" as a synonym for "fertilizer." It was only after my arrival at the job site and some indoctrination that I discovered this fact. Another truth uncovered that day was that fertilizer could be synthesized, it did not all come from animals as I had been led to believe from my summer experiences on my grandfather's farm as a barefoot boy in the cow pasture, barnyard, and chicken coop.

My first duty in this laboratory was daily analyses of the previous day's plant production of various grades of fertilizer. I became an expert in the determination of phosphorus content in fertilizers using the old (now discredited) volumetric ammonium phosphomolybdate method carried for so many decades as an official AOAC method. From phosphorus analyses I moved rapidly to Kjeldahl nitrogen determinations and then to the "Cadillac" of all fertilizer analyses, potassium, by means of the gravimetric Lindo-Gladding method (still considered the most accurate of all potassium methods in the hands of an experienced analyst). Eventually, I was exposed to the analyses of the other recognized plant nutrients, i.e., calcium, magnesium, sulfur, boron, etc.

From the very first day I was constantly referred to the "bible," the *Official Methods of Analysis of the AOAC.* I became enamored of this text and marveled at its coverage and detail. The latest edition was always available on the laboratory bookshelf and took a prominent location next to *Scott's Standard Methods of Analysis.*

My first of many participations in an AOAC collaborative

study was during my second year of employment. Dr. M. P. Etheredge, the Mississippi State Chemist at that time, was the Associate Referee and the study concerned itself with the formaldehyde titration of ammonium nitrate as well as the effect of high concentrations of chloride in the presence of nitrates upon total nitrogen determinations.

I had always been interested in organic chemistry, especially organic synthesis and analysis, so when I was selected to head a project for laboratory staffing and facilities for pesticide formulation quality control I was very pleased. Perhaps the most renowned U.S. pesticide analysts at that time were in the laboratories of the Bureau of Chemistry in the California Department of Agriculture in Sacramento. The research director of my company was a close friend of the bureau chief and it was agreed that I should spend several weeks in those laboratories actually working beside these brilliant chemists. It was a memorable learning experience and one in which AOAC methods were always held up as the epitome of excellence.

I returned from California to plan, install, and staff the first pesticide chemical control laboratory for my company's pesticide formulations produced throughout North America. Following that year, at the age of 27, I attended my first AOAC meeting amidst the splendor of the Shoreham Hotel in Washington, DC.

In those days the analysts of pesticide formulations had very few instruments of sophistication. Those available included the infrared spectrophotometer, the polarograph, ultraviolet and visible spectrophotometers, and rudimentary gas chromatographs. Total elemental analyses were common, utilizing combustion tubes, Parr bombs, and sodium fusion techniques. Separation techniques were not well known other than those employing thin-layer chromatography and liquid-liquid chromatography on large silica-packed partition columns which had some specific uses. Colorimetry was used in many cases for specificity and quantitation but this, as well as some of the other techniques, required reference standards difficult to come by.

Given all the roadblocks of that day it was still a pleasure to match wits against a complex mixture for the answers. I still recall the excitement of watching the beautiful series of colors (red to purple to blue to green) which developed when

Presented at the 102nd AOAC Annual International Meeting and Exposition, August 29–September 1, 1988, at Palm Beach, FL.

Pyrethrin I (chrysanthemum monocarboxylic acid) reacted with Deniges reagent. I knew when this occurred that I had made a clean separation from Pyrethrin II, the dicarboxylic acid.

Obtaining separation of the gamma isomer of hexachlorocyclohexane from other isomers on a partition column marked with D & C Violet No. 2 dye was always challenging. Experienced analysts learned to differentiate one isomer from another by the unique crystal formation of each isomer upon evaporation of the *n*-hexane solvent from the fractions as they eluted from the column. In some cases we also separated the isomers of hexachlorocyclohexane from DDT in this same manner.

I was attending my fifth AOAC meeting when Dr. Romeo Payfer, Agriculture Canada, resigned his associate refereeship following a report on rotenone, a specific insecticide found concentrated in sizable quantities in the roots of Cubé and Derris plants. Dr. T. H. Harris, USDA, the General Referee, upon the urging of Dr. Payfer, promptly asked if I would assume the refereeship. After clearance with my company superiors I agreed and thus began a more intimate relationship with AOAC. My first report and collaborative study on rotenone occurred the following year. Three other reports followed and a method using ultraviolet measurements was adopted as official first action.

I took a short course in statistical analysis the first year I was an Associate Referee. I was so imbued with this concept I applied statistical analysis of variance to evaluate the collaborative results that year and had the audacity to write the AOAC Executive Director and expound to him upon the advantages of such practice. I later discovered to my horror that the analysis of variance tables and conclusions derived therefrom and published as part of my report were in error.

In 1966 I joined the staff of the Indiana State Chemist at Purdue University and two years later became General Referee on Fertilizers, a position I held for 17 years. My position as rotenone referee was terminated by resignation in 1971. In 1976 I accepted an appointment to the Editorial Board which I chaired from 1980 until 1986. In 1983 I was elected to the Board of Directors. In addition, and during these appointments, I also found time to serve as AOAC liaison to the ISO Technical Committee on 134 Fertilizers and Soil Conditioners, the AOAC Safety Committee, the International Coordination Committee, and the Long Range Planning Committee.

So, 40 years after I first opened that copy of *Official Methods of Analysis* I stand here before you, the members of this prestigious scientific association, as your President. The amazing thing to me is that I have never consciously sought any of these positions to which I was appointed and elected. At the risk of appearing blasphemous, and perhaps with tongue in cheek concerning any comparison, my thoughts at this moment may be similar to those which Moses must have had as he gathered the thousands of his followers together on the banks of the Jordan River after 40 years of wandering in the wilderness to remind them of their heritage and to tell them of the wonderful things awaiting their crossing into the land of milk and honey. Moses had been told by God that he would not be allowed to cross the river to enjoy the fruits of his journey. I have not received such a message. Therefore, I hope I can be allowed to complete my journey well into the future.

As the first of the next 100 AOAC presidents I have been privileged to lead the Association on to new ground in accordance with the Long Range Plan of 1987, revised bylaws, and directives of the current Board of Directors. These moves have been extensive and will affect the Association for years to come. I will discuss some of them at this point.

Equalization of Membership

Changes made in 1987 to the bylaws required equalization of sustaining and individual memberships as well as voting rights. There is now no differentiation between corporate, academia, or regulatory (government) benefits derived from newly obtained sustaining memberships at the same level of contributions. The minimum base annual fee for sustaining members is now $750. For those who contribute $1000 or more, additional benefits in terms of publications and individual memberships as well as technical assistance will be provided.

The category of "associate member" without vote has been deleted and only one class of individual membership now prevails, including members from industry, formerly restricted. All individual members of the AOAC are now provided identical voting privileges.

International Relationships

Liaisons with international and other organizations have been reviewed and improved through changes in liaison appointments, and newly established or discontinued liaisons. Liaison officers are now required to report annually to the President through the Assistant Executive Director. In most cases the appointed liaison officer is a referee in the area of the liaison.

It is important that we recognize our international role in the standards-setting arena. AOAC is looked upon as a premier organization throughout the world in this regard. We must guard this reputation, play upon it, promote it, and use it to our best advantage. Any attempt at dissolving this relationship will certainly be detrimental to the Association.

Regional Sections

I think my comments regarding regional sections as expressed in the January 1988 issue of *The Referee* are still pertinent and I repeat here an excerpt from that publication.

AOAC regional sections offer opportunities for local analysts of high repute to both speak and participate in AOAC. Regional section members can profit personally and professionally from one-on-one contact with colleagues at regional and international levels. Just as professional baseball has a feeder system from minor to major leagues, AOAC must learn to exploit the regional section relationship for membership growth and development.

Regional section officers must recognize the important role they play in this scenario. Their responsibility is to enlighten section members regarding the relationship between the section and the parent AOAC, highlight the purpose of AOAC, and encourage AOAC members to become involved at the section level. In return, AOAC must find resources to support section growth, programming, leadership, and direction.

We welcome the new Central Regional Section and wish it well. The first meeting of this section is scheduled for November 10 in Marysville, Ohio. It is my distinct pleasure and privilege to have been requested to attend this session.

Closer ties between regional sections and the parent AOAC have been strengthened this past year through action of the Board of Directors. In its effort to become more proactive and visible, all meetings of regional sections with the exception of one have been attended by at least one Board member as the official representative of AOAC.

AOAC Europe has expressed an interest in becoming a regional section and I understand that this group is in the

process of developing bylaws to harmonize with the guidelines adopted by the Board from recommendations of the Committee on Regional Sections.

Past President Johnson visited Mexico City last January and extended an invitation to a gathering of interested individuals to form a regional section in that country. Such a development appears likely in the near future.

Association Management

The Long Range Plan called for a "strengthening of the total management staff." Executive Director Ronald Christensen is committed to this task. Your President is confident that a tightening of the entire staff operation has occurred since Mr. Christensen's employment and that improved efficiency will result. A new and current employee manual has been developed, and has received legal review and Board adoption. The manual has been made available to all staff. Within the constraints of the budget, all staff are provided opportunities to improve skills within their area of expertise.

New Headquarters Site

The current site of our headquarters is in disrepair, too small and not conducive to expansion, poorly furnished, and otherwise not adequate. Our lease on this property terminates as of the end of April 1989.

Following a prolonged search a new site has been located in Arlington, Virginia, and a proposed 10-year lease agreement has been approved by your Board of Directors at this meeting. The new facilities will likely be occupied in January of 1989. Floor space described in the lease will be 50% greater than present holdings. We will also have the option to expand into adjacent vacant space at a later date. We have been fortunate in obtaining lease terms which are very reasonable. The new site will be highly visible, accessible, accommodating, morale boosting, and a showcase of pride for the staff and membership alike.

However, we must not allow ourselves to become complacent about housing. We need to continually look well into the future and plan for our own building. To do this will possibly require the establishment of a foundation to raise funds for such a purpose. It is my hope that certain industrial leaders will come forth following proper contact and explanation to serve as the founders and directors of this foundation.

Restructured Board of Directors

Last year's change in our bylaws directed an enlargement of the Board of Directors to nine persons. Elections this year have dealt with that requirement. The enlargement also provides implied representation upon the Board for all members regardless of their employment or nationality. While this year's nominating committee listed two members from industry as nominees, is it too remote for us to consider the possible inclusion in the future of a nominee from outside North America? I would think this is entirely possible and reasonable given the fact that approximately 13% of our individual members reside outside North America.

In his presidential address of three years ago, Richard Ronk played upon the benefits to be derived from the separation of activities and responsibilities of the three AOAC boards, the Board of Directors, the Official Methods Board, and the Editorial Board. Prerogatives of each junior board are governed in bylaw provisions and more specifically within terms of reference.

For the most part I agree with Mr. Ronk. However, on occasion, conflicts can and do arise between the Board of Directors and the other boards, as well as between committees. Most conflicts occur over "turf" matters and presumed authority.

Inasmuch as the future success of AOAC is almost completely dependent upon method validation and publication it is crucial that the two junior boards be ever conscious of activities, sentiments, and actions of the overall governing board, the Board of Directors, and vice versa, as well. While an attempt over the years has been made to maintain liaison through staff and, more recently, by distribution of meeting minutes to the Board of Directors, I believe an additional step is required. I suggest a bylaw amendment providing for appointment from within the members of the Board of Directors as chairmen of the Official Methods Board and the Editorial Board. An alternative solution might be to allow the appointed chairman of each board to serve as ex officio to the Board of Directors without vote. I recognize the extensive work load this suggestion implies upon a volunteer chairman but I believe it is necessary.

Publications

The Long Range Plan noted the need for updating the AOAC Handbook. I am pleased to announce that this is under way and the first draft is complete.

One of the most exciting events this year has been the initiation of electronic on-line access to the *Journal of the AOAC* through STN International, the Scientific and Technical Network. Details for subscribing to and using this computerized access were discussed in the July issue of *The Referee.*

Perhaps you have not noted the new flyer on AOAC publications and the reference therein to the AOAC publication *Use of Statistics to Develop and Evaluate Analytical Methods.* This text received the American Statistical Association 1987 W. J. Youden Award in Interlaboratory Testing, not a small achievement! Certainly, it is a tribute to the quality of this text and to the author and editor.

It is proposed that the Editorial Board consider seriously and soon the division of the next edition of *Official Methods of Analysis,* possibly into three volumes. One volume might contain all non-food methods and the other two could be divided between methods related to Methods Committee Foods I and methods common to Methods Committee Foods II. It is important, I believe, to maintain the hardback version, as opposed to other formats, in order to maintain a quality publication and for archival purposes.

I ask, too, that the Editorial Board give special attention and consideration to the recommendations of the Journal Review and Evaluation Committee which worked so very hard in the past two years and verified the high regard held by the scientific world for this journal.

Beyond these items there is a real need to expand our publication area to provide more products and services. Publications provide our major source of revenue and as government grants and contracts diminish this source becomes more important than ever. I believe there is an untapped reservoir of proprietary methods for analyses used for process control which would be useful and marketable if produced in monograph form. A possible publication on laboratory safety has been discussed and will be considered in the future. Authors and editors should take note.

Workshops

We continue to do well with the quality assurance workshop. Another one on the subject of sampling is to be offered

in the fall of this year. New workshops need to be developed and staffed by other directors and volunteers.

Accreditation

A task force has been named to give some direction for possible involvement of AOAC in laboratory accreditation. The need is apparent throughout the world and is awaiting a champion. AOAC could be that champion if financial and legal obstacles can be overcome. I believe opportunities abound in the accreditation of regulatory, private, and industrial/trade laboratories, and resulting benefits can accrue to AOAC. We have the scientific and human resources to engage in a modified, perhaps cooperative, program to gain experience for a successful future operation.

Methods

This area is a continuing operation and one in which we excel. We must, however, recognize the need to reduce the time interval between introduction of a method for study and presentation to the Association for adoption. This is a matter of concern for all but only the Official Methods Board can resolve the matter and I encourage it to do so with all haste.

I wish to express my personal thanks to each member of the staff and Board of Directors for the strong support and cooperation provided me throughout this year. As they alone know, it has been a year of many changes and unforeseen events. I also wish to express my appreciation to my colleagues at Purdue University and to my superiors for allowing me the opportunity to be away from my assigned duties on so many occasions required by this position during the year.

It's been a great experience and I would not have traded it for anything.

Finally, I paraphrase a quotation attributed to Abraham Lincoln, one of America's great presidents, "I do the very best I can, the very best I know how. If the end brings me out right, all that is said against me will not matter. If the end brings me out wrong, ten thousand angels swearing on my behalf will make no difference."

CHEMICAL CONTAMINANTS MONITORING

Radionuclides in Domestic and Imported Foods in the United States, 1983–1986

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Findings in the Food and Drug Administration's Radionuclides in Foods program are summarized for samples collected between October 1, 1982, and September 30, 1986. All radionuclide findings for Total Diet and reactor samples were either in Action Range I or low in Range II of the surveillance and control recommendations given by the Federal Radiation Council. The only long-range trend noted was a continuation of the general decline in dietary intake of ^{90}Sr since 1961. Imported food samples were analyzed for contamination after the Chernobyl nuclear accident. The findings for imported foods indicate that the surveillance efforts successfully targeted contaminated foods, and that contamination levels were below levels of concern for all but one oregano and 3 cheese samples.

The U.S. Food and Drug Administration (FDA) has monitored radionuclides in the food supply for more than 25 years, beginning in 1961 (1–3). Although contamination of the environment through weapons testing is not as prevalent as when the monitoring began, the risk of radionuclide contamination continues, especially because of numerous other uses of radioactive materials. In addition, public concern for the health risks associated with radionuclide contaminants has become very strong, especially following the nuclear incidents that occurred in March, 1979, at the Three Mile Island nuclear power station and in April, 1986, at the Chernobyl nuclear power station.

FDA's Radionuclides in Foods program provides on-going monitoring of the food supply to establish baseline levels and show long-range trends. Maintaining the necessary expertise through this program continually enables FDA to react quickly to major nuclear incidents, such as the Chernobyl incident. The activities of the program are coordinated with the radionuclide monitoring efforts of the U.S. Environmental Protection Agency and U.S. Department of Agriculture (USDA). FDA's Center for Food Safety and Applied Nutrition coordinates the program's activities, using the Office of Regional Operations for sample collection and analysis and the Center for Devices and Radiological Health for health physics guidance.

Samples

During the period covered by this paper, 3 main types of samples were examined: Total Diet Study (TDS) foods, reactor survey foods, and imported foods. TDS samples represented the general U.S. food supply and were obtained through FDA's TDS program (4). One complete TDS market basket consisting of 234 individual foods has been analyzed each fiscal year since the structure of the TDS market basket was modified in 1982 (5). Although this paper deals primarily with data generated during fiscal years 1983 through 1986 (October 1982 through September 1986; noted as FY83–FY86), the findings from the restructured market basket analyzed during FY82 (October 1981 through September 1982) have not been presented elsewhere and are included in this evaluation. During these 5 fiscal years, more than 1100 TDS food samples were analyzed for ^{137}Cs, ^{3}H, ^{131}I, ^{106}Ru, and ^{90}Sr. All TDS findings reported here are for samples collected before the Chernobyl nuclear accident.

Food originating from the vicinities of nuclear reactors included raw vegetables, food crops (primarily fruits), fish, and milk. Approximately 500 samples were collected from the vicinities of a total of 11 nuclear reactors. Reactor sites were selected to include those from many geographical regions and were varied from year to year. All reactor survey samples were analyzed for ^{137}Cs, ^{131}I, and ^{106}Ru. Half of the samples were analyzed for ^{3}H and ^{90}Sr.

Imported foods have been monitored during selected years. They were not monitored during FY83–85; however, extensive sample collection and analysis efforts were begun in May 1986 to monitor for contamination resulting from the Chernobyl nuclear accident. Samples were collected at points of entry during routine import inspections. The collection of samples depended on country of origin and food type. This monitoring effort is continuing and is planned to extend at least through FY88.

Experimental

All radionuclide analyses were performed at FDA's Winchester Engineering and Analytical Center in Winchester, MA. The analytical methods used are described in the literature (6–10). A high efficiency sodium iodide (NaI) detector was used for gamma-ray counting in the determination of ^{137}Cs, ^{131}I, and ^{106}Ru in the TDS and reactor survey samples. When an interference was indicated, the results were verified using a lithium-drifted germanium (Ge(Li)) detector. All imported foods were analyzed using the Ge(Li) detector. Liquid scintillation was used to determine ^{3}H, and radiochemical separation followed by beta counting was used to determine ^{90}Sr.

By definition, the detection limit was set equal to 3 times the standard deviation of the analytical uncertainty. Because of large variations in food density and, in some cases, the quantities available for analysis, detection limits covered a wide range. However, detection limits for most foods were approximately 2 Bq/kg for ^{131}I, ^{137}Cs, and ^{106}Ru, 0.08 Bq/kg for ^{90}Sr, and 7.5 Bq/kg for ^{3}H.

Results

In the interpretation of the analytical results, FDA follows the surveillance and control program recommendations given by the U.S. Federal Radiation Council (FRC) (11) for intake of radionuclides. The surveillance and control recommendations were based on the derived intake ranges shown in Table 1. Contamination control actions are not recommended by the FRC until radioisotope concentrations approach the Range III level.

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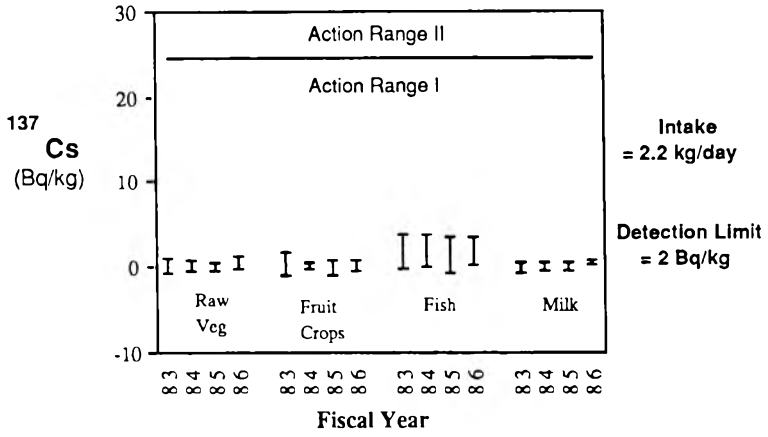

Figure 1. Cesium-137 in reactor survey samples.

Total Diet Study

As noted above, the TDS findings include data from 5 fiscal years, FY82–86, and all findings, including those for FY86, were for samples collected before the Chernobyl accident. ^{131}I and ^{106}Ru were not found in any of the TDS samples. ^{137}Cs was found in only one sample of honey. This finding was not considered unusual because ^{137}Cs has been found in other honey samples analyzed under the Radionuclides in Foods program. During FY82–86, 7 TDS and reactor survey samples of honey were analyzed; ^{137}Cs was found in 3 of them. These ^{137}Cs levels were all low in Action Range I, and the ^{90}Sr concentrations were within Action Range I or low in Range II. The intakes are discussed below in greater detail in the section on trends.

Reactor Survey

FDA's reactor survey findings for the FY83–86 period were comparable to those from earlier years. ^{131}I and ^{106}Ru levels were below detection limits in all samples. ^{137}Cs concentrations were below detection limits in all milk samples and in nearly all raw vegetable and food crop samples. The exceptions were one tomato sample and one honey sample in which the ^{137}Cs concentrations (2.4 and 4.7 Bq/kg, respectively) were low in Action Range I and not considered unusual. Fish was the only food for which there were frequent findings (approximately 38%) of ^{137}Cs. Figure 1 summarizes the ^{137}Cs findings for the reactor survey samples. The ^{137}Cs results are very low and all within Action Range I; no definitive control measures are recommended below the 240 Bq/kg (6600 pCi/kg) level. The frequency of detection and concentration range for ^{137}Cs in fish have not changed significantly since FDA began monitoring reactor sites.

Tritium in the reactor survey samples ranged from below detection limits to 89 Bq/kg with the higher levels found in fish (Figure 2). The elevated ^{3}H concentrations during FY83 and FY85 were high enough above detection limits to suggest that releases from the power plants were being observed. However, no additional surveillance was warranted because the observed concentrations would have to be 100–1000 times as high to indicate a need for specific action according to the FRC recommendations. The observed levels of ^{90}Sr were comparable to those found in the TDS samples. Nearly all were within Action Range I; the highest level was approximately 2 Bq/kg, which is low in Range II.

All reactor survey results are consistent with those reported in the annual radiological environmental monitoring program reports submitted to the Nuclear Regulatory Commission (NRC) by the individual power utility companies. These annual reports, required by the NRC, summarize the findings of very extensive monitoring programs and include data on various foods collected near the reactor sites.

Imported Foods

In May 1986, within 2 weeks after the Chernobyl accident, an FDA/USDA-Food Safety and Inspection Service (FSIS) Task Force, set up by the Director of FDA's Center for Food Safety and Applied Nutrition, recommended the radioactivity levels of concern shown in Table 2 (13). The radionuclides ^{134}Cs, ^{137}Cs, and ^{131}I were to be used as indicators of contamination with the levels of concern being derived from the Preventative Action Guides (14). Shipments with food containing radioactivity levels exceeding a level of concern were not to be allowed into the United States.

Approximately 500 import samples were collected and analyzed by the end of FY86; about 25% of them had radioactive contamination attributable to the Chernobyl accident. ^{131}I was useful as an indicator for only a few weeks after the accident. No appreciable ^{131}I activity was found in

Table 1. Radiation protection guides (RPGs) and derived intake action ranges for selected radionuclides, as recommended for the average of the general population

Radionuclide	Target	RPG dose, mSv/yr	Action ranges[a] for derived intakes, Bq/day		
			I	II	III
^{131}I	thyroid	5	0–0.37	0.37–3.7	3.7–37
^{137}Cs[b]	whole body	1.7	0–54	54–540	540–5400
^{90}Sr	bone	5	0–0.74	0.74–7.4	7.4–74
^{3}H[b]	whole body	1.7	0–7400	7400–7.4×10^4	7.4×10^4–7.4×10^5

[a] Range I required no specific action; Range II, surveillance and routine control of upward trends toward Range III; Range III, surveillance and controls to reduce exposure to Range II (11); 1 Bq is approximately 27 pCi.

[b] ^{137}Cs and ^{3}H were not considered by the Federal Radiation Council. The ranges were derived by using the radionuclide concentrations in water tabulated by the National Committee on Radiation Protection (12) for occupational exposure, $\times 1/30$ to apply to the average of the general population.

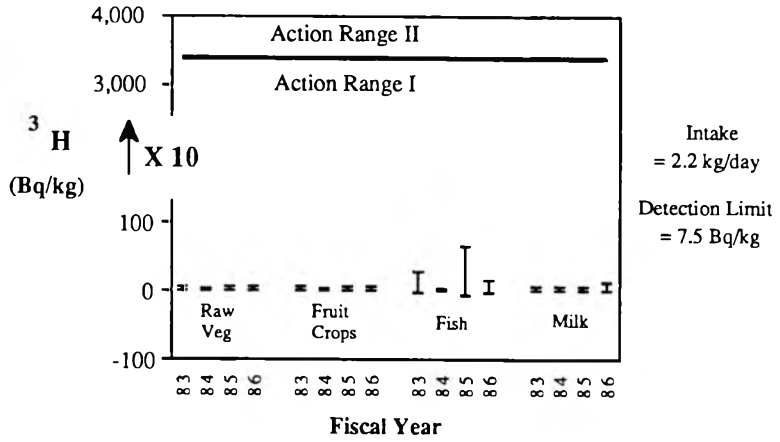

Figure 2. Tritium in reactor survey samples.

any sample collected after June 1986. Before that time, however, contamination exceeding the level of concern was found in 2 Italian cheese samples. This caused the shipments from which these samples were taken to be denied entry into the United States. The cesium radioactivity levels were usually less than 30% of the levels of concern. Contamination exceeding the level of concern for cesium was found only in one Austrian cheese sample and one Turkish oregano sample; the shipments from which these samples were taken were also denied entry into the United States. The cesium isotopes continue to receive the most attention because they were predicted to contribute the most significant radioactivity dose. The analytical findings support this prediction, and cesium isotopes continue to be used as contamination indicators. Although cesium isotopes were present at low levels that did not constitute a significant dose, FDA has observed many other radionuclides that are likely to have resulted from the Chernobyl accident. To date, a total of 33 radionuclides have been identified and are shown in Table 3.

Discussion

The radionuclide concentrations found in the FY83–86 reactor survey and TDS samples were comparable to those of previous years. No long-term trends have been noted for ^{131}I, ^{137}Cs, ^{106}Ru, or ^{3}H in either TDS or reactor survey samples. The only significant trend noted was for ^{90}Sr in the TDS samples as shown in Figure 3. The plot suggests that the ^{90}Sr intake continued the general decrease that has occurred since 1965. Also evident in Figure 3 is that modification of the TDS market basket affected the predicted ^{90}Sr intake. The current program accommodates several age/sex groups, and the ^{90}Sr intake calculations were based on the 14- to 16-year-old male group. This is the age/sex group closest to the 16- to 19-year-old male group that was used for the old program. During FY82 both types of market baskets were collected and analyzed. Although small sample-to-sample differences were expected, the plot suggests that the change in market basket structure resulted in a measurable difference in the predicted ^{90}Sr intake. In spite of the change in market basket format, the results nevertheless suggest that the ^{90}Sr intake is approaching a level intake of approximately 0.1 Bq/day.

Table 2. Radioactivity levels of concern (Bq/kg) recommended by the FDA/FSIS task force[a]

Radionuclide	Infant foods[b]	Other foods[b]
^{131}I	56 (1500)	300 (8000)
^{134}Cs + ^{137}Cs	370 (10 000)	370 (10 000)

[a] Ref. 13.
[b] Values in pCi/kg in parentheses.

Conclusions

Radionuclide analysis of the TDS samples did not show any significant change in the radionuclide content of the general domestic food supply during recent years; however, the findings reported here include no results from TDS samples collected since the Chernobyl nuclear accident. ^{131}I and ^{106}Ru were below detection limits in all TDS samples, and ^{137}Cs was below detection in all but one sample, in which it was very low. The gradual decrease in ^{90}Sr intake that has occurred since the mid-1960s appears to be leveling off at approximately 0.1 Bq/day.

Results obtained from the reactor survey samples were comparable to those from earlier years. ^{131}I and ^{106}Ru were below detection limits in all samples. ^{137}Cs was below detection limits in all milk samples and in nearly all raw vegetable and food crop samples. ^{137}Cs was frequently found only in fish (in 38% of the samples). The observed concentrations were low and considered to be at baseline levels. ^{90}Sr levels were comparable to those found in the TDS samples. The levels were in Action Range I or low in Action Range II of the surveillance and control recommendations given by the FRC. Tritium was near or below detection limits for all reactor survey samples. The highest concentrations, which

Table 3. Radioisotopes identified in imported foods collected after the Chernobyl nuclear accident

^{110m}Ag	^{99}Mo
^{241}Am	^{95}Nb
^{140}Ba	^{239}Np
^{14}C	^{238}Pu
^{115}Cd	^{239}Pu
^{141}Ce	^{240}Pu
^{144}Ce	^{103}Ru
^{242}Cm	^{106}Ru
^{60}Co	^{125}Sb
^{134}Cs	^{127}Sb
^{136}Cs	^{89}Sr
^{137}Cs	^{90}Sr
^{3}H	^{129m}Te
^{131}I	^{131m}Te
^{132}I	^{132}Te
^{133}I	^{95}Zr
^{140}La	

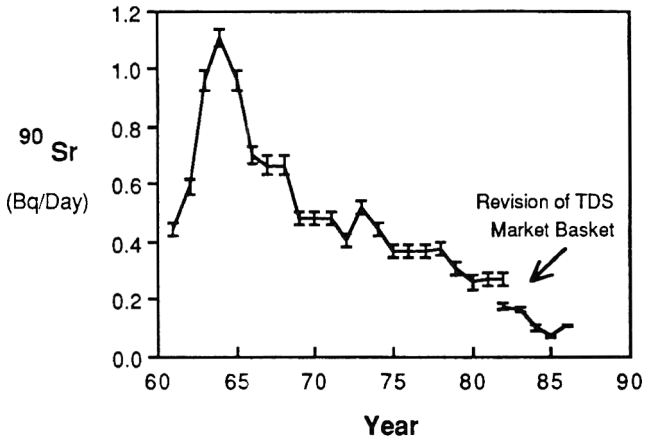

Figure 3. Strontium-90 in Total Diet Study samples, 1961–1986.

were in fish, were low in Range I and would have to be 100–1000 times as high to indicate a need for any specific control action. All reactor survey results are consistent with those obtained by the electric power utilities, who conduct extensive radiological environmental monitoring programs.

The imported food results showed the presence of contamination that probably originated from the Chernobyl accident. However, for the vast majority of samples the contamination was below FDA's level of concern, with only one oregano and 3 cheese shipments needing to be detained. Although the incidence and levels of contamination in imported foods are expected to decline, systematic surveillance for radionuclide contaminants from Chernobyl will continue, along with the other food-monitoring efforts.

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Investigations on *N*-Nitrosopyrrolidine in Dry-Cured Bacon

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Dry-cured or "country-style" bacon is a low volume specialty product typically made by small producers whose production practices vary widely. These practices include the direct application of dry-cure formulations containing varying concentrations of salt, sugar, flavoring agents, sodium nitrite, and sometimes sodium nitrate, and the use of lengthy curing and processing times. Because of the possibility of generating higher levels of *N*-nitrosopyrrolidine (NPYR) after frying in this product type compared with pump-cured bacon, an investigation was carried out on dry-cured bacon obtained from cooperating state or federally inspected establishments. Three different samples from each of the 16 plants were analyzed. Only one sample from each of 2 different producers exceeded the Food Safety and Inspection Service (FSIS) action level of 17 ppb NPYR, indicating that the majority of samples tested were in compliance. A significant correlation ($P < 0.01$) was found between residual $NaNO_2$ prior to frying and NPYR after frying. The elimination of added nitrate in the dry-cure formulations is recommended.

An estimated 15 million pounds of dry-cured or "country-style" bacon is manufactured annually in the United States (J. D. Kemp, University of Kentucky, personal communication, 1987). This specialty product, which is usually associated with the southeastern part of the United States, is typically made by small producers whose processing practices vary widely. These practices include the use of cure formulations that contain varying concentrations of NaCl, sugar, and $NaNO_2$ sometimes in combination with $NaNO_3$, as well as different flavoring agents. These components are applied directly to the pork bellies in a dry form; then the bellies are held to permit diffusion of the salts throughout the product prior to smokehouse treatment. The heterogeneous nature of the cure application may cause localized areas of the product to have a high nitrite content. The more lengthy curing time for dry-cured bacon compared with that for brine-pumped bacon may produce more amine precursor through microbial-enzymatic action, thereby creating the potential for greater formation of the carcinogen *N*-nitrosopyrrolidine (NPYR).

Pensabene et al. found from 39 to 89 ppb NPYR in fried dry-cured bacon in the 7 commercial samples that they tested (1). A higher incidence and concentration of NPYR (trace to 320 ppb) was found in dry-cured bacon from 15 different producers compared with levels in other dry-cured products that included ham, picnics, shoulders, and dried beef (2). In 1981, A Food Safety and Inspection Service (FSIS) report indicated a 15% incidence of fried dry-cured bacon that exceeded the 17 ppb action level ("Dry-Cured Bacon Survey Report," U.S. Dept of Agriculture, FSIS internal report, unpublished, 1981). Although this was lower than the incidence found in earlier, more limited studies ("Study to Survey Nitrosamine Levels in Dry-Cured Bacon, Hams and Shoulders," U.S. Dept of Agriculture, FSIS internal report, unpublished, 1980), dry-cured bacon was still considered a potential problem. All of these findings identified dry-cured bacon as the one product type that should warrant further investigation. For this reason, we carried out a survey of dry-cured bacon obtained from a number of cooperating state and federally inspected establishments where the ingredient composition, conditions, and other processing details were available. The objective was to use the results to identify those processing parameters and practices that contributed to high NPYR formation and to make recommendations to processors that would enable them to reduce the nitrosamine levels should it become necessary.

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The mention of firm names or trade products does not imply endorsement or recommendation by the U.S. Dept of Agriculture over other firms or similar products not mentioned.

Experimental

Caution: *N*-Nitrosamines are potential carcinogens. Exercise care in handling these compounds.

Reagents and Apparatus

(a) *Dry-cured bacon.*—Dry-cured bacon from 3 different bellies was obtained from 16 processors (48 samples) immediately after production, and samples were shipped to ERRC within 1 day.

(b) *N-Nitrosoazetidine (NAZET) internal standard.*—0.10 μg NAZET/mL dichloromethane (DCM).

(c) *N-Nitrosohexamethyleneimine (NHMI) internal standard.*—0.10 μg NHMI/mL DCM.

(d) *N-Nitrosopyrrolidine (NPYR) standard solution.*—0.10 μg NPYR and 0.10 μg NAZET/mL DCM or 0.10 μg NPYR and 0.10 μg NHMI/mL DCM. NPYR, NAZET, and NHMI were synthesized from their corresponding amines and sodium nitrite following the general method described previously (3).

(e) *Gas chromatograph–thermal energy analyzer (GC-TEA).*—Operating conditions: 2.7 m × 3.2 mm stainless steel column packed with 15% Carbowax 20 M-TPA on 60–80 mesh Gas-Chrom P; He carrier gas 35 mL/min; injector 200°C; TEA furnace 450°C; TEA vacuum 1.5 mm; liquid nitrogen cold trap; column 180°C isothermal for fried bacon extracts, 190°C isothermal for bacon-dripping extracts.

(f) *Other reagents and apparatus.*—As previously described (4–10).

Procedures

(a) *Bacon frying.*—Rind-free bacon (⅛ in./slice) was fried in preheated Farberware electric frying pan for 4.5 min (2.25 min/side) at calibrated temperature of 171°C (340°F). Both the fried edible portion and rendered drippings were retained for nitrosamine analysis.

(b) *NPYR in fried bacon.*—Complete details of procedure for analysis of NPYR in fried bacon have been described previously in secs **24.054-24.058** (4). Analyze all samples in duplicate. Briefly, prepare glass column containing acidified Celite. Add to this column ground mixture of fried bacon, anhydrous sodium sulfate, and Celite. Rinse column mixture with pentane–DCM, then elute nitrosamines from column with DCM. Concentrate sample to 1.0 mL and quantitate on GC-TEA system.

(c) *NPYR in bacon drippings.*—Complete details of pro-

Table 1. Nitrite, nitrate, and *N*-nitrosopyrrolidine in dry-cured bacon

Producer	Days in cure	Raw bacon				NPYR, ppb, in cooked bacon			
		$NaNO_2$, ppm		$NaNO_3$, ppm		Fried		Drippings	
		Range	Av.	Range	Av.	Range	Av.	Range	Av.
1	10	22.3–33.6	27.9	42.8–88.2	54.7	3.5–5.1	4.2	18.1–22.8	20.4
2	10	4.2–26.0	12.2	5.9–72.1	27.4	2.6–4.4	3.4	10.9–16.6	13.5
3	3	27.8–49.3	38.8	51.2–77.8	66.5	6.6–14.9	10.5	32.7–43.4	37.2
4	13	10.4–30.7	20.2	34.2–68.5	46.5	4.4–8.3	6.8	15.4–28.2	22.4
5	4.5	20.2–39.6	29.5	109.8–200.1	153.6	5.8–8.5	7.0	13.9–21.1	16.7
6	7	102.3–288.7	181.5	55.6–137.2	89.0	7.0–13.1	10.4	36.0–54.5	42.1
7	5	41.0–53.6	45.9	41.4–66.1	53.3	4.9–5.8	5.3	20.0–23.7	21.3
8	4	21.6–37.2	28.5	0.0–100.5	28.7	5.3–9.3	7.8	6.5–9.7	7.6
9	6	12.2–19.8	15.4	54.8–104.9	83.3	4.5–6.2	5.5	13.5–20.5	16.8
10	7	15.0–29.7	20.9	0.4–31.7	14.5	6.7–18.0	11.3	21.0–33.7	26.4
11	7	22.0–36.2	26.6	30.6–55.1	39.4	4.4–6.8	5.4	18.3–35.5	28.3
12	4	13.6–39.3	28.0	7.6–65.8	31.8	5.5–13.4	8.6	19.4–36.7	26.0
13	12	44.9–50.8	47.9	42.0–87.2	60.1	12.6–22.3	15.9	17.4–26.3	22.5
14[a,b]	56	2.2–5.8	3.4	370.2–766.8	525.9	4.8–10.1	6.6	17.0–28.9	22.1
15	33	11.3–73.8	33.1	36.8–144.7	74.7	9.9–11.9	10.7	24.3–40.2	32.9
16	14	9.4–42.0	21.6	5.2–44.6	28.9	5.3–8.1	6.4	18.0–26.2	22.8

[a] Pork side meat.
[b] Also contained *N*-nitrosopiperidine, 3.3–6.1 ppb.

cedure for analysis of NPYR in bacon drippings have been described previously (5). Briefly, steam-distill drippings from 5N sodium hydroxide. Extract aqueous distillate with DCM, then wash DCM extract with acid and base to remove interfering compounds. Concentrate sample to 1.0 mL and quantitate on GC-TEA system.

(d) *Sodium nitrite–nitrate.*—Determine values on 10.0 g uncooked sample-sodium nitrite by modified Griess-Saltzman procedure (6); sodium nitrate was determined by cadmium reduction method (7).

(e) *Fat, moisture, protein.*—The following AOAC methods (4) were used: fat by Soxhlet extraction, sec. **24.005**; moisture by oven drying method, sec. **24.003**; protein by Kjeldahl procedure, secs **24.038-24.040**.

(f) *pH.*—pH was determined on 10.0 g uncooked sample as in secs **28.025-28.026**.

(g) *Peroxide value.*—Peroxide value (PV) was determined on 20.0 g uncooked sample by using modification of procedure for determining PV in fats and oils, secs **28.025-28.026** (8). Briefly, homogenize sample for 2 min with 10 g anhydrous sodium sulfate and exactly 100 mL $CHCl_3$ in a 250 mL Virtis flask. Filter homogenate through Whatman No. 2 paper containing 10 g anhydrous Na_2SO_4. Pipet 25 mL aliquot into 250 mL flask, add 30 mL acetic acid and 2 mL KI solution, and place flask in dark for exactly 2 min. After 2 min, add 50 mL water and 2 mL starch solution, and titrate to clear end point with sodium thiosulfate.

(h) *Sodium chloride.*—Salt value was determined on 10.0 g uncooked sample following modification of Mohr method for chloride (9). Briefly, homogenize sample for 5 min with 50 mL hot water in 250 mL Virtis flask. Using 50 mL hot water, transfer sample to 250 mL beaker and heat on steam bath for 30 min. Filter hot sample through glass wool using another 50 mL hot water into 250 mL Erlenmeyer flask. Add potassium chromate and titrate.

(i) *Water activity.*—Water activity was determined using Rotronic Hygroskop DT instrument.

Statistical Analysis

The General Linear Models (GLM) procedure of Statistical Analysis System PC software distributed by SAS Institute, Inc., was used to interpret results according to methods of Snedecor and Cochran (10).

Results and Discussion

Because dry-cured bacon has a lower water activity, a_w, than pump-cured bacon, there is less water to evaporate during frying. The temperature-dependent reaction would be more rapidly attained in the dry-cured product, which could result in higher NPYR values under the same cooking conditions. The details of the experiments leading to the reference standard of cooking for dry-cured bacon, taking into account such factors as degrees of doneness, slice thickness, frying time and temperature, yields, proximate analysis, and water activity, are described in the FSIS internal report, "Dry-Cured Bacon Survey," 1981. Thus, the FSIS frying protocol for this product of 340°F for 4.5 min (2.25 min/side) employing ⅛ in. slices was used for the present study instead of 370°F for 6 min normally used for pump-cured bacon. To help evaluate reproducibility of the NPYR values and to determine if they were representative, the analyses were performed in duplicate on 3 different dry-cured bacon samples from each establishment. The solid phase extraction method used for the determination of NPYR was compared previously against 2 other methods for this type of product and was shown not to produce artifactual nitrosamines (11).

The range and mean of residual $NaNO_2$ and of $NaNO_3$ in the uncooked and NPYR in the fried bacon and its cooked-out drippings from each establishment are shown in Table 1. The overall range for NPYR in fried bacon was 2.6–22.3 ppb and the mean was 7.9 ppb. Analysis of the data showed a highly significant ($P < 0.01$) correlation between residual $NaNO_2$ in uncooked bacon and NPYR in the edible fried portion and cooked out drippings. This finding is in agreement with that first found by Pensabene et al. with respect to fried pump-cured bacon (12). Only single samples from 2 different producers (10, 13) exceeded the FSIS action level of 17 ppb NPYR. One producer's bacon (6) contained 102.3–288.7 ppm residual $NaNO_2$ compared with an overall average of 36.1 ppm and had a higher NaCl content than the other samples tested. Only 7.0–13.1 ppb NPYR was found in the fried bacon from this producer despite the indication that the incorrect amount of cure/amount meat was used.

The overall NPYR results from the corresponding drippings ranged from 6.5 to 54.5 ppb, with a mean of 23.6 ppb. This is consistent with results reported by Sen (13) who found the concentration of volatile nitrosamines in cooked-out ba-

con fat to be more than twice that present in cooked bacon. This is largely because the NPYR precursor(s) are located almost exclusively in the adipose, not lean, tissue (14, 15). The total NPYR yield from adipose tissue, including NPYR released (volatilized) during cooking, is at least 12 times in excess of that derived from lean tissue (16). There is also some indication that the nitrosating species is not N_2O_3 generated directly from nitrite, but generated indirectly through a lipid-NO_x reaction product (17). Analysis of variance (ANOVA) of the data showed that the repeatability for NPYR was 0.48 ppb in the edible fried bacon and 1.12 ppb NPYR in the drippings.

Interestingly, bacon from company 12 which had the typical oxidized fat-like off odor associated with dry-cured meats averaged only 8.6 ppb NPYR. Samples from company 14 were actually a pepper-coated, dry-cured product-pork side meat. The fried product and drippings contained an average of 6.6 and 22.1 ppb NPYR, respectively. All 3 samples also contained 3.3–6.1 ppb *N*-nitrosopiperidine (NPIP) in the drippings despite the low residual nitrite (2.2–5.8 ppm) available at the time of frying. NPIP has been detected in cured products processed with a cure premix containing both nitrite and black pepper that had reacted to form the nitrosamine prior to its addition to the meat (18). Because the pork side meat was a single sample, we purchased 3 samples of the same product of one company to obtain additional data. They contained 15.7, 8.8, and 20.9 ppm residual $NaNO_2$ and gave 29.1, 1.5, and 21.5 ppb NPYR in the edible portion, and 47.0–49.6 ppb in the drippings with 2.4 ppb NPIP in the latter sample. The second sample yielded insufficient drippings for analysis. Given the fact that 2 of the 3 samples had the highest NPYR values encountered in any of the dry-cured bacon and that this product type is used exclusively for cooking and flavoring purposes, additional investigation is warranted.

Peroxide value (PV) was determined in at least one of the 3 samples from each producer to assess the degree of oxidized fat present. The values ranged from 0.50 to 1.90 except for pork side meat, where values for those 3 samples ranged from 5.65 to 6.25. No significant correlations were found between PV values and NPYR in either the fried bacon or its drippings.

No significant correlations were found between NPYR (edible portion and drippings) and days in cure of the product. Excluding the pork side meat from company 14, whose curing time was 56 days, the conventional dry-cured bacon curing time varied widely from 3 to 33 days with a mean of 9 days. It is interesting that 6 of the processors used a curing time of less than 7 days. The curing time previously used for making traditional dry-cured bacon was 2 days per pound of belly, which typically weigh 15–20 lb (19). It appears that most of the current producers of dry-cured bacon have successfully employed shorter curing times. This may, in part, help account for the recent relatively low NPYR values because there is less opportunity for the bacterial/enzymatic degradation of meat components to form the nitrosamine precursor(s). It would also help explain the wide range among the water activities (a_w), from 0.90 to 0.99 with a mean value of 0.94. The a_w results show a wide variation between processors and between samples from the same producer. As expected, there was a significant correlation ($P < 0.05$) between a_w and NaCl content. The range of 1.05–5.08% NaCl and mean of 2.34% suggests that processors are using less salt than previously used for this product type.

One producer (No. 8) with the lowest NPYR in the drippings (average 7.6 ppb), but not in the fried bacon (average 7.8 ppb) used the reductant sodium ascorbate, which has been shown to be effective in reducing NPYR in pump-cured bacon. The dry-cure premix also contained sodium carbonate as a buffering agent to prevent the rapid destruction of nitrite prior to use. Sodium carbonate would tend to raise the pH of the product slightly (pH 6.6) so that presumably less nitrosating species N_2O_3 from nitrous acid was available at the time of frying. Company 10 who also used a commercial premix containing nitrite and sodium carbonate, but no ascorbate, had samples (pH 6.3) with much higher levels of NPYR in fried bacon and its drippings than those from company 8. When the values for pork side meat are eliminated, pH values for the rest of the dry-cured bacon ranged from 5.4 to 6.7 with a mean of 6.0. Because of the buffering ability of the meat itself, the use of carbonate buffer, in the amounts used in the premix (1%), had little effect on the overall pH levels of the product. The effect of ascorbate on NPYR formation in company 8 samples was not apparent since none of the other producers used this reductant or its isomer erythorbate. Many of these producers had bacon that contained lower NPYR. Ascorbate has not been used extensively in dry-cured bacon production. Perhaps this is due to its limited solubility in adipose tissue. Nevertheless, ascorbate/erythorbate should be effective in reducing residual nitrite and, thus, NPYR, if it could be preserved in the premix.

The use of $NaNO_3$ introduces yet another factor insofar as nitrosamine formation is concerned. A number of factors affect the microbial/enzymatic conversion of nitrate to nitrite. These include the composition of bacterial flora and processing and post-processing time/temperature. Considerably within- and between-plant variation would make control of this conversion extremely difficult. In this study, only 3 (companies 5, 9, and 14) of the 16 processors claimed to use $NaNO_3$ in combination with $NaNO_2$ in the cure premix. Taken without the values from company 14, who produced pork side meat, the residual $NaNO_3$ content was generally low, ranging from none detected to 200 ppm with a mean of 57 ppm. Statistical analyses showed no significant correlation between $NaNO_3$ and NPYR levels in either fried dry-cured bacon or its drippings. From 4.8 to 7.8 ppb NPYR was detected in the 2 bacon processors who used $NaNO_3$. This finding, based on a limited number of samples, contrasts with an earlier study in which up to 280 ppb NPYR was found in fried bacon in 12 of 15 samples from 7 of 15 producers who added $NaNO_3$ in the cure (2). This was one of the studies that helped identify dry-cured bacon as the one product type requiring further investigation. The fried bacon from the few producers who employed $NaNO_3$ in this study did not contain higher NPYR than the majority who used $NaNO_2$ alone. Generally short curing times (4–6 days) would help explain the lack of significant nitrate-to-nitrite conversion.

Despite claims by some processors that nitrate is essential to produce "good, high quality" dry-cured bacon and ham, a majority no longer use nitrate. There is overwhelming evidence that nitrite is the source of the nitrosating agent. Nitrite concentrations, higher than those needed for color and flavor development, are needed for *Clostridium botulinum* inhibition. Nitrate is generally considered nonessential in these respects, serving only as an unreliable source of nitrite (20, 21). Recognition of these factors led the Expert Panel on Nitrates, Nitrites and Nitrosamines to recommend that the use of nitrate salts be discontinued in curing all meat and poultry products, except for fermented sausage and dry-cured products (22). In 1978, nitrate was specifically prohibited from use in pump-cured bacon (23).

This study presents evidence for the principal association

between residual $NaNO_2$ prior to and NPYR after frying. Therefore, it is essential to control ingoing and thus residual nitrite. Although somewhat inconsistent with the current finding that no correlation exists between nitrate and NPYR levels, we nevertheless recommend the elimination of nitrate to avoid the *potential* for an additional source of nitrite for nitrosamine formation. There appears to be no need for using nitrate in a dry-cured formulation for making bacon, except if there were compelling quality or safety considerations that are unique to specific producers.

In conclusion, it is recognized that dry-cured bacon is a unique product. Clearly, modern curing practices within the industry have undergone substantial changes; these changes allow better control of the amount of ingoing nitrite through the use of commercial premixes and the elimination of added nitrate.

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Survey of Aflatoxins, Ochratoxin A, Zearalenone, and Sterigmatocystin in Some Brazilian Foods by Using Multi-toxin Thin-Layer Chromatographic Method

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A previously published method for ochratoxin A was evaluated and proved appropriate for simultaneous determination of aflatoxins, ochratoxin A, sterigmatocystin, and zearalenone, with considerable savings in time and reagent costs. The detection limits were 2, 5, 15, and 55 μg/kg, respectively. The recoveries and coefficients of variation obtained with artificially contaminated samples were 91–101% and 0–16% for aflatoxin B_1, 98–117% and 0–17% for sterigmatocystin, and 96–107% and 0–17% for zearalenone, respectively. The coefficients of variation for naturally contaminated samples (aflatoxins in rice and ochratoxin A in beans) ranged from 0 to 8%. The method was used to survey 296 samples that included 10 cultivars of dried beans, 8 types of corn products, 3 types of cassava flour, and both polished and parboiled rice between May 1985 and June 1986 in Campinas, Brazil. Only aflatoxin B_1 (9 samples, 20–52 μg/kg), aflatoxin G_1 (4 samples, 18–31 μg/kg), and ochratoxin A (5 samples, 32–160 μg/kg) were found. The average contamination percentage was 4.7%; beans showed the highest (6.6%) and rice showed the lowest (3.3%) incidence rates. Zearalenone and sterigmatocystin were not detected. Positive samples were confirmed by chemical derivatization, corroborated by development in 3 solvent systems.

Little information concerning mycotoxin contamination in foods consumed in Brazil has been gathered to date. A few surveys, mostly on the incidence of aflatoxins, have been conducted, and with one exception all took place in the state of São Paulo. Peanuts and peanut products have been found to be the most susceptible commodity (1–4). A total of 279 samples of other food products such as wheat, soy and cassava flour, and corn and corn products were examined (4–8). Sixty-six of these samples were also checked for patulin and sterigmatocystin. Only 2 samples were found contaminated (corn flour, 14 and 18 μg/kg aflatoxin B_1) (6).

The present survey was aimed at foods, raw and processed, as sold to consumers. It is hoped that when a sufficient number of surveys have been accomplished, either at country or state level, the possible existence and extent of a mycotoxin problem can be verified. Efforts and resources may then be concentrated toward solving the problem of the implicated commodities, and subsequent studies would include surveys at the various stages of production. This approach is contrary to the Food and Agriculture (FAO) guidelines for mycotoxin surveillance (9), which recommend that surveys be directed first at products in the earliest stages of production. If examination of the basic raw products reveals a marked contamination problem, then FAO suggests that further surveys of derived products be undertaken. In developing countries,

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considering the infrastructure required to conduct surveys at harvest and storage times, the FAO strategy is costly and difficult to implement.

The present survey included 296 samples, including 172 different branded and 53 unbranded products. The types of foods chosen represent an important portion of the Brazilian daily diet. In the corn group, 8 different types of products were analyzed (130 samples). For cassava products (45 samples), only flour was chosen; none of the cassava starch products was chosen due to a lesser likelihood of contamination in pure starch material. Raw dried beans, encompassing 10 different cultivars (61 samples), and rice, polished (52 samples) and parboiled (8 samples), were examined. Peanuts were not included in this study because even though peanuts have an already identified problem, they do not represent a major food item. Samples included foods produced in São Paulo and other states, as well as foods imported from other countries. It was not possible to identify the imported items, but normally they constitute a very minor portion of the products offered to the consumer.

Expenses incurred in evaluating food safety in developing countries must be kept low. A low cost multi-mycotoxin method that could be used to survey many consumer products is desirable. Thus, a previously published method for ochratoxin A (10) was evaluated for the simultaneous determination of aflatoxin B_1, B_2, G_1, and G_2, zearalenone, sterigmatocystin, and ochratoxin A.

Experimental

Apparatus and Reagents

(a) *Blender.*—Waring, with 1 qt jar. Explosion-proof not necessary.

(b) *TLC apparatus.*—Stahl-type (Desaga GmbH, Heidelberg, GFR).

(c) *TLC plates.*—20 × 20 cm glass, coated with 0.25 mm silica gel G 60 (E. Merck, Darmstadt, GFR). With scriber, divide plates into twenty 1 cm strips. Activate plates before use in 110°C oven for 1 h.

(d) *Ultraviolet light.*—Longwave tube, 366 nm (Sylvania F8T5 BLB/USA) in suitable stand.

(e) *Hyflo Super-Cel.*—Or equivalent product.

(f) *Extraction solvents.*—*(1)* Methanol (E. Merck). *(2)* Potassium chloride solution, 4%, 40.00 g KCl/L. *(3)* Chloroform (E. Merck).

(g) *Clarifying agents.*—*(1)* Cupric sulfate solution, 10%, 100.00 g $CuSO_4$/L. *(2)* Ammonium sulfate solution, 30%, 300.00 g $(NH_4)_2SO_4$/L (E. Merck).

(h) *Ochratoxin A standard solutions.*—10 and 1 μg/mL benzene (Makor Chemicals Ltd, Israel). Prepare from stock solution. Determine exact concentration as described in *Official Methods of Analysis* (11).

(i) *Short wavelength tube and filter.*—34-00004-01 and 38-0035-01 s/w in UVSL-11 mounting (Ultra-Violet Products Ltd, Div. of UVP Inc., Cambridge, UK), or equivalent, for zearalenone confirmation.

(j) *TLC developing solvents.*—Toluene-ethyl acetate-formic acid (60 + 40 + 0.5); acetone-chloroform (1 + 9); toluene-ethyl acetate-formic acid (5 + 4 + 1); ethyl ether; toluene-ethyl acetate-chloroform-formic acid (35 + 25 + 25 + 10); hexane-ethyl acetate-acetic acid (18 + 2 + 1); benzene-methanol-acetic acid (90 + 5 + 5).

(k) *Mycotoxin standard solutions.*—All in benzene. Aflatoxins B_1, B_2, G_1, and G_2, 2.5 μg/mL each; zearalenone, 70 μg/mL; sterigmatocystin, 10 μg/mL (all from Makor Chemicals Ltd, Israel). Prepare from stock solutions. Determine exact concentration as described in *Official Methods of Analysis,* secs **26.004, 26.005, 26.009, 26.113, 26.140** (11).

(l) *Aluminum chloride solution.*—Dissolve 20 g $AlCl_3 \cdot 6 H_2O$ in 100 mL 75% ethanol.

Sample Collection and Preparation

Samples were purchased at random from retail stores, including large supermarkets and small neighborhood grocery shops, in Campinas, Brazil, from May 1985 to June 1986. For whole grains, such as dried beans, rice, and corn, 5 kg samples were collected. For processed foods, 1 kg samples were used.

Grain samples were ground to 20 mesh in a Wiley-type mill. For each sample of canned sweet corn, 5 cans of the same lot (200 g drained weight each) were opened and drained; the corn was ground in an electric meat grinder. All ground samples as well as processed foods such as corn meal, cassava flour, corn flour, and corn grits were thoroughly mixed with an aluminum spatula before analytical samples were taken.

Extraction and Cleanup

The method used was based on a previously published procedure for ochratoxin A (10). It involves extraction with methanol–4% KCl (9 + 1) and cleanup with a clarifying agent and Hyflo Super-Cel followed by 2 partitions to chloroform. For the present multi-toxin procedure, larger aliquots of chloroform extracts were collected and combined for screening and quantitation procedures. Also, cupric sulfate and ammonium sulfate solutions were used as clarifying agents for beans and rice samples and corn and cassava products, respectively (10).

Screening and Quantitation by Thin-Layer Chromatography

In 15 mL vial, combine 5 mL each of first and second chloroform extractions. Evaporate just to dryness in 80°C water bath. Dissolve residue in 200 μL benzene, and place closed vial in ultrasonic bath 30 s to ensure complete dissolution.

For screening, twice spot 5 μL sample extracts on TLC plate 2 cm from bottom. Spot standards separately and on top of each second sample spot. Place plate in unsaturated tank with toluene-ethyl acetate-formic acid (60 + 40 + 0.5) as developing solvent. Remove plate after 10 cm development, and let dry. Inspect plate under longwave ultraviolet light for aflatoxins and ochratoxin A. Spray plate with $AlCl_3$ solution. Heat 5 min at 110°C, and observe under longwave ultraviolet light for possible presence of zearalenone and sterigmatocystin. Samples with spots that match both color and R_f values of standard spots are considered presumptive positive samples and can be submitted for quantitation and confirmation procedures.

For quantitation, spot 3.5, 5.0, and 6.5 μL sample extracts and standards on TLC plate 2 cm from bottom. Develop plate with solvent most appropriate for the suspected mycotoxin: acetone-chloroform (1 + 9) for aflatoxins, toluene-ethyl acetate-formic acid (5 + 4 + 1) for ochratoxin A, and toluene-ethyl acetate-formic acid (60 + 40 + 0.5) for sterigmatocystin and zearalenone. For the latter, spray plate with aluminum chloride solution (10), and heat as described above. Compare spot intensities. Perform calculations according to **26.031** (11).

Confirmation

Prior to chemical derivatization, spot sample extracts and standards on 2 plates as described above. Develop each plate

Table 1. Recovery and repeatability data for mycotoxins in artificially contaminated samples[a]

Product	Aflatoxin B_1			Sterigmatocystin			Zearalenone		
	Amount added, μg/kg	Av. rec., %	CV, %	Amount added, μg/kg	Av. rec., %	CV, %	Amount added, μg/kg	Av. rec., %	CV, %
Yellow corn meal	4.7	100	0	35	—[b]	—[b]	165	100	0
	29.5	91	11	65	—[b]	—[b]	1031	107	0
	76.7	95	8	218	107	17	2680	96	0
Cassava flour	4.7	100	0	35	100	0	165	—[b]	—[b]
	29.5	96	0	65	107	0	1031	107	0
	76.7	99	0	218	128	0	2680	96	0
Polished rice	4.7	100	0	35	100	0	165	100	0
	29.5	96	0	65	107	16	1031	107	0
	76.7	98	0	218	117	0	2680	96	0
Dried black beans	4.7	—[b]	—[b]	35	100	16	165	100	0
	29.5	91	1	65	98	17	1031	107	0
	76.7	90	16	218	107	0	2680	107	17
Raw peanuts	4.7	100	0	35	100	0	165	—[b]	—[b]
	29.5	101	9	65	107	0	1031	107	0
	76.7	94	8	218	112	14	2680	96	0

[a] Based on 3 determinations for each level.
[b] Inappropriate for quantitation.

in a different solvent system as corroborative evidence and as means of eliminating false presumptive samples. The following solvent systems can be employed: toluene-ethyl acetate-formic acid (5 + 4 + 1) and hexane-ethyl acetate-acetic acid (18 + 3 + 1) for ochratoxin A, toluene-ethyl acetate-chloroform-formic acid (35 + 25 + 25 + 10) and benzene-methanol-acetic acid (90 + 5 + 5) for sterigmatocystin and zearalenone, and chloroform-acetone (9 + 1) followed by a second development in ethyl ether or benzene-methanol-acetic acid (90 + 5 + 5) for aflatoxins. Submit samples not eliminated as negative in this stage for chemical derivatization, i.e., for aflatoxins, and sterigmatocystin, and ochratoxin A as in **26.076-26.083**, **26.118**, and **26.138**, respectively (11). For zearalenone, no chemical derivatization is currently indicated in *Official Methods of Analysis* (11). The plates can be examined under short and longwave ultraviolet light, sprayed with aluminum chloride solution, heated, and observed under longwave ultraviolet light as in **26.146** (11).

Results and Discussion

An evaluation of the method was conducted on naturally and artificially contaminated samples. Samples of yellow corn meal, raw peanuts, dried black beans, polished rice, and cassava flour were spiked at 3 levels of contamination as described previously (10). Results obtained from triplicate determinations at each level are shown in Table 1. Recoveries and coefficients of variation ranged from 91–101% and 0–16% for aflatoxin B_1, to 98–117% and 0–17% for sterigmatocystin, and 96–107% and 0–17% for zearalenone, respectively. Ochratoxin A recoveries and coefficients of variation with artificially contaminated samples have already been reported (10). Repeatability was also verified for aflatoxins and ochratoxin A in naturally contaminated samples (Table 2). Samples naturally contaminated with sterigmatocystin were not available. In the case of zearalenone, small amounts of 3 visibly molded feed corn samples, not part of the survey, were quantitated in duplicates, and levels of 7, 11, and 11 μg/kg were obtained. Scientists from another institution analyzed 53 contaminated peanut samples for aflatoxins by the proposed method and the CB method, **26.026-26.031** (11), obtaining 96% agreement of the results (private communication, G. M. Prado and S. V. Melo, 1987).

The detection limits of the method were 2, 5, 15, and 55 μg/kg for aflatoxin B_1, ochratoxin A, sterigmatocystin, and zearalenone, respectively. The corresponding quantitation limits, defined as the lowest concentration at which confirmation and reproducible quantitation are possible, were 4, 10, 35, and 165 μg/kg, respectively, in most of the commodities evaluated. The quantitation limits were higher for aflatoxin B_1 in beans, for zearalenone in cassava and peanuts, and for sterigmatocystin in corn (Table 1).

Taking into account the prices of locally purchased reagents, the cost of performing these simultaneous determinations by the proposed method would be 10-fold less expensive than the sum of the equivalent determinations accomplished by individual methods (11) (for aflatoxins, the CB method was quoted). In terms of time, the proposed method allows results to be obtained roughly 7 times faster. In contrast with most mycotoxin methods (12–19), the proposed method can be used both for screening and quantitation with additional savings in time.

During the survey, the usefulness of developing thin-layer chromatograms in different solvent systems as corroborating tests became evident. For aflatoxin and ochratoxin A presumptive positive samples, which maintained the same R_f values for suspected spots and standards in 3 developing solvents, all proved positive when submitted for chemical derivatization. Zearalenone, however, posed a problem. Five presumptive positive samples were found in the survey. They were checked simultaneously by the current **26.146** (11) confirmation procedure and by development in 2 additional solvent systems. In 2 of these samples (rice), zearalenone-like

Table 2. Repeatability data for aflatoxins and ochratoxin A in naturally contaminated rice and beans (μg/kg)

Mycotoxin	Sample 1	2	3	4	5	Av.
Rice						
Aflatoxin B_1	38	(62)[a]	38	38	38	38
Aflatoxin B_2	13	13	13	13	13	13
Aflatoxin G_1	18	18	18	18	18	18
Beans						
Ochratoxin A	136	136	113	136	127	130

[a] Outlier.

Table 3. Incidence of mycotoxin contamination of foods in Campinas, Brazil, from May 1985 to June 1986

Commodity		Samples, total	Branded samples	Unbranded samples	Positive samples
Corn	canned sweet	32	18	—	—
	flour	27	18	2	—
	white meal	6	6	—	—
	yellow meal	18	11	1	—
	instant yellow meal	13	8	—	—
	popcorn	15	8	5	5
	yellow grits	7	4	—	—
	dried white	12	6	4	1
	total	130	76	12	6
Cassava flour	raw	33	18	7	2
	roasted	9	6	1	—
	seasoned and roasted	3	3	—	—
	total	45	27	8	2
Rice	polished	52	40	5	2
	parboiled	8	5	—	—
	total	60	45	5	2
Dried beans	black	14	7	7	—
	white	4	4	—	—
	Jalo	3	2	1	—
	Mulatinho	3	0	3	1
	Carioca	9	3	5	—
	Carioquinha	10	5	5	2
	Rajado	7	5	2	—
	Rosinha	3	1	2	1
	Roxinho	5	3	2	—
	blackeyed peas	3	—	3	—
	total	61	24	30	4

spots continued to be observed under ultraviolet light, at both 254 and 366 nm, and after $AlCl_3$ treatment, at 366 nm. These samples proved negative by development in different TLC systems. This indicated the need for a chemical confirmatory test for this mycotoxin.

The mycotoxin contamination of corn, rice, beans, and cassava products in Campinas, Brazil, from May 1985 to June 1986 was tolerable. Of the 296 samples analyzed, 14 samples were found positive, corresponding to an incidence rate of 4.7% (Table 3). Beans exhibited the highest (6.6%) incidence. Corn (4.6%) and cassava (4.4%) presented contamination close to the overall incidence percentage. Considering the commodities individually, popcorn appeared with the highest proportion of contamination (one third of the samples), suggesting the need for better evaluation of this type of corn.

The mycotoxin levels in the positive samples are presented in Table 4. Only aflatoxin B_1 and ochratoxin A were found at levels of 20–52 μg/kg and 32–160 μg/kg, respectively. In 4 of the aflatoxin positive samples, aflatoxin G_1 was also found at 18–31 μg/kg. Zearalenone and sterigmatocystin were not detected in any sample.

Table 4. Levels of mycotoxin contamination in positive samples

Commodity	Contaminant	Level of contamination, μg/kg
Popcorn	aflatoxin	38 B_1
Popcorn	aflatoxin	47 B_1
Popcorn	aflatoxin	38 B_1
Popcorn	aflatoxin	20 B_1
Popcorn	aflatoxins	32 B_1, 18 G_1, 8 G_2
Dried white corn	ochratoxin A	32
Raw cassava flour	ochratoxin A	65
Raw cassava flour	ochratoxin A	32
Polished rice	aflatoxins	38 B_1, 15 B_2, 20 G_1
Polished rice	aflatoxin	26 B_1
Dried Carloquinha bean	ochratoxin A	94
Dried Rosinha bean	ochratoxin A	160
Dried Carloquinha bean	aflatoxins	39 B_1, 21 G_1, 4 G_2
Dried Mulatinho bean	aflatoxins	52 B_1, 31 G_1, 8 G_2

The low cost of the mycotoxin procedure made it possible in 1 year to more than double the number of food samples other than peanuts and its products, reportedly analyzed for mycotoxins in the country since 1970. Ochratoxin A and zearalenone have not been included in earlier reports, and some of the commodities such as popcorn, beans, and rice were not investigated previously.

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OILS AND FATS

Determination of Polysorbates in Foods by Colorimetry with Confirmation by Infrared Spectrophotometry, Thin-Layer Chromatography, and Gas Chromatography

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A method is presented for the detection of polysorbates (PSs) in 8 kinds of processed foods by colorimetric and thin-layer chromatographic (TLC) techniques. The PSs are extracted from processed foods with a mixture of methylene chloride and ethanol by using an Extrelut column. The extract is further purified by using a silica gel column. The PS extract is complexed with cobalt-thiocyanate (Co-thiocyanate) reagent and is determined spectrophotometrically at 620 nm. The recoveries and coefficients of variation for 8 kinds of processed foods fortified with 0.1% PS 80 were 67.9-94.6% and 4.0-11.3%, respectively. The detection limit of TLC corresponded to 50 mg PS 80/kg. PS identity was confirmed by infrared spectrophotometry of PS extract, and gas chromatography of fatty acids and thin layer chromatography of POE-sorbitan residues after saponification.

Polysorbates (PSs), which are complex mixtures of polyoxyethylated fatty acid esters of sorbitan, are widely used as emulsifiers, but they are not permitted in processed foods in Japan. A number of analytical methods for the detection and/or determination of PSs have been described. Hall (1) determined PS 80 in bakery products and frozen desserts by a gravimetric method. Ishiwata et al. (2) used cobalt-thiocyanate (Co-thiocyanate) reagent for the quantitative colorimetric method and the qualitative thin-layer chromatographic (TLC) analysis. Daniels et al. (3) isolated PS 60 in salad dressing by a silica gel column and determined it with the colorimetric method. They also confirmed the presence of PS 60 by using TLC. Brumley et al. (4) characterized PSs by using OH^- negative ion chemical ionization mass spectrometry. These methods, if applied to a wide variety of foods, would require laborious and time-consuming extraction of PS from foods and partition of PS from sample solutions.

In the present paper, we describe a simple method for the detection and confirmation of PSs in foods. The method is based on 2 procedures. The first is a screening test composed of Extrelut extraction, silica gel column cleanup, and TLC separation. The other is a confirmatory test composed of infrared spectrophotometry, gas chromatographic analysis (5) of the fatty acid components, and TLC analysis of the polyoxyethylene (POE)-sorbitan residue.

METHOD

Apparatus

(a) *Spectrophotometer.*—Shimadzu Model MPS-5000 (Shimadzu Corp., Kyoto, Japan), visible and ultraviolet range.

(b) *Precoated TLC plate.*—Kieselgel 60 (E. Merck, Darmstadt, FRG), Art. 5721.

(c) *Infrared spectrophotometer.*—Jasco A-3 (Japan Spectroscopic Co., Ltd, Tokyo, Japan).

(d) *Gas chromatograph.*—Shimadzu Model GC-4BMPF (Shimadzu Corp.), with flame ionization detector. Operating conditions: column 150°C; detector and injector 200°C; glass GC column, 200 cm × 3 mm id, packed with 5% Advance-DS on Chromosorb W(AW-DMCS) (Shimadzu Corp.), nitrogen flow ca 38 mL/min.

(e) *Recorder-integrator.*—Chromatopack-1A (Shimadzu Corp.).

Reagents

Use reagent grade chemicals.

(a) *Co-thiocyanate reagent.*—Dissolve 17.4 g ammonium thiocyanate and 2.8 g cobalt nitrate (Waku Pure Chemical Industries Ltd, Osaka, Japan) in 100 mL water as described by Ishiwata et al. (2).

(b) *PS standard solutions.*—PS 20, 40, 60, 65, 80 (Nikko Chemicals, Osaka, Japan), 5 mg/mL each in methylene chloride. Separate standard solutions for each PS.

(c) *Column adsorbent.*—Extrelut 20 (E. Merck), Art. 11738.

(d) *Developing solvents for TLC.*—*(1) Solvent A.*—Chloroform–methanol–acetic acid–water (68 + 12 + 12 + 3). *(2) Solvent B.*—*n*-Propanol–chloroform–methanol–NH_4OH (10 + 10 + 5 + 2). *(3) Solvent C.*—Chloroform–acetone–isoamyl acetate–isoamyl alcohol–propionic acid–water (90 + 20 + 13 + 5 + 2 + 5). Solvents A and B were discussed by Ishiwata et al. (2).

(e) *Silica gel.*—Kieselgel 60 with 10% water (E. Merck). Heat Kieselgel 1 h at 110°C. To 100 g Kieselgel, add 10 mL water, mix thoroughly, and store in airtight jar.

(f) *Fatty acid methyl ester standards.*—Qualitative Mixture AI208Y and 110Y (Gasukuro Kogyo Inc., Tokyo, Japan). Use for GC confirmatory test.

Sample Extraction

Weigh 8.5 g Extrelut in 100 mL beaker. Add 5 or 10 g sample (chop sample in small pieces) and 10 mL methylene chloride–ethanol (1 + 1). Mix thoroughly and let stand 15 min. Fill 5 g Extrelut and then sample-Extrelut mixture into column. Elute with 150 mL methylene chloride–ethanol (1 + 1), and collect eluate in 500 mL round-bottom flask. Evaporate solvent under vacuum by using rotary evaporator at 40°C. Dissolve residue in 10 mL tetrahydrofuran, and place 5 min in ultrasonic bath.

Silica Gel Chromatography

Insert glass wool plug in glass column (30 cm × 1.5 cm id). Add 10 g silica gel mixed with *n*-hexane. Prewash column with *n*-hexane. Transfer tetrahydrofuran solution of sample into column; wash container with 50 mL ethyl ether–chloroform (80 + 20) and add wash to column. Elute PS from column with 100 mL acetonitrile–water (3 + 1), and collect in 500 mL round-bottom flask. Evaporate solvent under vacuum to dryness. Suspend residue with 10 mL water (test solution).

Screening Test

(a) *Co-thiocyanate colorimetry.*—To 50 mL separatory funnel, add 10 mL test solution, 7 g NaCl, 10 mL methylene chloride, and 10 mL Co-thiocyanate reagent as described by Ishiwata et al. (2). Shake vigorously 5 min, and let layers

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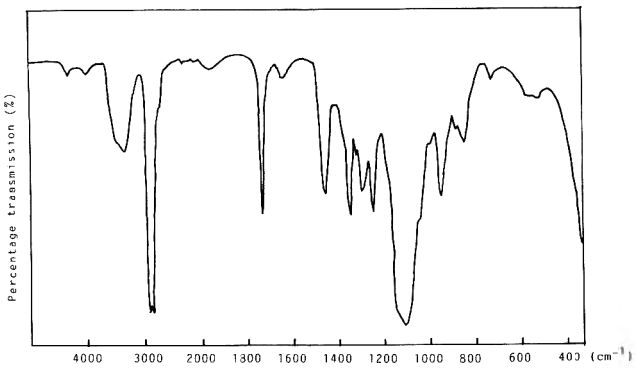

Figure 1. Infrared absorption spectrum of PSs recovered from a Chinese noodle soup. The 1120 cm^{-1} and 1740–1725 cm^{-1} bands are caused by the presence of POE and ester groups, respectively.

separate. Measure absorbance of methylene chloride layer at 620 nm.

(b) *TLC analysis.*—If methylene chloride layer changes to blue, evaporate the layer under vacuum by using rotary evaporator. Dissolve residue in 200 μL methylene chloride. Spot 5 μL test solution and 5 μL PS 80 standard solution 2 cm apart. Equilibrate TLC plate in chamber with developing solvent A or B and develop 10 cm. After development, spray Co-thiocyanate reagent on TLC plate.

Quantitative Test

(a) *Calibration curve.*—Dilute aliquots of PS 80 standard solution with methylene chloride to prepare solutions containing 1.25–5 mg PS 80/10 mL. To individual 50 mL separatory funnels, add 10 mL of a prepared standard solution, 7 g NaCl, and 10 mL water. To each funnel, add 10 mL Co-thiocyanate reagent, shake vigorously 5 min, and let layers separate. Measure absorbance of methylene chloride layers at 620 nm. Plot calibration curve of absorbance corresponding to PS 80 standard concentration.

(b) *Colorimetric quantitation.*—To 50 mL separatory funnel, add 10 mL test solution, 10 mL methylene chloride, 7 g NaCl, and 10 mL Co-thiocyanate reagent. Shake vigorously 5 min and let layers separate. Read absorbance at 620 nm and calculate PS concentration from standard curve as follows:

$$C = C' \times (1000/W)$$

where C = concentration of PS in sample, mg/kg; C′ = amount of PS corresponding to standard curve, mg; and W = weight of sample, g.

Confirmatory Test

(a) *Infrared spectrophotometric analysis.*—Add 10 mL test solution, 7 g NaCl, and 10 mL methylene chloride to 50 mL separatory funnel, and shake vigorously 5 min. Let layers separate, and dry methylene chloride layer with anhydrous sodium sulfate. Evaporate solvent under vacuum to near dryness. Place sample solution on KBr plate, and evaporate solvent under vacuum. Sandwich sample with another KBr plate. Record infrared spectrum with "window technique."

(b) *Confirmation of fatty acid residue and their components of PS.*—Extract test solution (>20 mg PS) twice with 10 mL methylene chloride, and dry combined methylene chloride layers with anhydrous sodium sulfate. Evaporate solvent under vacuum to dryness, and dissolve residue in small amount of methylene chloride. Streak methylene chloride layer on first preparative TLC plate (Kieselgel 60) and develop with solvent C. Spray Co-thiocyanate reagent, and scrape off blue silica gel zone. Extract PS from adsorbent with 30 mL methanol–chloroform (1 + 1), 30 mL methanol, and 30 mL acetonitrile–water (3 + 1) in that order. Evaporate combined extracts under vacuum to dryness, and dissolve residue in small amount of methylene chloride. Streak methylene chloride solution on second preparative TLC plate and develop with solvent A. Spray with Co-thiocyanate reagent; scrape blue silica gel zone and extract as described above. Transfer combined extracts to 300 mL round-bottom flask, and evaporate under vacuum to dryness.

Add 50 mL 10N KOH–ethanol (1 + 19) to 300 mL round-bottom flask containing separated PS fraction. Reflux 1 h at 100 °C. Evaporate solvent under vacuum to dryness and add 60 mL 0.5N HCl. Reflux for 1 h (6).

Extract fatty acid components with 25 mL ethyl ether 3 times. Use combined upper ethyl ether layers for confirmation of fatty acid components and lower aqueous layer for confirmation of POE-sorbitan residue. Remove remaining HCl from ethyl ether phase by washing twice with water. Dry solution over anhydrous sodium sulfate. Evaporate solvent under vacuum to dryness at 30°C, and methylate with diazomethane. Evaporate solvent under vacuum to dryness at 30°C. Add 4 mL *n*-hexane, and inject 2 μL sample solution into gas chromatograph and compare peaks with those for fatty acid methyl ester standards.

(c) *Confirmation of POE-sorbitan residue.*—Neutralize lower aqueous layer with 0.5N KOH. Add 20 mL Co-thiocyanate reagent and 45 g NaCl. Extract twice with 10 mL methylene chloride, and dry combined extracts over anhydrous sodium sulfate. Evaporate solvent under vacuum to dryness at 40°C, and dissolve residue in small amount of methylene chloride. Spot sample extract on TLC plate, and develop 10 cm with solvent A or B as described above. Observe blue spots by spraying with Co-thiocyanate reagent. Compare these spots with spots of POE-sorbitan residues obtained from saponification of 5 kinds of PSs (PS 20–PS 80).

Table 1. Recovery of PS 80 from processed foods

Foodstuffs	No. of samples	Added PS 80, mg	Recovery, %	CV, %
Chinese noodle soup	4	5	88.1	4.0
Chocolate	10	5	88.2	6.0
Mayonnaise	3	5	77.0	11.3
Butter	1	5	89.6	—
Margarine	2	5	94.0	—
Peanut butter	3	5	73.1	9.7
Pickled dill cucumber	1	5	94.6	—
Salad dressings[a]	6	5	67.9	8.3

[a] Salad dressings include 1 French, 1 Italian, 1 non-oil, and 3 semisolid types.

Results and Discussion

The use of Extrelut adsorbent gave a simple, rapid preparation because PSs can be extracted without emulsion and the procedure can be applied to various kinds of foods.

In the silica gel column chromatography, oily substances were separated from the PS extract with 50 mL ethyl ether–chloroform (80 + 20). No PSs were observed in this fraction. PSs were eluted quantitatively from the column with 100 mL acetonitrile–water (3 + 1).

The screening tests included colorimetry and TLC analyses. The TLC analyses were used to substantiate the results of colorimetric analysis. The TLC analyses could not differentiate among 5 kinds of PSs. Recovery for the screening test

Table 2. Comparison of fatty acid components among standard polysorbates in Chinese noodle soups

Polysorbates	Fatty acid components (weight, %)						
	12:0	14:0	16:0	16:1	18:0	18:1	18:2
PS 20[a]	40.4	18.5	15.8		11.3	9.8	4.2
PS 20[b]	31.4	28.8	18.6		8.9	9.7	2.5
PS 40[a]		1.5	43.3		48.3	7.0	
PS 40[b]		2.3	45.9		51.0	0.8	
PS 60[a]		2.0	26.2	1.7	62.3	7.8	
PS 60[b]	1.3	3.2	41.9		51.6		1.9
PS 65[a]		1.5	28.2		70.3		
PS 65[b]		2.3	31.8		64.5	0.2	1.1
PS 80[a]		1.5	4.2	6.8	2.0	85.5	
PS 80[b]	4.0	3.2	5.9	6.1	1.5	77.8	1.5
PS SO[c]		1.2	24.6		9.5	61.1	3.6
Unknown PS[d]		2.4	15.3		8.4	61.3	12.5

[a] The fatty acid components of 5 kinds of PSs recovered from standard PSs.
[b] The fatty acid components of 5 kinds of PSs recovered from a spiked Chinese noodle soup.
[c] The fatty acid components of PS SO (made from a sesame oil) recovered from a spiked Chinese noodle soup.
[d] The fatty acid components recovered from unknown PS separated from a Chinese noodle soup.

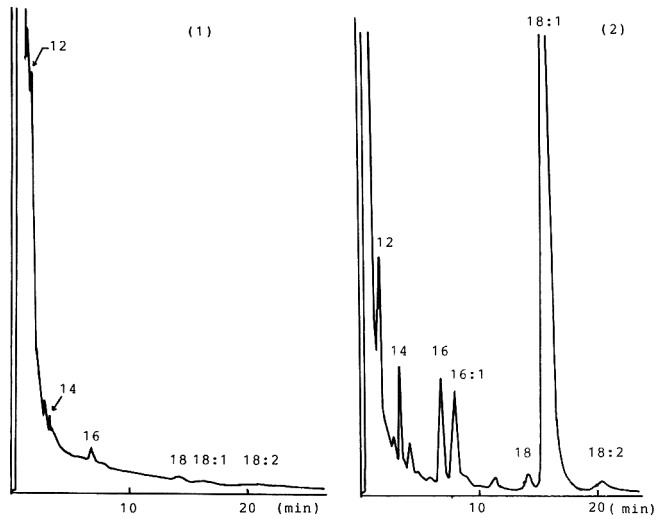

Figure 2. Chromatograms of fatty acids recovered from (1) PS-free Chinese noodle soup (sample weight 5 g) and (2) Chinese noodle soup spiked with PS 80 (sample weight 5 g, PS 80 spike 25 mg).

was determined by spiking 0.1% PS 80 to 8 kinds of PS-free processed foods (Table 1). The recoveries and coefficients of variation ranged from 67.9 to 94.6% and from 4.0 to 11.3%, respectively. In the TLC analyses, main spots of the standard PSs appeared with R_f values 0.71 ~ 0.82, 0.62 ~ 0.76 (solvent A), and 0.85 ~ 0.90 (solvent B). The detection limit was 50 mg PS 80/kg in the foodstuffs.

The confirmatory test was carried out by using infrared spectrometry. Figure 1 shows an infrared absorbance spectrum obtained from a Chinese noodle soup judged "PS positive" in the screening test. The 1120 cm^{-1} and 1740 ~ 1725 cm^{-1} bands are characterized by the presence of POE and ester groups, respectively.

Another confirmatory test relied on saponification of the PSs to fatty acids and POE-sorbitan residues. Table 2 shows the fatty acid components of 5 kinds of PSs (PS 20–PS 80) and those of 5 kinds of PSs recovered from a spiked Chinese noodle soup. When the preparative TLC procedures were omitted, oil from the foods interfered with the analyses of the fatty acid components. The 2 successive preparative TLC procedures allowed good separation of the PSs without interfering spots when the visualizing treatment on TLC was performed (developing with solvent A and B, spraying with 50% H_2SO_4 and heating at 150°C for 30 min). Figure 2 shows the chromatograms of the fatty acids recovered from a Chinese noodle soup free of PS and a Chinese noodle soup spiked with PS 80. The fatty acid components of the PSs recovered from the spiked Chinese noodle soup corresponded to those of the standard PSs.

Table 3 shows R_f values of the PSs (PS 20–PS 80) and other POE compounds. The R_f values of the PSs were decreased by 0.5 (solvent A) and 0.2 (solvent B) after saponification; whereas, the R_f values of other POE compounds (POE-alkyl ether, POE-nonylphenyl ether, and POE-octylphenyl ether used widely as detergents) stayed the same after the saponification treatment. The decrease of the R_f values after saponification made it possible to distinguish the PSs from other POE compounds.

A screening test was applied to 8 kinds of commercial processed foods. The PSs were detected in only one of 5 Chinese noodle soup samples examined. No PSs were detected in 10 chocolate, 3 mayonnaise, 1 butter, 2 margarine, 3 peanut butter, 1 pickled dill cucumber, or 6 salad dressing samples. The confirmatory tests were applied to the extract from the Chinese noodle soup judged "PS positive" in the screening test. The infrared absorbance spectrum and the R_f values of POE-sorbitan residue corresponded to those of the standard PSs. On the other hand the fatty acid components did not correspond to those of the standard PSs. The components were similar to a PS obtained from sesame oil. From these results, we expect that the PS contained in the Chinese noodle soup came from such a vegetable oil (Table 2).

The proposed method described in this paper is simple, rapid, and well suited for detecting and confirming PSs in various kinds of processed foods.

Table 3. R_f value of polysorbates and other POE-compounds

Compound	R_f[a]	
	Solvent A	Solvent B
Polysorbate	0.71–0.82 0.62–0.76	0.85–0.90
POE-sorbitan residue[b]	0.31, 0.15	0.69
POE-alkyl ether	0.70	0.80
POE-nonylphenyl ether	0.68–0.70	0.79–0.80
POE-octylphenyl ether	0.71	0.80

[a] Detection: Co-thiocyanate reagent.
[b] From saponification of polysorbate.

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ARTIFICIAL SWEETENERS

Determination of Aspartame in Beverages Using an Alcohol Oxidase Enzyme Electrode

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A new method for the determination of the artificial sweetener aspartame is described. α-Chymotrypsin is used to cleave the methyl ester group of aspartame, producing methanol hydrolytically. The methanol is detected using an electrode which is constructed by physically trapping yeast alcohol oxidase enzyme at the tip of a dissolved oxygen electrode. The decrease in oxygen concentration, which occurs as methanol is enzymatically oxidized to formaldehyde, is measured amperometrically. Aspartame levels in diet soft drinks as determined by the proposed method and by liquid chromatography are in excellent agreement. The relative standard deviation of the measurements is 0.83%. The methanol present in diet cola as a result of aspartame degradation can also be measured by using the electrode without α-chymotrypsin.

The artificial sweetener aspartame (*N*-L-α-aspartyl-L-phenylalanine-methyl ester), commonly known by the brand name NutraSweet®, has been marketed in the United States since its final approval by the U.S. Food and Drug Administration (FDA) in 1981. It is now featured in 160 food products. The use of aspartame increased to 13.0 million metric tons in 1986 compared with 200 000 tons in 1981. About 85% of diet soft drinks include the sweetener (1; USA Today, March 24, 1987; Food and Beverage Marketing, June 1987; Monsanto annual report, 1986).

Liquid chromatographic (LC) methods (2–9) are most commonly used for the analysis of aspartame in soft drinks and food products. Titration with lithium methoxide is the official FDA method (10).

The literature describes many analytical applications of enzyme electrodes that measure the decrease in dissolved oxygen accompanying the specific enzyme-catalyzed oxidation of substrate in the presence of molecular oxygen. The general reaction scheme is shown in equation 1:

$$\text{Substrate} + O_2 \xrightarrow{\text{oxidase enzyme}} \text{Products} + H_2O_2 \qquad (1)$$

Examples of analytes that have been measured in this way include uric acid (11), L-lactate (12), phenols (13), pyruvate (14), and sulfite (15).

The determination of alcohols by using alcohol oxidase enzyme electrodes has been reported (16–20). Recently, Hopkins (21) reviewed the use of alcohol oxidase probes coupled with linked enzyme systems for the determination of NADH, dehydrogenases and their substrates, and esterases and their substrates.

This paper describes the determination of aspartame following chymotrypsin-catalyzed esterase cleavage as shown in equation 2. The methanol formed is subsequently detected by using an alcohol oxidase enzyme electrode:

$$\text{L-Asp-L-Phe-methyl ester} \xrightarrow{\text{chymotrypsin}} \text{L-Asp-L-Phe} + \text{methanol} \qquad (2)$$

$$\text{Methanol} + O_2 \xrightarrow[\text{oxidase}]{\text{alcohol}} \text{formaldehyde} + H_2O_2 \qquad (3)$$

Results obtained by using the proposed method correlate well with LC analyses of aspartame in carbonated beverages.

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METHOD

Reagents

(a) *Aspartame.*—Sigma Chemical A-5139 (Sigma Chemical Co., St. Louis, MO 65178). Prepare 800 ppm standard solution daily in water. Accurately weigh 0.08 g aspartame to ±0.1 mg. Dissolve in ca 90 mL water, and dilute to 100 mL. Prepare additional standards by dilution in water.

(b) *Bovine α-chymotrypsin.*—Sigma Chemical C-4129 (Sigma Chemical Co.). Prepare fresh daily at 25 U/mL in assay buffer.

(c) *Assay buffer.*—0.05M potassium phosphate; pH 7.5.

(d) *Mobile phase (beverage analysis).*—Water–acetic acid–isopropyl alcohol. 15% acetic acid (v/v) buffered to pH 3.0 and modified with 3% isopropyl alcohol. Add 150 mL glacial acetic acid (v/v) to ca 700–750 mL LC purity water. Adjust pH to 3.0 with saturated sodium acetate solution. Add 30 mL LC grade isopropyl alcohol, and dilute to 1 L with water. Filter through 0.45 μm filter, and degas before use. Acetic acid and isopropanol concentrations may be varied slightly from column to column to achieve desired retention time and resolution.

(e) *Mobile phase (aspartame hydrolysis studies).*—0.01M KH_2PO_4, pH 2.5–acetonitrile (80 + 20, v/v).

Apparatus

(a) *Liquid chromatograph.*—Model 6000A solvent delivery system, WISP 710B auto-injector, Model 450 variable wavelength absorbance detector (Waters Chromatography Div., Millipore Corp., Milford, MA 01752), or equivalent; Hewlett-Packard 3390A recording integrator with input/output board for automatic run start.

(b) *Enzyme electrode.*—Provesta Multipurpose Bioanalyzer Model 98 (Provesta Corp., Bartlesville, OK 74004) equipped with dissolved oxygen probe, alcohol oxidase enzyme gel, membranes, and strip chart recorder.

(c) *Chromatographic conditions (beverage analysis.)*—μBondapak C_{18} stainless steel column, 300 × 4 mm id, 10 μm particle size; column temperature ambient; flow rate 2.0 mL/min; UV detector at 254 nm; injection volume 50 μL.

(d) *Chromatographic conditions (aspartame hydrolysis studies).*—Brownlee RP-300 C8 column, 100 × 4.6 mm id; column temperature ambient; flow rate 2.0 mL/min; UV detector at 215 nm; injection volume 100 μL.

Sample Preparation

(a) *Beverage analysis.*—Dissolve dry mixes completely as specified on label directions, using LC grade water. Degas carbonated beverages by sonicating under vacuum 20–25 min. Filter portion of sample using 0.45 μm cellulose acetate syringe-type filters, or equivalent.

(b) *Aspartame hydrolysis studies.*—To verify performance of analytical methods, add α-chymotrypsin to 200 ppm aspartame standard to final concentration of 0.4 U/mL. Incubate at ambient temperature, and withdraw samples for LC and enzyme electrode analysis at time intervals. To monitor heat-initiated aspartame degradation, seal samples of diet cola in gas-tight tubes, and heat in 80°C water bath. At

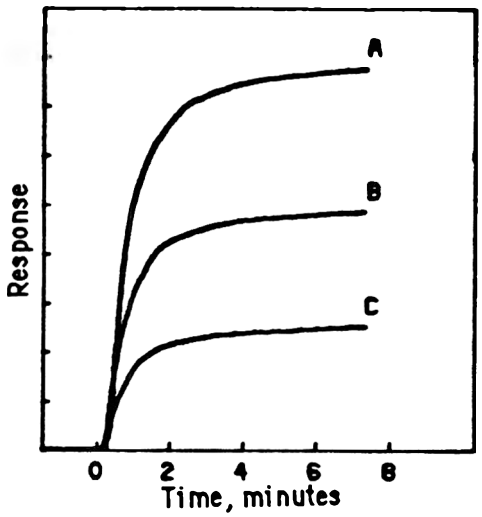

Figure 1. Typical response profiles of alcohol oxidase electrode in buffered α-chymotrypsin to (A) 600, (B) 400, and (C) 200 ppm aspartame.

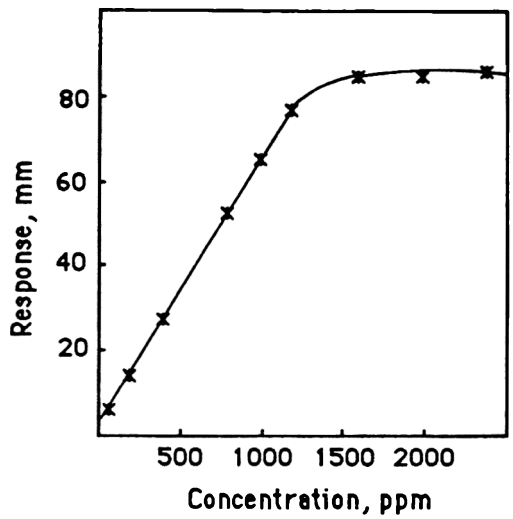

Figure 2. Calibration plot for aspartame obtained with alcohol oxidase electrode. Gain was 4.4, and injection volume was 200 μL.

time intervals, chill sample tube, degas under vacuum in sonic bath containing ice water, and analyze by LC and enzyme electrode.

Procedures

(a) *Preparation of enzyme electrode.*—Prepare electrode according to Bioanalyzer operator's manual. Rehydrate small amount of alcohol oxidase gel, and apply gel to center of wet membrane. Remove conical tip of dissolved oxygen electrode, and center gel and membrane on probe (gel toward inside). Replace tip sandwiching enzyme gel between Teflon membrane of oxygen electrode and retaining membrane.

(b) *Aspartame determination by enzyme electrode.*—Set Bioanalyzer gain at 6.8, and immerse probe tip in 3.8 mL stirred assay buffer at 25°C. Set chart speed at 30 cm/h, and adjust pen position to right margin of chart using zero control. Establish steady baseline. Add 200 μL sample or standard to assay buffer, and record response profile for 5 min. Response due to free alcohols or other interfering matrix components (R_m) is measured in this step. Rinse probe tip and immerse in 3.8 mL α-chymotrypsin solution. Re-establish baseline, and add 200 μL sample or standard as described above. Total response (R_t), which includes R_m and response due to aspartame (R_a) is measured in this step. Run series of aspartame standards (e.g., 800, 400, and 100 ppm), making single injection for R_m and duplicate injections for R_t.

Measure all responses on strip chart trace as distance in millimeters between initial baseline and response curve 5 min after sample addition. Calculate R_a for each standard or sample by subtracting R_m from R_t. Generate standard curve by entering response of standards as y value and aspartame concentration as x value in linear regression program of appropriate calculator or computer. Calculate aspartame in samples by entering R_a as y and solving for x.

(c) *LC of aspartame in beverages.*—Comparative data are generated by using procedures described by Webb and Beckman (9).

(d) *LC of aspartame hydrolysis products.*—The method of Verzella and Mangia (4) is used. Dipeptide (equation 2) and aspartame elute at 0.95 and 1.23 min, respectively.

Results and Discussion

Figure 1 shows the enzyme electrode response profile to increasing amounts of aspartame with α-chymotrypsin buffer. The signal begins to change 10–15 s after the addition of aspartame and approaches a steady state in 3–5 min. For routine sample analysis, linear standard curves ($y = 0.083x + 1.52$) are generated in the 80–800 ppm range using a Bioanalyzer gain setting of 6.8 and 200 μL injection volume. This working range includes the aspartame concentrations normally found in soft drinks. The linear correlation coefficient of the standard curves is 0.99. Samples containing higher levels of aspartame can be analyzed by sample dilution or by using smaller sample sizes. The response to increasing aspartame plateaus above 1200 ppm (Figure 2) as dissolved oxygen availability in the enzyme interface becomes limited.

The effect of α-chymotrypsin concentration on response was examined. Identical response profiles were achieved with 10–100 EU/mL. Below 10 EU/mL, response decreased and response time increased. Because the enzyme is inexpensive, an excess of α-chymotrypsin (25 EU/mL) was routinely used.

Figure 3 illustrates the effect of buffer pH on enzyme electrode response to ethanol and aspartame. The response to ethanol decreased only slightly throughout the pH range of 5.5–8.5. This result is consistent with the reported broad pH optimum of *Pichia pastoris* alcohol oxidase (22). Therefore, it is likely that the reduced response of the enzyme sensor to

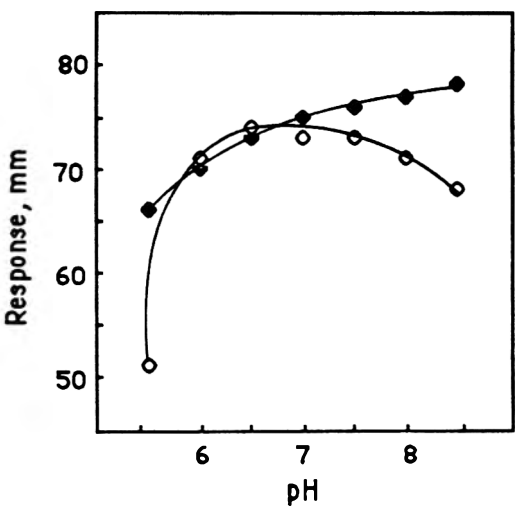

Figure 3. Effect of pH on alcohol oxidase electrode response to (◆) ethanol and (◇) aspartame (with chymotrypsin).

Table 1. Recovery of aspartame from beverage samples

Sample	Quantity added, ppm	Rec., %
Diet cola	100	96.6
	200	96.6
	300	100.3
Regular cola	100	100.3
	500	101.8
	800	99.0
Tea mix	100	97.3
	200	103.6
	300	104.9
Average		100.0

Table 3. Determination of aspartame and methanol during chymotrypsin treatment

	Aspartame by LC		Methanol by alcohol oxidase electrode	
Time, h	ppm	Change, μmole/mL	ppm	Change, μmole/mL
0	200	0	0	0
0.3	142	0.20	6.5	0.20
0.4	132	0.23	7.5	0.23
0.8	118	0.28	9.3	0.29
1.8	91	0.37	11.5	0.36
3.8	75	0.43	13.5	0.42
9.0	41	0.54	16.5	0.52

aspartame at low pH reflects the reduced activity of α-chymotrypsin. Both enzymes are fully active at the prescribed assay pH of 7.5.

To assess electrode stability, 3 alcohol oxidase enzyme electrodes were prepared and stored in (*1*) assay buffer at room temperature, (*2*) assay buffer containing 0.02% sodium azide at room temperature, or (*3*) assay buffer at 4°C. Each electrode was periodically challenged with a standard amount of aspartame and its response was recorded. Storage tests revealed that the probes could be stored 2 weeks at room temperature in buffer containing 0.02% sodium azide. Without the addition of sodium azide, the electrode failed when tested at 2 weeks. The electrode which was stored in buffer at 4°C retained full activity for over 7 weeks. Data shown in this report were obtained using probes prepared daily.

Precision data were generated by 8 determinations of aspartame in a diet cola. To correct for any signal drift, standards were analyzed before and after the series of sample injections, and the average response value was used to prepare the calibration curve. A single assay without α-chymotrypsin was included before each series of 4 sample injections with α-chymotrypsin. The average sample value was 502.9 ppm (SD = 4.2). A separate data set taken without these precautions yielded a relative standard deviation of 2.1% compared with 0.83% for the data reported.

Table 1 shows the recovery obtained by spiking samples with aspartame at 3 levels prior to analysis. Aspartame was added to the samples as a dry powder. Good recoveries were obtained in all cases.

Table 2 compares aspartame determinations in various beverages by the reported method and by LC analysis. As indicated in Table 2, the determination of aspartame in tea and powdered drink mix was not possible by using the described LC method due to other components which co-eluted with aspartame. If the fused peaks were integrated and total area was calculated as aspartame, a value of 2860 ppm for powdered drink mix was obtained compared with 546 ppm by enzyme electrode. LC analysis of these samples was later repeated at 50°C, using a photodiode array detector. In this case, aspartame in powdered drink mix eluted as a pure component calculated as 511 ppm. This suggests that the enzyme sensor could be used to analyze samples in which interfering components co-elute with aspartame from the LC column.

Table 2. Determination of aspartame (ppm) in beverages by alcohol oxidase electrode and LC analyses

Sample	Electrode[a]	LC[b]
Diet cola	507	514
Diet cola, caffeine free	439	436
Cola No. 2 with saccharin and aspartame	56	58
Nutrasweet solution	279	294
Regular cola	ND[c]	ND
Tea mix	280	——[d]
Powdered drink mix	564	——[d]

[a] Reported values are average of 4 determinations.
[b] Reported values are average of 2 determinations.
[c] ND = not determined.
[d] Aspartame not resolved from interfering component.

An abbreviated enzyme electrode procedure can be used. Rather than performing 2 separate determinations as described above, the sample is initially added to the analysis buffer and a steady baseline is established. Any matrix response (R_m) occurs during this step, and the signal re-equilibrates at a higher baseline than for buffer alone. A 200 μL aliquot of concentrated α-chymotrypsin (500 EU/mL) is then injected into the solution, and the response due to aspartame is recorded.

The chemistry of aspartame determination by enzyme electrode could be adapted to a microtiter plate assay with colorimetric readout. Solutions of the enzymes α-chymotrypsin and alcohol oxidase, and an appropriate chromogenic alcohol oxidase substrate could be used. In this way, a rapid, automated assay for use in quality control of diet beverage production might be developed.

The matrix response by enzyme electrode was typically 10–20% as great as total response. This suggests that low levels of free alcohol are present in these beverage samples. The ability to determine aspartame as the difference between total and matrix response depends on the absence of significant amounts of amino acid esters other than aspartame in the beverage samples. The excellent correlation of results obtained by the proposed method and LC analysis indicates that there is no significant α-chymotrypsin activity toward other sample components.

Verzella and Mangia (4) identified the primary degradation products of aspartame as the dipeptide L-α-aspartyl-L-phenylalanine and 5-benzyl-3,6-dioxo-2-piperazine acetic acid. To form these compounds, the methyl ester group of aspartame is hydrolyzed yielding a mole of methanol per mole of degradation product. The alcohol probe could be used with-

Table 4. Determination of aspartame and methanol in diet cola during heat treatment

	Aspartame by LC		Methanol by alcohol oxidase electrode	
Time, days	ppm	Change, μmole/mL	ppm	Change, μmole/mL
0	559	0	14.9	0
1	441	0.40	22.9	0.25
2	319	0.82	36.9	0.69
3	213	1.19	41.4	0.83
4	130	1.17	46.4	0.98

out α-chymotrypsin to determine the methanol formed by aspartame degradation in the presence of intact aspartame. A simplified system in which an aspartame standard was converted to dipeptide and methanol (equation 2) by α-chymotrypsin was used to show the correlation between enzyme electrode determinations of methanol produced and LC determinations of aspartame lost during this reaction. This allowed an evaluation of the methods in the absence of competing reactions which might occur during heat-initiated aspartame degradation in diet cola (Table 3). As expected, the disappearance of aspartame as determined by LC analysis was directly proportional to the appearance of methanol as determined by enzyme electrode. Results of a similar experiment in which aspartame and methanol were determined in diet cola after heating at 80°C for various times are shown in Table 4. Although the linear correlation coefficient of μmole methanol formed/min to μmole aspartame lost/min was 0.967, methanol values were significantly lower on a mole basis at each time point ($y = 0.82x - 0.046$; where y = μmoles methanol formed, x = μmoles aspartame lost). This result could be possibly explained by the loss of methanol during heating or analysis due to evaporation or chemical reaction with sample components. This hypothesis was rejected by spiking regular cola with 10 and 20 ppm methanol and by heating and by analyzing the samples as described above. Methanol recovery as determined by alcohol probe was 100 ± 10%. The lower methanol values could be due to aspartame degradation products which retain the methyl ester group. Structures of such compounds have been reported by Verzella and others (3, 4). The alcohol probe can be used to determine methanol resulting from aspartame degradation in diet soft drinks if a determination has been made on the fresh sample.

These studies demonstrate that the proposed enzyme electrode method can be used to determine aspartame in beverages with an acceptable degree of precision and accuracy. Results are comparable to those obtained by LC analysis. The method can be performed by using inexpensive instrumentation in situations in which LC equipment is not available.

Acknowledgment

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METHOD EVALUATION

Experimental Designs for Interlaboratory Precision Experiments: Comparison of ISO 5725 with Draft NEN 6303

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To determine the precision of standardized analytical methods, interlaboratory experiments are carried out in which several laboratories analyze identical samples from well homogenized batches of material. From the test results, estimates of the standard deviations under repeatability as well as under reproducibility conditions are calculated. In the present work, the experimental designs recommended in the International Standard ISO 5725 have been compared with a design proposed in the draft Netherlands Standard NEN 6303. This has been done by comparing their mathematical models as well as by applying them to the results of a recent collaborative study on the determination of heavy metals in edible oils and fats. The reproducibility standard deviation is estimated equally well with both Standards, but it appeared that the designs given in ISO 5725 can lead to serious underestimation (uniform-level design) or overestimation (split-level design) of the repeatability standard deviation. By using the design proposed in NEN 6303, these biases can be avoided. Hence, it is recommended that interlaboratory studies be organized according to the design of NEN 6303.

When presumably identical samples of material are analyzed according to a standardized test method, the results obtained should ideally be identical. Because of unavoidable variations in experimental conditions, however, differences will occur. Factors of a human, instrumental, or environmental nature may contribute to the variability of the test procedure. The larger the number of factors involved, the greater the variability of the test results will be.

Tests carried out with the same method on identical material are said to be under repeatability conditions if they are carried out by one well trained operator in one laboratory within a short period of time and using the same instrument(s) and equipment.

When tests are done with the same method on identical material in different laboratories (which implies different operators, different instruments and equipment, different environmental conditions), reproducibility conditions are said to apply. Thus, repeatability and reproducibility conditions are 2 extremes, the first leading to the minimum, the second to the maximum variability of the test results.

Precision is a general term for the variability in the results of repeated tests on identical samples, under specified experimental conditions (1). The generally accepted parameter for describing the dispersion of test results is the standard deviation. Hence, for a specified test procedure applied to samples of identical material, 2 precision parameters can be defined for the abovementioned extreme conditions: the repeatability standard deviation (σ_r); and the reproducibility standard deviation (σ_R).

In an interlaboratory precision experiment (also called collaborative study or ring test), tests on samples of identical material are carried out by several laboratories using a well defined test procedure which yields a single numerical figure as the test result. A single test result can also be a figure calculated from several observed values, provided this is specified in the test procedure. It is the aim of an interlaboratory precision experiment to find numerical estimates s_r for σ_r and s_R for σ_R.

In the field of interlaboratory precision tests, the International Standard ISO 5725 plays an important role (2). ISO 5725 recommends 2 experimental designs:

The uniform-level experiment (clause 6.1): "Samples from q batches of material, representing q different levels of the test property, are sent to p laboratories which each perform n tests under repeatability conditions at each level. These n tests are thus made on identical material."

The split-level experiment (clause 6.2): "Each level is split into 2 sub-levels, a and b, which are only slightly different. Each laboratory receives one sample from each of these sub-levels for testing."

In 1983, the Commission on Vegetable and Animal Oils and Fats of the Netherlands Standardization Institute (Nederlands Normalisatie Instituut, NNI) initiated the drafting of the Netherlands Standard NEN 6303: "Determination of repeatability and reproducibility of methods of analysis by interlaboratory tests" (3). The experimental design for an interlaboratory precision test according to draft NEN 6303 is as follows:

(*1*) The concentration range of the method of analysis to be tested is established. (*2*) Within the concentration range, a number of concentration levels is chosen which represent (as well as possible) the whole concentration range. As a rule, also in connection with the amount of samples and work involved, the number of levels is fixed at 3. (*3*) For each concentration level, 2 batches of the material to be investigated are chosen which show a small but significant difference in value of the quantity to be measured. Whenever possible, this difference should be 2 or 3 times the expected repeatability standard deviation. (Although the true value of this parameter is not known, at least a rough idea of its order of magnitude exists on the basis of previous experience.) (*4*) After the batches are carefully homogenized, 2 samples per participating laboratory are taken from each batch of material, which results in 4 samples per concentration level for each participant. (*5*) The 4 samples of a concentration level are given 4 successive code numbers in random order. (*6*) The $3 \times 4 = 12$ samples in total per participant are forwarded to the participating laboratories. (*7*) The samples of the same concentration level have to be analyzed singly in order of code number, simultaneously, or in succession as rapidly as possible under equal conditions (same operator, same instruments and equipment, etc.). (*8*) The samples of different concentration levels may, if necessary, be analyzed on different days, but should be analyzed by the same operator using the same instrument(s) and equipment.

The draft NEN 6303 design is in fact identical to the ISO 5725 split-level design except that 2 samples from each batch are investigated instead of one sample. The NEN 6303 design can therefore loosely be referred to as a "double split-level design."

Among the considerations which led to the drafting of NEN 6303 were some objections which had been raised to

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Table 1. Analysis of variance within a concentration level for ISO 5725 split-level design and draft NEN 6303 design[a]

Source of variation	Code	ISO 5725 E(MS)	ISO 5725 df	Draft NEN 6303 E(MS)	Draft NEN 6303 df
Between batches (fixed)	B_i		1		1
Between laboratories (random)	L_i	$\sigma^2 + 2\sigma^2_L$	k − 1	$\sigma^2_r + 4\sigma^2_L$	k − 1
Interaction L × B	LB_{ij}	$\sigma^2_r + \sigma^2_{LB}$	k − 1	$\sigma^2_r + 2\sigma^2_{LB}$	k − 1
Samples (random)	$S_{n(ij)}$	σ^2_r	0	σ^2_r	2k
Total			2k − 1		4k − 1

[a] E(MS) = expectation of mean squares; df = degrees of freedom.

the experimental designs of ISO 5725, which will be dealt with in subsequent sections.

Objections to the ISO 5725 Designs

Uniform-Level Experiment

According to this design, one batch of material at a specified concentration of the quantity to be measured is thoroughly homogenized. From this batch of material, each participating laboratory receives either one sample to be analyzed n times or n samples to be analyzed singly.

The tests within one laboratory are carried out under repeatability conditions, thus the n test results per laboratory supply a basis for estimating the repeatability standard deviation. One of the assumptions, then, is that the n tests have been performed independently of each other.

In ISO 5725, clause 10.4.1e reads: "It is essential that a group of n tests under repeatability conditions be performed independently as if they were n tests on different material. As a rule, however, the operator will know that he is testing identical material, but the point should be stressed to him in his instructions that the whole object of the experiment is to determine what differences in results can occur in actual testing. If it is feared that, despite this warning, previous results may influence subsequent test results and thus the repeatability variance, then a split-level experiment is considered the correct procedure."

It is our experience that indeed the independence of the n tests on the same material may be doubted. Especially in the case of an interlaboratory test, the temptation to work toward "good results" can hardly be resisted when it is known that identical samples are investigated, which of course leads to identical results. We think that it is the rule rather than the exception that the repeatability standard deviation will be underestimated when the uniform-level design of ISO 5725 is used.

Split-Level Experiments

According to the ISO 5725 split-level design, 2 nearly identical batches of material are chosen at each concentration level; one sample from each batch is analyzed singly by each participating laboratory. The NEN 6303 double split-level design is analogous to that of ISO 5725 except that 2 samples from each batch are investigated singly (see Table 1). Hence, the designs differ only in the number of investigated samples per batch, so that the mathematical model for the analysis of variance is the same in both cases except for a constant factor. Obviously, if k is the number of laboratories, in either design both the "between laboratories" mean square (MS) and the "interaction laboratory × batch" (L × B) mean square can be estimated with (k − 1) degrees of freedom. On the other hand, the "samples" mean square, which can be estimated with 2k degrees of freedom in the NEN 6303 design, appears to have nil degrees of freedom in the ISO 5725 split-level design and can therefore not be estimated. For this reason, the repeatability standard deviation σ_r in the ISO 5725 design is estimated from the Interaction L × B mean square. As can be clearly seen from Table 1, this is allowed only if $\sigma^2_{LB} = 0$. In the ISO 5725 split-level design, this is taken for granted, due to the absence of the possibility of checking the term σ^2_{LB}. Ample practical experience with results obtained with the draft NEN 6303 design, however, has shown that σ^2_{LB} contributes significantly to this source of variation in a considerable number of cases. It is therefore to be feared that application of the ISO 5725 split-level design may easily lead to overestimation of the repeatability standard deviation. In the NEN 6303 design, we always have an unbiased estimate for σ^2_r from the samples mean square MS(S) with 2k degrees of freedom, irrespective of whether σ^2_{LB} is or is not equal to zero. If $\sigma^2_{LB} = 0$, then MS(LB) is also an unbiased estimate of σ^2_r with k − 1 degrees of freedom.

Interlaboratory Test Showing Biases Due to ISO 5725 Designs

To gain an impression of the magnitude of the biases with which ISO 5725 designs estimate the numerical value of the repeatability standard deviation, an interlaboratory precision experiment on the determination of heavy metals in edible oils and fats will be considered, which has been carried out by Working group 5/83 of the Commission on Oils, Fats and Derivatives of IUPAC (4). The design of this experiment was an extension of the draft NEN 6303 design:

First, samples of liquid oils as well as solid fats were offered, which yields 2 series, both at 3 levels of metal concentration (low, medium, high), each level being designed as a double split-level according to NEN 6303, leading to 2 × 12 samples per laboratory. Moreover, 3 metals (copper, iron, and nickel) were measured in liquid oil, and 2 metals (copper and iron) were measured in solid fat. Second, participants were asked to carry out duplicate determinations in these 24 samples, and to report both results.

Table 2. Experimental design of study on heavy metals in oils (4)

Class			Experimental results	
Concn level	Batch	Sample	Liquid oil	Solid fat
1	1	1	x_1 x_2	x_{25} x_{26}
(High)		2	x_3 x_4	x_{27} x_{28}
	2	1	x_5 x_6	x_{29} x_{30}
		2	x_7 x_8	x_{31} x_{32}
2	1	1	x_9 x_{10}	x_{33} x_{34}
(Medium)		2	x_{11} x_{12}	x_{35} x_{36}
	2	1	x_{13} x_{14}	x_{37} x_{38}
		2	x_{15} x_{16}	x_{39} x_{40}
3	1	1	x_{17} x_{18}	x_{41} x_{42}
(Low)		2	x_{19} x_{20}	x_{43} x_{44}
	2	1	x_{21} x_{22}	x_{45} x_{46}
		2	x_{23} x_{24}	x_{47} x_{48}

Table 3. Model of analysis of variance per concentration level for study on heavy metals in oils (4) (Table 2)[a]

Source of variation	df	MS(..)	E[MS(..)]
Laboratories (L)	k − 1	MS(L)	$\sigma_A^2 + 2\sigma_S^2 + 8\sigma_L^2$
Batches (B)	1	MS(B)	
L × B interaction	k − 1	MS(LB)	$\sigma_A^2 + 2\sigma_S^2 + 4\sigma_{LB}^2$
Samples (within B)	2k	MS(S)	$\sigma_A^2 + 2\sigma_S^2$
Analyses (within S)	4k	MS(A)	σ_A^2
	8k − 1		

[a] E[MS(..)] = expectation of mean square (..).
$\sigma_r^2 = \sigma_A^2 + \sigma_S^2$, hence s_r^2 = [MS(A) + MS(S)]/2.
$\sigma_R^2 = \sigma_r^2 + \sigma_{LB}^2 + \sigma_L^2$, hence s_R^2 = [MS(L) + 2 MS(LB) + MS(S) + 4 MS(A)]/8.

This design implies that the operator carried out 2 kinds of duplicates in the same test, viz.: 24 × 2 duplicate determinations (paired 2 by 2), in which the operator was aware of the fact that the results of a pair should be equal (known duplicates); 12 × 2 duplicate samples, the identity of which had been hidden by randomization and coding of the samples (hidden duplicates).

The design of this experiment is given in Table 2, and the corresponding model of the analysis of variance is presented in Table 3. (*Note:* It should be realized that the model in Table 3 differs from the general draft NEN 6303 model (see Table 1) due to the more complicated design of this experiment!)

Underestimation of σ_r in ISO 5725 Uniform-Level Experiment

In Table 2, the vertical pairs of results (x_1;x_3), (x_5;x_7), etc., are hidden duplicates according to the draft NEN 6303 design; the horizontal pairs (x_1;x_2), (x_3;x_4), etc., are known duplicates. Table 4 gives the numerical results of the analyses of variance of the copper determinations. (The results of iron and nickel are not presented here, but these lead to the same conclusions.)

The calculations pertain to the results from 25 laboratories. From Table 4 it follows that the standard deviation s_r, estimated from the hidden duplicate samples, is without exception considerably larger than the standard deviation s_A, estimated from the known duplicate analyses. On average, s_r is about 1.7 × s_A, which implies that the open-duplicates system as recommended in the ISO 5725 uniform-level design has led to an evident underestimation of σ_r.

In a recent paper in this journal (5), Hamaker stated that "A serious warning is imperative here. It has been observed and confirmed by special experiments that operators are inclined, consciously or unconsciously, to censor replicate test results so as to bring the data closer together. Consequently, σ_r may be underestimated, sometimes by as much as 50%." This is in agreement with the value 1.7 as found by us.

Overestimation of σ_r in ISO 5725 Split-Level Experiment

A second conclusion from Table 4 is that at the high and medium concentration levels, the interaction mean square MS(LB) appears to be significantly larger than the samples mean square MS(S) in 4 of 6 cases at the 95% or 99% confidence level. This means that in 4 of 6 cases the laboratory × batches interaction is not negligibly small as compared with the repeatability standard deviation, so that application of the ISO 5725 split-level design would have led to serious overestimation of σ_r in those cases. In the latter design we have to estimate σ^2_r from the interaction mean square MS(LB) (which has the expectation $\sigma^2_r + \sigma^2_{LB}$) assuming that σ^2_{LB} is negligibly small as compared with σ^2_r, because in this design the samples mean square (expectation σ^2_r) has zero degrees of freedom. If the assumption is false, however, σ^2_r will be overestimated to an unknown extent. To gain an idea of the magnitude of this overestimation, we can use again the results of the interlaboratory study on heavy metals in edible oils (4).

Considering the structure of Table 2 once more, we may assert that, e.g., the set of pairs of results (x_1;x_5) of all participating laboratories together can be seen as the outcome of a split-level experiment according to the design of ISO 5725 for the high concentration level of copper in liquid oil. The same is true for the sets of pairs (x_9;x_{13}), (x_{17};x_{21}), (x_{25};x_{29}), (x_{33};x_{37}), and (x_{41};x_{45}) for the medium and low levels in liquid oil and for the 3 levels in solid fat, respectively.

In this way we obtain 6 data sets that are organized according to the ISO 5725 split-level structure. These data sets have been analyzed statistically according to the ISO 5725 rules, as follows:

To the cell differences d_i and the cell averages $\bar{y}_i$, Dixon's outlier test was applied. For the differences, this test led to the rejection of 2 results of liquid oil (both at medium level) and 2 results of solid fat (one at medium level, one at low level). To keep the data as comparable as possible with those obtained with NEN 6303, these 4 rejected results have been replaced by the results obtained with the second (identical) sample.

Subsequently, the estimates of the standard deviations of repeatability (s_r) and of reproducibility (s_R) have been calculated. The results of these calculations have been tabulated

Table 4. Analysis of copper concentrations in edible oils and fats (25 laboratories) for study on heavy metals in oils (4)

MS term:	MS(L)[a]	MS(LB)[a]	MS(S)[a]	MS(A)[a]	Derived quantities[b]			
E[MS(..)]:	$\sigma_A^2 + 2\sigma_S^2 + 8\sigma_L^2$	$\sigma_A^2 + 2\sigma_S^2 + 4\sigma_{LB}^2$	$\sigma_A^2 + 2\sigma_S^2$	σ_A^2	s_A	s_r	s_R	s_{LB}^2[a]
Oil:								
High level	249 099	42 448	20 075	3001	0.0055	0.0107	0.0214	5593
Medium level	77 037	25 241	10 579	1785	0.0042	0.0079	0.0135	3666
Low level	26 718	5809	5053	698	0.0026	0.0054	0.0076	189
Fat:								
High level	407 968	69 553	15 016	5281	0.0073	0.0101	0.0270	13 634
Medium level	267 558	9086	4601	1482	0.0038	0.0055	0.0192	1121
Low level	34 491	4187	3226	892	0.0030	0.0045	0.0079	240

s_r^2 = [MS(A) + MS(S)]/2.
s_R^2 = [MS(L) + 2 MS(LB) + MS(S) + 4 MS(A)]/8.
s_{LB}^2 = [MS(LB) − MS(S)]/4.
s_A^2 = MS(A).
[a] The tabulated results have to be multiplied by 10^{-8}; e.g., MS(L) = 0.00249099 for oil, high level.
[b] The derived quantities s_A, s_r, and s_R are given in mg/kg (ppm).

Table 5. Comparison of results for copper concentration in edible oils and fats (4) according to draft NEN 6303 and split-level ISO 5725 designs[a]

Concn level	m		s_r			s_R		
	NEN 6303	ISO 5725	NEN 6303	ISO 5725	ISO/NEN	NEN 6303	ISO 5725	ISO/NEN
Oil:								
High	0.143	0.143	0.0107	0.0152	1.42	0.0214	0.0219	1.02
Medium	0.085	0.085	0.0079	0.0106	1.34	0.0135	0.0133	0.99
Low	0.036	0.035	0.0054	0.0065	1.20	0.0076	0.0080	1.05
Fat:								
High	0.153	0.149	0.0101	0.0151	1.50	0.0270	0.0274	1.01
Medium	0.102	0.102	0.0055	0.0065	1.18	0.0192	0.0197	1.03
Low	0.040	0.041	0.0045	0.0040	0.89	0.0079	0.0079	1.00

[a] m = mean concentration level; s_r = standard deviation of repeatability; s_R = standard deviation of reproducibility.

in Table 5, where, for comparison, the corresponding results from NEN 6303 have also been presented. For the mean values (m) and for the standard deviations of reproducibility (s_R), the agreement between the results of both designs is excellent. The repeatability standard deviations (s_r) obtained with ISO 5725 split-level experiments, however, appear to be (much) higher than those obtained with NEN 6303 experiments in 5 of 6 cases. This phenomenon has to be ascribed to the incorporation of the interaction term σ^2_{LB} into the ISO 5725 estimate of σ^2_r, by which this estimate tends to be systematically too high, as indicated already on theoretical grounds in the split-level experiment.

Discussion and Conclusions

In the NEN 6303 (double split-level) design, 2 independent mean squares are calculated, viz., the MS(LB) with expectation $\sigma^2_r + 2\,\sigma^2_{LB}$, and MS(S) with expectation σ^2_r. From an F-test on the ratio MS(LB)/MS(S), it can be judged whether or not σ^2_{LB} can be assumed to be zero or negligible. In ISO 5725 (split-level design), however, the mean square MS(S) cannot be calculated; it has nil degrees of freedom according to Table 1. Hence, we can only assume that the interaction term σ^2_{LB} in the MS(LB) mean square is negligible as compared with σ^2_r, because this design offers no possibilities to compare the magnitudes of σ^2_{LB} and σ^2_r. With NEN 6303, we can always calculate an unbiased estimate of σ^2_r from MS(S), irrespective of whether $\sigma^2_{LB} = 0$ or $\sigma^2_{LB} > 0$. On the other hand, with ISO 5725, we have no other choice than to use MS(LB) for estimating σ^2_r, without knowing the magnitude of σ^2_{LB} and thus with the risk that σ^2_{LB} is appreciably greater than zero.

In the latter case, the repeatability standard deviation will be overestimated to an unknown extent, without the analyst ever knowing that this has occurred! From the numerical example given above (ISO 5725 split-level experiment) it appears that this overestimation can be as high as 50%. On the other hand, the open-duplicates system as applied in the ISO 5725 uniform level design may lead to an underestimation of the repeatability standard deviation of the same magnitude according to the example of the ISO 5725 uniform-level experiment. It is for these reasons that in NEN 6303 we have tried to avoid the disadvantages of the ISO designs. It may indeed be argued that with NEN 6303 the number of samples to be analyzed is twice that in the corresponding ISO designs. However, as it is the aim of an interlaboratory precision experiment to arrive at realistic estimates of precision, we should design it in such a way that even the possibility of bias is avoided. Hence, we believe that on these grounds a design as proposed in NEN 6303 has to be preferred.

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MYCOTOXINS

Limited Survey of Deoxynivalenol in Wheat and Corn in the United States

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A limited survey was conducted over a 2-year period to determine the incidence and levels of deoxynivalenol (DON) in corn and wheat grown in selected areas of the United States. Samples of corn (198) and wheat (247) were collected and analyzed by a gas chromatographic method. Sixty-six percent of the corn samples collected in 1984 and 30% of the corn samples collected in 1985 contained DON. The average concentration of DON in corn, by state, ranged from 0.11 to 1.20 μg/g; the maximum concentration was 2.47 μg/g. Only 2 of the 247 samples of wheat contained DON at a concentration >2 μg/g, which is the level of concern suggested by the Food and Drug Administration for wheat entering the milling process for human consumption.

Deoxynivalenol (DON, vomitoxin) is one of a group of closely related secondary fungal metabolites called trichothecenes. The trichothecenes are associated predominantly with some species of *Fusarium,* which, under certain climatic conditions, invade grains in the field and in storage. Many members of this group have been implicated in human and animal toxicoses. DON causes feed refusal and emesis in swine (1, 2). It is associated with "pink scab" or "tombstone" kernels of such small grains as wheat and with pink ear rot of corn, both of which are caused by *Fusarium graminearum* (*Gibberella zeae*). Infection of wheat and corn by this fungus usually occurs when there is cool, wet weather before harvest (3). This situation slows the maturation of the grain and, thereby, provides conditions favorable for attack by the mold. Some damage of this type occurs in wheat and corn each year; however, the severity of the infection by the mold depends on weather conditions.

During the spring of 1982, pink scab in wheat reached epidemic proportions in certain areas of the United States; extensive damage occurred in Missouri, Kansas, Iowa, and Nebraska. DON levels as high as 9 μg/g (average 3.6 μg/g) were found in some samples of mold-damaged wheat from these states (4). That finding caused concern for the possible presence of DON in corn harvested in the same and adjacent states. Thus, a small number of samples of shelled corn, collected at various grader stations within the major corn growing region, were analyzed and found to contain up to 2.14 μg DON/g (average 0.35 μg/g) (5). Samples of bread were collected from 23 bakeries in 11 states and analyzed for DON. Of the 45 samples analyzed, the toxin was found at levels of 0.050–0.240 μg/g in 23 samples and 0.013–0.046 μg/g in 16 samples; the remaining 6 samples were negative (unpublished data, Mycotoxin Analytical Laboratory, Food and Drug Administration, New Orleans, LA, 1983). In view of these findings, a limited survey of corn and wheat was initiated.

Design of Survey

The survey was designed to obtain information on the incidence and levels of DON in corn and wheat designated for human consumption in commercial channels in specific areas of the United States over a 2-year period. Corn was collected from 11 states in the corn belt and 2 southeastern states; wheat was collected from 7 (1984) or 9 (1985) states in the Midwest and South. Ten lb (4.5 kg) samples of each commodity were obtained by random probes of commercial lots of corn and wheat that had been previously graded by U.S. Department of Agriculture graders or private contractors. The samples were collected, shipped, and stored in paper bags to minimize moisture buildup that could encourage mold growth. A total of 198 samples of shelled corn and 247 samples of wheat were collected and analyzed over the 2-year period.

The samples were sent to the Food and Drug Administration's (FDA) Mycotoxin Analytical Laboratory in New Orleans, LA, for analysis. The analytical method involved a rapid and efficient cleanup procedure and determination by gas chromatography with an electron-capture detector to quantitate the heptafluorobutyryl derivative of DON in the grain (6). The amount of DON in each sample was determined by comparing the peak area of the derivatized sample with the peak area of derivatized DON standards. Recovery studies were done on selected samples, some of which were negative for DON and some were positive. When pure DON was added to selected positive samples, the peak height for DON was additive in each case. Thereby, the compound measured was indicated as being, indeed, DON. Recovery of DON added to 19 corn samples and 17 wheat samples averaged 89.3 and 95.2%, respectively.

Results and Discussion

The results of this survey are summarized in Tables 1 and 2. Among the 92 samples of corn collected in 1984, 61 (66%) were positive for DON (Table 1); the average concentration, by state, ranged from 0.11 to 1.20 μg/g. The samples collected in Minnesota and Wisconsin had the highest incidence and levels; the maximum level found was 1.87 μg/g. Three of the samples were obtained from grain elevators in Minneapolis, and 2 were obtained from grain companies in Wisconsin.

Of the 123 samples of wheat collected in 1984, 75 (61%) were positive for DON (Table 1); the average concentration, by state, ranged from 0.22 to 0.98 μg/g. The samples collected in Louisiana were the most severely contaminated; the maximum level was 2.29 μg/g. The samples were collected at various grain elevators in Louisiana; however, the lots originated with wheat growers in the Midwest.

Thirty-two (30%) of the 106 samples of corn collected in 1985 contained DON (Table 2); the average concentration, by state, ranged from 0.18 to 0.80 μg/g. The samples collected in Ohio, Michigan, and Indiana were the most severely contaminated; the maximum level found was 2.47 μg/g.

Of the 124 samples of wheat collected in 1985, 57 (46%) contained DON (Table 2); the average concentration ranged from 0.24 to 0.79 μg/g. The samples with the highest contamination levels were collected in Iowa and Missouri; the maximum level found was 2.65 μg/g.

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Table 1. Shelled corn and wheat designated for human consumption—number of samples collected from states and summary of analytical results—1984 data

States	No. of samples	DON found			
		No.	%	Av., μg/g	Range
Shelled corn					
GA	7	2	28	0.24	0.14–0.34
NC	8	5	63	0.29	0.10–0.54
IL	11	4	36	0.35	0.17–0.62
OH	12	6	50	0.26	0.10–0.85
SD	3	0			
MI	4	4	100	0.46	0.34–0.65
IN	10	7	70	0.11	0.10–0.15
MN	3	3	100	0.59	0.38–0.80
WI	2	2	100	1.20	0.53–1.87
KS	8	8	100	0.41	0.10–0.91
NE	8	5	63	0.25	0.14–0.37
MO	11	10	91	0.32	0.12–0.47
IA	5	5	100	0.26	0.10–0.46
Total	92	61			
Wheat					
MI	4	1	25	—	0.25
IN	2	2	100	0.55	0.48–0.62
KS	31	14	45	0.54	0.10–1.85
NE	25	17	68	0.22	0.10–0.38
LA	7	7	100	0.98	0.52–2.29
MO	51	34	66	0.64	0.11–1.90
IA	3	0			
Total	123	75			

Table 2. Shelled corn and wheat designated for human consumption—number of samples collected from states and summary of analytical results—1985 data

States	No. of samples	DON found			
		No.	%	Av., μg/g	Range
Shelled corn					
GA	8	0			
NC	9	1	11	—	0.28
IL	15	4	27	0.40	0.17–0.81
OH	12	10	83	0.60	0.10–2.47
SD	3	0			
MI	3	2	67	0.80	0.31–1.28
IN	5	4	80	0.50	0.18–1.13
MN	3	3	100	0.18	0.15–0.23
WI	5	4	80	0.62	0.38–0.80
KS	10	0			
NE	17	2	12	0.19	0.16–0.22
MO	9	1	11	—	0.20
IA	7	1	14	—	0.13
Total	106	32			
Wheat					
MI	3	0			
IN	1	1	100	—	0.65
KS	31	11	35	0.36	0.14–0.84
NE	19	14	74	0.24	0.10–0.44
MN	6	1	17	—	0.55
LA	4	0			
MO	47	25	53	0.58	0.11–2.65
IA	13	5	38	0.79	0.19–1.75
Total	124	57			

The incidence of DON contamination of corn and wheat was significantly lower in 1985 than in 1984; however, the levels observed were not significantly different. The severity of the infection of corn or wheat by *Fusarium* organisms depends on weather conditions; hence, the differences noted in results for 1984 and 1985 may be a normal representation.

There was no correlation between the grade of the corn or wheat analyzed and the level of toxin found. This result is understandable because, in the survey, no attempt was made to selectively collect obviously mold-damaged corn or wheat as had been done for wheat in the limited 1982 survey (4). In that survey, a correlation was observed between the percent of total damaged kernels (a grading factor in wheat samples) and the level of DON found.

FDA has not established a guideline or tolerance for DON; however, an "advisory" was issued in 1982 (7). The advisory to federal and state officials recommended a level of concern of 1 μg DON/g in finished wheat products for human consumption, 2 μg/g for wheat entering the milling process, and 4 μg/g for wheat and wheat milling by-products used in animal feeds; no advisory has been issued for corn. Of the 247 samples of wheat analyzed in this survey, only 2 samples contained DON at a level >2 μg/g.

DON is distributed into different components of grains during the milling process (8). Attempts have been made to remove DON from naturally contaminated corn and wheat by physical and chemical treatments (9–12); none of the treatments was completely effective. DON is quite stable to heat; therefore, it was not completely destroyed by baking products made from contaminated wheat flour (9, 13–16). Limited surveys have been conducted on the incidence and levels of DON in wheat and corn food products in commercial channels in Canada (17) and the United States (5). The latter survey included snack foods in addition to baby foods, breakfast cereals, bread, corn meal, and wheat flour. DON was found in all food categories examined. The U.S. data revealed, however, that no DON was found in corn-based snack food (corn chips, corn snacks, and taco shells) and that the lowest DON contamination levels were found in breakfast cereals containing corn as the major ingredient. Limited data from Canada (18) suggest that corn syrup and corn starch may be free of DON. The data obtained in this survey serve as background information for future studies on DON in grains. More yearly surveys are needed to obtain a better idea of the year-to-year variation that occurs in the incidence and levels of the toxin in corn and wheat.

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FOOD ADULTERATION

Class Definition and Mixture Class Definition by Means of Construction of Convex Hull Boundaries: Application to Analysis for Animal Fat Adulteration

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A visual classification technique based on the construction of convex hull boundaries in combination with a principal component analysis is described. This combined technique was evaluated in the situation in which a distinction has to be made between 2 pure animal fat classes and the corresponding mixture class. In the first instance, a principal component analysis is carried out to ensure the 2-dimensional and thus visual aspect of the technique. Convex hulls are then constructed in the 2-dimensional principal component plane to delimit the boundaries of the different classes to be defined. The effectiveness of the constructed hull boundaries in the definition of class-membership was investigated by means of the classification of different simulated test samples. The results show that, at least for the tested applications, the technique is valid, although some false positive classifications occur. The detection of outliers especially seemed to pose problems. Therefore, some propositions are made of how to refine the developed hull technique to enhance the classification results.

The estimation of food adulteration may often pose serious problems in food quality control. Entire or partial substitutions of the original product with an allied product of (mostly) inferior and cheaper quality are sometimes difficult to detect. A first necessity in trying to solve this substitution problem is to verify whether the 2 kinds or classes of products, i.e., the original products on the one hand and the adulterant on the other hand, can be distinguished in an efficient way and whether mixtures of these 2 products can also be recognized. The solution of this problem is 2-fold. In the first instance, one has to select and analyze those parameters that are supposed to discriminate the 2 possible classes. In a second step, it will be necessary to apply multivariate mathematical techniques to define class characteristics on the basis of the determined parameter. Those class characteristics in turn will allow us to define class-membership of unknown samples.

The scope of this article is restricted to the description and evaluation of a possible mathematical way of solving the "substitution" or "mixture" problem in the supposition that the necessary analytical measurements have already been carried out and discriminating data are available. The technique in question makes use of the construction of 2-dimensional convex hull boundaries for the delimitation of the different classes or, in other words, for the definition of class characteristics. In comparison to other techniques that might be used for solving the same problem, emphasis should be put on the graphical and thus visual aspect of this convex hull technique.

With supervised learning techniques such as, for instance, SIMCA (1) and UNEQ (2), it is possible to develop separate mathematical models for each of the classes to be defined (i.e., the 2 pure classes and the mixture classes) and to define class-membership by fitting each test object to each of the developed models. Linear discriminant analysis (LDA) (3) creates linear boundaries to distinguish different possible classes; objects are classified according to their position with respect to those boundaries. Factor analytical techniques such as target transformation factor analysis, which is used, among others, to assign the influence of different emission sources in a pollution pattern (4) or partial least squares (5) might be more precise. However, in all applications of multivariate mathematical techniques, one starts by representing the data visually, i.e., in 2 dimensions, using techniques such as principal components analysis. This is necessary because one likes to have a visual idea of the shape of the developed class-models, the position of the different classes in relation to each other and of test objects in relation to the different classes, the presence of outliers, the homogeneity of the classes, etc. We reasoned that since this technique is used anyway, it would be useful to enhance it by including the possibility of classifying pure samples and, more important, distinguishing mixture samples. The convex hull method seemed to us to be a valid alternative to reach this goal. It was tested on the detection and classification of adulteration of fat samples from different animal species.

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Experimental

Data

A data set concerning the fatty acid composition of animal fat samples of different origin was taken as a starting point to evaluate the usefulness of the developed hull technique in the problem of class and mixture class definition. The original data set was made available by H. De Brabander. It lists the percent content of 7 fatty acids incorporated at the 2-position of triacylglycerol of 21 pork fat samples, 20 hen fat samples, 15 beef fat samples, and 14 horse fat samples. The fatty acids taken into consideration are myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3).

The analysis procedure is briefly described as follows: Triacylglycerols are extracted and isolated from meat tissue samples, or isolated from fat tissue samples by homogenization, melting, and filtering. The fatty acid composition in position 2 of the triacylglycerols is determined by *(1)* the reaction of pancreatic lipase on the triacylglycerols, *(2)* the separation of the reaction products by thin-layer chromatography, *(3)* the transesterification of the resulting 2-monoacylglycerols with sodium methylate, and *(4)* the quantitative gas chromatographic analysis of the resulting fatty acids. A more detailed description of the materials and methodology used for this fatty acid analysis can be found in ref. 6.

It is known that the fatty acid pattern and the relative

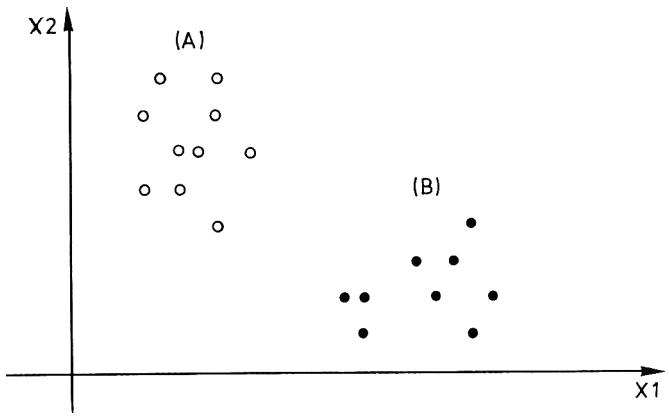

Figure 1a. Representation of objects of 2 different classes, A and B, in 2-dimensional plane defined by parameters X1 and X2.

distribution of fatty acids within the triacylglycerols is to some extent species-specific. However, because of differences in feeding regime and anatomical location of the fat, fatty acid contents may also vary within one animal species (6). Nevertheless, this data set seemed to be a suitable working sample, since it is our intention to evaluate the usefulness of the convex hull technique in defining class-membership rather than to determine whether the available data are the most discriminating in the distinction between the different animal fat species.

Philosophy of the Applied Hull Technique

Consider the situation where objects or samples known to belong to either class A or class B are characterized by their measurement values for 2 parameters X1 and X2. Both kinds of samples can be represented as points in a 2-dimensional plane defined by the 2 parameters, the coordinates of the objects being their measurement values for X1 and X2 (Figure 1a). A possible way of defining class characteristics in this 2-dimensional situation is to delimit the area around each of the 2 possible classes by constructing class boundaries. Class boundaries can be determined by computing the convex hull around the cloud of points representing the samples of a specific class. This procedure corresponds with successively connecting the most extreme points of each separate class with each other (Figure 1b). The convex hulls then serve as the criterion for the classification of unknown samples, a classification that is, however, not probabilistic since the convex hull technique can be seen as a nonparametric method. In fact, class-models may be described in 2 ways, that is, by parametric or by nonparametric methods.

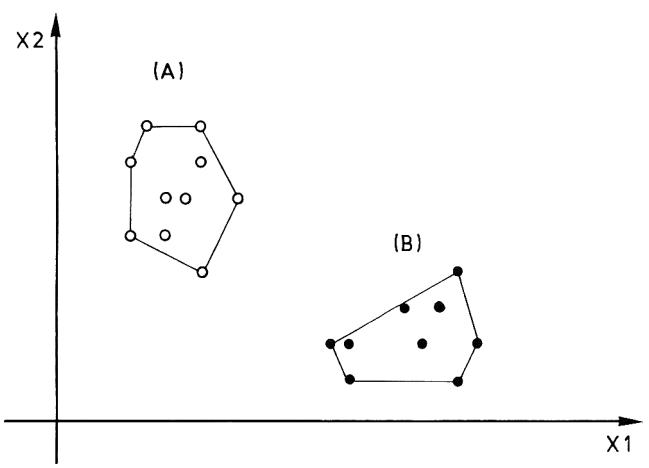

Figure 1b. Determination of class boundaries by construction of 2-dimensional convex hulls around each class.

Parametric techniques, i.e., techniques that define class-models or class boundaries on the basis of statistical parameters derived from the underlying distribution of the class samples (e.g., UNEQ, LDA . . .), have the advantage that the classification of test samples can be expressed in a probabilistic way. Each test object can be assigned a certain probability of belongingness to a specific class. A disadvantage is that the techniques are based on the assumption that the samples are bi- or multivariate normally distributed. Moreover, LDA also assumes equal within-group variance. These conditions are certainly not always fulfilled. If the underlying conditions are not fully satisfied, the classification rules derived from the technique are non-optimal and, consequently, classification results will not always be reliable. Nonparametric methods, on the contrary, do not make any assumptions with regard to the underlying distribution of the class samples, but the disadvantage of these methods is that the classification decision is perhaps too "clear-cut." A new sample is assigned to a specific class when it falls inside its class boundaries (in our case, the convex hull); otherwise it is considered to be an outlier with respect to that class.

Defining the convex hull around a 2-dimensional cloud of points is the same as finding the most extreme points in the corresponding class of objects. These extreme points form the vertices of the convex hull. A step-wise procedure for determining those vertices is summarized hereafter and is illustrated in Figure 2.

(1) Determine the centroid (c) of the class points. The 2 coordinates of the centroid are calculated as the class mean of each of the 2 respective parameters (Figure 2a). *(2)* Find the class point situated at maximal distance (d_{max}) of the centroid. This point defines the first vertex of the convex hull (v_1) (Figure 2a). *(3)* Determine the second vertex (v_2) by selecting the class point that forms a maximal angle (θ_{max1}) with the line that connects the centroid with the first vertex (Figure 2b). *(4)* Define consecutive hull vertices ($v_3 \ldots v_n$) according to the same principle, namely, by selecting the class point that forms a maximal angle with the line segment that connects the 2 former vertices (Figure 2c). *(5)* Repeat step 4 until the last vertex defined coincides with the first vertex ($v_n = v_1$). The construction of the convex hull around the cloud of points of a particular class is then completed (Figure 2d).

The procedure for defining class-membership of new samples with respect to the constructed hull boundaries can also be described in a step-wise manner (see also Figure 3):

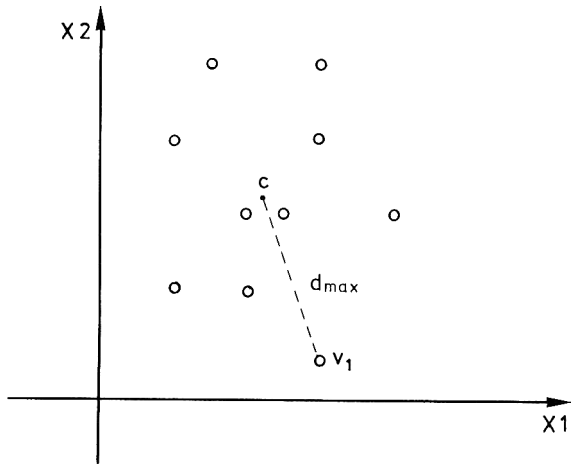

Figure 2a. Step-wise procedure for construction of convex hulls: Determination of centroid (c) and first vertex (v_1) by selecting class point with maximal distance (d_{max}) to centroid.

(1) Find the vertex to which the new point (T) is closest (v_c). *(2)* Determine the "direction" of the new point with respect to the closest vertex. More precisely, verify whether the new point lies in the wedge defined by vertices v_c and v_{c+1}. If this is the case, set v_d equal to v_{c+1}. Otherwise, that is, if the test point lies in the wedge defined by vertices v_c and v_{c-1}, set v_d equal to v_{c-1}. *(3)* Define whether the new point is (with respect to the line segment connecting vertices v_c and v_d) situated toward the centroid or away from it, or, in other words, whether the new point lies inside the triangle (c, v_c, v_d) or outside this triangle. When the new point lies inside this triangle, it is considered to be a member of the class under consideration. A description of an analogous procedure for defining convex hulls and for defining convex inclusion of new objects can be found in ref. 7.

Mixture class boundaries can also be described in terms of convex hulls. The procedure described hereafter is restricted to the situations where it can be assumed that the response of a linear combination of material from class A and from class B is equal to the linear combination of the responses of the material from both classes. This rules out situations where the combination of material from 2 classes interacts synergistically. If there is no interaction between the material from both classes, it can be stated that the mixture of a sample (a) from class A with a sample (b) from class B results in a new sample (c) with a composition intermediate to both original samples. Consequently, on the X1 vs X2

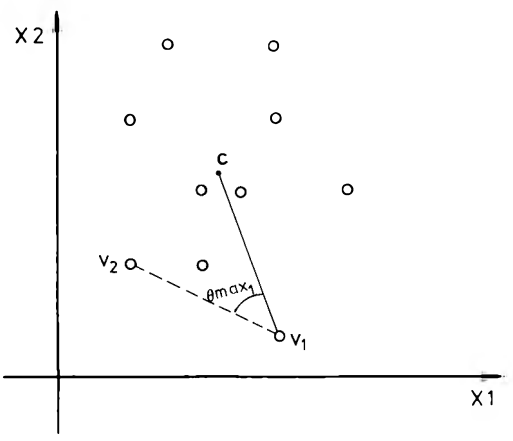

Figure 2b. Determination of second vertex (v_2) by selecting class point that forms maximal angle (θ_{max1}) with line that connects centroid with first vertex.

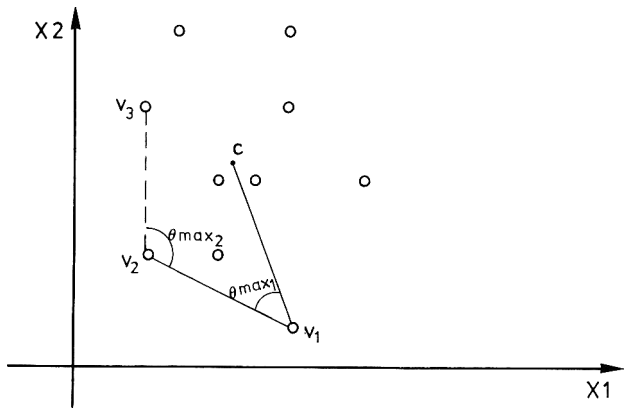

Figure 2c. Determination of consecutive vertices (v_3 . . .) by selecting class point that forms maximal angle (θ_{max2} . . .) with line segment that connects the 2 former vertices.

graph, the new mixture sample is situated on the line that connects both original samples (Figure 4a). This is true for all mixtures of A-samples with B-samples in every possible proportion. One observes that each of the possible "mixture lines" falls within the boundaries of a convex hull constructed around the total of points, that is, considering the points of class A and the points of class B as a whole (Figure 4b). Mixture class boundaries can thus be defined as the convex hull around the total of points belonging to both pure classes. The same procedure as described above can be applied to determine its hull vertices (i.e., the most extreme points) and to define class-membership.

The area occupied by the mixture class comprises thus the area occupied by class A, the area occupied by class B, plus the area between these 2 classes. Hence, since the mixture class includes both pure classes, it will not always be possible to distinguish a mixture (adulterated sample) and a pure sample. For instance, object d in Figure 4a can be a pure A-sample but it can just as well be a mixture sample obtained by mixing a large proportion of an A-sample with a small proportion of a B-sample. The distinction between a pure or a mixed sample is even less obvious as the 2 classes become more similar (small between-class distance) and as the classes become strongly heterogeneous (large within-class distance).

Evaluation Procedure

In testing the convex hull technique, we considered the 2-dimensional situation in which a distinction has to be made between 2 "pure" classes and the corresponding mixture class.

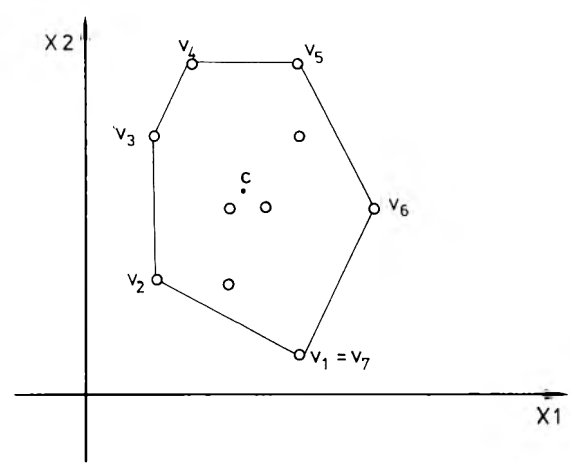

Figure 2d. Complete convex hull around points of a class.

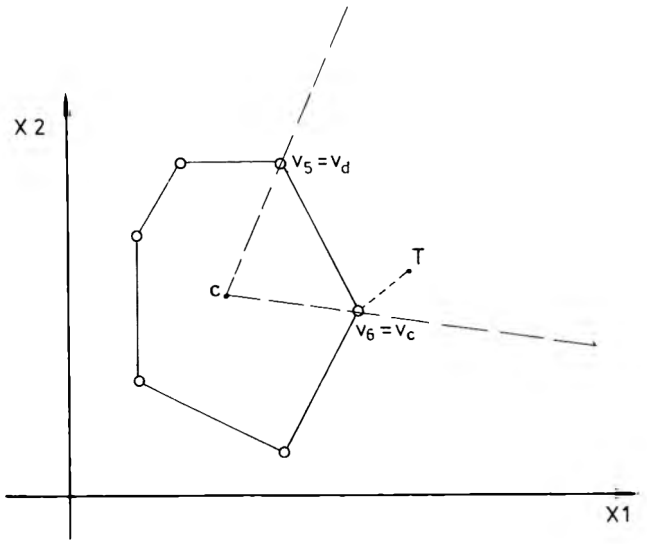

Figure 3. Definition of class-membership: T is closest to v_6, thus $v_6 = v_c$; T lies in wedge defined by vertices v_6 ($=v_c$) and v_5 ($=v_{c-1}$), thus $v_5 = v_d$; T falls outside triangle (c, v_c, v_d). Conclusion: T is outlier.

Starting from the total data set consisting of 4 different animal species (classes), every possible combination of 2 classes was considered. Convex hulls were constructed around each class (and mixture class) in each possible combination considering, respectively, the 21 pork, 20 chicken, 15 beef, or 14 horse fat samples from the original data set as representative samples.

Since in the original data set 7 parameters were determined (i.e., fatty acids incorporated at the 2-position of the triacylglycerols), it was necessary to reduce the number of dimensions before calculating the 2-dimensional class boundaries. We used principal component analysis to do this. Principal component analysis (PCA) can be seen as a visual dimension reduction technique with the objective of representing multidimensional data into a 2-dimensional space without losing too much of the original information residing in the data set. This goal is achieved by the computation of new variables (i.e., principal components or PCs) as orthogonal linear combinations of the original variables. The principal components are constructed in such a way that the first one explains more variation than the second, the second explains more than the third, etc. The dimension reduction results thus from the fact that most of the original variation in the data set is retained

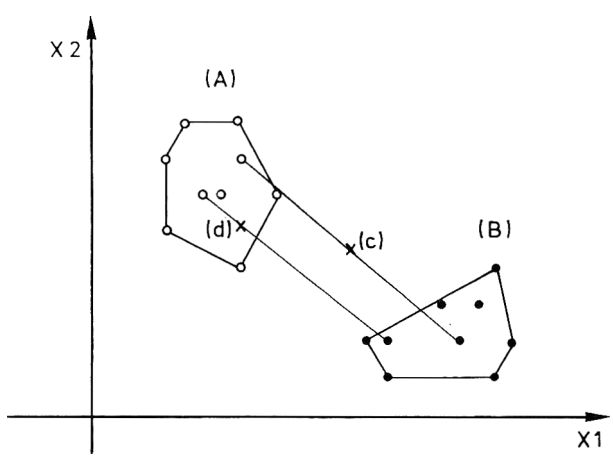

Figure 4a. Mixture samples (c) and (d) are situated on line that connects 2 original samples of which they are composed.

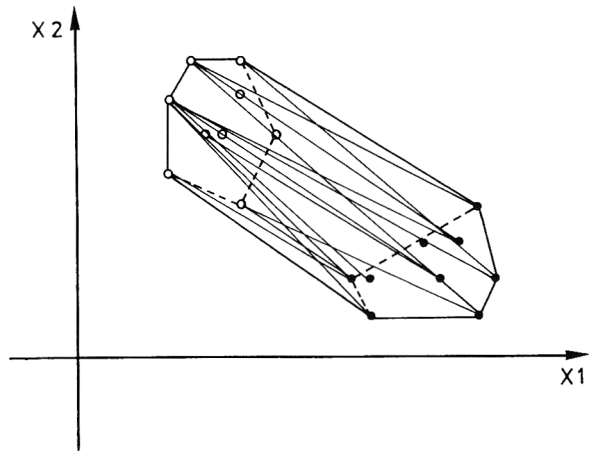

Figure 4b. Mixture class boundaries are defined as convex hull around total of points. (All possible mixture lines fall within these boundaries).

by the first few PCs. Hence, by carrying out a preliminary PC analysis on each subset of 2 classes, it becomes possible to represent the 2 classes into a 2-dimensional plane defined by the first 2 PCs and to construct the convex hulls around each class considering these 2 PCs as the new dimensions. New objects that have to be classified should then be projected in the corresponding PC plane.

The choice of PCA as the dimension reduction method is made somewhat arbitrarily. There is no specific reason for preferring it above other factorial methods except that it is the most commonly used and therefore also the best known and best understood method. For reasons of visualization, only the first 2 PCs are retained as the basic dimensions for constructing 2-dimensional convex hulls. However, it should be mentioned that 2 PCs might not always be sufficient to approximate adequately the multidimensional data set. Consequently, classification results derived from convex hulls constructed in a PC plane that poorly represents multidimensional reality might be unreliable. Nevertheless, experience shows that on most occasions the 2 first PCs fairly approximate the real dispersion among the objects so that the constructed convex hulls can be considered as reliable parameters for defining class characteristics.

The method was thus tried in 6 different combinations, namely, in the definition of the pure and mixed classes of beef and horse fat, beef and pork fat, beef and chicken fat, horse and pork fat, horse and chicken fat, and, finally, pork and chicken fat. For each subset of 2 classes, the effectiveness of the hull boundaries in the definition of class-membership was evaluated by means of the classification of test objects that were generated by simulating—arithmetically—combinations of the genuine animal fat samples of the original data set. Each test set consists, more precisely, of 4 pure samples, 6 mixture samples, and 4 outliers. Pure samples were simulated by combining 2 randomly chosen samples of the corresponding pure class with each other; mixture samples are imitated by combining (in different proportions) randomly chosen samples of each of the 2 pure classes with each other; whereas the samples that are supposed to be outliers are arbitrarily chosen among the 2 remaining classes.

Results and Discussion

The graphical reproductions of the different classes, the constructed convex hulls, and the positions of the test samples with respect to those hull boundaries are represented in Figures 5 and 6 for, respectively, the beef-horse classification

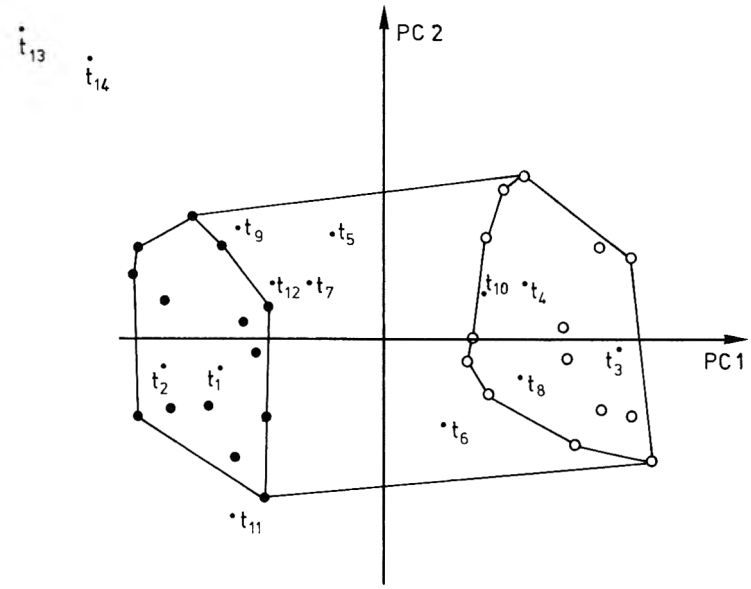

Figure 5. Beef-horse classification: 2-dimensional representation (PC1 vs PC2) of constructed convex hulls and position of test samples toward the hull boundaries. ● = beef samples; ○ = horse samples; t = test samples.

and the pork-chicken classification. The plots are PC1 vs PC2 plots. For reasons of classification, 3 different areas were determined on each of the 2-dimensional PC plots. The first area is defined as the area delimited by the convex hull boundaries constructed around the first pure class. It should comprehend all corresponding pure samples and possibly also some of the mixture samples (for reasons described above). The second area is defined as the area delimited by the convex hull around the second pure class. Again, it should include all corresponding pure samples and possibly some of the mixture samples. Finally, the third area is defined as the area comprehended by the convex hull around the complete mixture class minus the area comprehended by both pure classes. It defines an area in which only mixture samples should fall. Samples that fall outside these 3 areas are defined as outliers.

The detailed classification results obtained in each of the 2 abovementioned situations are represented in Tables 1 and 2. A positive sign means that the test object falls inside the corresponding area, a negative sign indicates that the test object falls outside it. Test objects that are "wrongly" classified are bracketed.

A general description of the results obtained in each of the 6 situations is given below:

Hull boundaries.—In each of the 6 combinations, the 2 pure classes are totally separated from each other. There is no overlap between the 2 corresponding convex hulls. However, one observes that in some situations (e.g., pork-chicken

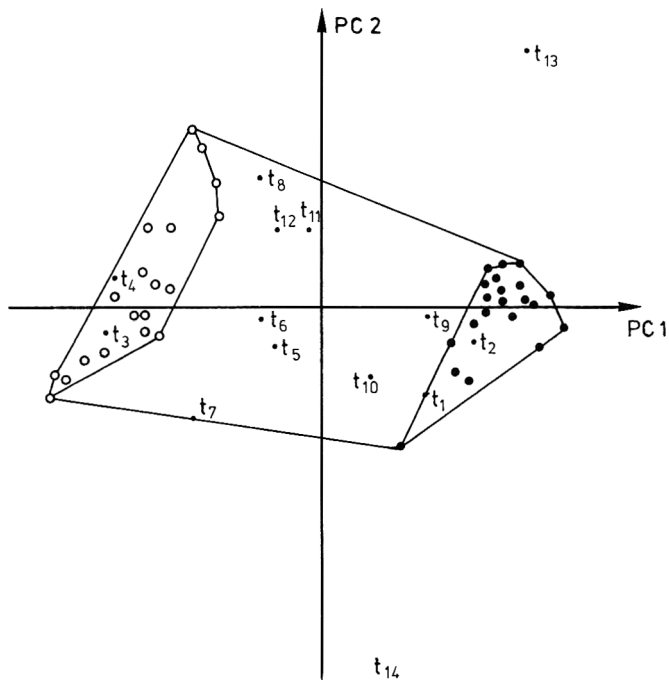

Figure 6. Pork-chicken classification: 2-dimensional representation (PC1 vs PC2) of constructed convex hulls and position of test samples toward hull boundaries. ● = pork samples; ○ = chicken samples; t = test samples.

Table 1. Areas of classification for beef, horse, and mixtures[a]

Test objects[b]	Area I: beef class	Area II: horse class	Area III: mixture class
T1 = Pure beef	+	−	−
T2 = Pure beef	+	−	−
T3 = Pure horse	−	+	−
T4 = Pure horse	−	+	−
T5 = Beef/horse: 1/1	−	−	+
T6 = Beef/horse: 1/1	−	−	+
T7 = Beef/horse: 2/1	−	−	+
T8 = Beef/horse: 1/2	−	[+]	−
T9 = Beef/horse: 3/1	−	−	+
T10 = Beef/horse: 1/3	−	[+]	−
T11 = Chicken	−	−	−
T12 = Chicken	−	−	[+]
T13 = Pork	−	−	−
T14 = Pork	−	−	−

[a] + = test object falls inside test area; − = test object falls outside test area; brackets = "wrong" classification.

[b] Simulation of mixture samples: Beef/horse:1/3, for example, is mixture sample consisting of 25% beef fat and 75% horse fat. It was simulated (arithmetically) by adding fatty acid percentages of one beef sample and 3 horse samples (randomly chosen among samples of original data set) and dividing resulting value by 4. Same remark accounts for all other mixture samples.

Table 2. Areas of classification for pork, chicken, and mixtures[a]

Test objects[b]	Area I: pork class	Area II: chicken class	Area III: mixture class
T1 = Pure pork	+	−	−
T2 = Pure pork	+	−	−
T3 = Pure chicken	−	+	−
T4 = Pure chicken	−	+	−
T5 = Pork/chicken: 1/1	−	−	+
T6 = Pork/chicken: 1/1	−	−	+
T7 = Pork/chicken: 2/1	−	−	+
T8 = Pork/chicken: 1/2	−	−	+
T9 = Pork/chicken: 3/1	−	−	+
T10 = Pork/chicken: 1/3	−	−	+
T11 = Beef	−	−	[+]
T12 = Beef	−	−	[+]
T13 = Horse	−	−	−
T14 = Horse	−	−	−

[a,b] See Table 1.

(see Figure 6)), the classes are rather heterogeneous resulting in convex hulls of an abnormal or stretched-out shape. It can be expected that with such widely spread hull boundaries, the classification results will not always be optimal.

Classification results.—(1) In each of the 6 situations, the 4 pure samples are correctly classified into the corresponding pure class.

(2) Thirty-three of the total 36 simulated mixture samples can unambiguously be defined as such since they all fall inside area III, i.e., the area delimited by the boundaries of the complete mixture class minus both pure classes. Three mixtures (2 for the beef-horse classification, 1 for the beef-chicken classification) are, however, classified into the area of one of the corresponding pure classes. Since the complete mixture class cannot totally be distinguished from the pure classes, this result can be expected, especially for mixtures of pure samples in unequal proportions and/or for mixtures of extreme pure samples (i.e., samples situated near the class boundaries). This is the case for the 3 mixture samples that are classified into a pure class: For instance, the 2 mixture samples that fall inside the pure horse class consist of a large proportion of horse fat (see Table 1) and, moreover, the horse fat samples, of which the mixtures are partly composed, are situated near the rightmost horse class boundaries.

(3) In 5 of the 6 situations, one or 2 of the samples that are supposed to be outliers are found inside the mixture class boundaries. These wrong classification results might be due to the fact that the mixture class boundaries are too liberal and cover too large a space (which may happen when the classes are heterogeneous). Another possibility is that, perhaps, the starting parameters are not discriminating enough to make a distinction between more than 2 different animal fat species and their corresponding mixtures.

In summary, it can be noticed that the developed hull technique leads to some false positive but no false negative results. In other words, some test samples are classified *inside* specific hull boundaries while in fact they should not be, but there are no samples that fall *outside* specific boundaries when they should be classified inside them. Therefore, it could be stated that, at least in this application, the method is probably sensitive but perhaps not selective enough.

Outlier detection especially seems to pose a problem. Possibly, the method could be improved by incorporating more sophisticated calculations for solving the abovementioned problem. The calculation of residuals, such as proposed and incorporated in the SIMCA method (1), seemed to us a possible help in solving the outlier problem. The residuals can be seen as a measure for the distance of an object toward the calculated PC plane. If the residuals of an individual object are found to be too large compared to the "mean" residuals of the global matrix to the PC plane, the object in question can be defined as an outlier. Classes would then be 3-dimensional: the 2-dimensional convex hull sandwiched between 2 boundaries in the third dimension, the boundaries being determined by allowable residuals. Preliminary calculations carried out on this subject prove that, although the outlier problem is not entirely solved, the evaluation of these residuals in combination with the 2-dimensional convex hull technique is certainly worth further investigation.

Conclusion

The research work carried out until now is not complete since, for instance, the effect of mixture samples composed of a known animal fat with an unknown animal fat is not yet investigated. However, it seems clear that the convex hull technique in combination with a principal component analysis can be considered as a mathematically simple and easily interpretable visual classification method giving good results. It must not be seen as a miracle technique bringing solutions to all kinds of problems. We would rather present it as an easily applicable technique that can be used besides other methods but not to the exclusion of other methods.

Certainly, further refinements could be implemented. For instance, the construction of ellipses instead of convex hulls can be seen as an analogous but probabilistic method. Boundaries of the corresponding mixture class could then possibly be defined as the common tangents to the 2 pure elliptical classes or perhaps the mixture class could be described as an ellipse of which the foci are the centroids of both pure classes. Those possibilities, however, have not been investigated. The technique could also be made more selective by the incorporation of supplementary and more sophisticated calculations for outlier detection. To solve the problem of selectiveness and accuracy, one also could consider the possibility of constructing convex hulls in more than 2 dimensions, but then the advantage of mathematical simplicity and visuality disappears.

Acknowledgment

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COLORS

Analysis of Natural Coloring Matters in Food. III. Application of Methylation with Diazomethane for the Detection of Lac Color[1]

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A new method has been proposed for detection of lac color in food. Lac color is a natural color additive derived from a secretion of the insect *Coccus Laccae (Laccifer lacca* Kerr). It is extracted from food with methanolic oxalic acid and eluted from a column of Amberlite XAD-2 with the same solvent. The fraction containing the lac color is treated with diazomethane to produce 2 reddish-orange markers. The marker species in the reaction mixture are detected by both thin-layer chromatography and reverse-phase liquid chromatography.

Stick lac is a secretion of the insect *Coccus laccae* (*Laccifer lacca* Kerr) which parasitizes on twigs of certain host trees, especially legumes indigenous to India and Southeast Asia. Colored extract from stick lac is used for a natural color additive called lac color, and a residual resinous fraction is valuable as an industrial shellac.

The color constituents of stick lac, laccaic acid A(A_1) (1–3), B (4, 5), C (6), D (7), and E (6), have been isolated and determined structurally. All have an anthraquinone moiety with dicarboxylic acids, except for laccaic acid D, which has only a monocarboxylic acid (7). Lac color is a water-soluble red color additive, and it is added to enhance some foods in Japan, including tomato ketchup, strawberry jam, candy, and beverages (8).

Useful methods have been developed by the authors for the detection of gardenia yellow color (9) and cochineal color (10) in food. In the course of an experiment, however, we had difficulty in judging the addition of lac color to food. Using the older method (11), R_f values obtained on a cellulose plate could not be compared without chemical treatments because of the appearance of several different types of spots on the plate. Also, none of the methods (11–17) that have been published for analyzing natural coloring matters in food contribute toward the resolution of this problem. Hence, the development of a new method became urgent.

Despite its chemical hazard and toxicity, diazomethane is frequently used as a methylating reagent for acidic compounds such as carboxylic acids and phenols because of its excellent reactivity. Because of the chemical structures of laccaic acids, diazomethane was applied to the methylation of lac color. Two conspicuous reddish-orange products obtained by methylation in tetrahydrofuran with ethereal diazomethane were completely distinguished from other spots on a silica gel plate. They were employed as marker species of lac color and were tentatively designated as LD-1 and LD-2; to detect the marker species, the addition of lac color to food was confirmed by using thin-layer chromatography (TLC) and liquid chromatography (LC).

METHOD

Apparatus

(a) *Liquid chromatograph.*—Shimadzu LC-6A pump (Shimadzu Ltd, Kyoto, Japan) equipped with Shimadzu SPD-1 detector, 100 μL loop attached to Rheodyne 7125 injector (Rheodyne Inc., Cotati, CA 94928), Shimadzu R-11 laboratory recorder, and/or Shimadzu C-R3A integrator used with a 25.0 × 0.4 cm id column packed with 5 μm particle size LiChromosorb RP-18 (E. Merck, Darmstadt, FRG). Operating conditions: mobile phase, acetonitrile–water (45 + 55), filtered (0.45 μm filter) and degassed before use; flow rate, 1.0 mL/min; detection, 495 nm, 16 × 10^{-2} absorbance units full scale (AUFS); ambient temperature (ca 22°C).

(b) *Spectrophotometer.*—Hitachi 333 automatic (Hitachi Ltd, Tokyo, Japan), suitable for registering both UV spectrum (200–350 nm) and visible spectrum (400–650 nm) and determining absorbance at 495 nm, equipped with quartz cells having 1 cm optical path length and a recorder.

(c) *Freeze dryer.*—Neocool DC-55B (Yamato Scientific Co., Ltd, Tokyo, Japan). Samples were frozen at −70°C and lyophilized at ca 10^{-3} torr.

Reagents and Materials

Reagents were analytical grade unless otherwise stated, and solvents were distilled before use or LC grade (Wako Pure Chemical Industry Ltd, Osaka, Japan). Water was deionized and distilled before use in all experiments.

(a) *Commercial (natural) lac color preparations.*—Lac color, powdered san red No. 3 (San-ei Chemical Industries, Ltd, Osaka, Japan); hi red SL-NA (Daiwa Kasei Co., Ltd, Saitama, Japan); stilac red (Aizen Co., Ltd, Tokyo, Japan); kidorenin S (Tokyo Tanabe Co., Ltd, Tokyo, Japan); laccaic acid (Tokyo Kasei Kogyo Co., Ltd, Tokyo, Japan); cochineal color, powdered san red No. 1 (San-ei Chemical Industries, Ltd); monascus color, powdered san red MR (San-ei Chemical Industries, Ltd); beet red color, powdered san beet N-2 (San-ei Chemical Industries, Ltd); corn color, san red No. 5 (San-ei Chemical Industries, Ltd); paprika color, hi orange LH (Daiwa Kasei Co., Ltd).

(b) *Native lac color preparation.*—Extract from stick lac as described in ref. 8.

(c) *Ethereal diazomethane.*—Prepare as described in refs. 18 and 19. *Caution:* Diazomethane is a highly toxic carcinogen and, under some conditions, explosive. Carefully prepare ethereal diazomethane in fume hood. Wear heavy gloves to prevent skin contact, and work behind safety screen.

(d) *TLC plates.—(1) Silica gel 60 F_{254}.*—20 × 20 cm, 0.25 mm layer thickness (E. Merck), chloroform–methanol–water (90 + 10 + 1) solvent. *(2) Cellulose plates.*—20 × 20 cm, 0.1 mm layer thickness (E. Merck), *n*–butanol–ethanol–10% acetic acid (6 + 1 + 3) solvent.

(e) *Column resin.*—Amberlite XAD-2 (Rohm and Haas Co., Philadelphia, PA 19105). Prewash resin with 10-fold volume methanol, 0.3M methanolic oxalic acid (0.3M oxalic acid in methanol), and methanol, successively. Keep prewashed resin in methanol.

(f) *Food.*—Red or orange colored milk beverage, tomato ketchup, jam, jelly, and candy with a label stating that they do not contain synthetic color additives.

(g) *LD-1 and LD-2 standard solutions.*—Prepare 25 μg LD-1 and 30 μg LD-2/mL, 50 μg LD-1 and 60 μg LD-2/mL, and 125 μg LD-1 and 150 μg LD-2/mL as 3 pairs of methanol

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[1] For parts I and II, see refs 9 and 10, respectively.

solutions of LD-1 and LD-2 standards. LD-1 and LD-2 standard solutions were prepared by using one of the commercial lac color preparations.

(h) *Native lac color solution.*—Prepare native lac color solution in water by using native lac color preparation. E% value of solution should be 0.50 at 495 nm after 5-fold dilution with water.

Methylation of Lac Color Preparation

Carry out methylation in fume hood. Accurately weigh 2 mg each of commercial and native lac color preparations, and dissolve in 10 mL tetrahydrofuran. Add 0.5 mL ethereal diazomethane, and let this solution stand 5 min at room temperature. Concentrate reaction mixture by evaporation, redissolve concentrate in 10 mL chloroform, and wash with 10 mL water. Evaporate lower phase to dryness after dehydration with anhydrous sodium sulfate. Then, redissolve concentrate in methanol, and dilute volume to exactly 5.0 mL for examination by TLC and LC.

Amberlite XAD-2 Column Cleanup

Place prewashed 30 mL Amberlite XAD-2 in 2.4 × 30.0 cm id glass column fitted with absorbent cotton. Wash column with 100 mL methanol followed by 100 mL water. Load 2 mL native lac color solution on top of column. Develop column with 100 mL each of water, methanol, and methanolic acid (0.1–300mM HCl in methanol, 1–1000mM oxalic acid in methanol, 1000–3000mM acetic acid in methanol, 3–1000mM malonic acid in methanol, or 100–1000mM glycolic acid in methanol), successively. Evaporate methanol and methanolic acid fractions, and lyophilize water fraction. Dissolve concentrate from methanolic acid fraction in 30 mL *n*-butanol, wash with 30 mL water to remove any acid, and then concentrate upper phase under reduced pressure. Redissolve concentrate in water, and dilute volume to exactly 5 mL. Determine absorbance at 495 nm by using spectrophotometer, and calculate percentage recovery by comparing with untreated standard solution.

Recovery of Lac Color from Model Milk Beverage

Spike 2 mL native lac color solution in 50 mL non-colored milk beverage, and stir 1 h at room temperature. Freeze milk beverage at −70°C, and lyophilize it by using freeze dryer. Extract residue with 100 mL 0.3M methanolic oxalic acid. Remove sediment by filtering under suction. Wash filtrate with 100 mL *n*-hexane. Evaporate lower phase to dryness. Use 5 mL water to redissolve concentrate. Then, load it on Amberlite XAD-2 column. Wash column with 100 mL water followed by 100 mL methanol. Elute lac color with 100 mL 0.3M methanolic oxalic acid. Follow procedure as previously described in *Amberlite XAD-2 Column Cleanup.* Calculate recovery at 495 nm by using spectrophotometer.

Detection of Lac Color from Commercially Available Foods

For tomato ketchup, weigh 50 g sample, extract it with 200 mL 0.3M methanolic oxalic acid. Wash filtrate with 200 mL *n*-hexane, and remove methanol under reduced pressure. Redissolve concentrate in 5 mL water, and pass through Amberlite XAD-2 column. Then, wash column with 100 mL each of water and methanol, successively. Elute lac color with 100 mL 0.3M methanolic oxalic acid, and concentrate eluate with rotary evaporator. Redissolve concentrate in 30 mL *n*-butanol, and wash with 30 mL water. Remove *n*-butanol under reduced pressure, and methylate concentrate as previously described under *Methylation of Lac Color Preparation.* Redissolve reaction mixture in methanol, and dilute volume to exactly 3.0 mL for examination by TLC and LC.

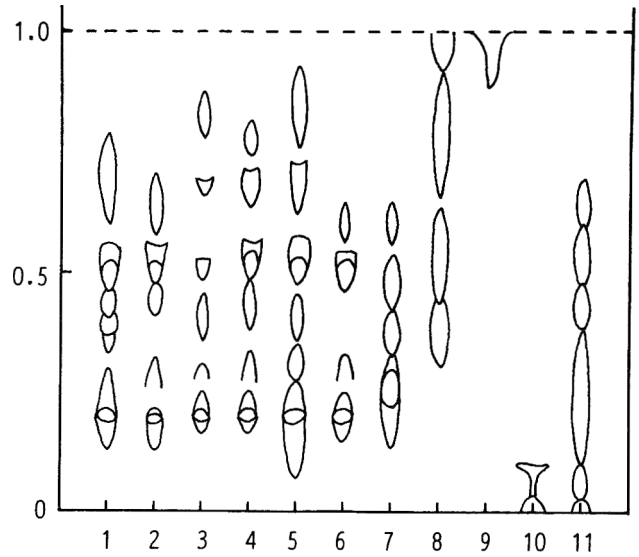

Figure 1. TLC chromatogram of intact natural red colors: 1–4, lac color from different manufacturers; 5, lac color prepared in the laboratory; 6, laccaic acid from commercial source; 7, cochineal color; 8, monascus color; 9, paprika color; 10, beet red color; and 11, corn color. Merck TLC plate cellulose. Solvent, *n*-butanol-ethanol-10% acetic acid (6 + 1 + 3).

For jam, jelly, and candy, weigh 50 g sample, dissolve in 100 mL 0.3M oxalic acid solution. Remove any insoluble matter by using filtration. Add 100 mL *n*-butanol, shake vigorously, and let phases separate. Then, concentrate upper phase to dryness. Redissolve concentrate in 5 mL water, and purify through Amberlite XAD-2 column as previously described. Prepare 3.0 mL sample solution in methanol for examination by TLC and LC. For milk beverage, extract, purify, and methylate as previously described, except use 100 mL sample. Finally, prepare 3.0 mL sample solution in methanol.

Quantitation

Inject 10 μL each of the 3 LD-1 and LD-2 standard solutions into liquid chromatograph. Construct calibration curves by plotting spectrophotometric response (peak height or peak area of integrator) vs amount of LD-1 or LD-2. Inject 5 or 10 μL sample solution. Calculate amount of LD-1 and LD-2 in sample solution from calibration curves. Represent concentration of lac color added to food as LD-1 and LD-2.

Results and Discussion

Several methods (11–17) for the detection of lac color in food have been reported; most use cellulose TLC or paper partition chromatography (PPC) and intact lac color as a spotting material. However, these methods are unsuitable because lac color cannot be clearly separated from other color additives on the chromatogram. Figure 1 shows a cellulose chromatogram of 5 commercial lac color preparations, one native lac color preparation, and some other natural red color preparations. More than 5 reddish-orange spots were observed from the native and commercial lac colors. The color of the spot at R_f 0.5 was more intense than that of the others. It was derived from 4 commercial preparations (see spots 1, 2, 4, and 6 in Figure 1) and one native preparation (see spot 5 in Figure 1) but not from one of the commercial preparations (see spot 3 in Figure 1).

Cochineal color yielded a chromatogram similar to that

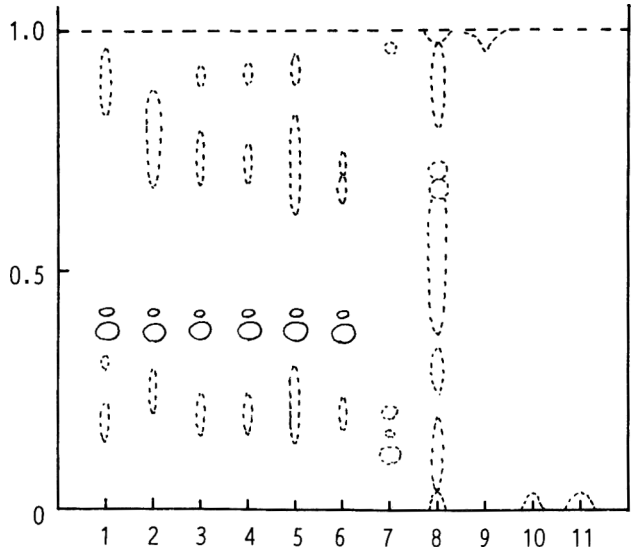

Figure 2. TLC chromatogram of reaction products of natural red colors methylated with ethereal diazomethane: numbering is the same as for Figure 1. Merck TLC plate silica gel 60 F_{254}. Solvent, chloroform–methanol–water (90 + 10 + 1).

for lac color in spot hue and pattern (see spot 7 in Figure 1); however, other natural red color yielded different chromatograms (see spots 8, 9, 10, and 11 in Figure 1).

It was quite difficult to judge the addition of lac color on the basis of R_f values on a cellulose plate because of the appearance of several spots from the lac color preparation and their nonuniformity on the plate. Considering chemical structures of color constituents (laccaic acids) in stick lac, it seemed that lac color could be more easily detected on a TLC plate by masking polar functional groups such as carboxyl and phenolic hydroxyl groups with a derivatizing reagent. Products of lac color methylated with ethereal diazomethane in tetrahydrofuran were examined, and their chromatogram is shown in Figure 2. Two conspicuous reddish-orange spots at R_f 0.41 (LD-1) and R_f 0.37 (LD-2) were observed when reaction products were developed on a silica gel plate with a chloroform–methanol–water (90 + 10 + 1) solution as the solvent. Both spots were derived from every commercial lac color (see spot 1, 2, 3, 4, and 6 in Figure 2) as well as the native lac color (see spot 5 in Figure 2). Therefore, the addition of lac color to food can be confirmed by the detection of LD-1 and LD-2 as marker species of lac color.

The methylated products are extracted not only from commercially available preparations but also from stick lac; therefore, they are not artifacts produced during the preparation of lac color but are inherent constituents in the stick lac. No compounds from other natural red color preparations had the same R_f values as either LD-1 or LD-2 when they were treated with the same procedure. As to reaction solvents, tetrahydrofuran gave better results than methanol, ethanol, and acetonitrile for the production of LD-1 and LD-2.

Table 1. Quantitation of LD-1 and LD-2 in lac color preparations[a]

	Source[b]	LD-1, mg/g	LD-2, mg/g
1	Commercial	97.77	487.26
2	Commercial	90.03	411.70
3	Commercial	100.55	386.75
4	Commercial	110.33	427.57
5	Native	107.71	492.51
6	Commercial	92.90	386.07

[a] Each value is the mean of triplicate determinations.
[b] Numbering is the same as for Figure 1.

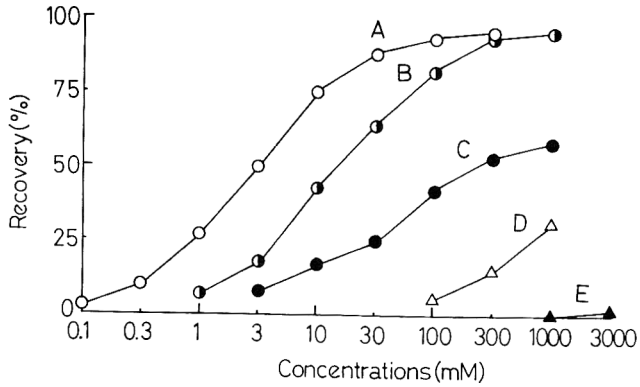

Figure 3. Effect of acids added to methanol on elution of lac color from an Amberlite XAD-2 column: A, methanolic HCl; B, methanolic oxalic acid; C, methanolic malonic acid; D, methanolic glycolic acid; and E, methanolic acetic acid. Each symbol represents mean of triplicate experiments.

An Amberlite XAD-2 column was used for the purification of lac color in the extract from food. Acid was added to the eluant because the color was not eluted with water, methanol, ethanol, or acetone. The effect of HCl, oxalic acid, malonic acid, glycolic acid, acetic acid added to methanol is shown in Figure 3. Recoveries greater than 95% were obtained by the addition of HCl or oxalic acid to methanol at a concentration of >0.3M (see curves A and B in Figure 3). Sixty and 35% of the color were eluted by the addition of 1M malonic and 1M glycolic acids, respectively (see curves C and D in Figure 3); however, acetic acid in excess of 3M concentration exhibited little effect on the elution of the color from a column (see curve E in Figure 3). Although recovery was quantitative for both HCl and oxalic acid added to methanol, oxalic acid was used in all experiments because irreversible transformation of color occurred during handling with HCl.

Recovery of color spiked in non-colored milk beverage ranged from 75 to 80% for 5 analyses. In addition, there were few differences of the spectra in the ultraviolet and visible regions between spiked and recovered lac color solutions. The elution profile of color from the column was not influenced by the oxalic acid used during the extraction stage.

One jelly treated according to the procedure described, yielded 2 conspicuous vermilion spots on a silica gel plate. Figure 4 shows LC chromatograms of the marker species, reaction products from a commercial lac color preparation, and the jelly. In the chromatograms of the commercial preparation and the jelly, 2 peaks elute with the same retention times as the marker species. It was confirmed that lac color was added to this jelly.

The detection limit of marker species was 100 ng for each under these operating conditions. The quantitation of LD-1 and LD-2 in lac color preparations and food is summarized in Tables 1 and 2, respectively. Although the ratio of content of LD-1 to LD-2 for individual lac color preparations was not constant (Table 1) because lac color is a naturally occurring constituent, the concentration of the former was always lower. If LD-1 is in excess of 0.5 μg/g food, we can

Table 2. Quantitation of LD-1 and LD-2 in jelly[a]

LD-1, μg/g	LD-2, μg/g
1.92	7.60
0.57	1.72
3.99	8.53

[a] Each value is the mean of triplicate determinations.

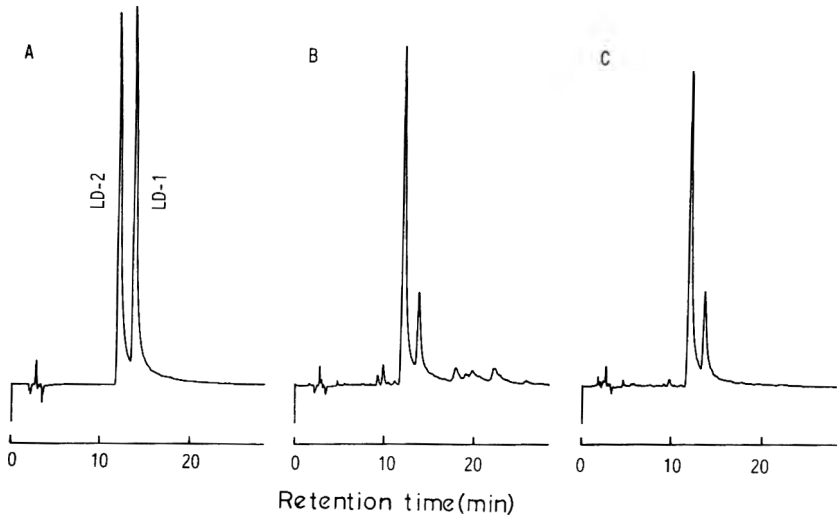

Figure 4. LC chromatograms of reaction products methylated with ethereal diazomethane: A, isolated LD-1 (125 μg/mL) and LD-2 (150 μg/mL); B, commercial lac color preparation (2 mg origin/5 mL); and C, jelly (50 g origin/3 mL). Injection volume, 10 μL.

observe definitely identifiable peaks of LD-1 and LD-2 by the proposed methodology.

Both marker species have been isolated and crystallized as reddish-orange needles by using one of the commercial lac color preparations. Many methylated derivatives of laccaic acids have been prepared to deduce the chemical structures of laccaic acids (1–7). However, our marker species are not identical to any methylated laccaic acids on the basis of physico-chemical characteristics (e.g., crystalline color, melting point, and nuclear magnetic resonance spectrum). Species must depend on the methylating reagent and reaction conditions. Studies on the chemical structures of the marker species are now in progress, and the details will be reported elsewhere.

Conclusion

This project demonstrated the applicability of the proposed method for detection of lac color in food. The TLC method offers a useful alternative for the qualitative determination of lac color. The LC method includes great selectivity, little interference by ingredients in food with the interpretation and measurement of the chromatographic peaks for LD-1 and LD-2, and ability to provide the information on quality control of natural coloring matters.

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VEGETABLE PRODUCTS

Liquid Chromatographic Determination of Acids and Sugars in Homolactic Cucumber Fermentations

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A rapid, high yield, and quantitative method for determination of sugars (glucose and fructose) and acids (malic, lactic, acetic, and citric) in fresh and fermented cucumber juices is described; the procedure solves the fructose and malic acid coelution problem. Samples are prepared by passing the juice directly through a disposable C_{18} cartridge. Compounds are separated by liquid chromatography on an Aminex HPX-87H chromatographic column, with 0.013N H_2SO_4 as mobile phase in the isocratic mode at 0.6 mL/min flow rate and 60°C column temperature. Compounds are then detected and quantitated by using ultraviolet and refractive index detectors connected in series, and comparing with succinic acid as internal standard. Values for both fresh and fermented cucumber juices were in good agreement with literature data.

The lactic fermentation process is a traditional vegetable conservation method. Recently, this process has been considerably improved by using controlled fermentation. Several studies (1–3) have shown the advantages of using *Lactobacillus plantarum* as a single starter in cucumber fermentation.

L. plantarum is an homofermentative microorganism which metabolizes low molecular weight sugars to produce lactic acid (1). Moreover, it can also metabolize malic acid, yielding lactic acid and CO_2 (4, 5).

The fermentable sugar content of cucumber is about 2% on a fresh weight basis, and consists essentially of glucose and fructose and traces of sucrose (6–8). Cucumber also contains malic and citric acids at concentrations ranging from 0.2 to 0.3% and from 0.023 to 0.035%, respectively, on a fresh weight basis (9).

Rapid acid production is essential for lowering pH and, thus, inhibiting the growth of undesirable bacteria during the initiation stage of fermentation (10). Likewise, conversion of fermentable sugars to lactic acid is essential in cucumber fermentation to prevent the possible later growth of pathogenic microorganisms (which could produce disease in consumers) and secondary yeast fermentations (which could deteriorate the product) (11). Another compound to take into account in the homolactic fermentation is malic acid, because it can be decarboxylated and contribute to bloater damage (12). Therefore, it is important to have a rapid and accurate analytical method to control the evolution of sugars and acids during the fermentation process; in addition, such a method could help in the selection of the more suitable lactic acid bacteria as starter cultures in vegetable fermentations.

Liquid chromatography has been applied to analysis of major substrates and products found in vegetable fermentations. Salvo and coworkers (13) described an LC method to determine glucose and fructose in fresh fruit from 7 Cucurbitaceae. Andersson and Hedlund (14) published LC methodology to determine organic acids in fermented vegetables. They used an Aminex HPX-87 chromatographic column and pointed out that sugars may interfere in the analysis because retention times for some sugars and acids are the same. Thus, glucose and tartaric acid appear as one peak if present together, and fructose interferes with the determination of malic acid. Recently, Ross and Chapital (15) described the simultaneous determination, on the Aminex column, of carbohydrates, alcohols, and organic acids in fermentation mixtures metabolized by intestinal microflora. The separation was monitored by ultraviolet and refractive index detectors in series. However, interference problems were not apparent in their samples because of the absence of malic and tartaric acids. McFeeters et al. (16) described an LC procedure for the determination of major substrates and products that commonly occur in vegetable heterolactic fermentations. Due to the complexity of these samples, they resolved the coelution problems by using 2 chromatographic columns and a dual guard column system.

The present work describes a rapid and accurate analytical method for the simultaneous determination of sugars and organic acids in fresh cucumber, as well as during its homolactic fermentation. Samples are prepared by passing fresh or fermented juice directly through a Sep-Pak C_{18} cartridge. Compounds are separated on an Aminex HPX-87 chromatographic column in protonated form, and detected by using ultraviolet (UV) and refractive index (RI) detectors connected in series. This detector combination allows the quantitation of the coeluting components, fructose and malic acid, by using their individual responses to both detectors and a simple mathematical algorithm. This combination also takes advantage of the greater sensitivity of the ultraviolet detector for organic acids and that of the refractive index detector for sugars.

Experimental

Apparatus

(a) *Liquid chromatograph.*—Model 721 (Waters Associates, Barcelona, Spain), equipped with Model U6K injector (Waters), Model 510 high-pressure pump (Waters), and column oven and temperature controller (Waters).

(b) *Detectors.*—UV Model 490 (Waters), monitoring at 210 nm, and RI Model LC-25 (Perkin Elmer Hispania S.A., Madrid, Spain) connected in series.

(c) *Integrators.*—Model 730 data module (Waters) and Model 3390A (Hewlett-Packard Española S.A., Barcelona, Spain) connected to UV and RI detectors, respectively.

(d) *Chromatographic column.*—Aminex HPX-87H, 300 mm × 7.8 mm id (Bio-Rad Laboratories, Richmond, CA). Operating conditions: isocratic mode, column temperature 60°C; flow rate 0.6 mL/min; injection volume 20 μL.

(e) *Membrane filters.*—0.45 μm porosity (Millipore Iberica S.A., Barcelona, Spain) for mobile phase and 0.22 μm porosity for standards and samples.

(f) *Disposable cartridge.*—Sep-Pak C_{18} (Millipore) activated with 3 mL acetonitrile (LC grade) followed by 5 mL water.

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Reagents

(a) *Mobile phase.*—0.013N H_2SO_4 solution, filtered and vacuum-sonicated for 15 min before use.

(b) *Internal standard solution (IS).*—2 g succinic acid (E. Merck, Darmstadt, FRG) dissolved in 100 mL water.

(c) *Standards.*—D(+)-Glucose, D(−)-fructose, and citric acid (Merck). L(−)-Malic and L(+)-lactic acids (Sigma Chemical Co., St. Louis, MO).

Preparation of Cucumber Juice

Cucumbers (Hyclos variety) were obtained from Ramiro Arnedo firm. Fruits were washed with tap water, dipped for 1 h in 50 ppm chlorine solution, and then washed again with tap water. Each cucumber fruit (7–9 cm diameter) was cut into 1 cm thick slices and frozen at −18°C. Pieces were thawed and put into a wine press for juice extraction. Juice was collected, heated to boiling (2 min) to inactivate enzymes, cooled, filtered, and then frozen until use.

Preparation of Fermented Cucumber Juice

Juice was fermented by using, as starter culture, a strain of *Lactobacillus plantarum,* isolated from brined cucumber in our laboratory. Cucumber juice was acidified, buffered (0.16% acetic acid and 0.4% trihidrate sodium acetate), sterilized, and inoculated to a final concentration of ca 10^6–10^7 bacteria/mL. Fermentation was performed at 28°C for 7 days.

Sample Preparation

Three mL fresh or fermented cucumber juice was percolated through an activated disposable cartridge to remove pigments. The first 1 mL eluate was discarded; then 1 mL eluate was mixed with 1 mL IS solution and filtered into a screw-cap glass vial. If the sample was not injected on the same day, it was stored at −18°C.

Calibration Graphs

Because of overlapping peaks for fructose and malic acid from the Aminex HPX-87H column, calibration graphs were prepared using 2 different standard solution series, one containing only sugars plus internal standard and the other one containing only acids plus internal standard.

Four aqueous standard solutions were prepared containing glucose, fructose, and internal standard in the ratios 20:20:10, 8:8:10, 4:4:10, and 2:2:10 (mg/mL). Four aqueous standard solutions were prepared containing citric acid, malic acid, lactic acid, acetic acid, and internal standard in the ratios 0.44:3.02:24.72:3.99:10, 0.16:1.21:9.89:1.60:10, 0.08:0.60:4.94:0.80:10, and 0.04:0.30:2.47:0.40:10 (mg/mL). Then 1 mL of each one of these solutions was filtered and 20 μL was injected into the chromatograph. The peak heights relative to internal standard (succinic acid) were determined and plotted against the relative concentration of the component for both detectors. A straight line was fitted by least-square regression analysis.

Quantitation of Fructose and Malic Acid

Because fructose and malic acid coeluted from the Aminex HPX-87H, they could not be determined by using a single detector. We used 2 detectors connected in series. Since both substances contribute to the final height of the coalesced peaks from the 2 detectors, the following equations are established:

$$H_{UV} = H_{UV}\,(\text{fructose}) + H_{UV}\,(\text{malic})$$
$$H_{RI} = H_{RI}\,(\text{fructose}) + H_{RI}\,(\text{malic})$$

where H_{UV} is the height of the coalesced peak relative to the internal standard from the UV detector and H_{RI} is its counterpart from the RI detector.

Using the calibration graphs obtained for each substance on both detectors, the following final equations are obtained:

$$W_{(\text{fructose})} = W_{IS}(a_1H_{UV} + b_1H_{RI} + c_1)$$
$$W_{(\text{malic})} = W_{IS}(a_2H_{UV} + b_2H_{RI} + c_2)$$

where $W_{(\text{fructose})}$ and $W_{(\text{malic})}$ are the weights (mg) of fructose and malic acid in 1 mL cucumber juice and W_{IS} is the added weight (mg) of internal standard. The coefficients a_1, b_1, c_1, a_2, b_2, and c_2 are related to the parameters of the calibration graphs in the following way:

$$a_1 = m_1n_1m_2/n_1m_2 - m_1n_2$$
$$b_1 = -m_1n_1n_2/n_1m_2 - m_1n_2$$
$$c_1 = n_1m_2p_1 - m_1n_2d_1 + m_1n_1(p_2 - d_2)/n_1m_2 - m_1n_2$$
$$a_2 = m_1n_2m_2/m_1n_2 - n_1m_2$$
$$b_2 = -n_1m_2n_2/m_1n_2 - n_1m_2$$
$$c_2 = m_1n_2p_2 - n_1m_2d_2 + m_2n_2(p_1 - d_1)/m_1n_2 - n_1m_2$$

where m_1 and p_1 are the fructose slope and intercept of the calibration graph from the UV detector, respectively; n_1 and d_1 are their counterparts from the RI detector; m_2 and p_2 are the malic acid slope and intercept of the calibration graph from the UV detector, respectively; n_2 and d_2 are their counterparts from the RI detector.

To evaluate the accuracy of this quantitation, a set of 7 aqueous standard solutions was prepared containing fructose, malic acid, and internal standard in the ratios (mg/mL) 20.04:3.00:10.00, 8.01:1.20:10.00, 4.01:0.60:10.00, 2.00:0.30:10.00, 2.96:2.99:10.00, 2.96:1.46:10.00, and 1.46:2.95:10.00. Then, 1 mL of each of these solutions was analyzed under the described conditions.

Correlations of calculated values by the equations vs real amounts were studied by least-square linear regression.

Evaluation of Matrix Effect

To evaluate the effect of a typical sample matrix on accuracy and precision of the analysis for glucose, fructose, and malic and citric acids, these compounds were added to a fermented cucumber juice, which contained citric and lactic acids and a small amount of fructose and malic acid, but no glucose. A set of 4 samples was prepared by adding the tested compounds (glucose, fructose, citric acid, malic acid) to 50 mL fermented cucumber juice in different amounts: 2.0:2.0:0.088:0.30, 0.8:0.8:0.035:0.12, 0.4:0.4:0.018:0.06, and 0.2:0.2:0.009:0.03 g. Since the fermented cucumber juice contains citric acid and a small amount of fructose and malic acid, a blank was considered.

To evaluate the matrix effect on lactic and acetic acids, these compounds were added to fresh cucumber juice which contained no lactic and acetic acids. A set of 4 samples was prepared by adding to 50 mL fresh cucumber juice different ratios of lactic and acetic acids: 2.50:0.67, 1.25:0.33, 0.50:0.13, and 0.25:0.07 g, respectively.

Samples and the blank were handled as indicated in sample preparation and each of these was injected 9 times.

Amounts of glucose in the spiked samples were calculated by using RI detector data. Amounts of citric, lactic, and acetic acids were calculated by using UV detector data. Amounts of fructose and malic acid were calculated from data from both detectors, using the deduced equations.

Correlations of analyzed vs added amounts of individual compounds were studied by least-square linear regression.

The reproducibility of the chromatographic method was

Table 1. Data for detector responses to aqueous calibration standards

Compound and detector	Slope	Intercept	Correlation coefficient (r)[a]
Citric acid RI	0.9753	−0.0036	0.9968
Citric acid UV	0.4627	−0.0008	0.9992
Glucose RI	0.6907	0.0032	0.9999
Malic acid RI	0.9362	−0.0097	0.9995
Malic acid UV	0.7056	−0.0095	0.9999
Fructose RI	0.6617	−0.0017	0.9999
Fructose UV	12.5004	0.0032	0.9991
Lactic acid RI	1.1622	−0.0249	0.9993
Lactic acid UV	0.8368	−0.0215	0.9999
Acetic acid RI	1.8157	−0.0022	0.9998
Acetic acid UV	1.1719	−0.0032	0.9999

[a] Correlation coefficient values were significant at 99% ($P < 0.01$).

studied over 30 determinations of each substance in fresh and fermented cucumber juices.

Results and Discussion

Standard Calibration Graphs

All tested compounds except glucose absorbed at 210 nm. Thus, glucose has been determined from the RI detector response. As can be seen in Table 1, the response of the RI detector to fructose is much greater than that of the UV detector; however, fructose absorbed appreciably at 210 nm. On the other hand, all UV slopes of the organic acids were lower than those obtained from the RI detector (Table 1). This was in agreement with other reports (15, 17). Thus, with these 2 detectors connected in series, it was possible to determine the sugars and organic acids simultaneously and take advantage of the greater detection level for organic acids by the UV detector. Intercepts (Table 1) were nearly zero and the correlation coefficients were close to unity in the concentration range studied.

Evaluation of Analytical Method and Resolution

Andersson and Hedlund (14) reported the analysis of organic acids in lactic acid-fermented vegetables by using LC separation on an Aminex HPX-87 column. They found that fructose and malic acid eluted with the same retention time; therefore, only organic acids and non-interfering sugars could be determined at the same time. An interference problem with ethanol noted by McFeeters and coworkers (16) did not occur with this column because ethanol was clearly separated from the major fermentation constituents (Rt = 22.5 min). In the present study, the fructose and malic acid are simultaneously determined using 2 detectors connected in series as described above. The quantitation is carried out by the deduced equations, for which the following coefficients were found: $a_1 = -0.52$, $b_1 = 0.69$, $c_1 = -0.002$, and $a_2 = 0.73$, $b_2 = -0.04$, $c_2 = -0.009$.

The accuracy in the quantitation of both compounds was checked by analyzing 7 sets of aqueous solutions containing different quantities of fructose and malic acid.

An ideal analysis should result in a linear relationship between real content and analyzed values with a slope of one and an intercept of zero. The actual relationship was linear with high correlation coefficients (r = 0.9994 for fructose and r = 0.9957 for malic acid) and statistically significant ($P < 0.01$); both slopes (1.018 and 1.048 for fructose and malic acid, respectively) were not significantly different from one, and the intercepts (0.121 and −0.103 for fructose and malic acid, respectively) were not significantly different from zero.

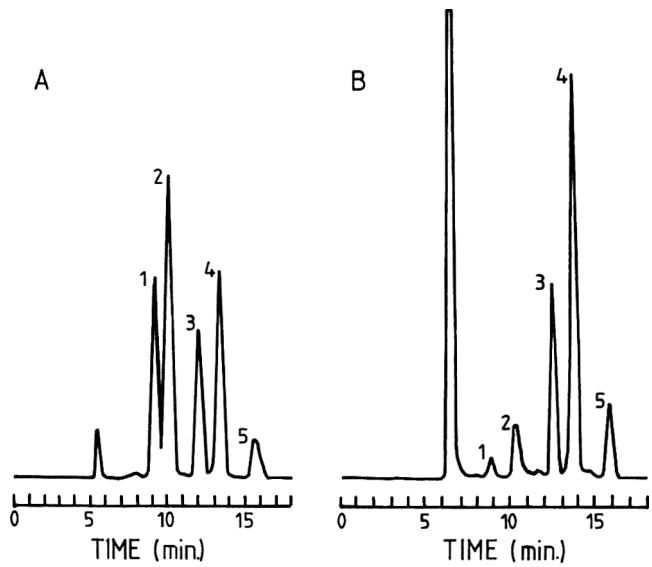

Figure 1. Chromatograms of fermented cucumber juice spiked with glucose, fructose, and citric, malic, succinic, and acetic acids. (A) Refractive index detection: 1, glucose; 2, fructose and malic acid; 3, succinic acid; 4, lactic acid; 5, acetic acid. (B) Ultraviolet detection (λ = 210 nm): 1, citric acid; 2, fructose and malic acid; 3, succinic acid; 4, lactic acid; 5, acetic acid.

These results confirm that both substances contribute in an additive way to the response of the 2 detectors within the range of the tested amounts.

Figure 1 shows the chromatograms of fermented cucumber juice spiked with glucose, fructose, and malic, citric, acetic, and succinic acids obtained from the RI (Figure 1A) and UV (Figure 1B) detectors and the Aminex HPX-87H column. Resolution was complete for all compounds with the exception of fructose and malic acid as mentioned above. Succinic acid was an optimum internal standard because it appears in the middle of the chromatogram without any interference, the response of both UV and RI detectors is excellent (Figure 1), and it does not occur in cucumber homolactic fermentation. Fleming and coworkers (18) also indicated too that only lactic acid was obtained as a product in homolactic fermentations. However, if any heterolactic bacteria growth occurs, mannitol may be formed. In this case, and in anaerobic conditions, *L. plantarum* could ferment the mannitol, using citric acid as an electron acceptor, to yield succinic acid and ethanol (19). Although this reaction did not normally occur in cucumber homolactic fermentation, this possibility must be considered by the analyst.

Matrix Effect

Table 2 summarizes the results obtained when detected values are compared with added weights by least square

Table 2. Regression analysis of analyzed vs added compounds in fresh and fermented cucumber juices

Compound	Intercept	Slope[a]	Correlation coefficient (r)
Glucose[b]	−0.02	1.02	0.992
Fructose[b]	0.03	1.06	0.989
Malic acid[b]	0.00	1.24**	0.989
Citric acid[b]	0.00	1.11*	0.982
Lactic acid[c]	−0.02	1.02	0.999
Acetic acid[c]	−0.01	1.03	0.994

[a] Slope is statistically significantly different from expected 1.00. (* $P < 0.05$ and ** $P < 0.01$.)

[b] Added to fermented cucumber juice.

[c] Added to fresh cucumber juice.

Table 3. Composition of sugars and organic acids in cucumber and reproducibility of chromatographic method

Compound	Content, %[a]	CV, %[a]	Literature data, %	Ref.
Glucose[b]	1.063 ± 0.025	6.2	1.14 ± 0.03	(6)
			1.0–1.2	(7)
Fructose[b]	1.052 ± 0.026	6.8	1.21 ± 0.02	(6)
			1.0–1.2	(7)
Malic[b]	0.236 ± 0.005	5.4	0.28–0.40	(9)
Citric[b]	0.025 ± 0.001	14.9	0.023–0.035	(9)
Lactic[c]	1.611 ± 0.026	4.3	1.16–1.20	(18)

[a] Based on 30 determinations in the same sample. Mean values and confidence interval ($P < 0.05$).

[b] Analyzed in fresh cucumber.

[c] Analyzed in fermented cucumber.

regression. The relationship between both weights was linear in all cases with high correlation coefficients ($r > 0.98$) and statistically significant ($P < 0.01$) in the concentration range studied. The intercept values were not significantly different from zero for all analyzed substances ($P < 0.05$). The glucose, fructose, and lactic and acetic acid slopes were not significantly different from 1 ($P < 0.05$). The slopes were significantly different from the expected value for the citric ($P < 0.05$) and malic acid ($P < 0.01$). The deviation from 1 of the citric and malic acid slopes coincides with the least linearity in the studied correlation. This might be due to their low content in the juice.

Application

Sugars and organic acids in fresh cucumbers and lactic acid in fermented cucumbers. — The values of sugar and major acid contents in fermented cucumber were in excellent agreement with published data (6, 7, 9, 20) (Table 3).

In the case of lactic acid, the comparison was more difficult because the strain employed for fermentation (*L. plantarum* isolated from brined cucumber in our laboratory) was different from that used by other authors.

The chromatographic method reproducibility (Table 3) was good in all cases. The variation coefficients were less than 7% except for citric acid. The very low content of citric acid (0.025%) in the analyzed samples explains its higher variation coefficient.

Conclusions

LC separation, using a strong cation-exchange column in the hydrogen form for the determination of sugars and organic acids that commonly occur in homolactic fermentation of cucumbers, was investigated. The simultaneous determination of glucose, fructose, and malic, citric, and lactic acids by using RI and UV detectors connected in series was possible. All the tested compounds eluted within 16 min. Considering how little sample preparation is required, the proposed procedure can be considered to be a rapid method. However, further studies are necessary for simultaneous analyses of major substrates and products in heterolactic vegetable fermentations.

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TECHNICAL COMMUNICATIONS

Application of Near Infrared Reflectance Spectroscopy to Determination of Fat in Cheddar Cheese

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Near infrared reflectance (NIR) spectroscopy was used to determine the fat content of Cheddar cheese. Through multiple linear regression, calibrations were developed for use with a commercial filter monochromator instrument. A 6-wavelength calibration, using 1734 nm as the primary indicator wavelength, was found to perform satisfactorily. Averaging readings from duplicate sample repacks reduced the standard error of performance (SEP) compared with the use of single readings. For a validation set of 23 samples, the correlation coefficient squared (r^2) between the NIR and Roese-Gottlieb methods was 0.92, with an SEP of 0.44%. Both sample temperature and age of the cheese influenced the NIR response.

Fat content is an important quality parameter for Cheddar and other hard cheeses. Current procedures for measuring fat in cheese usually involve a modification of the Babcock procedure, or solvent extraction as in the Roese-Gottlieb method. When used with solid dairy foods such as cheese, the Babcock procedure frequently lacks the degree of accuracy needed. Solvent extraction methods, although more accurate and reproducible, are quite time consuming. Therefore, rapid methods that can reliably measure the fat content of hard cheeses are urgently needed.

Near infrared reflectance (NIR) spectroscopy has recently been applied to the analysis of various components in solid dairy foods (1–3), including the fat content of cream, dried whole milk powders, and yogurt (3, 4). Frank and Birth (5) used a research instrument based on a Cary 14 scanning monochromator to measure fat and other constituents in cheese. They determined that NIR analysis has potential for successfully measuring the fat content of cheese, although results obtained from freeze-dried samples were much better than results obtained from samples containing full moisture. Ereifej and Markakis (6) also obtained a good correlation relating NIR readings to the fat content of commercial dried cheese powders. Frankhuizen and van der Veen (7) used a commercially available filter monochromator NIR instrument to measure fat and other constituents in several dairy products, including European-style soft cheeses. Using this system, fat contents of Edam and Gouda cheeses were successfully predicted, with correlations between NIR and gravimetric data equal to or greater than 0.9

On the basis of these results, there is significant interest in using NIR spectroscopy for analyzing American-style hard cheeses. Previous work in our laboratory has shown that a commercial NIR instrument can be successfully used to determine the moisture content of Cheddar cheese (8). The purpose of the present work was to evaluate the use of such a filter monochromator NIR spectrometer to measure the fat content of Cheddar cheese.

Experimental

Selection of Samples

A 40-member sample set was obtained for use in developing NIR calibrations for measuring fat in Cheddar cheese. Forty samples of mild, medium, and sharp Cheddar cheeses were collected from several different commercial processors and the University of Nebraska dairy processing plant. Samples were selected to maximize the range of aging times included in the calibration and to provide a uniform distribution of fat contents. An independent validation set was also obtained for testing the calibrations. This set included 23 samples of mild, medium, and sharp Cheddar cheeses, again representing several different commercial processors.

Determination of Fat by Solvent Extraction

Grate cheese sample so that individual shreds are ca 1 mm diameter × 20 mm long, and mix thoroughly. Determine fat content by using a modification of the Roese-Gottlieb extraction procedure, according to AOAC method **16.284** (9).

Spectroscopic Measurement

The technique for preparing cheese samples for spectroscopic measurement has been previously described (8). Samples are equilibrated to 25°C prior to placing in the instrument. Reflectance measurements, expressed as log (1/R), are obtained from the samples at 10 wavelengths using a Technicon InfraAlyzer® 300C NIR spectrometer (Technicon Industrial Systems, Tarrytown, NY). A reverse stepwise linear regression is then applied to the data to select optimum wavelengths and corresponding calibration constants for predicting fat content. The instrumentation and calibration procedures have also been previously described in detail (8).

Results and Discussion

Both the calibration and validation sample sets were selected to be representative of Cheddar cheese normally encountered in commerce, and to provide the maximum degree of diversity within those limits. Samples from the University of Nebraska dairy plant were added to the calibration set to ensure inclusion of an adequately wide range of aging times. The fat contents of samples in the calibration set ranged from 29.8 to 35.5%; samples in the validation set contained from 29.9 to 34.6% fat.

The sample handling technique previously found to provide reliable results for NIR moisture measurement (8) also worked well for the fat determination. On the basis of results of the reverse stepwise linear regression, calibrations utilizing 6, 5, or 4 wavelengths appeared to have potential for measuring fat (Table 1). The coefficients of multiple determination (R^2) for the 6, 5, and 4 wavelength calibrations are 0.891, 0.885, and 0.870, respectively. Inclusion of more than 6 wavelengths in the calibration did not measurably improve the correlation statistics. It is desirable to minimize the number of wavelengths in an NIR calibration, so calibrations utilizing more than 6 wavelengths were not evaluated further. Inclusion of less than 4 wavelengths resulted in a significant degradation of the correlation statistics; therefore, calibrations with fewer than 4 wavelengths received no additional testing.

For each of the 3 calibrations subjected to further evalu-

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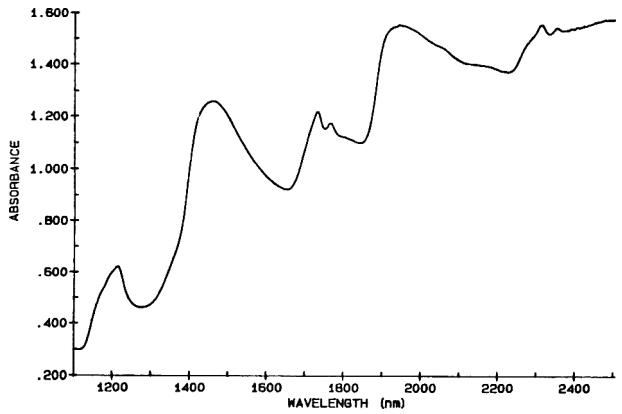

Figure 1. Near infrared reflectance spectrum of typical Cheddar cheese sample. Ordinate values are expressed as log (1/R).

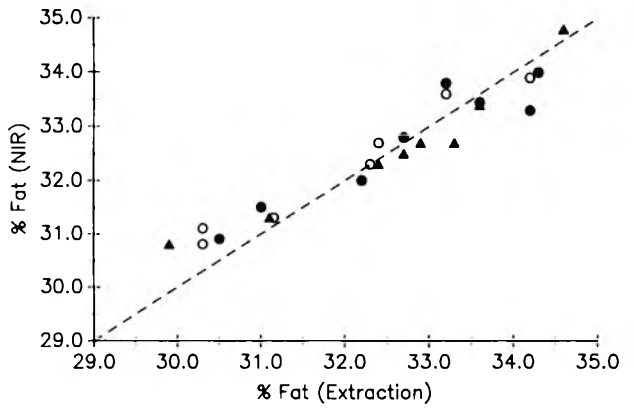

Figure 2. Scatter diagram comparing fat contents of 23 commercial Cheddar cheese samples, as determined by NIR, with values determined for those samples by Roese-Gottlieb solvent extraction procedure: mild (○), medium (▲), and sharp (●) Cheddar cheeses.

ation, the wavelengths selected by the regression program on the basis of statistical significance coincide with absorption bands of importance in the spectrum of cheese (Figure 1). Each calibration in Table 1 uses 1734 nm as the primary indicator wavelength for measuring fat. This wavelength responds to an absorption band that arises from the first overtone of a —CH stretching motion in the methylene groups of the fatty acids (10). The 1734 nm wavelength remains the primary indicator wavelength even if measurements at 2310 nm are included in the calibration. The 2310 nm wavelength responds to an intense absorption band that is associated with the lipids in a sample, and arises from a combined —CH stretch and deformation. However, because of its high content of water and lipid, cheese absorbs radiation above 1900 nm very strongly. In regions of such strong absorption, changes in reflectance are frequently not linear with changes in concentration, thus the reason for using the 1734 nm wavelength. All of the calibrations include measurements at 1940 nm and/or 1445 nm. These 2 wavelengths respond to absorption bands associated with the —OH groups of water. Because these 2 water bands are dominant features in the NIR spectrum of cheese (Figure 1), the influence of water must be taken into account in any calibration used for measuring fat.

The 3 calibrations listed in Table 1 were tested using the 23 member validation sample set. In contrast with previous experience with NIR moisture measurement (8), averaging readings from duplicate packs of each sample measurably reduced the standard error of performance (SEP) of NIR fat determination, reducing the SEP obtained with the 6 wavelength calibration from 0.56 to 0.44%. This indicates that the NIR fat determination is more susceptible to sample heterogeneity and packing variability than is the moisture determination. Therefore, in evaluating the fat calibrations, readings from duplicate packs of each sample were averaged to obtain the NIR value. The cheese from each sample pack was then analyzed in duplicate by the Roese-Gottlieb procedure, and these 4 determinations were averaged to obtain the reference value.

Based on the 23 member validation set, the correlation coefficients squared (r^2) between the Roese-Gottlieb method and the 6, 5, and 4 wavelength calibrations were 0.92, 0.90, and 0.83, respectively. The standard errors of performance were 0.44, 0.49, and 0.60%, respectively. Inclusion of additional wavelengths in the calibration allowed the instrument to better compensate for overall spectral variation. Figure 2 shows a comparison of the NIR data obtained with the 6 wavelength calibration with the data obtained by the classical method. The NIR results indicate that the mild Cheddar samples have a +0.2% bias compared with the extraction values, while the medium and sharp Cheddar samples show no significant bias. This indicates that lipolysis and other changes that occur during aging may affect the reflectance properties of cheese. While the observed differences were not large, results might be improved by developing separate calibrations for cheeses with short aging times.

The data in Figure 2 further indicate that the NIR results for lower fat (<31%) samples are consistently high. This occurs even though samples in this range were included in the calibration set. Preliminary regression results with a limited number of lower fat samples indicate that narrower range calibrations can be developed which will more accurately predict samples in the 29–31% fat range. Perhaps samples can be analyzed using the broad range calibration, and those shown to have a lower fat content can then be reanalyzed using a calibration optimized for the lower range, thereby obtaining more accurate results.

Our previous work showed that temperature changes affected the NIR moisture determination (8). Since measurements that respond to changes in the water bands are included in the fat calibrations, it can be expected that temperature fluctuations will affect the reliability of the NIR fat determination as well. To test the effect of temperature, the fat content of a Cheddar cheese sample was determined at several different temperatures using the 6 wavelength NIR calibration. For a 12°C increase in temperature, the apparent

Table 1. Filter locations and corresponding coefficients for 6, 5, and 4 wavelength calibrations used in determining fat content of Cheddar cheese

Filter location	Wavelength, nm	Constants 6λ	5λ	4λ
Intercept:[a]		39.34	37.13	48.48
01	2208			
02	2310	−25.81		
03	2139			
04	1818			
05	2180			
06	1734	179.88	153.41	142.15
07	2100	−40.27	−54.40	−26.81
08	1940	33.56	20.18	
09	1445	−58.91	−48.27	−48.29
10	1680	−100.12	−79.33	−83.74

[a] Intercept value will vary among instruments and must be determined for each specific instrument.

fat content of the sample, as measured by NIR, decreased by over 1%. Because of this temperature dependency, the precautions used for measuring moisture in cheese by NIR must also be applied to the fat determination, including equilibrating all samples to a uniform temperature prior to analysis. Also, samples used to calibrate the instrument must be equilibrated to the same temperature as those samples that are to be predicted.

In conclusion, the results of this study indicate that NIR spectroscopy has the potential for successful, rapid measurement of fat in American-style hard cheeses. Although the agreement between the classical and NIR fat determination methods is not as close as the agreement previously obtained for the moisture determination, it appears that with appropriate precautions the technique can be used successfully.

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461	462	463	464	465	466	467	468	469	470
471	472	473	474	475	476	477	478	479	480
481	482	483	484	485	486	487	488	489	490

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- ☐ A. Food
- ☐ B. Feeds
- ☐ C. Fertilizers
- ☐ D. Pharmaceuticals
- ☐ E. Cosmetics
- ☐ F. Environment
- ☐ G. Veterinary and/or animal science
- ☐ H. Forensics
- ☐ I. Toxicology
- ☐ J. General Analytical Chemistry
- ☐ K. General Microbiology
- ☐ L. Medical
- ☐ M. Other

PRIMARY JOB FUNCTION:
- ☐ A. Laboratory Director/Manager
- ☐ B. Laboratory Scientist
- ☐ C. Purchasing
- ☐ D. Sales/Service/Mkt
- ☐ E. Other_____

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COLLEGE/UNIVERSITY
- ☐ E. Research/Development
- ☐ F. Teaching

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Liquid Chromatographic Determination of Ivermectin in Bovine Serum

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U.S. Department of Agriculture, Agricultural Research Service,
U.S. Livestock Insects Laboratory, PO Box 232, Kerrville, TX 78029-0232

A rapid, sensitive method is described for the determination of ivermectin concentrations in bovine serum. Ivermectin is extracted by passing a sample through a reverse-phase C_{18} cartridge. A silica-packed cartridge is used to purify the extract further. Ivermectin is quantitated by liquid chromatography with detection at 245 nm. Recoveries were 95 ± 4% for samples fortified with 20 ppb ivermectin. Concentrations as low as 2 ppb can be detected in 5 g samples.

Ivermectin, an extremely potent, broad spectrum parasiticide, is one of a group of natural products (1). Ivermectin is already widely used on cattle for the control of many internal and external parasites and will likely find widespread use in other meat-producing livestock. The current recommended dosage is 200 μg/kg body weight administered in a subcutaneous injection. Determination of ivermectin concentrations in serum of treated animals is important for several reasons, but of particular interest to us is the minimum concentration required for the control of target pests and the use of serum concentrations to monitor performance of various controlled-release systems. Previously published analytical methods are labor intensive and require relatively large amounts of glassware and solvent (2–4). The liquid chromatographic (LC) procedure described in the present report requires only minutes per sample cleanup and will detect concentrations as low as 2 ppb in 5 mL serum samples.

Experimental

Apparatus and Reagents

(a) *Liquid chromatograph.*—Waters Model 6000A solvent delivery system, Waters Model U6K injector, and Waters Model 480 variable wavelength detector set at 245 nm (Waters Chromatography Div., Millipore Corp., Milford, MA 01757). Hewlett-Packard Model 3390A reporting integrator (Hewlett-Packard Co., Analytical Group, Palo Alto, CA 94303).

(b) *Analytical column.*—8 mm id × 10 cm 4 μm NOVA-PAK®, Radial PAK® Liquid Chromatography Cartridge (Waters).

(c) *Solvents.*—LC grade (J. T. Baker Inc., Phillipsburg, NJ 08865).

(d) *Mobile phase.*—Acetonitrile–methanol–water (45 + 45 + 10) at flow rate of 1.0 mL/min.

(e) *Cleanup cartridges.*—Sep-Pak silica and C_{18} cartridges (Waters). Purge C_{18} cartridge with 4 mL acetonitrile–water (1 + 1) and silica cartridge with 4 mL acetonitrile followed by 4 mL methylene chloride.

Extraction and Cleanup

Mix 5 mL bovine serum with 5 mL acetonitrile–water (1 + 1). Draw resulting mixture through C_{18} cartridge, and flush cartridge with 4 mL acetonitrile–water (1 + 1). Then, using empty 5 mL syringe, blow out excess solvent with 1 or 2 rapid puffs. Attach C_{18} cartridge to silica cartridge by using tip cut from disposable plastic syringe as coupling. Elute ivermectin from C_{18} cartridge onto silica cartridge with 4 mL acetonitrile–methylene chloride (1 + 9). Discard C_{18} cartridge, and elute ivermectin from silica cartridge with 4 mL acetonitrile. Evaporate eluate with stream of nitrogen, and reconstitute residue with 1 mL mobile phase.

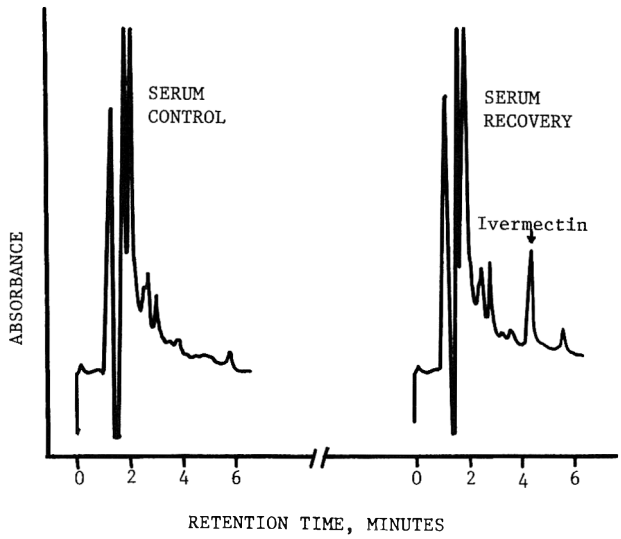

Figure 1. Liquid chromatograms of extracts from control serum and serum spiked with 10 ppb ivermectin.

Results and Discussion

Samples of serum were fortified with ivermectin to concentrations of 10, 20, or 50 ppb. Recoveries repeated 3 times at each concentration ranged from 95 ± 4% for the 50 and 20 ppb samples to 91 ± 2% at 10 ppb. A 10 ng injection of ivermectin standard in 100 μL acetonitrile produced a 16 mm peak with a retention time of approximately 4 min when detector attenuation was set at 0.002 AUFS (Figure 1). A standard curve plot was linear in the 1–200 ng range.

Careful manipulation of solvent strength and polarity allows the entire extraction and cleanup to be performed without a single transfer or concentration step. Recovery of ivermectin from fortified samples was severely diminished unless acetonitrile was added to the serum. Of course, if the acetonitrile concentration is too high, protein in the serum will be denatured and will plug the cleanup cartridge or elute the ivermectin prematurely from the cartridge. The acetonitrile–methylene chloride solution used to elute ivermectin from the C_{18} cartridge also removes some interfering compounds from the silica cartridge which were coeluted from the C_{18} cartridge.

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For research only. Mention of a pesticide or proprietary product does not mean recommendation for use or endorsement by the Agricultural Research Service, U.S. Department of Agriculture.

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Development of Serological Ovine Field Test (SOFT) by Modified Agar-Gel Immunodiffusion

MARK E. CUTRUFELLI, RICHARD P. MAGEAU, BERNARD SCHWAB, and RALPH W. JOHNSTON[1]
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A serological ovine field test (SOFT) has been developed for detection of lamb or sheep tissue in a wide variety of raw meat products. The test is an adaptation of previously developed field screening immunodiffusion tests for beef, poultry, and pork detection. The SOFT test was demonstrated to be specific, sensitive, and accurate in the analysis of 104 samples.

A serological ovine field test (SOFT) has been developed as a screen for detection of sheep (or lamb) tissue substitution or adulteration in any raw whole, ground, or formulated meat product. The test employs agar-gel immunodiffusion plates with a printed template for accurate placement of stabile freeze-dried reagent paper discs and sample discs saturated in meat tissue fluid. Within 18–24 h at room temperature, fusion of a sample immunoprecipitin line with a reference band formed in the agar between reference antigen and antibody discs indicates the presence of sheep tissue in the sample. This basic procedure was established and described in previous publications on the ORBIT (1), PROFIT (2), and PRIME (3) tests for respective identification of beef, poultry, and pork tissues. Subsequent to a collaborative study (4), the method was adopted AOAC official first action (5). The present paper reports an adaptation of the method, requiring suitable reagent modifications, for detection of sheep tissue in raw meat products.

Experimental

Reagent Modifications

Prepare reference anti-ovine antibody discs by impregnating blank filter paper discs (BBL No. 31039, Becton, Dickinson and Co., PO Box 243, Cockeysville, MD 21030) with 40 μL calf anti-ovine serum (Environmental Diagnostics, Inc., PO Box 908, Burlington, NC 27215). Prepare ovine reference antigen discs by impregnating additional blank paper discs with 40 μL ovine serum albumin Fraction V (No. A3264, Sigma) at 0.05% concentration in phosphate-buffered saline pH 7.2. Let both sets of discs absorb their reagents and freeze-dry overnight as previously described (1). For weak antiserum, recharge antibody discs with 40 μL calf anti-ovine, and relyophilize. Prepare immunodiffusion plates as previously described (1), substituting crystal violet, certified biological stain (No. C581-10, Fisher), for Lanaperl fast pink R dye. Prepare 1% stock solution of crystal violet stain by dissolving 0.1 g stain in 1 mL 95% ethanol, mix, and add water to 10 mL. Sterilize through 0.22 μm Millipore filter. Add sufficient 1% stock solution to hot sterile agar to effect a final crystal violet stain concentration of 1:240 000 which distinguishes SOFT from ORBIT, PROFIT, and PRIME plates.

Reaction Characteristics

Specificity for the SOFT test was determined by reacting blank paper sample discs saturated in homologous (ovine) and heterologous species meat tissue fluids against reference antibody discs. Sensitivity, as applied to ground meat mixtures, was assessed by testing prepared sample composites of known amounts of sheep adulterant tissue added to ground red meat base tissue. Three replicates were tested at each adulterant level (1–22% by weight). Presence of a visible sample immunoprecipitin band that completely fused with the reference band was taken as evidence of detection at a given percentage level of adulterant.

Shelf Stability

Longevity of reagent discs was evaluated by storing some prepared calf anti-ovine antibody discs and ovine reference antigen discs in glass vials with screw caps under conditions of room temperature and refrigeration (4°C). These discs were tested periodically to note any loss of immunoprecipitin band intensity.

Sample Analysis

To determine the accuracy and reliability of the SOFT test, 64 meat samples (Table 1) of a wide variety were first analyzed using the AOAC method (5) with reagent modifications for ovine detection described above. Forty additional unknown samples (Table 1) were then analyzed in a blind in-house laboratory trial. Species origin of all 104 samples was confirmed by Ouchterlony agar-gel immunodiffusion technique (6).

Results and Discussion

In specificity determinations using reference antibody discs prepared with calf anti-ovine serum, the following SOFT test

Table 1. Results of pre-trial and blind laboratory trial of serological ovine field test on a variety of meat product samples

Product	Species composition[a]	No. of samples	No. of samples with ovine	No. of positive samples
Pre-laboratory trial:				
Bologna emulsion	bovine	2	0	0
Frank emulsion	bovine	4	0	0
Gyro	ovine, bovine	8	8	8
Gyro	bovine	4	0	0
Sausage	ovine, pig, bovine	4	4	4
Whole lamb	ovine	32	32	32
Ground lamb	ovine	2	2	2
Ground lamb	ovine, (bovine)	2	2	2
Ground beef	bovine	2	0	0
Ground beef	bovine, (ovine)	2	2	2
Whole deer	deer	2	0	0
Total		64	50	50
Blind laboratory trial:				
Bologna emulsion	bovine	1	0	0
Frank emulsion	bovine	2	0	0
Gyro	ovine, bovine	3	3	3
Gyro	bovine	1	0	0
Sausage	ovine, pig, bovine	2	2	2
Whole lamb	ovine	21	21	21
Ground beef	bovine	6	0	0
Whole pork	pig	4	0	0
Total		40	26	26

[a] Identity of species in all samples was confirmed by Ouchterlony agar-gel immunodiffusion technique (6) using anti-species sera and extracts of authentic reference tissue. Species given in parentheses represent known adulterant tissue present in the test samples for the pre-laboratory trial.

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[1] Microbiology Division, Washington, DC 20250.

reactions occurred for whole and ground tissue samples of known species origin: sheep (+), goat (+), horse (−), bovine (−), pig (−), deer (−), chicken (−), turkey (−), red kangaroo (*Macropus rufus*) (−). Use of a calf host for production of antiserum precluded the possibility of cross reactivity with closely related bovine tissue.

The cross reaction with goat tissue was expected because of the known close relationship of albumin proteins in ovine and caprine species tissues. Products containing goat tissue are unusual in the United States; therefore, this cross reaction should not pose a serious limitation to the intended use of the SOFT test as a screening procedure in this country.

Sensitivity determinations indicated that adulterant sheep tissue was detectable at the 3% level in either beef or pork tissue bases (data not shown). Immunoprecipitin bands were, as expected, very weak at the endpoints.

Tests of shelf stability revealed that SOFT reagent antigen and antibody discs stored for one year at 4°C produced immunoprecipitin bands of intensity equal to that of freshly prepared reagent discs. Reagent discs stored at room temperature for 4 months lost considerable reactivity. Decreased immunoprecipitin band intensity was primarily attributable to decline in the antigen discs. Therefore it is recommended that reference reagent discs be stored in the refrigerator for maximum shelf life.

Results of the pre-trial and blind laboratory trial of analyses of samples are shown in Table 1. Of 104 total samples analyzed, 76 contained ovine proteins and gave positive SOFT reactions. Twenty-eight samples, devoid of ovine proteins, gave negative reactions. The absence of any false positive or false negative reactions for these samples demonstrates the accuracy and reliability of the SOFT test. However, since the test is intended as a screening procedure, it is recommended that positive results always be confirmed by using the traditional Ouchterlony immunodiffusion technique (6) or by using isoelectric focusing (7), especially when legal action is considered for violative results.

The SOFT test, as well as its predecessors, the ORBIT, PROFIT, and PRIME tests, are commercially available in kit form at this time. The tests are used internationally by the U.S. Department of Agriculture, other national governments, and commercial laboratories to assure accurate labeling of meat products.

Acknowledgment

The authors thank Darlene Worsham for her help in preparing this manuscript.

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GENERAL REFEREE REPORTS: COMMITTEE ON PESTICIDE FORMULATIONS AND DISINFECTANTS

Disinfectants

ARAM BELOIAN
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Use-Dilution Test.—The Associate Referees have done no further work on this test method. Preliminary studies designed to modify the carrier of the test bacteria are being carried out at the microbiology laboratory of the Biology and Economics Analysis Division of the Office of Pesticide Programs, U.S. Environmental Protection Agency (EPA). These studies are intended to replace the steel carrier with a glass carrier and reduce the numbers of test bacteria so that each initial bacterial count is nearly equal to one another. Past studies at the University of North Carolina (UNC) have shown a 10-fold difference in bacterial numbers grown in broth for *Salmonella choleraesuis* (0.5–2 million/mL), *Staphylococcus aureus* (1–5 million/mL), and *Pseudomonas aeruginosa* (13–20 million/mL). The original intent of these studies was to provide this modified procedure to the Associate Referee at UNC so that another collaborative test of the Use-Dilution Method could be carried out. Because of equipment breakdowns at the EPA laboratory, extending over 3 months, studies were not completed in time for UNC to do another collaborative study.

In the interim, the Chemical Specialties Manufacturers Association (an industry group that includes disinfectants manufacturers) has proposed to the Office of Pesticide Programs to co-fund studies at UNC to improve the Use-Dilution Method or replace it with a new test method. This work is due to begin in October 1988 and continue for the next 2 years. It is anticipated that with increased funding and closer monitoring of experimental statistical design, work on the Use-Dilution Method should be completed and the test will be accepted or rejected accordingly.

Since the Associate Referee for this topic believes (based on data developed to date) that the Use-Dilution Method should be abandoned and the Office of Pesticide Programs, the regulatory agency with purview over disinfectants, strongly believes that the Use-Dilution Method should be retained as the test of choice in measuring the efficacy of disinfectants, an unresolved controversy exists. Another industry group, together with commercial test laboratories, has been formed to conduct further tests with the Use-Dilution Method. This group believes the Use-Dilution Method should be retained with new pass-fail criteria and is against using a suspension test to measure efficacy of disinfectants. A series of collaborative tests is needed to determine whether to keep the Use-Dilution Method or abandon it, or to define new pass-fail criteria. All collaborative efforts by any and all groups will be done through the AOAC Committee on Pesticide Formulations and Disinfectants.

Tuberculocidal Test.—No report was received from the Associate Referee. Studies on the AOAC tuberculocidal test and alternative test procedures are expected to be carried out in the future after resolution of the Use-Dilution Test. Continued study is recommended.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Pesticide Formulations: Fungicides and Disinfectants

PETER D. BLAND
ICI Americas Inc., 1200 S 47th St, Richmond, CA 94804

No collaborative studies were carried out during the year. The following is a status report of selected topics in this section:

Anilazine.—This method was presented at the 1987 session and was granted interim first action. It is recommended for official first action.

Benomyl.—Associate Referee Mikio Chiba reports no progress during the year. He plans to refine the method for analysis of benomyl and MBC in Benlate formulations for collaboration.

Triadimefon.—No further comments have been received on this method. It is recommended for official final action.

Water-Soluble Copper and Water-Insoluble Copper.—This topic should be deleted.

No further progress is reported on the topics Carboxin and Oxycarboxin, Chlorothalonil, Dithiocarbamate Fungicides, and Triphenyltin. The following topics are open: Copper Naphthenate, Dinocap, Dioxins, *o*-Phenylphenol, Quaternary Ammonium Compounds, and Thiram. Anyone interested in serving as Associate Referee should contact the General Referee.

Recommendations

(1) Adopt as official first action the LC method for anilazine.

(2) Adopt as official final action the official first action method for oxythioquinox, **6.B34-6.B39.**

(3) Adopt as official final action the official first action method for triadimefon, **6.A31-6.A36.**

(4) Discontinue the topic Water-Soluble Copper in Water-Insoluble Copper Fungicides.

(5) Advertise the Associate Referee openings in the newsletter.

(6) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Section numbers refer to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68,** 369–411 (A methods); *J. Assoc. Off. Anal. Chem.* (1986) **69,** 349–390 (B methods).

Pesticide Formulations: Herbicides I

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One collaborative study was carried out during the year, and several have been planned for 1988/1989. The conflict on the method for bentazon was resolved, and BASF Corp. is repeating its study under the auspices of CIPAC to meet AOAC guidelines.

The following is a status report of selected topics in this section:

Alachlor, Butachlor, and Propachlor.—The GC method for microencapsulated alachlor was adopted official final action in 1987. Associate Referee David Tomkins plans a collaborative study on a method to analyze alachlor/atrazine mixtures.

Amitrol.—This topic is open. No further action is recommended until EPA resolves the status of this compound.

Bentazon.—Associate Referee Thomas M. Schmitt reports that his parent company in Europe is planning to repeat its collaborative study under the auspices of CIPAC following AOAC rejection of the original study. It met in June with AOAC to discuss protocols.

Bromoxynil.—Associate Referee Lawrence J. Helfant reports that various studies have been completed on modifications to the existing GC method. These modifications address other esters of bromoxynil and combination products with phenoxy esters and triazine compounds. Alternative LC methods have also been studied to determine a bromoxynil equivalent in combination products. The Associate Referee plans a collaborative study on the modified GC method for additional esters of bromoxynil and combination products. He also plans to collaboratively study the alternative LC methodology.

Cyanazine.—The method has been converted from CIPAC to AOAC format, and statistics were recalculated to meet AOAC guidelines. The method will be posted at the 1989 session.

Dichlobenil.—Associate Referee A. A. de Reyke has developed a capillary GC method for technical and formulated material. He plans a collaborative study on the method under the auspices of CIPAC.

Fomesafen.—Associate Referee Stephen J. Eitelman has completed a collaborative study on the LC method for fomesafen. He will be unable to present this at the 1988 session but will present it at the 1989 session.

Metolachlor.—Associate Referee Arthur H. Hofberg proposes to conduct a collaborative study on a capillary GC method during 1988/1989.

Metribuzin.—Associate Referee William Betker recommends this topic for deletion since the work on the method is completed. At the 1987 meeting, the methods committee recommended continued work in this area to evaluate formulations containing mixtures of active ingredients.

Pesticides in Fertilizers.—No further progress is reported in this area.

Propanil.—No further progress reported in this area. Steve Gazaway was appointed as Associate Referee on this project.

The topics Cacodylic Acid and Sodium Chlorate are open. Anyone interested in serving as Associate Referee should contact the General Referee.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Pesticide Formulations: Herbicides II

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Associate Referees were not able to initiate or complete any collaborative studies this year. Registrations, reregistrations, and requests from EPA and several states as well as reorganizations in several companies have prevented the allocation of personnel to collaborative studies. In-house efforts to improve existing methods and develop new ones continue, however, and several Associate Referees plan to initiate collaborative studies in late 1988 or early 1989.

Associate Referees are needed for Bensulide, Substituted Urea Herbicides, Aryzalin, and Dinoseb. The status of current assignments is as follows:

Bensulide (Betasan).—W. Ja has completed in-house work on an improved LC method. Because of changes in his responsibilities, he will be unable to do any AOAC studies in the future. He hopes to find an in-house specialist who would be willing to take over his Associate Referee responsibilities.

Bromacil.—Paul K. Tseng has completed his in-house testing of an LC method. He will initiate a collaborative study in 1989 if time permits.

Fluometuron (Cotoran®).—Arthur H. Hofberg has completed all in-house testing of an improved LC method. The collaborative study planned for 1988 was delayed because of other priorities. It is now scheduled for 1989.

Methazole (Probe).—Benjamin A. Belkind has made modifications to his proposed collaborative study, based on recommendations from the General Referee. He will initiate the study in early 1989 if time permits.

Naptalam (Alanap).—Milton Parkins has changed responsibilities and will be unable to do AOAC studies. He hopes to find someone in his company who may be interested in completing this work once reorganizations are completed.

Sulfonylurea (Metasulfuron-Methyl, Sulfometuron-Methyl, and Chlorsulfuron).—Glenn A. Sherwood returns to a new responsibility that will enable him to devote time to AOAC collaborative studies. He has in-house LC methods that are ready for studies. He expects to initiate these studies in 1988.

Thiocarbamates.—W. Ja will be unable to continue this work because of changes in his responsibilities. He hopes to find an in-house specialist who would be willing to take over this topic.

Recommendations

(1) Appoint Associate Referees for the compounds listed.
(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Pesticide Formulations: Organohalogen Insecticides; Other Insecticides, Synergists, and Repellants

DAVID F. TOMKINS
Monsanto Co., PO Box 473, Muscatine, IA 52761

Organohalogen Insecticides

No actions were taken and no recommendations are presented. The topics *Chlordane, Hepachlor,* and *Methyl Bromide* are open. Anyone interested in serving as Associate Referee should contact the General Referee.

Other Insecticides, Synergists, and Repellants

Cyhalothrin.—Associate Referee Stephen J. Eitelman has planned a collaborative study for 1989.

Cypermethrin.—The cypermethrin method is recommended for official final action.

Cyromazine.—Associate Referee Arthur H. Hofberg has planned a collaborative study for 1989.

Permethrin.—This topic is open. Anyone interested in serving as Associate Referee should contact the General Referee.

p,p'-DDT.—A collaborative study for *p,p'*-DDT, Technical and DDT Formulations, is in progress. Associate Referee Frederick C. Churchill has initated the study and is awaiting the results from 3 remaining laboratories.

Recommendations

(1) Adopt as official final action the official first action method for cypermethrin, **6.B01-6.B05**, based on several years of routine use by several laboratories with no adverse comments.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Section numbers refer to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

Pesticide Formulations: Carbamate and Substituted Urea Insecticides; Other Organophosphorus Insecticides; and Rodenticides and Miscellaneous Pesticides

G. MARSHALL GENTRY
Florida Department of Agriculture and Consumer Services, Tallahassee, FL 32399-1650

The following is a summation of activities this past year.

Carbamate and Substituted Urea Insecticides

Aldicarb and Isomers.—Karin A. Mede indicated there was no change needed in the current methodology.

Bendiocarb.—Peter Carter did not see a need for changes in the present LC methodology.

Carbaryl.—Mede did not anticipate any changes in current methodology.

Carbofuran.—Edward J. Kikta indicated that the present LC method was adequate.

Methomyl.—James L. Conway will make minor corrections in the 1983 collaborative study and this method should be ready for adoption.

Mexacarbate (Zectran).—Mede requested to be relieved of her duties for this compound.

Oxamyl.—Glenn A. Sherwood will continue study.

Other Organophosphorus Insecticides

Crotoxyphos.—Wendy King will continue study on this compound.

Naled.—Robert H. Iwamoto will continue study on this compound.

Nemacur.—Carl Gregg has a collaborative study in progress and should conclude this study during the next year.

Rodenticide and Miscellaneous Pesticides

Brodifacoum.—Jeff Parker has been appointed Associate Referee for this compound. The present method for brodifacoum with the exception of wax bait formulations should be considered for final action. Study should continue for the wax bait formulations.

Recommendations

(1) Adopt as official final action the official first action method for brodifacoum, **6.597-6.601**.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed.

Pesticide Formulations: CIPAC Studies

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AOAC and the Collaborative International Pesticide Analytical Council (CIPAC) agreed in 1971 to carry out common collaborative studies and to standardize common methods for pesticide formulations where possible. The joint status of AOAC/CIPAC or CIPAC/AOAC would be given to these methods, depending on which organization conducted the collaborative study. Since 1971, AOAC has adopted more than 20 methods which were collaboratively studied under CIPAC leadership, and many AOAC-directed studies have resulted in methods that have likewise been adopted by CIPAC.

During the past few years, James Launer greatly facilitated the adoption of CIPAC methods through his efforts to rewrite them into AOAC format and to resolve questions that arose during review by the Committee on Pesticides and Disinfectants, i.e., he essentially served as the AOAC General Referee for CIPAC studies. This past year, Launer was recognized for his efforts by the creation of a new General Referee area, CIPAC Studies, to which he was the first appointee. Last year was also Launer's final year as an active AOAC General Referee. The new General Referee for CIPAC Studies would like to both salute and thank Jim Launer for his superb performance in the transfer of CIPAC methods to AOAC and for his long and dedicated service in this and other capacities as an AOAC General Referee.

A paper covering the design and conduct of collaborative studies by CIPAC has been published and should be noted by those interested in the organization and its operation (Henriet, J., & Martijn, A. (1986) *J. Assoc. Off. Anal. Chem.* **69**, 408–409). Formulation chemists may also be interested to note that CIPAC has developed a draft entitled, "Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods." This document was reviewed and revised during the 32nd meeting of CIPAC this June in Geneva, and it will be further circulated for review of the revisions and comment. The proposed CIPAC guidelines include recommendations of the IUPAC Workshop on Harmonization of Collaborative Analytical Studies (May 4–5, 1987), and, thus, they are very similar to the guidelines recently adopted by the AOAC Board of Directors (*J. Assoc. Off. Anal. Chem.* **71**, 161–172 (1988)). The General Referee believes that the acceptance of CIPAC methods by AOAC will be promoted if the guidelines are adopted by CIPAC.

In the future, the General Referee will provide abstracts for publication by AOAC on those CIPAC methods that are adopted by AOAC but have not been submitted by the authors to the AOAC *Journal*. These abstracts will be sent

along with this annual report. Since there are no methods for which abstracts might be included this year, the following covers the status of methods adopted by CIPAC as full methods which have been or will be submitted to the AOAC for consideration.

Deltamethrin.—A GC method was studied by 14 collaborators through the analysis of 6 samples: a technical material, a ULV formulation (10%), an emulsifiable concentrate (2.5%), 2 wettable powders (2.5, 5.0%), and a dry powder (0.05%). The Methods Committee has previously reviewed this study and indicated a need for a few clarifications, including the potential interferences by deltamethrin isomers. The needed information has been obtained and will be submitted to the Committee.

Isoprocarb (MIPC).—The first CIPAC collaborative study on the analysis of this phenyl methyl carbamate insecticide was conducted in 1985 with an LC method applied to 2 samples: a technical material and a liquid (15%). When the results were reported at the 29th CIPAC meeting in Copenhagen, it was decided to add a solid formulation, and another study was conducted using the same method applied to a 75% wettable powder. Results of both studies will be referred to the Committee.

Maneb Plus Fentin (Triphenyltin Acetate).—This combination of fungicides is used mainly on potatoes, especially in The Netherlands. The recovery of maneb when formulated in combination with fentin acetate is incomplete using the standard dithiocarbamate carbon disulfide evolution method (**6.537-6.540**). In the CIPAC study, sulfuric acid was replaced by hydroiodic acid in glacial acetic acid (CIPAC document 3278/R). Sixteen laboratories participated in the study, consisting of the analysis of 5 samples containing maneb, 4 of which also contained fentin acetate. Results of the study will be submitted to the Committee for consideration.

Pencycuron.—This fungicide was determined by LC using nonaqueous absorption chromatography on a diol-modified silica gel. In this CIPAC study 10 participating laboratories analyzed a technical material and 3 different formulations: a wettable powder (26%), a flowable (22%), and a dustable powder (16%). Results of the study will be sent to the Committee for consideration.

Sethoxydim (Poast).—A normal-phase LC method for this systemic postemergence herbicide was subjected to a study, and data were reported by 20 laboratories on 3 samples: a 50% technical material and 20 and 12.5% emulsifiable concentrates. Results of the study will be referred to the Committee.

Vinclozolin.—A GC method was subjected to a study with 19 participating laboratories and the following samples of this fungicide: a 50% wettable powder, a 50% suspension concentrate, and a technical material. Results of the study will be sent to the Committee for consideration.

Methods for other pesticides for which studies were directed by CIPAC were also adopted as full methods at the 32nd meeting in Geneva. The General Referee will be seeking copies of these studies for consideration by AOAC: phsalone, chlorphoxim, and metazachlor.

Recommendations

(1) The Methods Committee should review future submissions prior to the rewriting of CIPAC methods in AOAC format so the General Referee can concentrate reformatting efforts on methods that are likely to be accepted for approval as interim official first action.

(2) Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed.

GENERAL REFEREE REPORTS: COMMITTEE ON DRUGS AND RELATED TOPICS

Cosmetics

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Aloe.—Associate Referee Alexis R. Eberendu is developing an analytical method for estimation of aloe gel in cosmetics and cosmetic raw materials. The approach now being investigated involves isolation of an acetylated polysaccharide using extraction and dialysis techniques. The isolated polysaccharide is then hydrolyzed with acid, and the resulting mixture of sugars is determined by liquid or gas chromatography. The sugar of interest is mannose. Addition of locust bean gum, guar gum, or glucomannon would be detected by a high galactose or glucose to mannose ratio.

Essential Oils and Fragrance Materials, Components.—Associate Referee Harris H. Wisneski reports that the gas chromatographic method for determination of musk ambrette in fragrances requires major modification before it can be submitted for collaborative study. It was found, using the previous method, that benzyl salicylate was not resolved from musk ambrette. To obtain the required separation, a 30 M WCOT gas chromatographic column was necessary. Although this column gave the required resolution, it was difficult to obtain reproducible recoveries on successive analyses. The cause of the recovery variations has been identified and, for the most part, corrected. The method is now being independently evaluated before being submitted for collaborative study.

Preservatives.—Due to the resignation of Associate Referee Ann R. Stack, the collaborative study for the liquid chromatographic determination of methyl, ethyl, propyl, butyl, and isobutyl p-hydroxybenzoates (parabens) will not be completed.

Recommendations

(1) Continue official first action status of the method for water and ethyl alcohol, **35.001-35.006**, and the method for soluble zirconium, **35.020-35.024**.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed.

Drugs I

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p-Aminobenzoic Acid and Salicylic Acid.—Richard D. Thompson completed his collaborative study on the LC determination of alkali salts of *p*-aminobenzoic acid and salicyclic acid in pharmaceutical formulations. Nine laboratories participated in the study. Each laboratory analyzed 6 formulations of coated tablets and an injectable product. The Associate Referee will present the results of the study at the 102nd AOAC Annual International Meeting.

Salicylic Acid in Acetylsalicylic Acid Preparations.—This is a new topic under the direction of Maria Ines R. M. Santoro. The method and the results obtained at the Universidade De Sao Paulo, Faculdade De Ciencias Farmaceuticas were presented by the Associate Referee at the 101st AOAC Annual International Meeting. The colorimetric method for salicylic acid is based on the reaction of ferric ion in acidic medium with salicyclic acid to produce a colored product with maximum absorption at 525 nm. The methodology was specifically developed for the determination of salicylic acid in liquid preparations of aspirin (acetylsalicylic acid) where the hydrolysis of aspirin to salicylic acid has been inhibited by the addition of other substances such as lysine or arginine. In coordination with Dr. Santoro, a minicollaborative study is in process at the FDA Winchester Engineering and Analytical Center.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Drugs II

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Belladonna Alkaloids.—LC methodology for the direct determination of the *d*- and *l*-hyoscyamine content is now available (1). In contrast, prior procedures require derivatization prior to the enantiomer determination (2–4).

Colchicine in Tablets.—A recent publication illustrated the feasibility of an LC method for the determination of colchicine in tablets containing colchicine in combination with probenecid (5).

Curare Alkaloids.—Associate Referee John R. Hohmann reported that he applied the new USP monograph (6) based on an LC procedure for metocurine iodide (7) and tubocurarine chloride (8) content in bulk drug and injections. With this procedure, the analysis of commercial tubocurarine chloride injections were reproducible. The results look promising in that the previous problems of interferences from decomposition products of the preservatives appear to be overcome, and he recommends that it be considered for a collaborative study. Identification of the other peaks detected should also be accomplished.

Dicyclomine Capsules.—Associate Referee Henry S. I. Tan reported his proposed capillary GC method for dicyclomine in capsules (9). He has shown that the GC method resolves any decomposition products from the dicyclomine and the internal standard. Excellent recoveries and low standard deviation were obtained. On the basis of these results, he is recommending that the proposed method be subjected to a collaborative study.

Morphine Sulfate in Morphine Sulfate Injection.—Associate Referee Ada C. Bello reported the results of a collaborative study at last year's AOAC meeting. The proposed LC method was demonstrated to be suitable for the determination of the morphine content of these products even where the samples contained decomposition products and some known contaminants (10). The Associate Referee recom-

mends that the proposed LC method be adopted official first action for determination of morphine sulfate and certain contaminants in bulk drug and injection. It is also now official in the USP (11,12).

Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine.—A new Associate Referee is needed for this topic.

Physostigmine and Its Salts.—Associate Referee Norlin W. Tymes evaluated a proposal to change the USP method (13) for physostigmine salicylate injection. The current USP method is based on his AOAC method, **38.074-38.080** (14). On the basis of these results, he is recommending no change in the method.

Pilocarpine.—It is recommended that this topic be discontinued.

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Recommendations

(1) Adopt as official first action the interim action LC method for determination of morphine sulfate and certain contaminants in injections and bulk drug material.

(2) Declare open any topic that has been inactive for an extended period (more than 2 years). Appoint new Associate Referees for the following topics: Belladonna Alkaloids; Pheniramine with Pyrilamine, Phenylpropanolamine and Phenylephrine; Rauwolfia Alkaloids.

(3) Discontinue the topic Pilocarpine.

(4) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed.

Drugs III

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Coumarin Anticoagulants.—The method is currently in official first action status.

Flucytosine.—The method is currently in official first action status.

Halogenated Hydroxyquinoline Drugs.—All comments regarding this manuscript have been replied to as of February, 1988. This method should be considered for interim official first action status.

Hydralazine.—This method is currently in official first action status.

Levodopa.—This method is currently in official first action status.

Medicinal Gases.—The proposed collaborative study of this method is currently being evaluated.

Metals in Bulk Drug Powders.—Study should continue on this topic.

Penicillins.—The manuscript titled "LC Determination of Penicillin V Potassium in Tablets" by Associate Referee Barry Mopper (FDA, New York Regional Laboratory) has been resubmitted for interim official first action status.

Salts of Organic Nitrogenous Bases.—No additional work has been done on this project. A collaborative study is still anticipated.

Recommendations

(1) Adopt as interim official first action the LC method for halogenated hydroxyquinoline drugs.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Drugs IV

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D-and L-Amphetamines.—Associate Referee Irving Wainer reported that no further plan for the topic has been made. The method, after official first action, **40.D01**, will be discussed at the Annual Committee Meeting on August 12 because of a disagreement.

Benzodiazepines.—Associate Referee Eileen Bargo is planning a collaborative study on flurazepam. For oxazepam in tablets and capsules, **40.014**, she has evaluated the bulk material and has yet to evaluate the tablet before submitting the method for official final action.

Diazepam.—Associate Referee Michael Tsougros is planning a collaborative study on diazepam in injectable and capsule formulations, using the same LC procedure specified for tablets.

Heroin.—Associate Referee Charles Clark is planning to set up a collaborative study for heroin HCl.

Recommendations

(1) Retain as official first action the D-and L-amphetamines method. Delete the statement ". . .however, suitable results can be obtained with resolution as low as 0.8" in **40.D05**. Advertise in the *Referee,* and invite a public critique on the method.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1988) **71,** 199–239 (D methods).

Drugs V

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Antihistamines, Selected Drug Combinations, Ion Exchange Chromatography.—The official final action method is cumbersone and not as specific as LC analysis. Many of the specified agents already can be analyzed more satisfactorily by LC; therefore, the Referee recommends designating method **36.108-36.114** as surplus.

Betamethasone.—Associate Referee David Krieger has been assigned to pesticide work and does not expect to return to pharmaceutical activity in the foreseeable future. Someone else will be assigned shortly.

Pentaerythritol Tetranitrate.—A collaborative study has been completed, and the data are being processed. Associate Referee Marvin Carlson is presenting his report as this meeting. So far, the results appear to be quite satisfactory.

Prednisolone.—The tablet method, **39.B01-39.B06**, has been official first action for 3 years and is being used regularly. Associate Referee James Brower has received no adverse comments. Adoption as official final action and discontinuation of the topic is recommended.

Progestins.—Associate Referee Larry K. Thornton hopes to try some studies with improved instrumentation and eventually run another collaborative study with a sufficient number of laboratories participating.

Steroid Acetates.—Associate Referee Linda Ng is extending the method adopted last year for elixirs, **39.D17-39.D30**, to tablets.

Recommendations

(1) Designate method **36.108-36.114** as surplus.

(2) Appoint an Associate Referee for Betamethasone.

(3) Adopt method **39.B01-39.B06** as official final action and discontinue study.

(4) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods); "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1988) **71**, 199–239 (D methods).

Drug Residues in Animal Tissues

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Two new topics were established during the last year: (*1*) clopidol in chicken tissues and (*2*) sulfonamides in milk (chromatographic methods). Five others were transferred to this General Referee subject area: (*1*) chloramphenicol residues in animal tissues, (*2*) tetracyclines in tissues (microbiological assay), (*3*) tetracyclines in tissues (chromatographic assay), (*4*) novobiocin in animal tissue, and (*5*) enzyme immunoassays for antimicrobial compounds.

Benzimidazoles in Cattle Tissues.—Associate Referee Leon LeVan developed an LC procedure for determining fenbendazole (FBZ), oxfendazole (OFZ), thiabendazole, 5-hydroxythiabendazole, and mebendazole in cattle tissues. The target sensitivity was 100 ppb for all drugs except fenbendazole which was 800 ppb. A collaborative study of this procedure by 7 laboratories, conducted under the joint sponsorship of FDA and AOAC, was completed in late 1985.

The results of the study were not good. Many of the sample vials, which contained the coded unknown liquids for each participant to add to the tissue samples, showed evidence of leakage. Two laboratories consistently generated very poor analytical data. Also, there appeared to be an error in the analytical data, generated by the Associate Referee, for the samples containing incurred FBZ and OFZ. The General Referee is considering recommending the procedure for FBZ and OFZ based on the data from the collaborators.

Ipronidazole in Turkey and Swine.—No Associate Referee report.

Screening Methods.—No current Associate Referee.

Clopidol in Chicken.—Associate Referee Claire Simmons reports that clopidol was recovered from fortified chicken liver and muscle samples by using a slight modification of the LC procedure of Mtema et al. (1). Recoveries averaged 90% at the 50 ppb level. Modifications are being investigated, including (*1*) elimination of the internal standard, (*2*) additional washes for the alumina column, and (*3*) the substitution of other mobile phases.

Sulfonamides in Milk (Chromatographic Methods).—Associate Referee John D. Weber is now conducting a collaborative study of an LC procedure that will quantitate sulfamethazine, sulfadimethoxine, and sulfaquinoxaline in the 5–20 ppb range and above in cow's milk. This procedure was developed with the assistance of another scientist in his laboratory. Seven laboratories are analyzing blind samples, including controls, fortified controls, and incurred milk. These samples contain 0–20 ppb sulfamethazine.

Chloramphenicol Residues in Tissues.—No Associate Referee report.

Tetracyclines in Tissues by Microbiology.—No Associate Referee report.

Tetracyclines in Tissues by Chromatography.—Associate Referee Raymond Ashworth reported that James D. MacNeil, Health and Welfare Canada, Food Protection and Inspection Branch, is scheduled in late 1988 to direct a collaborative study of the procedure of Matsumoto et al. (2) for determining tetracyclines in animal tissues.

Novobiocin in Animal Tissues.—Associate Referee William Moats reported that his procedure for determining novobiocin in animal tissue and milk was accepted for publication by the AOAC *Journal*. The analytical method passed laboratory trials in the FDA laboratory in Beltsville, MD. Whether or not to conduct a collaborative study in tissue is being studied; a recent FDA study suggests that getting novobiocin above the tolerance level in tissues by dosing animals is difficult. There appears to be more interest in official methods for milk.

Enzyme Immunoassay for Antimicrobial Compounds.—Associate Referee Nitin Thaker reported he was almost prepared to initiate a collaborative study of an enzyme immunoassay procedure in a card format for sulfamethazine (SM) in swine muscle when FSIS USDA changed its regulatory strategy and began using blood SM levels to estimate SM levels in muscle. He is almost prepared to initiate a collaborative study of the same procedure in serum. He requested that his topic be changed to permit a study in serum. Thaker also expressed interest in doing a study of chloramphenicol in urine.

Levamisole.—This topic was discontinued in 1987 but no report of the collaborative study had been written at that time.

A study of a nitrogen sensitive alkali flame ionization GC procedure by 6 laboratories was initiated in October 1983 under the joint sponsorship of the Food and Drug Administration and AOAC. Each laboratory was assigned 52 samples which included (*1*) control and fortified control of cattle, swine, and sheep liver and muscle samples and (*2*) incurred

liver from dosed animals of each of the 3 species. Fortified samples contained 0.05, 0.10, or 0.20 ppm levamisole. The incurred samples contained approximately 0.10 ppm levamisole.

In early 1984, 5 laboratories reported data from this study. Other than the observation that 1.5 days were required to complete the analyses of 6 samples, there were no adverse comments on the determinative procedure. Average recoveries, standard deviations, and percent interlaboratory coefficients of variation for the 5 reporting laboratories are summarized as follows:

Statistics	Cattle liver	Cattle muscle	Swine liver	Swine muscle	Sheep liver	Sheep muscle
Rec., %	85.5	80.2	78.8	76.0	80.2	82.9
Std dev.	10.0	8.3	13.4	5.2	6.7	17.3
CV, %	11.7	10.2	17.5	6.8	8.3	23.9

Similar CVs for the incurred sheep, swine, and cattle liver samples containing approximately 0.1 ppm levamisole are 23.9, 14.8, and 22.8, respectively. Additional details from this study are available from the General Referee.

The committee on Drugs and Related Topics will be asked to comment on whether this topic should be re-established.

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Recommendations

(1) Discontinue *Screening Methods* and Ipronidazole in Turkey and Swine.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Forensic Sciences

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Gunshot Residues.—Robert D. Koons (FBI-Quantico, VA) has been appointed the Associate Referee for gunshot residues and is currently conducting a collaborative study for the determination of antimony and barium in gunshot residue collection swabs. In this method, cotton swabs are extracted with a dilute nitric acid solution and analyzed by flameless atomic absorption spectrophotometry. Standards are extracted from swabs in the same manner as the samples, thus eliminating or reducing interferences.

No reports were received on other topics.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Immunochemistry

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Development of Quality Standards Criteria for Monoclonal Antibodies Used in Immunoassays.—This effort was originally proposed in 1987 for establishing quality standards and defining a key "reagent" that could be used in immunoassay validation and collaborative studies. Representative or model monoclonal antibody preparations were to be chosen in 1988 on the basis of work in the laboratories of Bruce Hammock, University of California at Davis, in collaboration with Alex Karu, University of California at Berkeley. Target compounds that were defined included: carbofurans, thiocarbamates, and certain bacterial toxins.

In 1988, the research conducted by these groups yielded 2 candidates for this evaluation, atrazines and the thiocarbamate herbicide, molinate. Work has progressed in regard to the production and characterization of atrazine and simazine polyclonal and monoclonal antibodies. Goodrow et al. have presented data that clearly demonstrate that polyclonal antibody preparations should be included in the antibody reagent validation studies. Monoclonal antibodies have been prepared by Karu, and representative clones are being selected. Once the representative clones have been selected, the monoclonal antibodies should be characterized in validation studies. Work presented by Harrison and others supports validation studies of both the polyclonal antibody preparation as well as the monoclonal antibody preparation(s) under development.

Guidelines for evaluation studies of immunoassays are in progress by the Environmental Protection Agency. These guidelines are in concert with the objectives of AOAC, and collaboration with EPA will be recommended for consideration by the AOAC methods committees. Protocol development for collaborative studies using the appropriate polyclonal antibodies for both the atrazine and molinate immunoassays should be considered in 1989 if there is concurrence with the California Department of Agriculture.

Development of Study and Collaborative Study of an Immunoassay for Pyridostigmine (3-Hydroxy-1-Methyl-Pyridinium Bromide Dimethylcarbamate).—This effort was originally proposed in the GR report in 1987 for evaluating a polyclonal antibody-based immunoassay under development by the Department of the Army. This product should be discontinued until further work is completed in regard to characterization of the antibody preparation.

Recommendations

(1) Discontinue study on immunoassay for pyridostigmine.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

GENERAL REFEREE REPORTS: COMMITTEE ON FOODS I

Coffee and Tea

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The Associate Referee on Ash in Instant Tea, Francis J. Farrell, has submitted a study on ash in instant tea to ISO, together with a study on moisture in instant tea which was carried out at the same time. Both methods were accepted by ISO. He will submit these reports to the Committee.

John W. Newton, Associate Referee on Methyl Xanthines in Coffee and Tea, reported that he had no time to work on this project.

Daniel Zuccarrello, Associate Referee for Caffeine in Coffee, made no report.

B. Denis Page, Associate Referee on Solvent Residues in Decaffeinated Coffee and Tea, reported that he had no time to work on his method, but has a schedule to finish the method this coming year.

Recommendations

(1) Appoint an Associate Referee for Water Extract in Tea.

(2) Discontinue study on an LC method for caffeine in coffee.

(3) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Dairy Chemistry

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Adulteration of Dairy Products with Vegetable Fat.—Graham MacEachern had no report; David Firestone asked to assist the Associate Referee on free fatty acid analysis.

Babcock, Mojonnier, and Kjeldahl Tests.—David M. Barbano responded to requests for glassware labeling modifications. He also requested that **16.020** be changed to assure that milk fat is fluid at sampling, and that action be taken to replace the Babcock test as a reference method for calibration of IR instrumentation.

Calcium, Phosphorus, and Magnesium in Cheese.—Roger Pollman conducted a second collaborative study, and the results are being analyzed. The statistics have improved significantly, and a paper will be submitted for publication in the AOAC *Journal*.

Fat in Butter, Direct Method.—D. Engebretsen suggested in 1986 and 1987 that method **16.233** be repealed in favor of the Kohman method (*Standard Methods for Evaluation of Dairy Products*, 15th Ed., Method 18.9C) due to significantly lower estimates for fat. Attempts to obtain an Associate Referee have not been successful.

Fat in Milk, Gerber.—Dick Kleyn reports that a publication is pending in the AOAC *Journal*.

Fat in Milk, Robotic Mojonnier.—Robert L. Bradley is conducting a collaborative study on the Zymark robotic system.

Fat in Milk, Udy Turbidity Test.—Doyle Udy presented data at the ADSA meetings in 1988.

Ice Cream and Frozen Desserts.—Margreet Lauwaars requested returning the text of **16.309** from surplus status because of the Codex requirements.

Iodine.—Dave Sertl reports that as many as 17 laboratories are collaborating on a study to be completed within a few months.

Lactose by Chromatographic Determination.—Leslie West is publishing a method in the AOAC *Journal*, but requests replacement as the Associate Referee. Ike J. Jeon, Kansas State University, has volunteered to help with this study.

Lactose by Enzymatic Determination.—D. Kleyn reported that no studies are under way in this area.

Mid-Infrared Instrumentation.—D. Biggs is preparing documentation for the AOAC *Journal*. D. M. Barbano is also preparing a symposium paper on standardization of this methodology for publication in the *Journal of Dairy Science*.

Moisture in Cheese (Microwave).—Judy Arey requests official status on the microwave oven method for cheddar and cottage cheese and requests that the current write-up be changed to reflect the data generated in recent studies. R. A. Case is preparing a comparative study on methods for moisture in cheese for publication in the AOAC *Journal*.

Nonfat Milk Solids.—D. M. Barbano requested a minor modification of the method for determining the total solids of raw milk. He conducted a collaborative study and recommends that an additional method for determining total solids be adopted.

Protein in Milk (Kjeldahl).—W. Horwitz requested Committee consideration of the collaborative study published in the AOAC *Journal* by Remy Grappin on the use of copper catalyst. D. M. Barbano is conducting a collaborative study on the use of mercury and copper catalysts in the Kjeldahl method. The study should be completed this year.

Phosphatase (Rapid Method).—G. K. Murthy completed collaborative studies on both the AOAC and APHA methods.

Somatic Cells in Milk (Fossomatic).—Charles Lowden is conducting studies to obtain approval for the Fossomatic 360 somatic cell counter.

Whey Proteins in Nonfat Dry Milk.—A method has been recommended again for official first action status.

No reports were received on the following topics: Babcock Test and Babcock Glassware; Chloramphenicol Residues in Milk; Chloride Meters; Cryoscopy of Milk; Nitrates in Cheese; Phosphorus; Protein Reducing Substances Test; Protein Consitituents in Processed Dairy Products; Tyramine.

Recommendations

(1) Approve the changes suggested in the Babcock and Mojonnier tests for fat.

(2) Approve changes in **16.020** to assure temperature of 38°C in milk samples.

(3) Replace Babcock with ether extraction reference method for calibration of mid-infrared instrumentation.

(4) Repeal method **16.233**.

(5) Return from surplus the text of **16.309**.

(6) Adopt as official first action the microwave methods for moisture in cheddar and cottage cheese.

(7) Approve modifications to method for total solids in raw milk.

(8) Adopt as official first action the additional method for total solids in raw milk.

(9) Adopt as official first action the use of copper catalyst in the Kjeldahl method for protein in milk.

(10) Adopt as official first action the APHA modified rapid colorimetric method for phosphatase in cheese.

(11) Adopt as official first action the Olieman method for measurement of whey proteins in nonfat dry milk.

(12) Include the precision statistics by Crudgington in the method for fat in dry milk.

(13) Adopt as official first action the determination of phosphorus in processed cheese, *IDF Bulletin* 207, pp. 41–93.

(14) Adopt as official first action the enzymatic determination of lactic acid and lactates content of dried milk, *IDF Bulletin* 207, pp. 94–121.

(15) Adopt as official first action the flame photometric determination of sodium, potassium, and calcium contents of dried milk, *IDF Bulletin* 207, pp. 122–132.

(16) Add precision values to thermistor method **16.096** for "Determination of the Freezing Point of Milk by Means of the Thermistor Cryoscope," *IDF Bulletin* 207, pp. 198–207.

(17) Request that T. Peeler be invited to provide precision parameters for inclusion in *Official Methods of Analysis.*

(18) Establish a procedure whereby methods previously approved by APHA Technical Committee working on *Standard Methods for Examination of Dairy Products* may be included in *Official Methods of Analysis.*

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed.

Fish and Other Marine Products

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Flow Injection Analysis.—Jim Hungerford has been appointed Associate Referee on this new topic. He has developed a method for determining histamine in fishery products by flow injection analysis (FIA). Preliminary results indicate that the method is rapid and accurate. Work is now under way on devising a system to remove interfering histidine. An FIA method has also been developed for rapid determination of total volatile acids (TVA) and total volatile bases (TVB). TVA and possibly TVB will be useful indicators of decomposition in fishery products. The General Referee concurs with this assessment and recommends further study; eventually, collaborative studies should be conducted for each of the 3 FIA methods.

LC Method for PSP Toxins.—Associate Referee John Sullivan has resigned and Jim Hungerford has been appointed. A collaborative study will be initiated within the next few months, in 2 phases: For phase 1, potential collaborators will be sent practice samples consisting of shellfish extracts naturally contaminated with levels of PSP ranging from <80 to >80 $\mu g/100$ g, negative shellfish controls, standards, shellfish tissues naturally contaminated, and tissues spiked with various levels of purified toxins. The General Referee concurs with this approach and recommends continued study.

Drained Weight of Block Frozen, Raw, Peeled Shrimp.—Associate Referee Michael Blattner has been transferred and a collaborative study was completed by Gerald J. Maus. Each of the 7 collaborators was sent a total of thirty 5 lb blocks of frozen, raw, peeled, shrimp. Blocks were sent to each participant in 3 groups of 6 and one group of 12. Results are now being evaluated; a report will be submitted and a recommendation for official action will be made. The General Referee concurs with this approach and recommends continued study.

Coprostanol.—James Stewart has asked to be removed as Associate Referee. The General Referee concurs with this request.

Although there were no reports on the topics Total Volatile Amines by GC, Fish Content in Coated Products, Ammonia in Seafood, Coprostanol, Decomposition of Crabmeat, Ethanol in Seafood, Decomposition by Gas and Liquid Chromatography, and Amines in Seafood by TLC, the General Referee recommends that study be continued.

Recommendations

(1) Appoint an Associate Referee on the topic of Coprostanol and continue study.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Food Additives

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Antioxidants.—The Associate Referee reports that the LC-diode array detection method for determining 7 antioxidants (PG, THBP, THBQ, NDGA, BHA, Ionox-100, and BHT) has been extended to other matrixes similar to dried snack foods. The procedure has been written up and submitted to AOAC for publication.

Brominated Vegetable Oils (BVOs).—Little work has been done by the Associate Referee during this past year due to several emergency projects. The topic will be reviewed and a status report will be presented to the General Referee. Continuance of the project will depend on the conclusions of the report, current needs, and availability of an alternative Associate Referee.

Indirect Additives from Food Packaging.—The following is a summary report of the activities worked on during the past year in the Associate Referee's laboratory:

(1) Heptane Alternative Fatty Food Simulants for Polyethylene Terephthalate (PET) Polymers: Methods have been successfully developed to determine a number of residues in PET polymers and to monitor their migration into several fatty food simulants including corn oil. Migration studies simulating low temperature applications of PET were conducted as a prelude to higher temperature tests. A computer program has been obtained to help evaluate the migration of the unstable migrants and their decomposition products.

Low temperature (49°C) migration experiments have been conducted on PET using water, heptane, corn oil, and 8%, 50%, and 95% ethanol solutions. Reduction and interpretation of data are made more difficult by complex degradative reactions of the migrating adjuvants. Progress has been made in speeding this process by adapting mathematical models and computer programs.

While interpretation of the raw data is incomplete, the preliminary indication is that corn oil is a better fatty food

simulant for PET at 49°C and higher temperatures than ethanol solutions. Ethanol extracts more aggressively than corn oil, results in increased crystallization of the polymer, and enters into side reactions with some of the migrants. A report on this study will be issued in the near future. Meanwhile, high temperature adjuvant migration studies with PET have been initiated and presently have a higher priority.

(2) Migration Characteristics of Susceptor Food Packaging: Due to a dramatic increase in the use of heat susceptors in microwave packaging by the food processing industry and the lack of data on the performance integrity of such packaging at proposed use temperatures, work was initiated to evaluate the migration characteristics of susceptor food packaging. Physical observations indicate that aluminized PET susceptor film degrades when used at the high temperatures attained during normal microwave heating. For example, a study was conducted on susceptors made from paperboard-supported aluminized PET film, where the plastic film is in direct contact with the food. Various foods were prepared following the cooking instructions on the packages. Using the recommended conditions and susceptors, it was found that the PET consistently degraded, cracked, and often melted. When this happens, there is direct contact between the food and adhesive-coated paper that serves to support the aluminized PET film.

Initial attempts to determine the maximum temperatures reached by the susceptor packages employed Hermet® temperature indicator strips. The study indicated that under some cooking conditions, temperatures exceed 500°F, which is the limit of the temperature test strips used. The accuracy of these strips is unknown. Our data conflict with industry temperature claims but appear supported by our physical observations of PET (melting point 525°F) degradation. Susceptor surface temperatures during microwave use will be studied in greater detail as soon as newly purchased equipment arrives. This will provide sound data with which to address differences in temperatures reported by industry and our own laboratory.

Migration of non-volatiles from the PET film component of the susceptor packaging and similar migration from dual ovenable PET trays under microwave conditions have been compared in preliminary experiments using corn oil. The migration of low molecular weight oligomer (cyclic tris ethylene terephthalate (cyclic trimer)) from susceptor packaging greatly exceeds that from microwaved dual ovenable trays. About 60% of the estimated available trimer (about 1% initial residue level) in the susceptor PET film migrates. Preliminary data also indicate that in the presence of an oil-water emulsion, there may be a small increase in extraction efficiency. Additional extraction studies are in progress to confirm these observations.

In addition to degrading the PET film, the intense heat generated by susceptor packaging causes the paper support to char, thus releasing volatile decomposition products, including furfural. Volatile decomposition products from the paper are released along with other volatiles from the hot adhesive that attaches the PET film to the paper support. Efforts are under way to identify and quantitate the levels of migrating volatiles. In addition to furfural, other volatiles that have been identified so far include isopropyl alcohol, 2-butoxyethanol, 2-ethyl-1-hexanol, and benzene. No one susceptor material includes all of these chemicals.

(3) Method Validation: An intralaboratory validation of the ASTM method (D4443-84) for vinyl chloride residual monomer in PVC articles and resin was conducted. The method was validated using automated headspace analyzers (Perkin-Elmer, Models F-42 and HS100/Sigma 2000 chromatographs). The column used was a ⅛ in. stainless steel 0.19% picric acid on Carbopac C. Samples of PVC resin, bottles, films, laminates, and coated foil were analyzed numerous times to obtain statistical data. The results were confirmed using FDA's research method for vinyl chloride monomer. The study suffered from not having enough samples, such as laminates, with VCM levels as low as the proposed regulatory limits.

Based on the samples available, the study confirmed that ASTM Standard Method D-4443 appears adequate for determining VCM levels at the regulatory limits (5 and 10 ppb) in resin, bottles, bottle compound, and plasticized film. Laminates were successfully analyzed at about 25 ppb. The procedure does not appear appropriate for analyzing PVC-coated aluminum foil when the concentration of VCM in the coating is in the low ppb range. Additional validation work is planned but has been postponed while FDA conducts an environmental impact study on the proposed vinyl chloride regulation.

This method was used to conduct a very limited survey of PVC food packaging on the local market. Only 5 unique food packages (excluding oil bottles) were found that were readily identified as PVC. Of these, 2 were made from calendered PVC with VCM levels exceeding 60 ppb. The remaining samples were within FDA proposed guidelines.

(4) Method Development: Benzene in Petroleum Wax: An FDA Laboratory Information Bulletin was prepared describing the modification of the benzene in polypropylene method for the determination of benzene in paraffin wax.

(5) High Temperature Migration: High temperature migration studies performed by Arthur D. Little under contract to FDA should be completed and a report of the work should be available in late summer. The study monitored radiolabeled Irganox 1010 and Irganox 1076 migration from low density polyethylene, high density polyethylene, and polypropylene into water, 8% ethanol, 95% ethanol, and corn oil at elevated temperatures of 49°C, 77°C, 95°C, 121°C, and 135°C as appropriate to the individual polymer. Limited studies were conducted using foods such as chicken broth and milk to correlate with the food simulant data.

Nitrosamines in Foods: Associate Referee N. P. Sen reports the publication of a review on methods for determining nonvolatile *N*-nitroso compounds in foods [*Food Addit. Contam.* **4,** 375–383 (1987)]. He also reports the development of a method for the determination of 2-(hydroxymethyl)-*N*-nitrosothiazolidine (NMNThZ) in smoked bacon and other cured meats. The method consists of extraction of the sample with acetonitrile–glacial acetic acid (100 + 1) in the presence of small amounts of ascorbyl palmitate and sulfamic acid, removal of fats and lipids by liquid-liquid partitioning with iso-octane, cleanup on basic alumina containing 6% water, and analysis of the extract by LC-TEA. Detection limit of the method is about 1–2 ppb. Recoveries of HMNThZ added to fried bacon at 20–50 ppb levels ranged between 80 and 95%. Identity of HMNThZ was confirmed by GC-TEA and GC-MS, both after derivatization with *N,O*-bis(trimethylsilyl)acetamide.

Other activities include research conducted in the area of *N*-nitroso compounds in the FDA laboratory, and can be summarized as follows:

(1) LC-TEA Post-Column Reactor: A post-column reactor to facilitate the detection of *N*-nitroso compounds by LC-TEA has been developed. *N*-Nitroso compounds are reduced with KI in an acidic environment to liberate nitric oxide which is detected by TEA. The system response is linear up to 500 ng; at a signal-to-noise ratio of 3:1, about 1–2 ng can be measured, equivalent to 1 ppb in a 25 g sample. Molar responses relative to NPRO have been calculated for 9 *N*-nitroso compounds. The detection system responds to nitrite and therefore it must be removed from samples prior to analysis. Neither aromatic nor alkyl nitro compounds elicit positive responses. The repeatability of the system was tested for NPRO and NTMU and found to give CVs of 7.6 and 1.5%, respectively. The system can be used with or without an LC column, thereby giving individual or total data. A few samples have been analyzed by the system for total *N*-nitroso compounds. A sample of malt gave 65 ppb, a rubber nipple

extract contained 185 ppb, while a cosmetic contained >2000 ppb. The post-column reactor will be used to screen samples with potential *N*-nitroso compounds. Those products indicating a potential for *N*-nitroso compound contamination will be further studied using an LC column.

(2) Fish-Meat-Collaborative Study of Two-Column Method: A collaborative study sponsored by USDA of their 2-column method for determination of volatile *N*-nitrosamines in minced fish meat/surimi frankfurters has recently been completed. Results of the study are not available at this time.

Sulfiting Agents in Foods.—Associate Referee C. Warner reported on the documentation of the formaldehyde/reverse-phase LC procedure for determining sulfites in foods. The basic principle of the method involves capture and stabilization of the sulfite in food as the bisulfite addition product of formaldehyde (HMS). The sample extract is then subjected to cleanup with a solid-phase extractor column. This procedure was validated with samples fortified after the addition of formaldehyde (post-column reagent spikes) as well as reverse isotope dilution assay (RIDA). The average recovery in the range of 5–20 ppm in table grapes was 98%. The stability of HMS during the chromatographic separation was investigated as a function of the pH of the mobile phase. Based upon these studies, pH 4.7 was selected because HMS dissociates only 2% during chromatographic separation; at pH 6.5, dissociation was approximately 35%. The pH of the sample extractant was also investigated. Conversion of the reversibly bound forms of sulfite proceeds very slowly at pH 3.75; at pH 7, the capture of reversibly bound sulfite vs HMS is complete within 5 min.

Recommendations

(1) Adopt as official first action the optimized Monier-Williams method for determining sulfites in foods.

(2) Appoint Associate Referees for Anticaking Agents; Dilauryl Thiodipropionate; and Propylene Chlorohydrin.

(3) Continue study on all other topics.

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Meat, Poultry, and Meat and Poultry Products

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The following 4 topics were discontinued during the past year: Bioassay Methods for Meat and Poultry Products, Histological Identification Methods, NMR Systems for Meat Analysis, and Species Identification by "ORBIT" and "PROFIT." Chemical Antibiotic Methods was switched to a more appropriate group of topics under the General Referee Charlie Barnes for Drug Residues in Animal Tissues. A vacancy still exists for the topic Automated Methods. No reports were received on 3-Methyl Histidine or Non-Meat Proteins in Meat.

Two collaborative studies were successfully completed this year, "Total Fat in Meat Using the Soxtec Fat Analyzer" and "Hydroxyproline as a Collagen Indicator."

The hydroxyproline study was conducted by Kurt Kolar at the Swedish Meat Research Institute, Analytical Services in Kavlinge, Sweden. AOAC statistical data forms have been submitted to the committee statistician for review, and a contributed paper is being prepared which will include both the analytical methodology and the results of the 18 collaborators. After the peer review process, it should be possible to recommend the procedure for interim first action. This project is being encouraged through the cooperative agreement between AOAC and the Nordic Committee on Food Analysis (NMKL). Another current NMKL project involving the analysis of meat is determination of total fat using 3 procedures, Foss-Let, Soxtec, and NMR. The first technique is already an AOAC official final action method. The Soxtec method to be studied is a modification of the System HT, which requires acid hydrolysis. This is not the same system studied by the Associate Referee for total fat in meat (see below). The NMR method is a new procedure. NMKL is also working on interlaboratory studies for the determination of trace metals (especially tin) in meats by atomic absorption, glutamic acid and monosodium glutamate determinations in meat, fish products, and soups by an enzymatic method, a gravimetric determination of ash in food, a spectrophotometric determination of sulfite in food, and meat analytical sample preparation by mechanical meat chopper and food processor.

Even though the topic of species identification by ORBIT and PROFIT has been discontinued, work continues on this type of test at the USDA-FSIS facilities in Beltsville, MD. Three more tests have been developed using similar technology, Pork Rapid Identification Method (PRIME), Serological Ovine Field Test (SOFT), and Rapid Equine Serological Test (REST). A technical communication for PRIME was published and one for SOFT is being prepared. USDA is combining all these tests under one title, Species Identification Field Tests (SIFT). For more specific information regarding these tests, contact Richard Mageau or Ronald Berger, USDA-FSIS-Science, Microbiology Division, Building 322, Agriculture Research Center (EAST), Beltsville, MD 20705.

Crude Protein Analysis of Meat (Peroxymonosulfuric Acid Digestion and Improved Nesslerization).—Associate Referee David Christians continues study on this topic. The statistical analysis for the successfully collaborated method, determination of phosphorus in meat and meat products using the Digesdahl system, has been completed. The method has been submitted to Committee on Foods I for first action consideration.

Fat in Meat Products.—Associate Referee Max Foster has successfully completed a collaborative study for the analysis of total fat in meat using the Soxtec adaptation of the Randall ether extraction methodology. The data evaluation and manuscript will be presented at the poster sessions of the annual meeting; the AR recommends the procedure for consideration as interim first action.

Immunochemical Identification of Additives in Meat Products.—Associate Referee Ronald Berger and coworkers developed and published a method for the detection of poultry and pork in cooked and canned meat foods by enzyme-linked immunosorbent assays (ELISA). Reagents for the test are now commercially available from American Bacteriological and Chemical Research Corp. (ABC), 3437 SW 24th Ave, Gainesville, FL 32607. ABC is currently a USDA interim recognized laboratory to conduct this test for its clients. Because of limited resources and higher priorities, Mr. Berger cannot conduct a collaborative study for this test during the coming year.

LC Methods for Meat Products.—Because of high workloads and other priorities, Associate Referee M. Sher Ali has not been able to conduct collaborative studies for LC methods he developed for sugars and carbamate pesticides in meat. Work continues on this topic, however, mostly in the area of residue analyses.

Microwave Methods for Meat Analysis.—The Associate Referee, David Fish, reports that a mini collaborative study of the CEM fat extraction equipment, substituting Freon-12 for methylene chloride as the extracting solvent for meats,

indicated a low bias for Freon-12 compared to methylene chloride. No further collaboration for Freon-12 is anticipated.

CEM Corp. has developed a rapid microwave digestion system for nitrogen (protein) determination for meat samples. The current system is capable of digesting one sample at a time in about 6–9 min. Last year in a study with USDA, this system was found to compare favorably with the official Kjeldahl digestion (mercury catalyst) method, **24.027**. Development work continues for a multiple sample unit. When this work is completed, a collaborative study will be considered.

Minimum Processing Temperature.—The Associate Referee, Grover Pickel, reports that a method is urgently needed for poultry products and "rare" cooked specialty meats. The USDA Agriculture Research Service (ARS) has compiled a chronological history of the search for a method to accurately deduce the internal cooking temperature of meat and poultry products. After examination of the chronology, it is evident that nearly every new and upcoming physiometric or electronic idealogy has been employed to answer this question to no avail. ARS is presently investigating the utility of a new concept based on the hydrophobicity of the inner-folded amino acid "R" groups. This involves the denaturation of the suspect enzymes and the consequential loss of the tertiary and quaternary structure of these enzymes, exposing their hydrophobic "R" groups to further reactivity. The denatured enzymes are then reacted with a chromophore and quantitated spectrophotometrically.

ARS is also working out details on an LC (ion exchange) method. This method looks at an 8.3 gradient minute peak, unidentified to date, that seems to show linearity over the temperature range 140–160°F. This is a rapid LC method that uses the juice of a sample directly injected onto the LC column. ARS will also be ironing out details on the pyruvate kinase method that they developed. Adequate information has not been gathered to determine the utility of this method which involves the derivatization of tissue extract, causing a loss of fluorescence when pyruvate kinase activity is present. The samples are visualized on gridded filter paper under a longwave fluorescent light. The greater the loss of fluorescence compared to a "standard" or unheated extract, the higher the internal temperature achieved in the product.

Mr. Pickel has recently been offered the extended use of an elecrophoresis unit. He may be able to use this instrumentation for the separation and identification of enzymes, amino acids, and proteins involved in the analysis of internal temperature. Plans are also being made to obtain GPC LC columns for the separation and identification of enzymes and proteins with UV/vis and fluorescence detection.

A manuscript is being prepared for the AOAC *Journal* on the collaborative study conducted last year by Webb Technical Group, Inc., of Raleigh, North Carolina, on the use of test kits to determine the internal temperatures achieved in canned hams. The results of this study indicated the lack of sufficient sensitivity for this application of the Boeringher Mannheim Diagnostics acid phosphatase colorimetric test kit. The use of the APIZYM test strip from Analytab Products in Plainview, New York, showed some promise for three of the 19 enzymes present on the strip. Further study would be required to confirm this.

Nitrates and Nitrites.—Associate Referee Juan Muniz reports that he is planning a collaborative study in 1989 to include the current colorimetric nitrite determination in meat, **24.044**, and a cadmium reduction column procedure for both nitrite and nitrate in meat. The study will incorporate the use of a sample blank which is not a part of either method **24.044** or the nitrate *m*-xylenol procedure **24.041**. If successful, the cadmium reduction column method could be used in lieu of the *m*-xylenol nitrate test which has rather poor repeatability and reproducibility.

Nitrosamines in Bacon.—Associate Referee Walter Fiddler reports that most efforts this year have been devoted to the collaborative study for nitrosamines in minced fish. This study is being conducted under the General Referee for Food Additives, Thomas Fazio. A poster presentation will be given at the annual meeting. The manuscript on the collaborative survey conducted last year for nitrospyrrolidine (NPYR) in dry cured bacon after frying has been submitted to the AOAC *Journal* for publication. Another paper, "Nitrosothiazolidine and Nitrosothiazolidine Carboxylic Acid in Dry Cured Bacon," has been submitted to *Journal of Food Safety* for publication.

Protein in Meat and Meat Products—Mercury vs Copper Catalysts.—Associate Referee Carolyn Henry reports that last year's collaborative study conducted under USDA contract with Webb Technical Group, Inc., has been evaluated. The manuscript and statistical data evaluation has been submitted to Committee on Foods I for consideration. In general, it appears that a copper-based catalyst of the specified formulation could be used as an alternative to the mercury catalyst now specified in methods **24.027** (**2.057**) and **24.038**, provided that Kjeldahl or block digestor units are properly calibrated as in **2.056(a)**, and that digestion be continued for 45 min after clearing rather than the specified 30 min. Failure to do so will result in a 0.1–0.2% low bias compared to the mercury catalyst when protein contents of meat products are calculated.

Sample Preparation Techniques for Meat Analysis.—Sylvan Eisenberg, the Associate Referee, reports that the collaborative study results of the preparation of meat samples for analysis, using a food processor as an alternative to the food chopper specified in **24.001**, has been submitted to the Committee on Foods I for consideration. If adopted, a statement is needed to the effect that extra care is required when food processors are used for the preparation of high fat samples and/or samples containing amounts of connective tissue so as not to lose fat on inner surfaces or connective tissue around blades of the instrument.

Serological Identification of Animal and Poultry.—The Associate Referee, Arthur Marin, reports that he was not able to work on this topic this year. If workloads and laboratory priorities permit, he may be able to study the USDA-developed serological methods, SIFT.

Specific Ion Electrode Applications.—Associate Referee Randy Simpson reports that, because of a change in assignments, he has not been able to provide the additional statistical evaluations requested by the Committee on Foods I since the collaborative study was conducted for the determination of sodium, potassium, and chloride in processed meat products using ion-selective electrode analysis. Because the methodology employed in this collaborative study appeared to be successful, the General Referee recommends that the data from the manuscript be evaluated by the Committee statistician so that this project can be completed and this valuable technology can be considered for official status.

Recommendations

(1) Change the topic Automated Methods for Meat Analysis to Robotic Methods for Meat Analysis and appoint an Associate Referee.

(2) Consider for interim first action the method for the determination of phosphorus in meat and meat products using the Digesdahl System.

(3) Consider for interim first action the method for the determination of total fat in meat using the Soxtec adoption of the Randall ether extraction procedure.

(4) Appoint an Associate Referee and consider for interim first action (when final manuscript is received) the NMKL collaboratively studied method for hydroxyproline as a collagen indicator.

(5) Consider for interim first action the use of a specified

copper catalyst for protein analysis in meat as an alternative to the mercury catalyst described in **2.057**, with appropriate statements on burner calibrations and digestion times required to avoid low bias.

(6) Combine under the Associate Referee for Immunochemical Identification of Additives, the topic Immunological Methods for Meat and Poultry Products and change the title to the latter.

(7) Consider for interim first action the alternative use of food processors for meat sample preparations with appropriate statements on the care required for high fat and/or connective tissue samples to avoid loss of fat on inner surfaces and connective tissue around blades.

(8) Complete the statistical evaluation in Committee for the collaborative study on specific ion electrode technology for determining sodium, potassium, and chloride content of meat products, and consider the method for interim first action. Appoint a new Associate Referee.

(9) Appoint an Associate Referee for a new topic entitled Total Nitrogen and Alpha-Amino Nitrogen and Total Creatinine in Meat Bouillons and Consommés.

(10) Appoint an Associate Referee for a new topic entitled Glutamic Acid and Monosodium Glutamate in Meat.

(11) Discontinue the topic Fat in Meats (redundant to topic Total Fat in Meat).

(12) Continue study on all topics.

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Mycotoxins

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The past year has seen further activity in collaborative testing of enzyme-linked immunosorbent assay (ELISA) procedures for mycotoxins. The new Associate Referee on Immunochemical Methods for Mycotoxins is James J. Pestka of Michigan State University. One other change in the list of Associate Referees is the appointment of Mary W. Trucksess of the Food and Drug Administration (FDA) as Associate Referee on Aflatoxin Methods and the resignation of Douglas L. Park, University of Arizona. Dr. Park has actively worked on Aflatoxin Methods for 5 years and in 1987 received the Committee on Foods I Associate Referee Award.

Reverse-phase liquid chromatography (LC) as a potential technique for multimycotoxin analysis has been further extended. Data have been published for 182 mycotoxins and other fungal metabolites using UV-visible photodiode array detection and alkylphenone retention indices (1). Application of the technique to analysis of fungal cultures was illustrated with *Fusarium* extracts. Also, reverse-phase LC-mass spectrometry (MS) has been carried out at normal flow rates with standard bore columns using a moving belt interface; separation of trichothecenes was demonstrated (2).

Potential new mycotoxin problems include the detection of the alkaloid echinulin, which is a metabolite of the *Aspergillus glaucus* group of fungi, in feed refused by swine (3); isolation of a hemorrhagic mycotoxin (wortmannin) from *Fusarium oxysporum* (4); and the presence in high incidence of aflatoxin-like substances with affinity to a monoclonal aflatoxin B_1 antibody in the urine of normal people in Denmark, the United States, and Finland (5).

Mycotoxins in Foods is a recently published book edited by Palle Krogh which features chapters by several well known mycotoxin researchers (6).

The final draft of Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis has been prepared by the AOAC Committee on Interlaboratory Studies (7). Changes from the draft previously published (8) include the following: the required minimum number of laboratories submitting valid data is now 8; following application first of the Cochran outlier test for removal of laboratories showing extreme within-laboratory variation, Grubbs single value and pair value tests are used for removal of outlier laboratories with extreme averages (tables of critical values for both Grubbs tests and the Cochran test are appended to the Guidelines); and the maximum outlier rate is given as 2/9 laboratories, when a minimum design is used. Attention of Associate Referees conducting collaborative studies is also drawn to the forms for recording and calculation of precision data, which were distributed by the Official Methods Board in January 1988.

Two new collaborative studies have been initiated and carried out during the past year. Reports of the Associate Referees are given below.

Aflatoxin M.—Associate Referee Robert D. Stubblefield (USDA, Peoria, IL) reports that he is working on a method utilizing an immunosorbent affinity column and he has been evaluating these columns and ELISA kits for aflatoxin M_1 as they have become available. He has no recommendations to make on any of these at the present time. Two recent papers describe the application of immunoaffinity chromatography in a method for determination of aflatoxin M_1 in skim milk (9) and of ELISA to determine aflatoxin M_1 in human milk and raw cow's milk (10).

Performance characteristics have been reviewed for methods for determination of aflatoxin M_1 in milk and milk products which have been tested collaboratively or included in check sample series, as well as methods with claimed detection limits in the low parts per trillion range (11). The availability of 3 full cream milk powder reference materials, certified for their aflatoxin M_1 content is noted (12, 13); difficulties encountered in determination of aflatoxin M_1 were discussed (12). The Associate Referee has published a paper on the optimum conditions for formation of the aflatoxin M_1-trifluoroacetic acid derivative (14). Losses of aflatoxin M_1 in milk caused by addition of formaldehyde have also been studied (15); this preservative should not be used for milk samples in collaborative studies.

The Associate Referee repeats his recommendations of last year on the status of official AOAC methods for aflatoxins M (16).

Aflatoxin Methods.—Associate Referee Douglas L. Park (University of Arizona, Tucson, AZ) reports that the ELISA procedure (17) for determining aflatoxin B_1 in cottonseed products and mixed feeds has now been granted interim official first action status.

The visual and semiquantitative spectrophotometric ELISA procedure for aflatoxin B_1 in corn and peanut products (18) is recommended for adoption as an official first action screening method for determining the presence or absence of aflatoxin B_1 at $\geq$20 ng/g in corn and roasted peanuts. Results from this follow-up collaborative study were unacceptable for raw peanuts and further study is recommended for this commodity.

The liquid chromatographic method for determination of aflatoxins B_1, B_2, G_1, and G_2 in corn, peanut butter, and raw peanuts, which was collaboratively studied last year (19), is recommended as official first action for corn and peanut butter at concentrations of $\geq$13 ng total aflatoxins/g. Continued

study on application of the method to raw peanuts is recommended.

Dr. Park also recommends adoption as official first action of the solvent-efficient TLC method with densitometric determination (20) for determination of aflatoxins B_1, B_2, G_1, and G_2 in corn, peanut butter, and raw peanuts at concentrations of ≥26 ng total aflatoxins/g for peanut butter and ≥13 ng total aflatoxins/g for corn and raw peanuts; however, variation for aflatoxin G_1 was generally greater compared to aflatoxin B_1. With visual comparison, concentrations should be ≥26 ng total aflatoxins/g for raw peanuts and >26 ng/g for corn and peanut butter.

Results obtained from collaborative study of an ELISA card screening method (21) for aflatoxins B_1, B_2, and G_1 in corn, cottonseed, mixed feed, peanut butter, and raw peanuts were variable. Percentages of correct responses from participating laboratories ranged from 36 to 88%. Correct responses according to commodity ranged from 42 to 88%. Samples containing <5 ng aflatoxin/g were correctly reported most of the time (81–88%). The number of false negative results reported for all products was unacceptable. A yellow pigment in the cottonseed extract interfered with reading the results on the card (see below).

New Associate Referee Mary W. Trucksess (FDA, Washington, DC) notes several publications that have appeared recently that underline the rapid progress in development of ELISA methods for aflatoxins (22–25) (see also under *Immunochemical Methods for Mycotoxins*). Several test kits are available commercially; 2 kits were evaluated. Both kits used a similar extraction step but different application procedures. In the first procedure, the extract is applied to a cup (cup method), while in the second procedure, the extract is applied to a card (card method).

Applying the manufacturer's cup method to 50 samples (corn, raw peanuts, peanut butter, cottonseed, poultry feed) with added total aflatoxins, B_1, B_2, and G_1 at levels of 30 and 20 ng/g, all samples tested negative. Subsequently, the method was modified (26) and a collaborative study to evaluate this modified method was conducted. Corn, raw peanuts, peanut butter, and poultry feed samples were spiked in duplicate with total aflatoxins B_1, B_2, and G_1 at 30, 20, and 10 ng/g, respectively. Samples of cottonseed were spiked at 60, 20, and 10 ng/g, respectively. The artificially contaminated samples, control samples (<2 ng/g), naturally contaminated corn (101 ng/g) and peanut meal (69 ng/g) samples, and practice samples were sent to 12 laboratories in the United States, France, Canada, Denmark, Japan, and The Netherlands. All collaborators have completed their analyses and submitted reports. They all were able to detect aflatoxins in the naturally contaminated peanut and corn samples. Ninety-three percent of the samples at a level of 30 ng/g were found positive, while 42% of the samples having 10 ng/g aflatoxins were found positive. With the exception of poultry feed, 86% of the samples at a level of 20 ng/g were found positive. All control samples tested negative. A complete report of the study has been prepared. The method is recommended for adoption as official first action for screening for aflatoxins in corn, cottonseed, peanuts, peanut butter, and poultry feed at ≥20 ng/g.

The card method referred to above was unable to detect added aflatoxins at a level of 20 ng/g in corn, peanuts, and poultry feed. The procedure was modified. The manufacturer has not given approval of the modification and the collaborative study has therefore been delayed.

The use of an affinity column to isolate aflatoxins (27) is under investigation. The aflatoxins are extracted from grains with aqueous methanol and the extract is passed through the affinity column. After the column is washed with water, the aflatoxins are eluted with either methanol or acetonitrile. Quantitation can be done by either TLC densitometric analysis or LC fluorometric measurement. A collaborative study of this method will be conducted next spring.

Due to the large number of immunochemical kits on the market for mycotoxins, both of the Associate Referees for Aflatoxin Methods suggest that the Associate Referee for Immunochemical Methods screen requests from alternative sources for antibodies, kits, etc., to confirm that they are comparable to those used in the collaborative study of the procedure in question.

In other areas of aflatoxin analysis, papers have been published recently on the rapid determination of aflatoxins in raw peanuts or peanut butter by LC with postcolumn iodination (28–30), on determination of aflatoxins in feeds by HPTLC (31), on a comparison of 4 TLC methods for determination of aflatoxins in raisins (32), and on rapid detection of aflatoxins by pressure minicolumn chromatography (33). A bright greenish yellow fluorescence in figs was qualitatively correlated with natural aflatoxin contamination (34).

Alternaria Toxins.—Associate Referee Edgar E. Stinson (USDA, Philadelphia, PA) reports no new analytical methods for *Alternaria* toxins. The necessity of confirmatory tests when reporting the presence of alternariol monomethyl ether or zearalenone was emphasized by a report on the co-extraction of these 2 mycotoxins during analysis of sorghum-based mixed feeds using 3 common methods for zearalenone analysis (35). A single TLC analysis using benzene–acetone (95 + 5) did not result in sufficient separation for positive differentiation. This report recommended confirmation of the presence of alternariol methyl ether and zearalenone by using a UV or (preferably) fluorescence detection method.

A study of the metabolism of alternariol methyl ether in porcine liver and intestinal mucosa showed that there was conjugation with glucuronic acid (36).

Toxins produced by *Alternaria alternata* may be involved in the etiology of esophageal cancer in China. The annual national death rate from this cancer is 16/100 000, and in the most severely affected areas may reach 161/100 000. With an estimated population of one billion, this is approximately 160 000 deaths per year from what is a rare cancer in the western countries. *Alternaria alternata* is a common grain contaminant in China, with a higher incidence of contamination in areas of high esophageal cancer. Corn cultures of this species have been shown experimentally to be carcinogenic to rats (37, 38).

The Associate Referee has stocks of alternariol, alternariol methyl ether, and tenuazonic acid on hand. Samples for reference purposes are available.

Citrinin.—Associate Referee David M. Wilson (University of Georgia, Tifton, GA) notes no new developments or publications concerning methodology for citrinin. The proposed collaborative study would have to include addition of citrinin to the sample by the collaborators themselves before analysis. It has been shown that in aqueous solution at pH 7.4 citrinin exists not as the quinone methide but as a diastereoisomeric mixture of hydrates (39). Instability of citrinin in grains might be studied by techniques used recently to investigate conversion of citrinin to dihydrocitrinone and ochratoxin A by *Penicillium viridicatum* (40). Natural occurrence of citrinin in coconut products has been reported (41).

Cyclopiazonic Acids.—Associate Referee John Lansden (USDA, Dawson, GA) notes that cyclopiazonic acid has been determined by normal phase LC with a detection limit of 0.2 ng standard, corresponding to a lower limit for quantitation in corn of 0.1 μg/g (42). However, the LC solvent system is ethyl acetate–2-propanol–25% aqueous ammonia (55 + 20 + 5, v/v/v), which being highly basic would be expected to cause technical problems, including dissolution of the silica gel packing. A recent study has demonstrated that cyclopiazonic acid can occur in meat of chickens dosed with the toxin; analysis was by ligand-exchange reverse-phase LC (43).

Emodin and Related Anthraquinones.—This topic should be deleted. No Associate Referee has been found.

Ergot Alkaloids.—Associate Referee George M. Ware

(FDA, New Orleans, LA) reports that he plans to complete preparation for a collaborative study of a method for ergot alkaloid determination in wheat and rye. Initial plans have been delayed because of problems associated with unstable standard solutions and LC column deterioration due to high pH of the mobile phase. Recently, ergot alkaloid standard solutions have been stabilized by using a mixture of water + propylene glycol + ethylene glycol in various proportions. Using this mixture, ergot alkaloid standards could be stabilized for one month. Also, the problem associated with column deterioration has been eliminated. Stabilization of ergotamine by formic acid has been reported elsewhere (44).

The Associate Referee reports that the method of Klug et al. (45) for determination of ergot alkaloids in foods was published in full (in German) this year (46). The authors stated that it is suitable for the routine analysis of ergometrine, ergometrinine, ergosine, ergosinine, ergotamine, ergotaminine, ergocornine, ergocorninine, α-ergocryptine, α-ergocryptinine, β-ergocryptine, β-ergocryptinine, ergocristine, and ergocristinine in cereal products. The method consists of extraction, cleanup by solid phase extraction, and identification and quantitative determination of the alkaloids by LC, with confirmation by TLC and GC/MS. The authors reported that a market survey of cereal products showed that rye products were the most frequent products contaminated with ergot alkaloids. An LC method has also been developed for determination of ergot alkaloids in tall fescue (47). In a further study on the effect of food processing on ergot alkaloids, a reduction of 50% total alkaloid content during bread baking has been demonstrated (48).

The Associate Referee recommends that the collaborative study on a method to determine ergot alkaloids be completed. Also he plans to confirm the performance of the method for cereal products referred to above. With respect to future research, the Associate Referee recommends that a long term stability study for ergot alkaloid standards be initiated.

Immunochemical Methods.—Associate Referee James J. Pestka (Michigan State University, East Lansing, MI) has been involved in collaborative studies reported under *Aflatoxin Methods* and *Zearalenone.* He draws attention to a recent review that has relevance to assessing performance of mycotoxin immunoassay; it discusses the theory of diagnostic systems and the evaluation of their accuracy based on analysis in terms of the "relative operating characteristic" (49). This is a measure uninfluenced by decision biases and prior probabilities and it places performance of diverse systems on a common, easily interpreted scale. New commercial kits for mycotoxins continue to be introduced. Prior to implementation in routine screening, the user should critically assess (*1*) recommended sampling protocols and extraction efficiency, (*2*) sensitivity and specificity of the antibody employed in the kit, (*3*) reproducibility, (*4*) handling ease and endpoint reliability, and (*5*) shelf life (50). The availability of standard check samples that contain known levels of mycotoxins and represent various commodities would be desirable for regular assessment of the functional characteristics of these kits, because they inherently vary from lot to lot.

New advances and application of aflatoxin immunoassay continue to be reported. Optimal conditions for radioimmunoassay (RIA) of aflatoxin B_1 using iodine-125 as a marker and dextran-coated charcoal as a separation matrix were described (51–53). Coumarin, albumins, steroids and ethyl vanillin were reported by the authors (54) to interfere in this RIA and concentrations of various coumarins and phenolic acids causing erroneously high or false positive values have been determined (55). New monoclonal antibodies to aflatoxin B_1 and aflatoxin M_1 have been applied to ELISA of aflatoxin B_1 in corn, cottonseed, feed, and peanut butter and aflatoxin M_1 in milk (22, 25). RIA, ELISA, and immunoaffinity chromatography (56) continue to be evaluated as probes in the assessment of aflatoxin B_1 exposure in areas exhibiting a high incidence of primary liver cancer (57–59).

An ochratoxin A specific monoclonal antibody was used in a competitive solid-phase IgG radioimmunoassay on protein A-Sepharose CL-4B, with ^{14}C-ochratoxin A as a tracer (60). Porcine kidneys were extracted with 0.5% phosphoric acid in chloroform and a 2-step cleanup was achieved on a Sep-Pak C-18 cartridge and a Sep-Pak silica cartridge. Radioimmunoassay with the monoclonal antibody coupled to protein A-Sepharose CL-4B allowed the detection of ochratoxin A in porcine kidneys at a concentration as low as 0.2 ng/g.

Recent applications of RIA and ELISA to analysis of trichothecenes include analysis of urine (61), tissue (62), corn (63), and *Fusarium* mutants blocked in T-2 toxin biosynthesis (64). A new monoclonal antibody against T-2 toxin was produced using a mouse immunized by subcutaneous injections into the shoulder with large immunogen doses (65). Strong cross reaction with the T-2 toxin metabolites 3′-OH T-2 toxin and 3′-OH HT-2 toxin was noted by the authors. An antibody against group A trichothecenes was produced after immunization of rabbits with an immunogen prepared by conjugation of T-2 toxin to bovine serum albumin at the C-8 position (63). Three new immunogens which were prepared by conjugation of the carboxymethyl oxime derivatives of HT-2 toxin, T-2 tetraol, and T-2 tetraol tetraacetate to bovine serum albumin were used for the production of antibodies against the major metabolites of T-2 toxin (66). A new homogenous competition inhibition assay was devised for T-2 toxin, based on complement-mediated lysis of liposomes (67). The authors reported that this assay was sensitive to T-2 toxin levels as low as 2 ng, which is 10-fold more sensitive than ELISA using the same antibodies. A repetitive hit-and-run fluoroimmunoassay was also developed for T-2 toxin (68). A monoclonal antibody for T-2 toxin was converted to a Fab′-fluorescein derivative and specifically complexed onto a T-2 agarose gel. Fifteen successive doses of T-2 toxin ranging from 1 to 50 ng were then repetitively and linearly detected using a column packed with a small volume (0.2 mL) of this gel without recharging with Fab′-fluorescein. For these assays, the effluent from the column was monitored with a spectrofluorometer.

A competitive indirect ELISA employing a monoclonal antibody detected zearalenone at 0.5 ng/mL and was cross reactive with the zearalenols (69).

The Associate Referee has recently reviewed mycotoxin immunoassays and their application to analysis of foodstuffs (70).

Ochratoxins.—Associate Referee Stanley Nesheim (FDA, Washington, DC) has been unable to conduct a collaborative study on determination of ochratoxin A in barley, corn, and swine tissue. The problem of ochratoxin A contamination has been brought closer to home by a report of its occurrence in serum of pigs from a western Canadian packing house (49 of 1200 samples contained 20–229 ng/mL) (71). Other recently reported sources of natural occurrence of ochratoxin A include randomly sampled German pig serum, pig kidneys, and sausage (72, 73); commercial roast coffee (74); and human serum (incidence 57%), kidneys, and milk from the Federal Republic of Germany (72, 75). A significantly higher incidence of ochratoxin A has been found in blood serum of patients with urinary systemic tumors and/or endemic nephropathy living in an endemic area of Bulgaria than in people from a nonendemic area (76). Sensitive LC methodology is now available that allows detection of as little as 0.1 ng ochratoxin A/g (mL) in cereals, tissue, and serum (72) and 0.02 ng/mL in human milk (75). A 2-year study recently completed by the National Toxicology Program of the National Institutes of Health showed that ochratoxin A is a potent carcinogen in rats (77).

Penicillic Acid.—Associate Referee Charles W. Thorpe (FDA, Washington, DC) reports no further work on this topic. Inclusion of penicillic acid in a multimycotoxin LC method for analysis of cocoa beans is noted (78).

Penicillium islandicum Toxins.—This topic should be dropped. No Associate Referee has been found.

Secalonic Acids.—No Associate Referee has been found for this topic, which should be dropped.

Sterigmatocystin.—Associate Referee Octave J. Francis, Jr (FDA, New Orleans, LA) reports that work is still in progress to develop a more satisfactory method for determination of sterigmatocystin in cheese. He has been evaluating a multimycotoxin method that utilizes reverse-phase LC on a cyano column with UV detection at 245 nm (78) and combination of this determination step with the extraction and cleanup procedures of the collaboratively studied cheese method (79). No other analytical methods for sterigmatocystin have been published recently. Interest in sterigmatocystin is still high, however, and recently published papers include studies on its production (80) and binding with macromolecules (81).

Tree Nuts.—Associate Referee Vincent P. DeProssimo (FDA, Brooklyn, NY) reports no further work on this topic.

Trichothecenes.—Associate Referee Robert M. Eppley (FDA, Washington, DC) reports that the development and evaluation of methods for trichothecenes have continued at a steady pace (2, 82–93). Emphasis on screening techniques has been evident and several publications described methods designed to detect and quantitate more than one trichothecene (82, 83, 85–88). Immunoassay procedures have received considerable attention (61–63, 65, 66, 90–93) (see also under *Immunochemical Methods for Mycotoxins*). Recently a monoclonal antibody against deoxynivalenol (DON) was prepared and used for ELISA (93); however, commercial kits for the detection of DON have not yet become available. DON is the most frequently reported trichothecene in grains, and a reliable sample screening procedure (such as an immunoassay kit) would be widely used. Long term storage stability of DON reference solutions has been studied (94).

The second greatest need is for a relatively simple multitrichothecene screening method which could be adapted to quantitation when positive samples are detected.

The Associate Referee recommends that studies on method development and confirmation of identity be continued and that a precollaborative study of a multitrichothecene method be initiated. The chosen method should be able to detect the following trichothecenes in grains: DON, 3-and/or 15-acetyl-DON, nivalenol, fusarenon-X, T-2 toxin, diacetoxyscirpenol, and trichothecin. However, it is not necessary that all of these be included in the first study. The first phase of the study would only require detection and rough approximation of quantities present in the sample.

Xanthomegnin and Related Naphthoquinones.—Associate Referee Allen S. Carman, Jr (FDA, New Orleans, LA) reports that he has conducted some method development work on an LC screening method for xanthomegnin, viomellein, and ochratoxin. A problem not yet solved to date is that viomellein elutes as a split peak using the solvent system developed by the author. This may be due to racemization of this compound caused by its initial cleanup and isolation on silica gel. Several other solvent systems tried did not give sufficient separation of these mycotoxins from co-extractives. This work is being continued.

Xanthomegnin and viomellein (2 peaks) were included in the previously mentioned paper by Frisvad and Thrane (1) on LC of numerous mycotoxins, using UV-visible photodiode array detection and alkylphenone retention indices. Xanthomegnin is not detectable by UV absorbance at 254 nm (95).

Zearalenone.—Associate Referee Glenn A. Bennett (USDA, Peoria, IL) reports that an international collaborative study on the detection of zearalenone by an ELISA procedure has been conducted. Twenty-three collaborators in the United States, Canada, Sweden, Italy, and The Netherlands participated in this study designed to screen corn, wheat, and feed for the presence of zearalenone at ≥500 ng/g. Fifteen collaborators determined the presence (≥500 ng/g) or absence (<500 ng/g) of zearalenone by visually comparing color development in sample extracts to color development in toxin-free extracts spiked to contain 500 ng/g zearalenone. Eight collaborators determined color development in sample extracts spectrophometrically and extrapolated zearalenone levels from standard curves constructed from toxin-free extracts spiked to contain known levels of zearalenone (200, 500, 1000, 1500, 3000 ng/g). Each collaborator assayed, in duplicate, 18 samples of corn, wheat, and feed. Each sample set contained blind duplicates of spiked and naturally contaminated samples. Levels of toxin in naturally contaminated samples were determined by LC analysis (**20.A09-26.A16**) of triplicate 50 g samples.

Preliminary examination of all data indicate that the ELISA procedure can detect zearalenone in corn, wheat, and feed at levels >500 ng/g. One false positive (2%) and 3 false negatives (7%) were reported for 0 ng/g and 800 ng/g levels, respectively. Two of the false negatives were reported by the same collaborator. Thirty-three percent (15/46) reported positive results for samples containing 250 ng/g zearalenone; 37% (17/46) of results were positive at 300 ng/g; 20% (9/46) were positive at 400 ng/g; 72% (33/46) were positive at 500 ng/g; 93% (42/45) were positive at 800 ng/g; and 100% were positive at 900, 1000, and 2000 ng/g levels. The spectrophotometric procedure did not provide more accurate results than the visual method. However, construction of standard curves with spectrophotometric data from extracts spiked to contain 200–3000 ng/g zearalenone appears to be suitable to evaluate the performance of the ELISA kits. These data indicate that the ELISA procedure can be used to screen corn, wheat, and feed for the presence of zearalenone at ≥800 ng/g levels. A complete report of the study and results obtained is being prepared.

An ELISA procedure has been used to survey grain-based food products for zearalenone (96). Zearalenone was found in 22% (17/79) of samples tested at an average level of 20 ng/g. Maximum levels of 120 and 130 ng/g were found in samples of corn meal and popcorn, respectively. Zearalenone was not found in wheat flour or baby food samples examined. The detection limit was reported to be 2.5 ng/g. A survey of grain sorghum grown in North Carolina between 1981 and 1985 indicates that zearalenone is a potential problem in this crop (97). A survey of 17 grower bins in 1983 revealed that zearalenone was present in all samples at an average concentration of 443 ng/g. Zearalenone has also been detected in bulk samples of soybeans from the Midwest that contained pink beans (98), in sorghum-based mixed feeds in South Africa (35), and in wheat from Queensland, Australia (99). Significant reduction in zearalenone concentrations was observed during the process of making tortillas from corn (100). A sensitive method for determination of zearalenone (and zeranol) in edible animal tissue uses LC with electrochemical detection and confirmation by GC-MS (101). Zearalenone has also been included in a multimycotoxin LC method for analysis of cocoa beans (78).

The Associate Referee recommends that the ELISA procedure to screen corn, wheat, and feed for the presence of zearalenone at ≥800 ng/g be adopted as official first action. Also, he recommends that additional studies on cross reactivities of zearalenone antibodies to zearalenone analogs and bound zearalenone be conducted.

Recommendations

(1) Adopt as official first action the ELISA screening method for aflatoxin B_1 in corn and roasted peanuts at ≥20 ng/g.

(2) Adopt as official first action the LC method for determination of aflatoxins B_1, B_2, G_1, and G_2 in corn and peanut butter at ≥13 ng total aflatoxins/g.

(3) Adopt as official first action the ELISA cup method

for screening for aflatoxins in corn (≥30 ng/g), cottonseed (≥20 ng/g), peanuts (≥30 ng/g), and peanut butter (≥20 ng/g).

(4) Adopt as official first action the ELISA screening method for zearalenone in corn, wheat, and feed at ≥800 ng/g.

(5) Drop the topics on *Emodin and Related Anthraquinones, Penicillium islandicum Toxins,* and *Secalonic Acids.*

(6) Continue study on all other topics.

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This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Section numbers refer to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68,** 369–411 (A methods).

Oils and Fats

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Antioxidants.—Associate Referee B. D. Page evaluated 3 procedures for extracting antioxidants from butter oil, using LC separation to evaluate extraction efficiency and possible interference from coextracted lipid material. (The study was carried out for the International Dairy Federation (IDF) Group E-43, Food Additives.) The extraction solvents studied [acetonitrile at 65°C; acetonitrile–2-propanol–ethanol (2 + 1 + 1); acetonitrile at room temperature] gave similar recoveries for each of the antioxidants if vigorous shaking was employed during extraction of the antioxidants. IDF is planning a collaborative study of the determination of BHA, BHT, and TBHQ in butter oil by AOAC **20.009-20.013**, and the Associate Referee anticipates inclusion of butter oil in the scope of the AOAC method. The Associate Referee also plans to continue studies of the LC separation and tailing phenomena of antioxidants and suggests that a caution is required regarding OG and Ionox-100 which coelute on most C-18 columns.

Emulsifers.—There was no Associate Referee activity during the past year.

Hydrogenated Fats.—Associate Referee R. A. DePalma completed a collaborative study of a capillary column GC method for determination of C-18 *trans* monoene and *cis, cis*-methylene interrupted unsaturated diene and triene levels as well as general fatty acid composition of vegetable oils, shortenings, and margarines. The method specifies use of a 60 m × 0.25 mm fused silica capillary column coated with SP-2340 cyano-silicone stationary phase. Six pairs of samples (shortening, vegetable oil, or margarine oil) were sent to collaborators. Within each pair of samples, one sample was fortified with methyl elaidate to elevate the *trans* level, and one sample was fortified with methyl linoleate to elevate the *cis-cis* level. Fortification levels ranged from 2 to 5%. Duplicate samples were prepared for one of the pairs. All samples were blind coded (each laboratory sample had a unique number code). Collaborative results were received from 14 laboratories. Preliminary review of the data indicates that repeatability and reproducibility were good. Recovery of analyte from the fortified samples was generally below 100%. A full report of the study is under preparation.

The IUPAC Commission on Oils, Fats and Derivatives drafted a capillary column GC method for determination of the fatty acid composition of edible oils and fats (1). Stationary phases of moderate polarity (polyglycol, polyester, or polar polysiloxane) are allowed rather than a specfic stationary phase. A collaborative study of the IUPAC method was initiated in early 1988. Six samples in duplicate (beef tallow, lard, beef and lard mixture, fish oil, hydrogenated fish oil, and a mixture of unhydrogenated and hydrogenated fish oil) were sent to collaborators with instructions to analyze each sample once, using normal capillary GC equipment and their usual operating conditions, provided they adhere to the general instructions in the IUPAC method.

Lower Fatty Acids.—Associate Referee G. Bigalli continued study of methodology for GC determination of lower fatty acids. The IUPAC Commission on Oils, Fats and Derivatives validated and adopted the Phillips and Sanders procedure for determination of butyric acid in fats containing butterfat (2). Portions of fat (100 mg) are saponified with ethanolic potassium hydroxide solution followed by acidification with phosphoric acid to liberate the fatty acids. The water-soluble fatty acids are separated from the water-insoluble fatty acids by filtration. Valeric acid (internal standard) is added to the filtrate containing the free butyric acid, and butyric acid is determined by gas chromatography (peak height measurements) using a stationary phase suitable for free fatty acid analysis. Butyric acid solution and valeric acid solution are used to construct a GC calibration curve. A preliminary study was carried out with 2 samples, a 100% butterfat and a 10% butterfat. The statistical results are shown in Table 1. Four samples were provided for a second study. Results of statistical analysis of the second study are shown in Table 2. The results of both studies indicate that the determination of butyric acid as the free acid by the Phillips and Sanders procedure can be carried out with an acceptable

Table 1. Statistical results[a] of preliminary IUPAC study of Phillips and Sanders procedure for butyric acid in fats containing butterfat

Statistic	100% Butterfat	10% Butterfat
No. of laboratories	12	6
Mean value ($\bar{x}$)	3.32	0.35
s_r (repeatability std dev.)	0.070	0.015
s_R (reproducibility std dev.)	0.232	0.055
RSD, ($s_r \times 100/\bar{x}$)	2.1	4.2
RSD, ($s_R \times 100/\bar{x}$)	7.0	15.8
r ($2.8 \times s_r$)	0.20	0.04
R ($2.8 \times s_R$)	0.65	0.15

[a] See reference 2.

degree of precision. The Referee recommends adoption of the method (IUPAC Method 2.310) as official first action. It is also recommended that the precision statement include the RSD_r and RSD_R values for samples 1, 3, and 4 shown in Table 2, which were obtained from analysis of butterfat or blends of butterfat and other fats or oils.

Marine Oils.—Associate Referee R. G. Ackman, in association with Jeanne D. Joseph, National Marine Fisheries Service, Charleston, SC, has completed collaborative study of a capillary column GC method for determination of the fatty acid composition of marine oils (3). The method specifies use of a bonded Carbowax-20M or equivalent polyglycol liquid phase, 25 m or more in length and 0.20–0.35 mm id. Directions are given for analysis of oils or ethyl esters using a 23:0 internal standard (23:0 methyl ester for oil samples converted to methyl esters, or 23:0 ethyl ester for ethyl ester samples). Theoretical correction factors (4–6) relative to 23:0 are used to calculate the levels of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) in each sample, expressed as mg EPA or DHA fatty acid per g sample. Six samples (marine oils in gelatin capsules) were sent to 15 collaborating laboratories with instructions to analyze each sample only once. One of the samples was a commercial ethyl ester preparation and 2 samples were duplicate oils. The collaborative data are under review.

Olive Oil Adulteration.—Associate Referee E. Fedeli and colleagues have developed standard procedures for determining sterol (7) and aliphatic alcohol (8) content of olive oils and other vegetable oils by capillary column GC. The Associate Referee has investigated use of capillary column GC for analysis of sterols and waxes in vegetable oils and plans to study methodology for organoleptic analysis of vegetable oils. Flor (9) used LC analysis to detect the presence of undeclared esterified olive oil in olive oil products. The ratio of the 4 major triglycerides when plotted as LOO/LOP vs 000/POO produced 2 distinctly separated lines, one of which was characteristic of esterified olive oil.

Table 2. Statistical results[a] of second IUPAC study of Phillips and Sanders procedure for butyric acid in fats containing butterfat

	Sample[b]			
Statistic	1	2	3	4
No. of laboratories	11	11	11	10
$\bar{x}$ (% m/m)	3.46	1.78	0.18	1.79
s_r	0.104	0.057	0.008	0.043
s_R	0.242	0.089	0.024	0.161
RSD_r	3.0	3.2	4.5	2.4
RSD_R	7.0	5.0	13.1	9.0
r ($2.8 \times s_r$)	0.29	0.16	0.23	0.12
R ($2.8 \times s_R$)	0.68	0.25	0.07	0.45

[a] Statistical analysis by R. Albert, Food and Drug Administration.

[b] Samples were (1) 100% butterfat, identical to that provided in the preliminary study (Table 1); (2) refined tallow containing 1.82% butyric acid; (3) refined tallow containing 5% sample 1; and (4) a blend of cream and vegetable oil containing about 50% milkfat.

Table 3. Statistical results[a] of IUPAC collaborative study (18) of headspace method for volatile hydrocarbon residues in vegetable oils

	Sample[b]			
Statistic	1	2	3	4
No. of laboratories after elimination of outliers[c]	16	16	9	10
$\bar{x}$ (mg/kg)	2.93	13.7	97	956
s_r	0.65	1.68	5.82	66.7
s_R	2.62	4.10	13.5	143
RSD_r	22.4	12.3	6.00	6.98
RSD_R	89.3	29.9	13.9	15.0
r ($2.8 \times s_r$)	1.8	4.7	16.3	187
R ($2.8 \times s_R$)	7.3	11.5	38	400

[a] Statistical analysis by R. Albert, Food and Drug Administration.

[b] Samples were (1) refined peanut oil containing 3.3 ppm technical hexane; (2) refined peanut oil containing 13.2 ppm technical hexane; (3) refined sunflower seed oil containing 100 ppm technical hexane; and (4) crude (pressed) rapeseed oil containing 1000 ppm technical hexane. Two additional samples included in the collaborative study were refined vegetable oils with negligible technical hexane content (less than 1.5 ppm residual solvent (mean value) reported by collaborators).

[c] Outliers from 2 laboratories eliminated for sample 3; outliers from 1 laboratory eliminated for sample 4.

Oxidized Fats.—Associate Referee M. M. Blumenthal reported a new colorimetric rapid test for monitoring the cumulative changes in frying oils (10). Snyder (11) used multiple headspace extraction for quantitative determination of volatiles produced from oxidized vegetable oils. The multiple headspace extraction technique includes a pressure and time-controlled injection onto a bonded capillary GC column, compared to the static headspace GC technique. Perkins and Pinter (12) compared the efficiency of 5 separation techniques [low temperature crystallization, countercurrent (batch type) distribution (aqueous ethanol/hexane), countercurrent distribution (acetonitrile/hexane), adsorption chromatography, and partition chromatography]. A batch-type distribution method using acetonitrile/hexane was the most effective in concentrating the polar products and removing the palmitate and stearate from oxidized vegetable oil. An LC system was also described for separating or "profiling" the oxidized products from abused fat. Dobarganes and Perez-Camino (13) described a procedure for analysis of heated fats, including used frying fats, which allows distinction of the 3 types of degradation: oxidative, thermal and hydrolytic.

Pork Fats and Other Fats.—The topic has no Associate Referee.

Sterols and Tocopherols.—Associate Referee R. J. Reina has reviewed LC methods for determination of tocopherols in oils and fats. Tocopherol composition is useful in the analysis of vegetable oil blends and an aid in detection of vegetable oil adulteration. Homberg and Bielefeld (14) examined the determination of sterols in fats and oils by GC analysis using betulin or cholestane as internal standard. They reported that errors can result from coextraction of other substances (methyl sterols, triterpenes) during isolation and silylation of the sterols. Betulin and cholestane were both satisfactory as internal standard. However, there was a greater chance for quantitative errors with cholestane. Maerker and coworkers (15) described the LC separation and quantitation of cholesterol oxidation products using a binary solvent system [hexane–2-propanol (97 + 3)] and a 3-part gradient with flame ionization detection.

Other Topics.—As noted in the previous General Referee report, and in the absence of adverse comments, the General Referee recommends that the following official first action methods be adopted official final action: **28.104-18.109**, betasitosterol in butter oil; **28.130-28.131**, foreign fats containing tristearin in lard; **28.120-28.123**, cyclopropene fatty acids in

oils; and **28.139-28.141**, chick edema factor (dioxins) in oils and fats.

The General Referee recommends that **28.B01-28.B05**, triglycerides in fats and oils, gas chromatographic method (16), be revised by adding the following at the end of section **28.B02(b)**: "(Equivalent results may be obtained with use of a short capillary column, i.e., 6 m or less.)" In the course of an IUPAC collaborative study of the GC method for triglycerides, 4 laboratories submitted results obtained using capillary columns. No significant difference was observed between the results obtained by use of the capillary or packed column (17).

The IUPAC Commission on Oils, Fats and Derivatives carried out collaborative studies of a direct injection method and a headspace method for GC determination of volatile hydrocarbon (hexane) residues in vegetable oils using either a packed or capillary column (18). The headspace method (method 2.607) was adopted by the Commission. A 5 g portion of vegetable oil is added to a vial, which is closed immediately with a septum and cap. Internal standard (*n*-hexane or cyclohexane) is added through the septum and the vial is shaken vigorously for 1 min. Then, the vial is heated for exactly 60 min at 80°C. A 1000 μL portion of headspace is withdrawn from the vial using a gas-tight syringe without removing the vial from the heating bath and is immediately injected into the GC system (oven temperature, 50°C; injector and detector temperatures, 100°C). The GC calibration factor is determined with hexane-free vegetable oil spiked with solvent. Hydrocarbons in the headspace of test portions are determined as hexane. A collaborative study of the headspace method and direct injection method was carried out in 1985. Six vegetable oils containing negligible (<1 mg/kg) to 1000 mg/kg of technical hexane were sent to collaborators. Results of statistical analysis of the collaborative data for the headspace method are shown in Table 3. Similar reproducibility relative standard deviations (27–30%) were obtained with hexane levels above 10 mg/kg. Mean values were in good agreement with the true level of hexane in the samples. The Referee recommends adoption of IUPAC Method 2.607 official first action.

Commission on Oils, Fats and Derivatives, Applied Chemistry Division, IUPAC.—The 41st meeting of the Commission was held at the University of St. Andrews, St. Andrews, Scotland, August 16–18, 1988. The meeting was chaired by the Commission chairman, Joyce Beare-Rogers, Food Directorate, Department of National Health and Welfare, Ottawa, Canada. The Commission reviewed reports submitted by the coordinators of 8 working groups covering 1987–1988 studies of methods for determination of mineral oil residues, color of commercial lecithins, lead in oils and fats by AAS, triglycerides as ECNs (equivalent carbon numbers) by RP-LC, polymerized triglycerides by gel permeation LC, phospholipids by LC, fatty acids (including n-3 and n-6 fatty acids in animal fats) by capillary GC, and benzo[a]pyrene (rapid method).

Results of collaborative studies of methods for determination of triglycerides by RP-LC and lead in oils and fats by AAS were satisfactory and these methods were adopted. Twenty laboratories from 12 countries participated in a third study of the LC triglyceride method. Four samples (palm–sunflower oil blend used as a training sample, olive oil, rapeseed oil, and palm oil) were sent to collaborators for analysis using a 5 μm C-18 column with refractive index detector (acetone–acetonitrile (50+50) elution solvent). Results from 16 laboratories were subjected to statistical analysis. For ECNs 40 to 48, reproducibility relative standard deviations (RSD_R) varied with concentration as follows: 0.5%, RSD_R = 21; 3–5%, RSD_R = 8–16; 5–10%, RSD_R = 3–6; 10–30%, RSD_R = 2–4; 30–65%; RSD_R = 1.6–2.5. RSD_R values for ECN 50 (concentration range, 5–10%) were 7–10%. All but 2 participating laboratories identified peaks eluting before ECN 38 as mono- and diglycerides. Good results were also obtained with the rapid method for benzo[a]pyrene. However, little progress was made with the method for determination of mineral oil residues.

Satisfactory collaborative studies were carried out of methods for determination of tocopherols and tocotrienols in vegetable oils and fats by LC (19) and copper, iron, and nickel in oils and fats by direct graphite furnace AAS (20), and they were adopted as methods **2.432** and **2.631**, respectively.

New topics to be considered by the Commission include determination of contaminants in oils shipped in bulk, halogenated solvents in olive oils, precision data for the peroxide value and acidity value methods, fat and oil refining, and phosphorus in oils. The Commission also agreed to support the ISO study of the proposed replacement of carbon tetrachloride with cyclohexane in the procedure for determination of iodine value and to cooperate with the Commission on Food Chemistry in a project for assessment of lipid oxidation. The Commission agreed to establish a joint committee with the Commission on Food Chemistry on the topic of lipid oxidation, and H. Wessels accepted the position of co-chairman of this committee.

Recommendations

(1) Adopt as official first action IUPAC Method 2.310, determination of butyric acid.

(2) Adopt as official first action IUPAC Method 2.607, determination of volatile hydrocarbon residues in fats and oils.

(3) Revise **28.B01-28.B05** to add the following at the end of section **28.B02(b)**: "(Equivalent results may be obtained with use of a short capillary column, i.e., 6 m or less.)."

(4) Adopt as official final action the following official first action methods: **28.104-28.109**, beta-sitosterol in butter oil; **29.120-28.123**, cyclopropene fatty acids in oils; **28.130-28.131**, foreign fats containing tristearin in lard; and **28.139-28.141**, chick edema factor (dioxins) in oils and fats.

(5) Continue study on all other topics.

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Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69,** 349–390 (B methods).

Plant Toxins

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Progress toward official analytical methods for intrinsic plant toxins has been primarily in the pre-collaborative study phase in several Associate Refereeships. Joint AOAC-IUPAC collaborative studies are being organized for the steroidal alkaloids in potatoes and for agaritine in mushrooms. Appointments of Associate Referees for Hypoglycin, for Hydrazines, for Phytoestrogens, and for Pyrrolizidine Alkaloids have been recommended. The needs for the establishment of Associate Refereeships for grayanotoxins in honey, for furanocoumarins in commercially important Umbelliferae species, for lathrogens, and for cyanogenic glucosides/cyanide in cassava are being considered.

Glucosinolates.—(D. Ian McGregor, Agriculture Canada, Saskatoon, Saskatchewan, Canada). There have been few new developments in the methods for glucosinolate analysis within the past year. While studies to optimize the LC method have continued, there has been no general agreement to adopt an official method either by the European Economic Community or the International Standards Organization [see (1)]. Therefore, if a sufficient number of collaborators can be obtained, a joint AOAC-IUPAC collaborative study will be carried out.

Hydrazines.—The General Referee recommends the appointment of Joseph M. Betz, Division of Contaminants Chemistry, FDA, Washington, DC, as Associate Referee for hydrazine derivatives. A procedure developed by Stijve, Fumeaux, and Philippossian (2) for the determination of agaritine in mushrooms has been modified and is being evaluated prior to collaborative study. A generous gift of agaritine by T. Stijve facilitated the evaluation of these procedures. However, unless a commercial source of agaritine is found, the additional quantity of reference standard that will be required for the collaborative study will have to be synthesized by the reported (2) procedures.

Hypoglycin in Ackee Fruit.—The General Referee recommends the appointment of G. William Chase, Center for Nutrient Analysis, FDA, Atlanta, GA, as Associate Referee in this subject area. An LC method for hypogylcin A in fresh and processed ackee has been developed (manuscript submitted for publication, private communication, W.O. Landen, Jr).

Phytoestrogens.—The General Referee recommends the appointment of Shia S. Kuan, Natural Toxins Research Center, FDA, New Orleans, LA, as Associate Referee for this subject area. A screening procedure for phytoestrogens in soybeans and processed soy products has been developed (manuscript in preparation, private communication, S. S. Kuan). A collaborative study of this procedure is being planned. This procedure is being modified for use with forage crops, including alfalfa and red clover.

Pyrrolizidine Alkaloids.—The General Referee recommends the appointment of Robert M. Eppley, Division of Contaminants Chemistry, FDA, Washington, DC, as Associate Referee for this subject area. The evaluation/modification of a TLC screening method for pyrrolizidine alkaloids (M. Heinitz, private communications) in comfrey is continuing. The isolation of reference standards of several of the comfrey alkaloids is also under way. A mass spectrometric confirmatory technique (3) has been developed for use with this TLC procedure, but will not be incorporated into the planned collaborative study.

Steroidal Alkaloids.—(Allen S. Carman, Natural Toxins Research Center, FDA, New Orleans, LA). A review of the literature for 1987–1988 reveals the following accessions. There is only one paper for this period dealing explicitly with the analytical determination of glycoalkaloids. Wang et al. (4) discuss the determination of solanine and 18 other alkaloids in organic solvents by potentiometric titration. The method is reportedly sensitive to the microgram level, is rapid, and yields recoveries greater than 96% in pharmaceutical preparations. Cham and Wilson (5) discuss the high performance liquid chromatography of glycoalkaloids obtained from *Solanum sodamaeum,* and Cham et al. (6) discuss the antitumor effects of these glycoalkaloids. Sahoo and Jain (7) describe the isolation of glycoalkaloids obtained from the seeds and callus of *S. glaucophyllum* in tissue culture.

Three laboratories describe the effects that variety and/or environment have on glycoalkaloid level. Varietal and environmental effects are evaluated in a paper by Uppal (8). The effects of light, temperature, and storage time for 5 potato varieties are reported by Komaitis and Ifanti-Papatrayianni (9). The method of irrigation and its effect is evaluated by Gosselin et al. (10).

Two laboratories report relationships between physical damage and glycoalkaloid level. Olsson (11) reports the effects of glycoalkaloids and impact damage on the resistance of potatoes to the molds *Fusarium solani* and *Phoma oxigua.* Mondy et al. (12) report the effects of bruising.

Several investigators report possible relationships between plant glycoalkaloid content and insect resistance: VanGelder and Deponti (13) of tomato regarding the whitefly, and Grassert and Lellbach (14) of insect resistant potato hybrids regarding the potato cyst eelworm. Hare (15) describes the effects of protein and glycoalkaloid concentration on the growth of the Colorado beetle. The toxic effects in hamsters of potato sprouts and of the alkaloids isolated from them is reported by Baker et al. (16).

Two papers discuss the synergism of alpha-solanine and alpha-chaconine regarding their effects on cells and their processes: Roddick et al. (17) concerning cell membrane destabilization, and Roddick and Rijenberg (18) concerning lysis of phospholipid sterol liposomes. Osman et al. (19) describe the metabolism of solanidine by microsomal fractions taken from *S. chaconese.*

The results of the 1986 screening of IR-1 *Solanum* accessions for foliar glycoalkaloid level is reported by Deahl and Sinden (20).

The Associate Referee reports that a collaborative study of the method developed in the Associate Referee's laboratory is being organized and will be conducted in coordination with IUPAC.

Recommendations

(1) Glucosinolates: Coordinate collaborative study of LC method with IUPAC; continue study in other areas.

(2) Hydrazines: Coordinate collaborative study of LC method for agaritine in mushrooms with IUPAC; continue study in other areas.

(3) Hypoglycin in Ackee Fruit: Conduct collaborative study of LC method.

(4) Phytoestrogens: Conduct collaborative study of LC method for phytoestrogens in soybean products; continue study in other areas.

(5) *Steroidal Alkaloids:* Conduct collaborative study of LC method for solanine and chaconine in potatoes in coordination with IUPAC; continue study in other areas.

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GENERAL REFEREE REPORTS: COMMITTEE ON FOODS II

Alcoholic Beverages

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AOAC-ASBC Liaison Officer Pete Gales has recommended for adoption as interim first action 3 ASBC methods for the fill of beer containers. The General Referee reviewed the data and concurred with his recommendation for adoption.

Ben Canas has resigned as Associate Referee for Ethyl Carbamate in Alcoholic Beverages due to a change in his work responsibilities. The General Referee has been appointed to the Associate Referee position because a collaborative study is being planned.

Barry Gump, Associate Referee for Sulfur Dioxide in Wine, is preparing the final report on the collaborative test conducted last year.

Work is continuing on the methods for LC determination of glycerol and sugar, and the enzymatic method for malic acid.

Recommendation

Continue study on all topics.

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Cereal and Cereal Products

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Iron in Flour.—Associate Referee Jim Martin reports that the study to reduce the 11-point standard curve for iron (**14.012**) to a 4-point curve is still in progress. Also, a comparison of iron values in cereals obtained by the colorimetric method (**14.012**) and by atomic absorption spectroscopy is currently being conducted by the General Referee. Based on these comparative data, a collaborative study may be initiated for an atomic absorption method for iron and other minerals in cereal products.

Fat Acidity in Flour.—Although a mini-collaborative study performed last year indicated no difference when fat acidity values derived by replacing toluene for benzene were compared, the study was expanded to determine repeatability and reproducibility associated with each solvent. Generally, the relative standard deviations (RSD) for repeatability and reproducibility were lower when toluene was used than when benzene was used in the determination of fat acidity values of selected flour samples. This evidence lends further support to the replacement of toluene for the more toxic benzene formerly prescribed in the fat acidity method (**14.069-14.071**).

In an effort to harmonize oil/fat analysis among AOAC, AOCS, AACC, and ISO, oil/fat values obtained using 4 different solvents (petroleum ether, ethyl ether, pentane, and hexane) on selected oilseeds and flours are being compared. Gary Granata, a graduate student in the Department of Human Nutrition and Hospitality Management, University of Alabama, is the analyst for this project. Oilseed samples are being provided by Roger Sinram, Chairman of the AOCS Seed and Meal Technical Committee.

L. Zygmunt, Associate Referee for Sugars in Cereals, has suggested making the following nonsubstantive changes in method **14.070-14.079**, which updates supply lists and equipment currently available for LC analysis: Section **14.076(a)**, insert ". . . include automatic injectors, 410 differential refractometer (Waters Associates, Inc., WISP 710B, R1 410, or equiv.), 100 × 4.6 mm (id) Spheri-5 amino cartridge column (Brownlee Laboratories or equiv.) and use specific . . . ." Section **14.076(c)**, insert ". . . 100 × 2 (id) mm (Waters Associates, Inc.), or 15 × 3.2 mm (id) 7 μm, amino guard cartridge (Brownlee Laboratories), or equiv. . . . ." Section **14.077(a)**, insert ". . . vac. Dissolve in alcohol–H_2O (1 + 1) to obtain . . . ." Section **14.077(b)**, insert "CH_3CN (LC grade) and H_2O (Milli-Q purified or equiv.) (80 + 20). Filter thru Whatman GF/F 0.7 μm glass fiber filter or thru 0.45 μm Pall Nylon 66 filter. Optionally . . . ." Section **14.078(b)**, insert ". . . to original wt. Filter portion of ext thru 0.45 μm Nylon syringe filter (Nalge or equiv.). If cloudy, centrf. 10 min at ≥2000 rpm. If still cloudy, recentrf. portion of ext 5 min at ≥3500 rpm and filter thru 0.45 μm Nylon syringe filter. If guard . . . ." Section **14.078(c)**, insert "Filter thru 0.45 μm Nylon syringe filter if necessary."

Consider as interim first action AACC Method 39-10, Near Infrared Reflectance Method for Protein Determination (scope: wheat of all classes), and AACC Method 39-11, Near Infrared Reflectance Method for Protein-Wheat Flour. These methods have been studied collaboratively and appear to meet established AOAC guidelines.

Recommendations

(1) Continue study of rapid iron analysis in cereal-based foods.

(2) Initiate collaborative study of iron in cereals by atomic absorption spectroscopy.

(3) Consider for adoption the AACC Methods 39-10, Near Infrared Reflectance Method for Protein Determination (scope: wheat of all classes), and 39-11, Near Infrared Reflectance Method for Protein-Wheat Flour.

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Color Additives

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The Associate Referee on Color in Candy and Beverages, M. Young, reports that her method "Rapid Identification of Color Additives, Using the C_{18} Cartridge: Collaborative Study" is published (*J. Assoc. Off. Anal. Chem.* (1988) **71,** 458–461). This official method also appeared in "Changes in Official Methods of Analysis," (14th edition, 4th supplement, *J. Assoc. Off. Anal. Chem.* (1988) **71,** 213–215).

The Associate Referee on Colors in Other Foods, N. Adamo, reports on the publication of a "Simple Method for the Analysis of Food Dyes Using Reversed-Phase Thin Layer

Plates" by O. Hisao, I. Yoshitomo, K. Norihisa, Y. Masuo, and I. Hiromosa (*J. Chromatogr.* (1987) **411,** 437–444). The method is a possible alternative for a presently used reverse-phase TLC system. A modification of the widely used liquid anion extraction method has appeared: "Qualitative Analysis of Synthetic Colorings in Food" by K. Spears and J. Marshall (*J. Assoc. Publ. Analysts* (1987) **25,** 47–54).

The Associate Referee on Colors in Cosmetics, S. Bell, reports on the recent publication "Determination of Organic Colorants in Cosmetic Products by High Performance Liquid Chromatography" by J. W. Wegener, J. C. Klamer, H. Govers, and U. Brinkman (*Chromatographia* (1987) **24,** 865–875). A total of 126 colorants were characterized by this ion-pairing LC method. The dyes are cross-referenced by Color Index number, EEC number, GRC number, Color Index name, and FDA name. In addition, a possible alternative TLC method to that presently used for lipstick analysis has been published, "Determination of Tar Pigments in Lipsticks" by K. Kijima and T. Nagayama (*Gekkan Yakuji* (1988) **30,** 75–79).

The Associate Referee on Intermediates in Certifiable Non-Azo Colors, A. Scher, has agreed to expand his topic area to include intermediates in all certifiable colors. He reports that 2 Japanese articles of interest were recently translated: "Studies on Organic Impurities in Synthetic Food Colors I. Intermediates and Side Reaction Products in Food Red 3 (Erythrosine)" by M. Kamikura (*Shokuhin Eiseigaku Zasshi* (1985) **26,** 243–53); and "Studies on Subsidiary Colors in Synthetic Food Colors II. Separation and Determination of Intermediates and Side Reaction Products in Food Red 3 (Erythrosine)" by M. Kamikura (*Shokuhin Eiseigaku Zasshi* (1985) **26,** 643–650). These publications address both the synthesis and analysis of intermediates in FD&C Red No. 3.

The Associate Referee on Subsidiary Colors in Certifiable Color Additives, J. Bailey, reports that he will no longer be able to serve in this capacity. We expect to continue the topic and recommend the appointment of another Associate Referee. He also reports on the recent translation of LC methods for the analysis of triphenyl methane dyes, modifications of which are possible replacements for the presently used TLC methods: "Structures of Subsidiary Colors Isolated from Food Green No. 3 and Their Separation and Determination by High Performance Liquid Chromatography" by M. Kamikura (*Shokuhin Eiseigaku Zasshi* (1986) **27,** 398–407) and "Structures of Subsidary Colors in Food Blue 1 and Their Separation and Determination by High Performance Liquid Chromatography" by M. Kamikura (*Shokuhin Eiseigaku Zasshi* (1986) **27,** 27–36.

Three new Associate Referee Topics in the area of color additives are being proposed. The first is Trace Organic Constituents of Certifiable Color Additives. The setting of low level specifications for carcinogenic aromatic amines and other nonsulfonated trace constituents of certifiable colors has spurred further interest in the identification and quantitation of these components. Due to the low part-per-million and part-per-billion levels of concern, methodology in this area has diverged somewhat from that applied to the analysis of certifiable colors for other intermediates and subsidiary dyes. Thus, a separate Associate Referee topic is warranted. Naomi Richfield-Fratz, who is with the Food and Drug Administration, Division of Colors and Cosmetics, is being recommended as a new Associate Referee for Trace Constituents of Certifiable Color Additives. She reports that a paper by N. Richfield-Fratz and J. Bailey has recently issued: "Determination of *p*-Cresidine in FD&C Red No. 40 by the Diazotization and Coupling Procedure Followed by Reversed-Phase High Performance Liquid Chromatography" (*J. Chromatogr.* (1987) **405,** 283–294).

A second new Associate Refereeship is being proposed: Anthocyanin Colors Exempt From Certification. Increased use of colorants of natural origin, such as the fruit and vegetable-derived anthocyanins, has generated a need for the development and evaluation of applicable methodology for their characterization and determination. Ronald Wrolstad, of the Department of Food Science, Oregon State University, is being recommended as an Associate Referee for this topic area.

The third new proposed Associate Refereeship is on Carotenoid Colors Exempt From Certification. Many fruit and vegetable juice extracts and derivatives, as well as spice oleoresins, belong to the carotenoid class of colorants. An Associate Referee for this topic has not been found. It is recommended that this vacancy be advertised by AOAC to attract suitable potential candidates.

Recommendations

(1) Establish 3 new Associate Referee topics: Trace Constituents of Certifiable Color Additives; Anthocyanin Color Additives Exempt from Certification; Carotenoid Color Additives Exempt from Certification.

(2) Confirm the appointments of N. Richfield-Fratz and R. Wrolstad as Associate Referees for the first 2 new topics, respectively.

(3) Advertise a vacancy for the third new Associate Referee topic listed above.

(4) Combine the Associate Referee topics Uncombined Intermediates in Water Soluble Azo Colors and Uncombined Intermediates in Non-Azo Certifiable Colors to a single topic, Uncombined Intermediates in Certifiable Colors.

(5) Confirm the appointment of A. Scher to the combined topic area.

(6) Eliminate as a separate topic LC of Certifiable Color Additives since LC is now being applied to many of the Associate Referee topic areas.

(7) Continue study on all other topics.

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Flavors

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Additives in Vanilla Flavoring.—Associate Referee Sydney Kahan has resubmitted a collaborative study of an LC method for determining flavor constituents in vanilla extract. Additional work was performed to establish that this method is accurate and that apparent discrepancies with the present method for vanillin in vanilla are due to bias in the latter method. It is recommended that the method be adopted official first action. It is further recommended that the current official method for vanillin in vanilla, **19.010**, be retitled "Rapid Method for Estimation of Vanillin in Vanilla Extract."

Glycyrrhizic Acid and Glycyrrhizic Acid Salts.—Associate Referee Peter Vora indicates that a collaborative study is being planned for the determination of glycyrrhizic acid and its salts in finished products containing licorice.

Vanillin and Ethyl Vanillin in Food.—Associate Referee Sydney Kahan indicates that a collaborative study is planned for the LC determination of vanillin and ethyl vanillin in imitation vanilla extract and other foodstuffs.

A method for the detection of petrochemical-based synthetic flavor chemicals in natural flavoring materials has been developed and is now in widespread use in the quality control of natural flavors. The method measures trace levels of natural carbon-14 in flavor chemicals by liquid scintillation counting. It is proposed that this method be submitted to collaborative study.

Recommendations

(1) Adopt the LC method for determination of flavor constituents in vanilla extract as official first action.

(2) Retitle the official method for vanillin in vanilla extract (**19.010**) "Rapid Method for Estimation of Vanillin in Vanilla Extract."

(3) Appoint an Associate Referee for measurement of carbon-14 in flavoring materials.

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Fruit and Fruit Products

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Adulteration of Apple Juice.—During the past year, John Heuser succeeded E. R. Elkins as Associate Referee in this subject area. Heuser reports that he has developed an LC method using a reverse-phase column and a chiral (optically active) eluant for the separation of the D- and L-malic acid isomers. Preliminary results were reported at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. Using this technique, it is possible to detect added food grade malic acid when it constitutes 10% or more of the total malic acid present in the juice. After a few more experiments are done, the work will be submitted to AOAC for publication and a collaborative study will be conducted. In addition, he also presented at the Institute of Food Technologists' meeting a paper on the detection of added citric acid in pineapple juice. He plans to publish a paper on this work. The Associate Referee recommends continued study and the General Referee concurs.

Adulteration of Orange Juice by Pulpwash and Dilution.—Associate Referee Donald Petrus reports that 2 methods for the detection of beet medium invert sugar addition to orange juice are currently being investigated. He also adds that preliminary results have been encouraging. The Associate Referee recommends continued study and the General Referee concurs.

Fruit Acids.—Associate Referee E. D. Coppola developed an LC method to determine major organic acids in apple juice and cranberry juice cocktail. This method was collaboratively studied and adopted official first action by AOAC (**22.B01-23.B05**) at its Annual International Meeting, October 27–31, 1985, at Washington, DC. This method has been subjected to the use and scrutiny of many scientists for over 2 years. No adverse feedback has been received from the many users which include several reputable independent analytical laboratories. Coppola recommends that the method be adopted official final action and study continue on this subject. The General Referee concurs in both of the Associate Referee's recommendations.

Fruit Juices, Identification and Characterization.—Associate Referee R. E. Wrolstad reports that a "Fruit Juice Adulteration Workshop" sponsored by General Physics Corp. and held at Washington, DC, July 20–21, 1988, provided a forum for discussions on detecting adulteration in fruit juices. Speakers included Kenneth Winters of Coastal Science Laboratory, Donald Petrus of Florida Department of Citrus, Ronald Wrolstad of Oregon State University, Sanford Kirksey of Procter and Gamble Co., Elia Coppola of Ocean Spray Cranberries, Inc., Samuel Page of FDA, Martin Stutsman of FDA, and Allan Brause of General Physics Corp. who organized the conference.

The workshop emphasized current analytical methods and their application in detecting adulteration. The potential of LC analysis of phenolics for determining authenticity was emphasized by Kirksey and Wrolstad. Commodities given attention included apple, red raspberry, strawberry, blackberry, and prune juice in addition to orange and other citrus juices. The need for better methods for detecting beet sugar as well as the need for an expanded compositional database was mentioned by several speakers.

The Associate Referee recommends continued study and the General Referee concurs in his recommendation.

Limonin in Grapefruit Juice.—Associate Referee Russell Rouseff of the Florida Department of Citrus (FDOC) reports that the interlaboratory study on the enzyme immunoassay method for limonin in grapefruit juice will be submitted to the AOAC *Journal* during the forthcoming year so that the results of the study can be publicized. Dr. Rouseff is considering making a recommendation that the method be adopted official first action. The commercially prepared kits used in the method are relatively expensive; because there is no funding to purchase additional test kits, no additional work is planned. The Associate Referee recommends that this project be terminated after the results of the interlaboratory study are published. The General Referee recommends continued study for this year.

Moisture in Dried Fruits.—Last year, Associate Referee Edward Steffen reported that for many years the dried fruit moisture tester of the Dried Fruit Association (DFA) has been an AOAC official method (**22.014-22.017**) for obtaining the moisture of prunes and raisins. However, the moisture tester has not been accepted as an AOAC method for other dried fruits, although charts are available for apples, apricots, dates, figs, peaches, and pears. There is pressure from the fig industry to have the moisture tester accepted as an AOAC method, and DFA is also interested in having their moisture tester accepted as an official AOAC method for other dried fruits.

One of the problems in conducting a collaborative study has been in obtaining samples with uniform moisture but that problem seems to have been largely corrected. In addition, samples with various amounts of moisture can now be supplied to collaborators. Steffen is presently looking for collaborators who have the DFA moisture machine and would be willing to participate in a collaborative study on figs. As soon as he has a reasonable number of collaborators, he plans to make up check samples and send them out.

The collaborative study is important, because the DFA moisture tester has been sold in both the domestic and international markets for a number of years and is widely used at present to obtain moisture values on more types of dried fruit than just prunes and raisins.

The Associate Referee recommends continued study, and the General Referee concurs in his recommendation.

Orange Juice Content.—No activity has been reported. The General Referee recommends that work continue on this subject.

Sodium Benzoate in Orange Juice.—Associate Referee H. R. Lee reports that in the national survey of commercial single-strength and reconstituted frozen concentrated orange juice, benzoic acid was found in the range of 0.6–30.2 ppm in 59 samples; 0.3–139.9 ppm sorbic acid was found in 7

samples. Eighty samples were found to contain benzoate below 0.5 ppm; at this level, it is uncertain whether benzoate occurs as adulteration with pulpwash, as a preservative, or is naturally present. Three samples showed both benzoate and sorbate. A total of 1574 samples were tested from July 1, 1987, through June 28, 1988.

The Associate Referee indicated that he plans to conduct a collaborative study within the forthcoming year and recommends continued study. The General Referee concurs in the Associate Referee's recommendation.

ICP Methods.—This refereeship was created during the past year and John Heuser of NFPA was appointed Associate Referee. Continued study is recommended.

Stable Carbon Isotope Ratio Analysis of Fruit Products.—This refereeship was started during the past year and Rae-Gabrielle Krueger, Krueger Food Laboratories, Inc., Cambridge, MA, was appointed Associate Referee.

Carbon SIRAs (stable isotope ratio analyses) as applied to adulteration detection are AOAC official methods for honey, maple syrup, orange products, and apple products. The method is based on differences in plant physiology, where fractionation of carbon during photosynthesis occurs in predictable and quantifiable fashion.

Two of the most important commercial sweeteners (cane sugar and corn syrup) can be differentiated by this method from most fruits and premium sweeteners, and mixtures of cane or corn with most fruits can be quantified, since mixing produces linear results. Based on these facts, carbon SIRA has become AOAC official methodology for determining the addition of cane or corn sugars to maple syrup, honey, and apple and orange products.

The method measures by isotope ratio mass spectrometry the $^{13}C/^{12}C$ ratio in a sample. The preparation of the sample and the method for obtaining ^{13}C values is identical for each method, and should probably remain so for future applications. In each method, the sample is combusted to CO_2 and water vapor at 800°C over CuO under flowing O_2; after the water vapor is trapped out and noncondensable gases are pumped away, the CO_2 is captured and run through a dual- or triple-collecting isotope ratio mass spectrometer. The methods differ after that point only in establishing a natural range and a criterion for determining adulteration.

The proposed method would be called something like "Carbon Stable Isotope Ratio Analysis of Fruit Products." Using the current AOAC official methods **22.109-22.112** and **22.133-22.116** for obtaining carbon SIRA values for apple and orange juices, the exact procedure would be very carefully specified, including oven temperature, combustion time, sample size, etc. Method **22.109-22.112** could be used with minor variations. A wide diversity of fruit samples would be obtained, and sent as blind duplicates to each of 8 laboratories involved in the study.

The method is largely theoretical and the database is already fairly large. This study, while contributing to the database, will *not* establish an interpretive base for any single fruit; it is simply intended to establish a method for obtaining reproducible carbon SIRA data.

In the proposed study, 10 juice sample types would be prepared from fresh fruits: tomato, grapefruit, lemon, pear, white grape, concord grape, raspberry, cranberry, cherry, and pineapple. All 10 samples plus 10 replicates would be sent to each of 8 laboratories with the specific method as annotated.

Recommendations

(1) Adopt as official final action the official first action LC method for determining quinic, malic, and citric acids in cranberry juice cocktail and apple juice, **22.B01-22.B05**.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

Nonalcoholic Beverages

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Leonard Valenti, Associate Referee for Quinine, obtained poor results in his collaborative study and is no longer working in a laboratory. He has resigned his position as Associate Referee.

Associate Referee for Methyl Xanthine in Nonalcoholic Beverages, John M. Newton, has not been able to devote time this year to this project.

Several quality control laboratories have used the AOAC method for determining methyl anthranilate in various products. They could not find a supplier for the reagent sodium-1-naphthol-2-sulfonate and had to use 1-naphthol-2-sulfonic acid potassium salt. One laboratory was using a chromatographic procedure for determining methyl anthranilate and felt that a modern method should be developed; the current official method was developed in 1928.

Recommendations

(1) Appoint a new Associate Referee for Quinine.

(2) Appoint an Associate Referee for the direct determination of methyl anthranilate in nonalcoholic beverages.

(3) Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Processed Vegetable Products

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Total Solids by Microwave Moisture Methods.—A technical communication [*J. Assoc. Off. Anal. Chem.* **70**, 758–759 (1987)] indicated that the reliability of the total solids analyzer is closely related to the performance of the microwave solids analyzer, and recommended certification of the microwave solids analyzer for use in method **32.A01-32.A04**. The General Referee offers the following comments: The study by Wang is certainly significant and interesting in that it describes difficulties which may be encountered when the microwave moisture analyzers are used without previous calibration. In the conduct of the collaborative study for this method, the participants were instructed to calibrate their ovens by using a supplied sample of tomato pulp of known solids content prior to analyzing the unknown samples. The benefit of this exercise was shown in the less than 2% coefficients of variation for samples comparable to those used by Wang. Wang reported CVs of 2.98–3.87%. Thus, it is agreed that it is essential that the oven be calibrated prior to

its first use and checked periodically thereafter. However, the best check would be a sample of tomato pulp analyzed by both the microwave and vacuum oven procedure. This performance check would be comparable to quality assurance procedures followed for other routine analysis methods. It is also believed that the omission of the 18–24 h warm-up period affects the accuracy of the microwave oven method. In the collaborative study for the method, 14 collaborators used 7 CEM AVC-MP and 7 CEM AVC-80 moisture analyzers. The equipment bias indicated by Wang was not observed in the data.

Interested persons are invited to submit comments and findings on this issue. Continued study of this topic is recommended.

LC Determination of Sugars in Processed Vegetables.—No response was received for the second consecutive year to efforts to obtain an Associate Referee for this topic. Interested persons are invited to comment and participate. Appointment of an Associate Referee is recommended.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411 (A methods).

Spices and Other Condiments

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Bulk Index of Spices.—Associate Referee Gary Oetting, Basic American Foods, is preparing a collaborative study proposal.

Ethylene Oxide in Spices.—Associate Referee Lynn Theiss, Durkee-French Foods, is evaluating a new method. A collaborative study will follow.

Moisture in Spices.—Associate Referee Lou Sanna, Santa Maria Chili, Inc., reports that the recommended method was adopted (**30.B01-30.B03**).

Curcumin in Tumeric.—Associate Referee Ted Lupina, Kalsec Co., reports that a collaborative study is in progress.

Piperine in Black Pepper.—Associate Referee Ted Lupina reports that studies continue.

Pungency of Capsicums and Their Oleoresins.—Patrick Hoffman and Bob Dull, McCormick & Co., report that a preliminary study on proposed methodology will begin August 1.

Vinegar.—A collaborative study was completed on a method for detecting corn- and sugar cane-derived vinegar in cider vinegar by using carbon stable isotope ratio analysis (SIRA). The method is also suitable for detecting corn- or sugar cane-derived vinegar in other varietal vinegars such as wine or malt vinegar. The level of reliable detection (>99.9% confidence) by this method is approximately 20%, although lower levels may be detected with diminished confidence. It is recommended that this method be adopted official first action.

Work is continuing on developing methods for chemically characterizing vinegars by a profile of their volatile constituents using capillary gas chromatography. A collaborative study may be recommended for this procedure in the coming year.

It is recommended that a collaborative study be conducted to evaluate a method for detecting petrochemical acetic acid in vinegar, and petrochemical ethanol in vinegar stocks, by measurement of ^{14}C-activity. This methodology is also applicable to the detection of petrochemical flavoring synthetics in natural flavor chemicals; therefore, it is recommended that a broadly based study be performed to evaluate this method for both applications. A detailed proposal for such a study is currently in preparation.

Recommendations

(1) Designate as surplus the following methods: **30.010**, **30.011**, **30.013**, **30.015**, **30.018**.

(2) In method **30.012**, replace diethyl ether with methylene chloride, and change title to Methylene Chloride Extract of Spices.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

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Sugars and Sugar Products

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Color, Turbidity, and Reflectance: Visual Appearance.—Associate Referee M. A. Clarke reports an increasing interest in determination of solid state color of sugars. New instrumentation, combining readings at several wavelengths, has recently become available and will be the object of future study.

Corn Syrup and Sugars.—No report from Raffaele Bernetti.

Enzymatic Methods.—Associate Referee Marc Mason reports no new developments.

Gas Chromatographic Methods.—Associate Referee Mary An Godshall will submit the Schaffler method for determination of sucrose in sugarcane juice (the official method of the International Commission for Uniform Methods of Sugar Analysis) when it is written in AOAC format.

Honey.—Associate Referee Jonathan W. White, Jr, reports that work is in progress to increase the accuracy of the isotope ratio method for detection of adulteration of honey.

Lactose.—Associate Referee Janice R. Saucerman reports no new developments.

Liquid Chromatographic Methods.—Associate Referee Charles W. S. Tsang reports a preliminary study on raffinose in beet sugars, comparing 3 LC methods with an enzymatic method available in kit form.

Maple Sap, Maple Syrup, and Maple Syrup Products.—(*1*) Associate Referee Lynn Whalen recommends that, in section **31.170**, Color Classification of Maple Products, the Berliner Maple Syrup Grading kit be included as an alternative to the VirTis grading set listed in that method. The VirTis kit is no longer in production, but many are still in use. The Berliner kit has been subjected to analysis by the USDA Agricultural Marketing Service, found to compare within USDA specifications that included the VirTis kit, and granted USDA License No. 33 for Maple Syrup Color Comparator. The Associate Referee recommends official first action for inclusion of this method; the General Referee supports this recommendation.

The Berliner system was presented by the Associate Ref-

eree as a poster at the 1987 AOAC meeting. The Associate Referee also recommends that the Berliner kit be listed as an alternative to the VirTis system in the forthcoming 15th edition of *Official Methods of Analysis* and suggests the wording to be used.

(*2*) Associate Referee Whalen recommends a nonsubstantive change in section **31.169(a)** (1) (Syrup sample preparation): from "filter sample through cotton wool" to "filter sample through cotton wool *or* centrifuge at 1000–3000 rpm for 20 min" on the basis that most maple products laboratories have a suitable centrifuge, whereas few any longer use cotton wool.

(*3*) Associate Referee Whalen has begun a study of the association of phenolic compounds with darkening (and grade change) of maple syrup. Initial results are reported as a poster at the 1988 AOAC meeting.

(*4*) Associate Referee Whalen notes that in section **31.188**, Corn Syrup and Cane Sugar in Maple Syrup—Carbon Ratio Mass Spectrometric Method, the alternative method listed for sample preparation can bias results and requests that the following note be appended to **31.188**, and be included in the method as it appears in the 15th edition:

Note: Results obtained by the alternative preparation method are biased approx. +1.0% relative to section **31.187**. All results obtained by this alternative preparative method must be corrected for this bias by adding an appropriate correction value (C). The correction may be determined by analyzing reference sample using both preparation procedures.

$$\delta^{13}C(\text{corrected}) = \delta^{13}C(\text{measured}) + C$$

The General Referee recommends addition, with references, as a nonsubstantive change.

(*5*) In reference to the 1987 "Changes in Official Methods," repeal of final action to first action on methods for lead numbers of maple products, a letter arguing against the repeal has been received. The General Referee maintains the opinion that these methods, which equate high levels of lead with authentic product, should be repealed. These methods employ large quantities of lead reagents.

Oligosaccharides in Sugars and Sugar Products.—Associate Referee George Steinle reports preliminary results on a planned collaborative study for analysis of raffinose in sugarbeet molasses: the analytical system of choice for the study is the ion chromatographic system, although LC systems using amino bonded columns with acetonitrile–water mobile phase, or cation exchange resin columns with water as mobile phase, can be used.

Polarimetric Methods for Measurement of Sugars.—See *Standardization of Methods of Sugar Analysis.*

Stable Carbon Isotope Ratio Analysis.—Associate Referee Landis Doner reports no new developments.

Standardization of Methods of Sugar Analysis.—Associate Referee Mary An Godshall, Liaison Officer for International Commission on Uniform Methods of Sugar Analysis, has submitted the following report:

The AOAC method for polarization of raw cane sugars is quite outdated, using an ICUMSA method from the 1930s. Over the years, ICUMSA has studied this subject extensively and adopted a newer method. The final changes made to the method occurred in 1986, when a New International Scale, measured in °Z was adopted. ICUMSA has dropped the use of any other scales, including those listed in the AOAC method. The new scale became effective July 1, 1988.

The basis for acceptance of the new scale was a study by the German Physikalisch-Technische Bundesanstalt in collaborating with Braunschweig Sugar Institute in 1973–1974. The new values were accepted by the British and U.S. Bureaus of Standards and subsequently by ICUMSA in 1986, and published in the ICUMSA Proceedings of that year.

Along with the acceptance of the new scale, a controversial decision was taken to modify part of the existing official method (*ICUMSA Methods,* Schneider, 1979). That is, the use of a correction factor that made the wet lead method equivalent to the (no longer accepted) dry lead method was dropped. It was an administrative decision by ICUMSA to have only one method (wet lead) and one way of reporting results.

A copy of the new official ICUMSA method is available from the General Referee.

A collaborative study on the permissible volume and basicity ranges for the lead solution was conducted. This study established repeatability and reproducibility estimates for the polarization method. The study confirmed the existing specifications for basic lead acetate and recommended no changes from those already in the method.

The AOAC method for color measurement is also very outdated. The method for measurement of sugar color at 420 nm, submitted to AOAC in 1984, remains in effect in ICUMSA. ICUMSA is continuing to study better methods for measuring the color, especially in the use of buffers to adjust the pH.

The Associate Referee recommends: (*1*) that AOAC update its polarization method; (*2*) that AOAC consider the official ICUMSA method.

No recommendations are made regarding color measurement at this time other than to note that better methods are available and in wide use.

Any recommendations from AOAC as to what is required to make the method conform to AOAC standards will be relayed to ICUMSA.

The General Referee recommends that method **31.020**, Sugars in Sugars and Syrups: Polarimetric Methods, be revised and updated.

Sugars in Cereals.—Associate Referee Lucien Zygmunt reports that an investigation of columns for LC analysis of fructose, glucose, sucrose, maltose, and lactose found that a cation-exchange column did not provide adequate resolution, but an amino column showed enough improvement in longevity that a change in method specification to this column will be recommended if reproducibility of column manufacture is demonstrated.

On the Interaction Chemicals CHO-682 lead-activated polymer column, the 5 sugars were separated, but the usually easy separation of sucrose and maltotriose could not be achieved, making this column inadequate for general analysis of mono- and disaccharides in foods made with corn syrups and maltodextrins containing saccharides of degree of polymerization 3.

The Rainin Instrument Co. aminopropyl bonded silica column analyzed over 500 injections of food extracts before resolution became inadequate, making longevity of this column substantially greater than that of the previously used Brownlee Labs amino column. Initially high water concentration (25 + 75 acetonitrile) allows the adjustment of the mobile phase with acetonitrile when resolution declines.

Sugars in Syrups.—Associate Referee Rose Goff reports that samples have been distributed for a collaborative study of sugars in cane, maple, and sorghum syrups, to determine contamination. An LC procedure using amino-bonded silane columns has been selected.

Sulfites in Sugars and Syrups.—Associate Referee Richard Riffer reports no new developments.

Weighing, Taring, and Sampling.—A new Associate Referee, Michael Steele, of R. Markey and Sons, has been appointed.

Formaldehyde in Maple Syrup.—The General Referee has received several complaints relating to method **31.203-31.206**. The method has been applied to sugar products other than maple syrup, and found to be unsuitable. There is no AOAC method for formaldehyde in sugars.

The reaction in this method can cause synthesis of form-

aldehyde from sucrose, thereby confusing results. The General Referee recommends that a note be added to this method: Not suitable for use on beet and cane sugars.

The following methods are recommended for surplus designation: **31.007**, has not been used in industry for many years and has been replaced by other AOAC methods; **31.012**, **31.013**, **31.015**, **31.016**, **31.017**, and **31.018**, not used because of inaccuracy; **31.037-31.044**, require use of asbestos, and replaced by **31.034** (this action affects methods **31.128**, **31.183**, and **31.237**(b)); **31.045**, no longer in use.

Recommendations

(1) Adopt as official first action in method **31.170** the inclusion of the Berliner Maple Syrup Grading kit as suitable test equipment.

(2) Adopt the nonsubstantive change recommended for methods **31.169** and **31.188**.

(3) Consider revision and update of method **31.020**, Sucrose in Sugars and Syrups: Polarimetric Methods.

(4) Add a note to method **31.203-31.206**, Formaldehyde in Maple Syrup, to state: Not suitable for beet or cane sugars.

(5) Designate as surplus methods **31.007**, **31.012**, **31.013**, **31.015-31.018**, **31.029**, and **31.037-31.044**.

(6) Continue study on all other topics.

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Vitamins and Other Nutrients

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Fifteen papers in this topic area were presented as a poster session at the 102nd annual meeting of the association. Nine relevant papers were also a part of the AOAC Europe IV/ASFILAB Seminar held in Neuilly-Sur-Seine, France. Ten additional nutrient papers were published in the AOAC *Journal* during the last year.

A statistical evaluation of official methods for nutrients collaboratively studied by the association was performed. Distribution has not been determined.

Alan J. Sheppard received the Association's Committee on Foods II Associate Referee of the Year Award. Lori V. Klatt of Hazleton Laboratories America, Inc., has been appointed Associate Referee for Cholesterol.

Recommendation

Continued study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

GENERAL REFEREE REPORTS: COMMITTEE ON RESIDUES

Metals and Other Elements

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Atomic Absorption Spectrophotometry (AAS).— Associate Referee Milan Ihnat reports that chemical characterization is in progress on 12 recently prepared candidate reference materials representing a number of important classes of foodstuffs and a wide range of composition with respect to elements and matrix. These materials are intended for analytical method quality control for the reliable determination of major, minor, and trace elements in foods, feeds, and other animal- and plant-based products and in particular will be useful for development and collaborative study of atomic absorption spectrometric methods. Work is also continuing on the investigation and assessment of parameters bearing on the development of an official multielement atomic spectrometric method of analysis.

Cadmium and Lead in Earthenware.—Associate Referee Benjamin Krinitz did not report on this topic.

Susan Hight at FDA reports that Pb, Cd, and other elements were leached from silver plated baby bowls using the AOAC method for Pb and Cd in earthenware, **25.024-25.027**, and were determined using an inductively coupled argon plasma (ICP) technique. Hight established analytical parameters for determining Al, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, V, and Zn in these leach solutions. The high levels of Ni (range 86.6–478 μg Ni/mL) caused spectral interferences for several elements and presented an analytical challenge which demonstrates the need for careful interpretation of ICP results from leach solutions. The Ni levels in these solutions interfered with Cd, Co, Sb, and Zn determinations at the analytical wavelengths commonly used for ICP analysis. In order to obtain reliable data it was necessary to select alternative wavelengths for these elements.

Emission Spectrochemical Methods.—Associate Referee Fred Fricke did not report on this topic.

Fluorine.—Associate Referee Robert Dabeka reports no plans for collaborative studies but recommends that laboratories contemplating fluoride determinations in foods use one of the following microdiffusion techniques combined with the fluoride-specific electrode: *Anal. Biochem.* (1965) **10,** 495–500 or *J. Assoc. Off. Anal. Chem.* (1981) **64,** 1021–1026. The latter method, which will not work for organofluorine compounds, has been collaboratively studied by his laboratory. Inquiries were made at the National Bureau of Standards (NBS) about fluoride methodology and whether validation of levels in the samples run in his collaborative study could be made. Unfortunately, the levels in the food samples are lower than the detection limits of their method. NBS is developing a reference material for higher levels of fluoride.

Dabeka also recommends that methods involving direct fluoride-specific electrode determination of fluoride in food slurries not be used because of the potential interference from organic matter. Since some analysts will obviously choose such methods for their simplicity, they should be made aware that (*1*) recovery studies will not reveal that any accuracy problem is present, (*2*) standard additions will not correct for the interferences, and (*3*) errors at lower concentrations of fluoride (below 5 μg/g) will be more significant than errors at higher concentrations.

Graphite Furnace-Atomic Absorption Spectrophotometry (GF-AAS).—Associate Referee Robert Dabeka reports that a precollaborative study of a coprecipitation GF-AAS method for lead and cadmium (*Can. J. Spectrosc.* (1986) **31,** 44–52) has revealed inadequate interlaboratory reproducibility. The method is currently being tested to better optimize precision and accuracy. In 2 interlaboratory studies, the 3 participating laboratories obtained the following results: Foods with high levels of lead (0.6–1.7 μg/g) and cadmium (0.01–1 μg/g)—reproducibility standard deviation: lead—3.8, 6.9, 6.7, 15, and 14%; cadmium—12.6, 27.2, 27.6, 21.8, and 37.8%. Foods with low levels of lead (0.016–0.121 μg/g) and cadmium (0.003–0.046 μg/g)—reproducibility standard deviation: lead—4.7, 13.9, and 50%; recovery 68–122%; cadmium—13.7, 20.7, and 36.9%; recovery 69–121%.

Hydride Generating Techniques.—Associate Referee Stephen Capar reports some progress on this topic. A continuous flow hydride generation apparatus has been assembled in his laboratory by George Alvarez and is currently being evaluated for the determination of As by atomic absorption spectrophotometry.

Lead in Calcium Supplements.—Associate Referee Patricia Maroney-Benassi reports that a method has been developed for determination of Pb in calcium supplements. The method uses a nitric/perchloric acid digestion and differential pulse anodic stripping voltammetry. The new method is an adaptation of *J. Assoc. Off. Anal. Chem.* (1979) **62,** 1054–1061 with major modifications being (*1*) use of a partial perchloric acid digestion, and (*2*) use of an acetate-tartrate buffer to remove tin interferences. A collaborative study of the method is planned for the near future.

Methyl Mercury in Fish and Shellfish.—Associate Referee Susan Hight reports that the rapid method for the determination of methyl mercury was approved official first action at the 1987 AOAC Annual International Meeting.

Neutron Activation Analysis (NAA).—Associate Referee William C. Cunningham has been appointed AOAC Liaison Officer with the American Society for Testing and Materials (ASTM) Task Group on Nuclear Methods of Chemical Analysis. He has been following that group's activities and studying issues related to the development of standard NAA methods. Cunningham has begun rewriting the AOAC collaborative study on Na in foods by NAA that was initiated a few years ago and hopes to use it to raise interest within the ASTM Task Group toward standardizing methods. The predominant interest in the Task Group to date is the development of guidelines as opposed to standard methods.

Polarography.—Associate Referee Susan Hight reports no developments in this topic area since the last report.

Organotin in Foods.—Associate Referee Allen Uhler did not report on this topic.

Organometallics in Fish.—Associate Referee Walter Holak completed his collaborative study on the liquid chromatographic—atomic absorption spectrophotometric method for methyl mercury in seafood and recommends the method be adopted official first action.

Other Topics.—The General Referee responded to a report sent to the AOAC on metal losses during dry ashing of marine mussel by H. Hennig of the National Research Institute for Oceanology, South Africa. He reported metal (Zn, Cd, Cu, Fe, Pb) losses occurring at ashing temperatures used by many AOAC methods. However, his report did not clearly state his analytical method. A review of AOAC methods and associated references indicated good recoveries of the metals. The General Referee's comments were sent to H. Hennig and a response is anticipated.

Recommendations

(1) Adopt as official first action the high performance liquid chromatographic—atomic absorption spectrophotometric method for methyl mercury in seafood.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed.

Multiresidue Methods (Interlaboratory Studies)

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Comprehensive Multiresidue Methodology.—(Associate Referee S. Mark Lee, California Department of Food and Agriculture.) The Associate Referee, recently appointed to this position, has proposed that a study be initiated to critically evaluate an existing multiresidue method. This comprehensive method, known as the CDFA Multiresidue Screening Method, has reportedly been used for several years by several laboratories but has never undergone testing in terms of establishing its expected accuracy and precision through an interlaboratory study. The ultimate goal of the Associate Referee is to establish these method performance parameters through the AOAC collaborative process.

The method is reported to be applicable to fresh fruit and vegetable sample types and recovers a wide range of pesticides in the organochlorine (OC) and organophosphorus (OP) classes as well as a limited number of the *N*-methyl carbamates. The principle steps of the method involve an acetonitrile extraction, an aqueous salting out step, and 3 separate cleanup/determinative steps for each class of pesticide. Programmed wide bore capillary gas chromatography is used with a variety of detectors and columns for the determination of the OC and OP classes; liquid chromatography is used to detect the carbamates.

Prior to drafting a collaborative study plan and protocol, the Associate Referee has proposed an extensive intralaboratory recovery study to provide some additional performance documentation of some suspect critical points in the analytical process. His expressed concern is that operational familiarity of the method by experienced analysts (SOPs) may be difficult to translate into a formal protocol. His proposed study is to initially test 15 representative pesticides, each at 3 levels in 4 different matrixes, through the method using carefully monitored conditions to help define the ruggedness of each analytical step.

The Associate Referee has also indicated that performance comparisons between the proposed CDFA method and that described in **29.A01–29.A04** would also be performed. In addition to recovery comparisons, the testing is intended to identify operational advantages and disadvantages between the 2 multiresidue method approaches.

Extraction of Low Moisture—High Fat Samples.—(Associate Referee Leon D. Sawyer, FDA, Washington, DC.) No substantive work on this topic was accomplished during the past year. The Associate Referee has recognized that further development of this topic under his refereeship is unlikely in the foreseeable future and requests the topic be reassigned to another Associate Referee.

Fumigants.—(Associate Referee James L. Daft, FDA, Kansas City, MO.) The Associate Referee submitted a collaborative study proposal for the determination of multifumigants in grain, milled and intermediate grain products, and citrus fruit. The proposal was based on the methodology published in *J. Assoc. Off. Anal Chem.* (1987) **70,** 734–739, by the Associate Referee. This methodology was developed as a rapid means to check for the presence of fumigants (and other volatile halocarbons) in a wide variety of food and feedstuffs. Recovery information submitted by the Associate Referee (and that published in the above reference) indicates that the proposed methodology, if collaborated, would not be expected to produce interlaboratory results within "normal" ranges of recovery and reproducibility expected of official multiresidue methods. Since the Associate Referee had previously published a multifumigant method (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 228–233) that demonstrated the potential for providing both accuracy and precision improvements over his current proposal, it was suggested that he reconsider the choice of methods to collaborate.

Miniaturization of Multiresidue Methodology.—(Associate Referee D. Ronald Erney, FDA, Detroit, MI.) No progress on this topic was achieved during the past year. The Associate Referee stated that every effort will be made in the forthcoming year to complete an interlaboratory trial involving 2 or 3 laboratories on his proposed miniaturization of the applicable sections of **29.011–29.018** for nonfatty foods. The trial would include the use of the miniaturized Florisil cleanup step described in **29.034**.

Organophosphorus Pesticide Residues.—(Associate Referee Ronald R. Laski, FDA, Buffalo, NY.) The Associate Referee reported on the successful recovery of 6 organophosphorus pesticide/metabolite chemicals through **29.A01–29.A04** followed by oxidation to "total sulfone" [Hill et al. (1984) *Analyst* **109,** 483–487] and determination by flame photometric detection (FPD). He reported that the oxidation procedure not only provided quantitative conversion/recovery (>90%), but it also provided significant cleanup of several fruit and vegetable extracts by eliminating extraneous FPD responses attributable to certain crop matrixes. Chemicals studied were: fenthion, fenthion oxygen analog/sulfoxide/sulfone, and phorate and its sulfone.

Immediate plans are to complete the recovery/conversion work with the remaining available phorate related chemicals: phorate sulfoxide and phorate oxygen analog/sulfoxide/sulfone. Further plans include publication of findings plus additional studies with more organophosphorus parent/metabolite combinations.

Sweep Codistillation.—(Associate Referee Barry G. Luke, Analytical Reference Laboratories Pty Ltd, North Melbourne, Victoria, Australia.) Two detailed reports on the use of sweep codistillation (SCD) by Australian laboratories were received from the Associate Referee during the past year. In the first report, the Associate Referee described a national laboratory accreditation program that involves all national, state, and private laboratories engaged in monitoring pesticide residues. This report included results from 51 laboratories that participated in a check sample study of 6 organochlorine pesticides in beef fat. The study was part of a proficiency testing program in which all laboratories must participate and perform satisfactorily to gain and retain accreditation. The Associate Referee reported that 46 of the 51 participating laboratories (not all seeking accreditation) used the commercially available Unitrex systems in their analysis and at least 2 other laboratories used similar systems that were custom designed.

Extensive interlaboratory data were obtained and thorough statistical treatment of the submitted data was performed. However, the Associate Referee noted that the requirements of the National Pesticide Residue Proficiency Testing Program (NPRPTP) are not consistent with those of AOAC, e.g., use of recovery-adjusted results and nonspecified methods. The Associate Referee expressed hope that with the assistance of the Committee on Residues these differences may

be overcome and an AOAC collaborative effort might be possible under the Australian NPRPTP.

In his follow-up report, the Associate Referee expanded on additional testing done under NPRPTP. In the beef fat study discussed above, the overall CVs obtained were: HCB (19.6%), BHC (24.7%), *p,p'*-DDE (12.1%), *p,p'*-DDT (27.4%), dieldrin (32%), and methoxychlor (22.3%). Many of the participating laboratories in this study were reported to be first-time users of the SCD technique. In an additional study involving 5 designated referee laboratories, the CVs obtained were (in the order above): 9.5, 6.0, 12.1, 13.7, 18.3, and 11.4%. All of the referee laboratories used SCD methods of analysis and were experienced users.

It was reported that approximately 600 000 samples of beef fat were analyzed by some 30 accredited laboratories in Australia during the last 12 months, 95% of these were analyzed by SCD techniques and 90% were analyzed with the commercial Unitrex system. The Associate Referee again expressed confidence that NPRPTP could produce an acceptable AOAC study and recommended that approval be given to cooperate with the Australian Quarantine Inspection Service (AQIS) Working Party in designing and conducting such a study. The Associate Referee stated that he personally would not be able to conduct such a study because of his added responsibilities in a new employment environment.

Specific research topics covered in the Associate Referee's second report included an investigation of using beadless tubes in the Unitrex system and a new recommendation regarding the amount of Florisil to use in the Unitrex trap. He reported that empty distillation tubes (glass beads removed) provide some advantages over the conventional packed ones. Included in the stated advantages were that equivalent or better recoveries were routinely obtained and that easier routine and less frequent rigorous (acid) cleaning of the distillation tubes was required. Recovery data of 14 organochlorine pesticides (beef fat) obtained with the beadless tubes were submitted with percent recovery and CV ranges between 90 and 109% and 7.0 and 11.1%, respectively. The recovery data summarized were from a series of 10 replicates (8 pesticides) and a series of 5 replicates (6 pesticides).

In regard to the Florisil media used for trapping in the Unitrex system, the Associate Referee has instituted an operational change that standardizes the amount added to the trap (0.8 g added by funnel) instead of the variable amounts that can be added by the currently recommended technique of vacuum packing. Variations in amounts added by vacuum packing occur because the volumes of the traps are not uniform.

Synthetic Pyrethroids.—(Associate Referee Darryl E. Johnson, FDA, Minneapolis, MN.) The former Associate Referee on Comprehensive Multiresidue Methods was recently assigned to this new topic. It was felt that this title is a more accurate reflection of the Associate Referee's efforts.

The Associate Referee has recently submitted a collaborative study proposal describing use of a modified version of **29.A01-29.A04** for the determination of technical permethrin, cypermethrin, fenvalerate, and decamethrin in peppers, apples, and a yet to be determined root crop. The Associate Referee originally proposed to study potatoes as a representative root crop, but has since determined that cypermethrin is not stable in chopped and frozen portions of this commodity. He has begun to investigate the use of beets as a possible substitute.

His proposed method of study differs from **29.A01-29.A04** in that it requires a Florisil cleanup step and a different determinative step and includes a small procedural change in the manner the partitioning solvents are added. The procedural change is a directed 10 s shaking step with the original 80 mL acetone extract and 100 mL petroleum ether prior to the addition of methylene chloride.

The Florisil cleanup step is incorporated with **29.A01-29.A04** by evaporating a 1.0 mL aliquot of the sample extract to dryness and transferring the residue (with hexane) to a column prepared according to **29.046**. The column is then eluted with 250 mL eluant II prepared according to **29.046** instructions. The Associate Referee reported that efforts to incorporate a commercially available bonded phase extraction column as either a replacement or supplement to the Florisil step described was unsuccessful because cleanup was not adequate.

The proposed determinative step utilizes gas chromatography (GC) with a wide bore methyl silicone capillary column and electron-capture detection. The wide bore column is operated in the packed column (high carrier flow) mode. This operational mode is reported to facilitate the quantitation of the mixed isomers that are present in permethrin and cypermethrin by chromatographing them as 1 unresolved peak. The 2 isomers of fenvalerate that are present are essentially separated under these GC conditions, and it is proposed that the summation of the 2 peak heights be used for quantitation.

The Associate Referee reports that the proposed wide bore capillary GC conditions offer no advantage over packed columns for quantitation, but adds that a confirmatory advantage exists in that peak retention comparisons (sample vs standard) can easily be made at near capillary chromatographic conditions by decreasing the carrier gas flow rate. Also, the higher resolving power achievable with wide bore chromatographic columns allows more flexibility to expand the method's applicability to other chemicals of this class.

Recommendations

(1) Proceed with the intralaboratory evaluation of the CDFA multiresidue method and the performance comparisons with **29.A01-29.A04** as planned. Draft a written report, preferably for AOAC publication, detailing the results of the evaluation along with all method comparative data obtained. If the results obtained in the evaluation/comparison testing appear satisfactory, proceed with plans to draft a collaborative study protocol for the CDFA method.

(2) Attempt to recruit a new Associate Referee for Extraction of Low Moisture-High Fat Samples. Change title by deleting "Extraction of" to describe more accurately the total method development work needed for these sample types.

(3) Submit a redrafted collaborative study proposal for selected multifumigant residues in whole grains and milled products for comment and review. The proposal must include suggested chemicals, fortification levels, and sample types to be studied along with detailed method instructions. It is recommended that the proposal be based on the method published in *J. Assoc. Off. Anal. Chem.* (1983) **66,** 228–233. Upon satisfactory completion of the review and comment process, proceed with an interlaboratory trial of the method and study design prior to initiation of a collaborative study.

(4) Prepare and submit a draft of detailed instructions and trial design for review and comment on the proposed interlaboratory trial of a miniaturized method for nonfatty foods based on the applicable sections of **29.011-29.018** and **29.034** methodology. Upon satisfactory completion of the review and comment process, proceed with the planned trial.

(5) Complete recovery/sulfone conversion testing for the remaining phorate-related chemicals as planned. Prepare a report of findings and observations for publication based on the results obtained for both the fenthion and phorate-related chemicals. Continue testing the application of the total sulfone oxidation procedure with additional organophosphorus parent and metabolite chemicals recovered through **29.A01-29.A04** methodology.

(6) Proceed to coordinate the design and organization of an interlaboratory study on the recovery of representative

organochlorine pesticides from beef fat using a defined method applicable to the commercially available Unitrex sweep codistillation system. The study should be designed with assistance from appropriate representatives of the Australian Quarantine Inspection Service (AQIS) and AOAC to assure that both the needs of the AQIS laboratory accreditation program and the collaborative study criteria of AOAC are met.

The Associate Referee should directly inform the manufacturer of the Unitrex system of his findings regarding addition of the Florisil trapping media and use of beadless distillation tubes to expedite the dissemination of this information to current and future users. This information should also be formalized through publication.

(7) Continue investigation of root crops and their interaction with cypermethrin and/or other synthetic pyrethroids upon freezer storage. Providing an acceptable root crop can be found and shown to be nonreactive with any of the pyrethroid chemicals proposed for study, it is recommended the current collaborative study proposal be redrafted and resubmitted for comment and review. A proposed fortification plan and supporting information on the levels, products, and recoveries obtained with the method should be submitted as well. Upon completion of a satisfactory review and comment process, the Associate Referee should initiate an interlaboratory trial prior to collaboration.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

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Organohalogen Pesticides

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There are currently 9 topic areas under the General Refereeship on organohalogen pesticides. One of these, Pentachlorophenol, is vacant. The current status of each topic area follows:

Chlordane.—(Wilbur Saxton, FDA, Seattle, WA.) The Associate Referee has had no opportunity to work on this topic in the past year. It does not appear likely that time will be allotted during the upcoming year for him to complete the testing of the methods under consideration.

Chlorinated Dioxins.—(David Firestone, FDA, Washington, DC.) The Associate Referee prepared an annual update on the activities in the field of method development for residues of dioxins and furans. His report includes reference to a 1986 survey (Clement and Lennox, *Chemosphere* (1986) **15**, 1941–1946) that found 55 laboratories engaged in dioxin/furan analysis. Trends in this type of analysis include: (*1*) efforts to assure good quality standards, (*2*) continued application of sophisticated separation and detection techniques, such as high resolution gas chromatography (GC) and high resolution mass spectrometry, (*3*) automation of methods, and (*4*) analysis of foods for dioxin/furan residues. Issues of regulatory importance, such as the definitions of limits of quantitation and the costs of individual analyses, are also of major concern in the field of dioxin/furan analysis.

Table 1. Recoveries through the Hopper method[a]

Compound	*N*	Level, ppm	Mean, %	SD	CV, %
2,4-D	186	0.1	72	25	34
2,4,5-T	270	0.04	79	25	31
PCP	275	0.02	70	21	31

[a] Data from FDA Total Diet Study. Over 150 different fatty and nonfatty commodities involved. Some 0% recoveries included.

The Associate Referee recommends continued monitoring of methods for dioxins and furans in food and environmental samples, but has not suggested a method that should be collaborated by AOAC.

Chlorophenoxy Alkyl Acids.—(Marvin Hopper, FDA, Total Diet Research Center, Kansas City, MO.) The Associate Referee was appointed during the year. He plans to collaborate his method for chlorophenoxy alkyl acid (CPA) residues. At this point, the method's several different extraction steps, each for a different sample type, are described in FDA's *Pesticide Analytical Manual,* Volume I, as is the gel permeation chromatography (GPC) used to clean up the extract. Methylation by ion pair alkylation is described by Hopper in *J. Agric. Food Chem.* (1987) **35**, 265–269. The method is also capable of determining residues of phenols, such as pentachlorophenol.

This method is used extensively as one of the routine analyses in FDA's Total Diet Study. Recovery tests are routinely incorporated into the program to assure analytical quality. Long-term use of the method has produced the results shown in Table 1.

Hopper will combine the current descriptions of the parts of this method into complete method directions as a first stage of preparation for collaborative study. Following that, he will find other laboratories to perform an interlaboratory study preparatory to full collaborative study. At the same time, he intends to examine further the adequacy of the extraction of incurred CPA residues from crops. Performance of this kind of experiment is dependent on obtaining crops with incurred residues, however.

Ethylene Oxide and Its Chlorohydrin.—(Ronald Ross, University of Kentucky College of Medicine, Lexington, KY.) The Associate Referee was appointed during the year. The General Referee informed him that interest in residues of this fumigant centered on spices and black walnuts, in which high levels of residues have been found. He was encouraged to study the analytical method of Scudamore and Heuser (*Pestic. Sci.* (1971) **2**, 80–91), as has been recommended previously by the General Referee and the Committee on Residues. Review of other methods that may be available for the residues was also encouraged. At this point, he has not reported on his plans for proceeding with this topic.

Methyl Bromide.—(Joseph Ford, USDA, Gulfport, MS, and Richard DePalma, Procter and Gamble, Cincinnati, OH.) The Co-Referees have made progress in 2 areas this year. A study of residue stability was undertaken as a preliminary step to collaborative study. Flour, raw peanuts, and raisins were fumigated with methyl bromide at a rate of 2 lb/1000 cu. ft, and samples were stored at −80°F in individual 4 oz jars. Samples removed from storage at 15, 50, 94, and 125 days after fumigation were analyzed by a method that traps volatile residues released from the sample by distillation. Stability of the residues over this period of time is indicated by the results of these experiments. The high residue levels (up to 190 ppm in peanuts) in these newly fumigated and unaerated samples is not typical, however, of the levels that might be expected in aerated products.

Ford's laboratory has continued to explore the suitability of 2 different thermal desorption devices for determining methyl bromide. He hopes to bring chromatograms and other pertinent data on the results of these experiments with him

to the annual meeting in August to provide further information about the advisability of pursuing these types of analyses for methyl bromide.

Pentachlorophenol.—(Vacant.) The former Associate Referee, George Yip, FDA, Washington, DC, retired during the past year. No further work has been done on the topic.

The method for CPAs currently being considered for collaboration is also capable of determining pentachlorophenol residues. This method includes a methylation step and GC of the methylated residue. In contrast, the official first action method for pentachlorophenol in gelatin, **29.A14-29.A18**, involves GC determination of the phenol itself, a step which appears to be susceptible to irreproducibility. The inability to improve upon the GC analysis has kept the first action method from being accorded final action status.

Polychlorinated Biphenyls.—(Leon Sawyer, FDA, Washington, DC.) The Referee is investigating the quantitation of PCBs by individual congener analysis, as proposed by Michael Mullin of the EPA Large Lakes Research Station, Grosse Ille, MI. Several different PCB quantitation techniques have been proposed, each of which measures only selected PCB congeners in the sample and compares them to the same congeners in the standard. Several national governments now regulate PCB residues by one of these techniques (see next referee topic below), but the choice of which congeners to include is not consistent among countries. The Associate Referee believes that it is useful to explore Mullin's technique, because it is based on *all* individual congeners present in the sample and the standard. This technique might then be used as a referee method against which the methods that rely on selected congeners could be compared.

At this point, the Referee has prepared a PCB quantitation standard by combining specified weight ratios of 4 Aroclors, along with known amounts of 2,4,6-trichlorobiphenyl and 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl. The latter 2 congeners are used as internal standards for measuring response and retention ratios of the other congeners. GC analysis on a capillary column, temperature-programmed over a 3 h run, resolved the 105 congeners in the mixture into 92 distinct chromatographic responses. Using this standard, 2 Aroclor 1254 reference materials were analyzed and found to contain 105% and 97%, respectively, of their stated amounts.

Additional study of the chromatographic systems and parameters continues, and the Referee is adapting a recently purchased data system to automate the calculations and provide an informative reporting format.

PCB Determination by Measurement of Specific Congeners.—(Kimmo Himberg, Technical Research Centre of Finland, Espoo, Finland.) The Associate Referee was appointed during the year. It is his intention to collaborate a method for quantitating PCBs using 9 specific individual congeners. The method, published as Research Note No. 684 by the Referee's laboratory, makes use of capillary GC analysis with electron-capture and mass-selective detectors to quantitate the specified congeners. The method is a determinative step only and does not include directions for extraction or cleanup techniques. Finnish law now regulates PCBs in certain foodstuffs by identifying this method as that to be used for quantitating the 7 congeners that appear in food.

The Referee intends to develop a collaborative study protocol acceptable to both the Nordic Committee on Methods of Food Analysis (NMKL) and AOAC. Milk fat, animal fat, vegetable oils, and fish are the substrates that are being considered for inclusion in the collaborative study. No further communication has been received from the Associate Referee since his appointment.

PCBs in Blood.—(Virlyn Burse, Centers for Disease Control, Atlanta, GA.) As recommended last year, the Associate Referee completed his written report of the collaborative study on a method for PCBs in blood serum. The manuscript was reviewed and approved by both the General Referee and the Committee Statistical Consultant. Minor changes were made by the author. The revised draft was submitted to AOAC and the Committee on Residues with the recommendation that the method be accepted as interim official first action.

Recommendations

(1) Perform method comparison studies on the several methods available for chlordane residues in fatty products; i.e., (a) the following sections of the official method: extraction by **29.011-29.012**, acetonitrile partitioning cleanup by **29.014**, Florisil column chromatographic clean up and residue separation by **29.046-29.048**; (b) extraction of fat as in (a), followed by GPC cleanup, **29.037-29.043**; and (c) extraction as in (a), followed by cleanup on the Unitrex system. Samples extracted and cleaned up by any of these methods can then be determined by the capillary GC system developed by the Associate Referee. Once such studies have been completed, the Referee should prepare to collaborate the method found to be most suitable for determination of chlordane residues in butter, fish, and poultry fat.

(2) Continue to monitor progress on the development of methods for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and for hexachloro-, heptachloro-, and octachloro-*p*-dioxins and dibenzofurans in foods. Continue to evaluate methods toward the ultimate goal of establishing one as an official method through APAC collaborative procedures.

(3) Combine the refereeships on chlorophenoxy alkyl acids and pentachlorophenol. Write a complete method description for the Hopper method. Study the extraction efficiency of the method using incurred residues. Prepare a protocol for an interlaboratory study and perform the study. Prepare for and perform a collaborative study on the method if the interlaboratory study is successful.

(4) Perform a literature search and method trials as necessary to select the best method for determination of ethylene oxide and its chlorohydrin in foods. Include the method of Scudamore and Heuser (1971) *Pestic. Sci.* **2**, 80–91.

(5) Continue study to select the best method for determining residues of methyl bromide in products such as nuts, dried fruit, and spices. Evaluate the ability to store and ship samples containing low levels of residue in preparation for interlaboratory study and collaborative study.

(6) Continue to study quantitation of polychlorinated biphenyls by the individual congener technique of Mullin to determine its suitability as a referee method. Evaluate the differences in quantitation that will be created by the various specific congener methods that have been proposed, and compare these to the existing official method for quantitation, **29.018**.

(7) Describe a complete method for PCB determination by measurement of specific congeners, including extraction and cleanup steps. Perform experiments to show the compatibility of the extraction, cleanup, and determinative techniques. Prepare a protocol for an interlaboratory study of this method. Perform the interlaboratory study, and if successful, prepare a collaborative study protocol acceptable to both AOAC and the NMKL.

(8) Assuming that the method for PCBs in blood serum is accepted as official first action, continue monitoring its use to judge its acceptability for final action in the future. Study this method to evaluate what changes would be needed to produce a method suitable for both PCBs and pesticides in blood serum.

(9) Continue the official first action status of the method for pentachlorophenol in gelatin, **29.A14-29.A18**.

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FL. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

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Organonitrogen Pesticides

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Reports were received from Associate Referees on 7 topics. One topic (Organotin Fungicides) was transferred to the General Referee for Metals and other Elements. Associate Referees are required for an additional 11 topics.

Anilazine.—An Associate Referee is required to select and conduct an interlaboratory trial of a method for Anilazine residues in fruit and vegetables.

Benzimidazole-Type Fungicides.—Associate Referee Mikio Chiba reports that due to other priorities within his organization, he has been unable to conduct the necessary ruggedness testing of this method for benomyl and carbandazim, and will be unable to do so in the foreseeable future.

Captan and Related Fungicides.—Associate Referee Dahlia Gilvydis reports that one laboratory has completed an interlaboratory trial of her method for captan, folpet, and captafol, and another laboratory has yet to report data. Recoveries were satisfactory for the 3 compounds spiked into various commodities at levels of from 0.2 to 25 ppm.

Carbamate Herbicides.—An Associate Referee is required to select and test a method for the carbamate herbicides asulam, desmedipham, and phenmedipham.

Carbamate Insecticides.—Associate Referee Richard Krause reports that his method for confirmation of phenolic carbamate residues by post-column hydrolysis and electrochemical detection has been published [*J. Chromatogr.* **442**, 333 (1988)], and that he has not conducted further research in the field.

Carbofuran.—An Associate Referee is required to conduct a collaborative study of a method for carbofuran phenolic metabolites and 3-hydroxy carbofuran glucoside residues in crops and for carbofuran and its carbamate and phenolic metabolites in milk and meat.

Chlorothalonil.—Interest in this topic was expressed by the Fermenta Plant Protection Co. who supplied the General Referee with documentation of residue methods for chlorothanonil and its 4-hydroxy metabolite. Representatives of the company were informed of the procedure for conducting a collaborative study and encouraged to contact the General Referee to appoint an Associate Referee who could provide recovery data and instructions to collaborators.

Daminozide and 1,1-Dimethylhydrazine (UDMH).—Associate Referee M. D. Parkins reports that the interlaboratory study of the method for daminozide involving alkaline hydrolysis, formation of the salicylaldehyde dimethylhydrazone, and quantitation by GC/MS technique indicated too large a variation in recovery for the method to be tested collaboratively. Subsequent development work led to the use of a ^{15}N-daminozide internal standard for this procedure which greatly improved the GC/MS quantitation.

The method for UDMH was also found to result in variable recoveries when subjected to interlaboratory trial. The incorporation of the isotopic internal standard (^{15}N-UDMH) gave satisfactory recoveries in spiking studies.

Organonitro Pesticides.—Associate Referee Richard Krause reports that the chromatography and electrochemical detection characteristics of 21 nitrophenyl pesticides and 3 nitrophenols have been investigated and the results have been submitted for publication. The recovery of several organonitro compounds through Florisil using methylene chloride–acetonitrile as eluant was studied. Dicloran, dinoseb, bifenox, binapacryl, and dinocap were completely recovered and 80% of the parathion was recovered but only 40% of DNOC, and 5–10% of 2-nitrophenol and 2,4-dinitrophenol were recovered.

Diquat and Paraquat.—Associate Referee Brian Worobey reports that data has been received from 6 collaborators and that one additional collaborator has yet to complete the study.

Dithiocarbamate Fungicides.—An Associate Referee is required to select a method for *o*-phenylphenol residues and conduct a collaborative study.

Maleic Hydrazide.—An Associate Referee is needed to study a GC or LC method for maleic hydrazide residues.

Sodium o-Phenylphenate.—An Associate Referee is required to select a method for the determination of dimethyl and ethylenebis-dithiocarbamates in foods.

Substituted Areas.—Associate Referee Ronald Luchtefeld reports that a 2 laboratory trial of his LC method for urea herbicides will be initiated this year. Ten compounds will be added to 2 commodities, potatoes and grapes, at levels of 0.05 and 1 ppm.

Thiolcarbamate Herbicides.—An Associate Referee is required to conduct a collaborative study of a residue method for thiolcarbamates, such as the steam distillation method described in *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. XIII (1984), for EPTC and butylate.

S-Triazines.—An Associate Referee is required to investigate the inclusion of atrazine and cyanazine into an existing multiresidue procedure and conduct a collaborative study.

Trifluralin.—An Associate Referee is required to study the inclusion of trifluralin into existing multiresidue methods.

Recommendations

(1) Appoint a new Associate Referee for benzimidazole-type fungicides to study a method that converts thiophanate methyl and benomyl to carbendazim and subsequently determines the latter and thiabendazole residues in foods.

(2) Obtain and analyze data from the interlaboratory trial of the method for captan, folpet, and captafol. If precision and accuracy are satisfactory, initiate a collaborative study.

(3) Conduct an interlaboratory study of the Associate Referee's method [*J. Chromatogr.* **442**, 333 (1988)] for the confirmation of phenyl carbamate insecticide residues.

(4) Conduct a 2 laboratory trial of the modified versions of methods for daminozide and UDMH using a ^{15}N-UDMH internal standard. Submit details of the revised methods and data from the trial to the General Referee for review. Prepare a set of instructions to collaborators and proposal for the numbers and types of commodities and spiking levels to be studied in a future collaborative study.

(5) Continue recovery studies of organonitro compounds through Florisil. Investigate the effect of coextractives on the detection system.

(6) Analyze the data, when complete, from the collaborative study of the method for diquat and paraquat in potatoes, and submit a report and recommendation.

(7) Conduct interlaboratory trial of the Associate Referee's method for substituted areas. If the data are satisfactory, prepare a protocol for the collaborative study and, after review by the General Referee and statistician, conduct a collaborative study.

(8) Transfer the topic of Trifluralin to Organonitrogen Pesticides for inclusion into that multiresidue procedure.

(9) Appoint Associate Referees to study anilazine, carbamate herbicides, carbofuran and metabolites, chlorothal-

onil, dithiocarbamate fungicides, sodium *o*-phenylphenate, thiolcarbamate herbicides, and *s*-triazines.

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Organophosphorus Pesticide Residues

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There are 6 Associate Referee topics assigned to this General Referee. A brief summary of Associate Referee activity during the past year follows:

Disulfoton.—(S. Szeto, Agriculture Canada, Vancouver.) The Associate Referee reports continued success utilizing the method for determination of disulfoton and its oxidative metabolites on a variety of crops (potato, lettuce, small fruits). The method consists of an organic extraction, charcoal column cleanup, oxidation of parent compound and metabolites to sulfones, and determination by GC-AFID (*J. Agric. Food Chem.* (1982) **30,** 1082–1086). He has not been successful in finding anyone interested in collaborating on the method.

Gel Permeation Chromatography.—(F. McCullough, ABC Laboratories, Columbia, MO.) The Associate Referee was appointed very recently. He will pick up the work of his colleague at ABC Labs, T. Spurgeon, who was the previous Associate Referee for this topic.

Phosphine.—(B. Puma, FDA, Washington, DC.) The Associate Referee was involved in other projects this year and had limited time for phosphine research.

Recommendations

(1) Continue to search for interested analysts to conduct an interlaboratory study of the Associate Referee's method for determination of disulfoton and its oxidative metabolites (*J. Agric. Food Chem.* (1982) **30,** 1082–1086). If results of interlaboratory study are satisfactory, submit protocol for collaborative study of the method for approval by the General Referee and Statistical Consultant to Committee on Residues.

(2) Appoint an Associate Referee to study efficiency of procedures for extracting field-incurred residues of organophosphorus pesticides and their metabolites from crops; extend the study of Watts (*J. Assoc. Off. Anal. Chem.* (1971) **54,** 953–958); and develop improved extraction procedures for incorporation into existing multiresidue methods.

(3) Continue study of GPC methods in general. Develop plan for collaborative study to extend official final action GPC method for organochlorine pesticides in animal fats, **29.037-29.043**, to determination of polychlorinated biphenyl, organophosphorus pesticide, and organophosphorus pesticide metabolite residues.

(4) Appoint an Associate Referee to study the topic of phorate and its metabolites in foods.

(5) Continue study to evaluate methods for determination of phosphine residues including the solvent soaking procedure for the extraction of fumigants in grains, **29.072**.

(6) Appoint an Associate Referee to study the topic of terbufos and its metabolites in foods.

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Radioactivity

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Cesium-137.—The collaborative study for the determination of cesium-137 in milk and other foods by gamma-ray spectroscopy will be resubmitted. The candidate for a new Associate Referee has not had approval by the Supervisor; therefore, search for an Associate Referee has now begun. The first order of business will be a study for the evaluation of methods for determining cesium-137 in milk, foods, and biological materials at lower levels.

Iodine-131.—The collaborative study for the determination of iodine-131 in milk and other goods by gamma-ray spectroscopy will be resubmitted. The collaborative study protocol for a method that was selected and tested for ruggedness was to have been prepared for testing (General Referee's report, *J. Assoc. Off. Anal. Chem.* (1986) **69,** 270–271). However, due to unforeseen circumstances, it has been delayed. A preliminary study for iodine-131 in water has been conducted as a prelude for the study for iodine-131 in milk. This study involved 3 levels of activity. The data are being evaluated to determine the feasibility of a low-level study for iodine-131 in milk.

Plutonium.—A new Associate Referee has resigned. Alfred Robinson of the U.S. Testing Co., Richland, WA, submitted a letter of resignation due to pressing work at his firm. A search has begun for a replacement.

Radium-228.—The collaborative study was completed and the data are being evaluated by the Associate Referee. A report is expected soon.

Strontium-89 and Strontium-90.—The Associate Referee has reported that no progress has been made this year. This is due to pressures from other activities. However, he plans to initiate a collaborative study protocol this coming year of the method described by Baratta and Reavey (*J. Agric. Food Chem.* (1969) **17,** 1337–1339) for determining strontium-89 and -90 in foods.

Tritium.—A search is now in process for an Associate Referee.

Recommendations

(1) Resubmit the collaborative study for cesium-137 and iodine-131 in milk and other foods by gamma-ray spectroscopy.

(2) Submit the collaborative study for low-level iodine-131 in milk.

(3) Submit the study for radium-228 when completed.

(4) Search for new Associate Referees for (a) Cesium-137, (b) Tritium, and (c) Plutonium.

(5) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

GENERAL REFEREE REPORTS: COMMITTEE ON MICROBIOLOGY AND EXTRANEOUS MATERIALS

Analytical Mycology and Microscopy

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(*1*) The Associate Referee for the topics *Geotrichum* Mold in Canned and Frozen Fruits, Vegetables, and Fruit Juices and Standardization of Plant Tissue Concentrations for Mold Counting has resigned. Replacements are sought to continue study on these topics.

(*2*) A method has been developed to detect adulterants in horseradish. The method relies on diagnostic histological features of horseradish root and its known adulterants, such as turnip root, parsnip root, potato tuber, and cornstarch, as viewed through a polarizing microscope with crossed polars and a first order red plate. Samples are being prepared for a collaborative study.

(*3*) The method for rot fragment count of comminuted tomato products, **44.224**, is recommended for surplus method status because it has been found that the method does not fulfill its purpose of indicating whether a product has been milled. The method had been revised by the Associate Referee on Tomato Rot Fragment Count (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 278; 402–403; 896–898); however, 2 statements were omitted from the published method. After the stained sample is poured over the No. 60 sieve, the beaker should be rinsed with an additional 200 mL water which is then poured directly over the sieve as before. After the rot counting slides are prepared, they are examined at 40–45× using transmitted light. The surplus method should reference the original method, **44.224**, 14th edition; the 3 articles above; and this report.

Editorial Change: Revise method **44.224**, Rot in Tomato Products (Comminuted) (as changed in "Changes in Official Methods" *J. Assoc. Off. Anal. Chem.* (1985) **68,** 402–403) as follows: After the sentence "Add 200 mL H_2O and pour directly from beaker onto a 3 in. diam. No. 60 sieve (Dual Manufacturing . . . Chicago, IL 60647).", add "Rinse beaker with addnl 200 mL H_2O and pour H_2O directly from beaker over sieve as before." After the sentence "Blow out last drop if necessary.", add "Examine each slide at 40–45×, using transmitted light."

Refs.: JAOAC (1985) **68,** 278; 402–403; 896–898.

(*4*) The Howard mold count method for mold in dehydrated tomato powder, **44.211**, has 2 omissions. The blending speed, which is not shown, should read "ca 3200 rpm" (*J. Assoc. Off. Anal. Chem.* (1972) **55,** 73–75; (1978) **61,** 992–993). In addition, the procedure for identification of powder as a spray-dried product was omitted. This procedure (see *FDA Microanalytical Manual,* Method M18R, May 1978), which is simply a microscopic observation to determine whether the product has been dried by spraying, is necessary in order to choose which FDA defect action level to use for the product. Because the tomato powder method was removed from the *FDA Microanalytical Manual* and is available now only as an AOAC method (**44.211**), this microscopic procedure should be included as part of that method.

Editorial Change: In method **44.211**, Mold in Tomato Power (Dehydrated), Howard Mold Count, change sentence 2 to read: "Blend 30 s at ca 3200 rpm . . . ." In addition, the following procedure for identification of powder as a spray-dried product should be added to the method as a first section.

(Tomato powder is produced by dehydrating concd tomato pulp. In prepg powder for mold counting, moisture content is disregarded and diln with H_2O is made to give mixt. with approx. tomato solids content of standzd prepn for mold count of tomato puree or paste, i.e., 8.5%.)

Microscopic Identification as Spray-Dried Product

Mold counts of spray-dried tomato powder show significantly higher counts than paste from which it is made because of breakage of mold hyphae aggregates. Use following procedure to det. whether powder represents spray-dried product.

Suitably mount a small portion of product on microscope slide in mineral oil or other non-aqueous mounting medium and examine microscopically at 100–200×. Spray-dried particles are translucent and contain air bubbles and numerous small granules within particles. Shape of particles ranges from spherical to elongate to irregular with rounded outlines and essentially no sharp angles. In rehydrated powder, practically no intact tomato cells are evident. Drum-dried or similarly processed powder or flakes are characterized by irregular-shaped particles with angular outlines and practically no embedded air bubbles.

Perform mold count detn as described in **44.211**.

(*5*) A clarification is needed for the calculation in **44.220-44.222**, *Geotrichum* mold in comminuted fruits and vegetables. In **44.222**, to express results in mycelial fragments per 100 mL prepn, first calculate the number of fragments per 1 mL prepn as the total number of fragments counted divided by number of mL examined (which is number of slides counted × 0.5 mL per slide). Multiply that quantity by 100 to calculate fragments per 100 mL prepn.

Editorial Change: In method **44.220-44.222**, Mold in Comminuted Fruits and Vegetables, *Geotrichum* Mold Count, the last sentence in **44.222** should read: "Express results in mycelial fragments per 100 mL prepn: $N = S \times 100$, where S = total mycelial fragments/mL sample prepn counted (0.5 mL/slide)."

(*6*) A clarification is also needed for the methods for *Geotrichum* mold, **44.216** and **44.219**. Analysts have reported some confusion in how to apply the equation in **44.216**. Variations in sample preparation occur, such as in the number of slides counted, or in having final sample preparation concentrations in excess of the 100 mL specified in **44.219**(c). Without knowing the derivation of the equation in **44.216**, it is difficult to modify it to suit one's situation. Therefore, **44.216** must be expanded so the equation is written in a more general form, and so that the derivation is clear. In addition, there is an error in the equation as written when it is applied to samples prepared by **44.219(c)**. Since mycelial fragment counts are expressed as number per 500 g product, counts per mL must be multiplied by the total volume of the preparation. In **44.219(c)**, the preparation (the portion centrifuged) represents only half of the original volume. Therefore, V should be the sum of the volume in both centrifuge tubes × 2. Also, the equation should allow for situations in which the original volume exceeds 100 mL.

Editorial Change: In method **44.214-44.216**, *Calculations,* **44.216**, should read:

Calc. mycelial fragments/500 g product:

$$N = (S/V\{\text{slides}\}) \times (500/W) \times V\{\text{diln}\}$$

where, S = total mycelial fragments counted; $V\{\text{slides}\}$ = total vol. counted (0.5 mL/slide); W = net wt of sample, g; and $V\{\text{diln}\}$ = vol. after final diln with stabilizer soln.

In method **44.219**, Mold in Vegetables, Fruits, and Juices (Canned), *Geotrichum* Mold Count, change sec. (c) to read:

(c) Transfer to g-s graduate. Dil. to ≥100 mL ($V\{\text{prepn}\}$) and mix well. Quickly pour off two 25 mL aliquots ($V\{\text{aliq.}\}$ = sum of aliquots taken) into sep. centrf. tubes and proceed as in (**b**). Keep final vols equal after dilg with stabilizer soln; $V\{\text{diln}\}$ = sum of vol. in both tubes. Proceed as in **44.214**, pipetting 1 slide from each dild aliquot. Calc. mycelial fragments/500 g product:

$$N = (S/V\{\text{slides}\}) \times (500/W) \times V\{\text{diln}\} \times (V\{\text{prepn}\}/V\{\text{aliq.}\})$$

where, S = total mycelial fragments counted; $V\{\text{slides}\}$ = total vol. counted (0.5 mL/slide); W = net wt of sample, g; $V\{\text{diln}\}$ = sum of vol. in both centrf. tubes after final diln with stabilizer soln; $V\{\text{prepn}\}$ = vol. before aliquots removed; and $V\{\text{aliq.}\}$ = sum of vol. of aliquots taken.

Recommendations

(1) Revise **44.211**, **44.216**, **44.219**, **44.222**, and **44.224** as described and declare **44.224** surplus.

(2) Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Microbiology. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed.

Extraneous Materials in Foods and Drugs

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Methods for mammalian feces in grain products, light filth in spirulina, and light filth in tofu were submitted for consideration as interim official first action.

Collaborative studies of methods for light filth in dried bean curd and for light filth in fish paste and sauces were conducted.

Methods for light filth in baked goods with fruit and nut tissues, light filth in chocolate products, brine extraction of light filth in tomato products, pancreatin digestion for filth in cheese, light filth in preserved and freeze-dried bean curd, light filth in fish sauce containing spices, light filth in canned fish and fish products, and a modification of the TLC method for urine stains are being considered for collaborative study in 1989.

The following Associate Referee topics and appointments were recommended: Light Filth in Ground Coffee, Flotation Method—Gerald E. Russell; Light Filth in Ground Basil, Flotation Method—Beverly Kent; Light Filth in Fish Paste and Sauces, Flotation Method—Larry E. Glaze; Light Filth in Grain Products, Flotation Method—Larry E. Glaze; Rodent Gnawing of Packaging Materials and Foods, Salivary Amylase Test—Roger L. Heitzman and Jack L. Boese; Filth in Onion and Garlic (Powdered and Granulated), Sedimentation Method—James Buhlert; Light Filth in Popcorn (Unpopped), Sieving Method—Alan T. Whiteman; Light Filth in Popcorn (Popped), Flotation Method—James Karpus; and Mammalian Feces in Spices, Chemical Detection Method—Harriet R. Gerber.

The following Associate Refereeships have been discontinued: Brine Extraction Techniques for Capsicum Products—Susan Schena; Adulteration of Botanicals by Foreign Plant Materials—Frank D'Amelio; Filth in Processed Meats—Phillip Alioto; Mite Contamination Profiles and Characterization of Damage to Foods—Diane M. Peace and Mary-Ann Gardiner; Mites in Stored Foods—Jack L. Boese; Filth in Dried Mushroom Products—Alan R. Olsen; Filth in Rye Bread—Richard R. Haynos; Soluble Insect and Other Animal Filth—George P. Hoskin; and Performance Evaluation of Methods—Jack L. Boese, James Karpus, and Alan Whiteman. Methods development research will continue on all other topics.

The continuing Associate Referee topics have been redefined in terms of analyte, matrix, and method. The revised topics are: Insect Infestation (Internal) of Whole Grains, Cracking Flotation Method—Richard L. Trauba; Mammalian Feces in Grain Products, Alkaline Phosphatase Detection Method—Harriet R. Gerber; Light Filth in Soybean Curd, Flotation Method—Marvin J. Nakashima; Light Filth in Spirulina, Flotation Method—Marvin J. Nakashima; Light Filth in Botanicals, Flotation Method—Marvin J. Nakashima; Light Filth in Tomatoes, Brine Extraction Method—Bernice B. Beavin (deletes mushrooms from the topic); Light Filth in Canned and Dried Soups, Flotation Method—Richard Klein; Filth in Cheese, Pancreatin Digestion Method—Mary-Ann Gardiner; Light Filth in Crabmeat, Shrimp, and Tuna (Canned), Brine Flotation Method—James D. Barnett; Light Filth in Baked Goods with Fruit and Nut Tissues, Flotation Method—Joseph K. Nagy; Urine Stains on Foods and Containers, Chemical Methods—Robert S. Ferrera; Light Filth in Chocolate and Chocolate Products, Flotation Method—C. Robert Graham (expands scope of original topic from cocoa powder and press cake); Light Filth in Fish (Canned) and Fish Products, Flotation Method—Wilfred A. Sumner; and Light Filth in Vegetable Products (Dehydrated), Flotation Method—Francis J. Farrell.

Recommendations

(1) Adopt as official final action the following official first action methods: Light Filth in Flour (Corn), **44.058(a)** and **44.059**; Filth in Eggs and Egg Products, **44.074**-**44.076**; Filth in Mushrooms, **44.115**; Light Filth in Ground Allspice, Flotation Method, **44.127**-**44.128**; and Excrement (Bird and Insect) on Food and Containers, Thin Layer Chromatographic Method for Uric Acid, **44.B07**-**44.B09**.

(2) Discontinue study on the following topics: Brine Extraction Techniques for Capsicum Products; Adulteration of Botanicals by Foreign Plant Materials; Filth in Processed Meats; Mite Contamination Profiles and Characterization of Damage to Foods; Mites in Stored Foods; Filth in Dried Mushroom Products; Filth in Rye Bread; Soluble Insect and Other Animal Filth; and Performance Evaluation of Methods.

(3) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Microbiology. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

Cosmetic Microbiology

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AOAC does not have an official method for evaluating the microbiological efficacy of cosmetic preservative formulations in situ. Such a method is needed because adequate chemical levels of preservatives need not necessarily mean adequate microbiological effectiveness particularly in used cosmetics.

Other organizations have their own official methods for preservative efficacy in liquid aqueous-base topical pharmaceutical products. Such methods have already been adapted and applied to cosmetics, so the logical approach for liquid (aqueous and non-aqueous) and cream products would be to collaboratively study application of one of the pharmaceutical methods to cosmetics analysis. Therefore, I propose as a first goal, to find an Associate Referee to carry out suitable preliminary studies and then a collaborative study of the *U.S. Pharmacopeia* (USP) method for pharmaceutical preservative efficacy as applied to liquid and cream cosmetics. This study should concentrate on eye-area products as much as possible. Also, and I feel this is very important, the study should be based on a single microbe challenge using *Pseudomonas aeruginosa*. Data from a single-challenge collaborative study using *P. aeruginosa* should provide a reliable standard against which to evaluate in subsequent studies: (*1*) multiple microbial pathogen challenges that include *P. aeruginosa*, (*2*) challenges with *P. aeruginosa* mixed with typical human skin microflora, and (*3*) challenges with other single microbial pathogens. Consideration of any of these aspects in the proposed collaborative study would make it overly cumbersome to conduct.

Although the USP method and related methods are slow, typically requiring a month to complete, they are not completely untested and, therefore, offer the most expedient route to establishing a first official method. It is likely that more rapid and sophisticated methods will be developed by others, and these could be evaluated against the proposed collaborative study method.

The second goal that I propose is more difficult and therefore more long-term. It is to establish another Associate Refereeship to do preliminary studies on a microbiological efficacy method for solid cosmetics. Such a method could be based on the published studies of R. Bhadauria and D. G. Ahearn (*Appl. Environ. Microbiol.* (1980) **39,** 665–667) on solid cosmetics.

A third goal is to find an Associate Referee willing to do preliminary studies on adaptation of the USP method mentioned above from an end-point-survival determination into a survival rate determination method. The aim here would be to evaluate the use of D-values and z-values as parameters of the biological efficacy of preservatives in order to develop a more rapid method.

The fourth and last goal is to consider new cosmetic preservative evaluation methods and new microbial recovery methods as they develop. But as already mentioned, such methods should always be evaluated against official methods based on microbial viability tests.

Of the 4 goals discussed, I feel the first has the most priority. Comments and criticisms from the Committee on Microbiology and from other interested persons would be most helpful to me.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Microbiology. See the report of the committee, this issue.

Dairy Microbiology

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During the past year 4 dairy microbiology topics were under active review:

(1) Use of Dry-Rehydratable Films to Enumerate Coliforms and Aerobic Bacteria in Dairy Products.—This method is in official final action status for raw and pasteurized milk (**46.B05-46.B07**). Collaborative studies utilizing chocolate milk, pasteurized cheese, nonfat dry milk, evaporated milk, and vanilla ice cream were completed and reviewed. A poster presentation will be given at the 1988 Annual Meeting.

(2) Rapid Detection of Listeria in Dairy Products by Using a DNA Probe.—This topic, which was established in 1987, currently has 2 methods under validation studies.

(3) Detection of Listeria in Dairy Products by Using Cultural Methods.—This topic, which was established in 1985, is progressing through method validation.

(4) Temperature-Independent Pectin Gel Method for Coliform Determination in Dairy Products.—Collaborative studies utilizing cream, cheddar cheese, cottage cheese, homogenized milk, raw milk, sour cream, and yogurt were completed and reviewed. A poster session will be presented at the 1988 Annual Meeting.

Development of rapid methods for detecting disease-causing organisms in foods continues to be the area of greatest need.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Microbiology. See the report of the committee, this issue.

Section numbers refer to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69,** 349–390 (B methods).

Drug and Device Related Microbiology

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Biological Indicator Testing and Standardization.—(Robert R. Berube, 3M Co., St. Paul, MN, and Gordon S. Oxborrow.) Evaluation of data from a collaborative study was completed. Significant between- and within-laboratory variability was noted. Significant difference was greatest between the bacterial growth media used.

Electronic Particle Counters.—(Gordon S. Oxborrow.) No progress was made this year.

Limulus Amebocyte Lysate Test for Endotoxin.—(Christine W. Twohy, FDA, Minneapolis, MN.) A statistical analysis of a 1984 collaborative study was completed and a paper was published. The methods used have been presented to the Methods Committee on Microbiological and Microanalytical Methods with a request for interim approval.

Packaging Integrity for Medical Devices.—(Ana M. Placencia. FDA, Minneapolis, MN.) A collaborative study is in progress to test the membrane agar plate strike-through method and the exposure chamber method. Study was also conducted to compare the above methods with a porometer. A system for ranking packaging materials was applied and a paper will be presented at the 1988 Annual Meeting. Studies

were conducted evaluating correlations between porosity, weight, and the above methods. A paper is being prepared.

Disinfectants/Sterilants Used for Medical Devices.—(James W. Danielson, FDA, Minneapolis, MN.) Improvements are being evaluated for the sporicidal method of testing disinfectants/sterilants using washed spore suspensions for inoculation of penicylinders. A paper, "Effects of Chemical Sterilants/Disinfectants on *B. subtilus* Spores Under Various Conditions," will be presented at the 1988 Annual Meeting. Evaluation of sporulation medium for replacement of present soil extract medium is being done.

Sterility Testing of Medical Devices.—(Michael Palmieri, FDA, Brooklyn, NY.) No progress was made this year.

Chemical Indicators.—(Marvin Hart, 3M Company, St. Paul, MN.) A collaborative study was completed evaluating a method for temperature-specific chemical indicators used in steam sterilization. Data are being evaluated and a paper prepared.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Microbiology. See the report of the committee, this issue.

Food Microbiology (Nondairy)

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Collaborative Studies

Enzymatic Methods for Escherichia coli.—Stephen Edberg of Yale University conducted an evaluation of the "autoanalysis colilert" (AC) procedure for the simultaneous enumeration of coliforms and *E. coli* in drinking water. Unlike classical enzyme assays which use ortho-nitrophenyl-beta-D-galactopyranoside(ONPG)and4-methylumbelliferyl-beta-D-glucuronide (MUG) to test only for the presence of enzymes, AC uses ONPG (for total coliforms) and MUG (for *E. coli*) as essential growth substances and as an indicator system (color formation and fluorescence).

The actual test is performed by adding the water sample to the AC powder formula contained in a tube. The formula contains, per L, the following ingredients: $(NH_4)_2SO_4$, 5 g; $MnSO_4$, 500 μg; $ZnSO_4$, 500 μg; $MgSO_4$, 100 mg; NaCl, 10 g; $CaCl_2$, 50 mg; KH_2PO_4, 900 mg; Na_2HPO_4, 6.2 g; Na_2SO_3, 40 mg; amphotericin B, 1 mg; ONPG, 500 mg; MUG, 75 mg; and solanium, 50 mg. After incubation for 24 h at 35°C, total coliforms in the water sample will turn the solution from colorless to yellow. The presence of *E. coli* will produce fluorescence in the same tube examined under longwave (366 nm) ultraviolet light.

In the conventional coliform enumeration procedure using lauryl tryptose broth and EC medium, the target analyte must proceed through several steps (transfer of lactose across the cell membrane, conversion of lactose to glucose, metabolism of glucose into pyruvate, and conversion of pyruvate into acid and/or gas). This series of steps may require up to 96 h to complete the process, providing ample opportunity for discrepant reactions to occur. With the AC procedure, however, each time a nutrient-indicator molecule is utilized, a chromatophore is produced, thereby providing results within 24 h.

Five water utility laboratories, each using samples from its own distribution system, compared the AC procedure and the 10-tube fermentation procedure of the American Public Health Association (*Standard Methods for the Examination of Water* and *Wastewater* (1985) 16th Ed., APHA, Washington, DC) for the enumeration of total coliforms and *E. coli* in drinking water. A total of 46 samples was analyzed. Within the design of the study, it was reported that the 2 procedures were equivalent. Because replicate (split) samples from a single source, i.e., laboratory conducting the collaborative study, were not used, it is recommended that the study be repeated according to AOAC guidelines.

Rapid Methods for the Enterobacteriaceae.—Associate Referee Russell Flowers of Silliker Laboratories conducted a collaborative study of the Q-TROL *Salmonella* Detection Kit (Dynatech Laboratories, Inc., Chantilly, VA). This system is based on a "sandwich" enzyme immunoassay (EIA). Monoclonal antibodies to *Salmonella* are coated onto the surface of wells of plastic microtiter plates. The introduction of a post enrichment containing *Salmonella* antigen will result in the formation of an antibody-antigen complex on the surface of the well. This complex is allowed to react subsequently with a second antibody that is conjugated to the enzyme, alkaline phosphatase, resulting in the formation of an antibody-antigen-antibody-enzyme complex. Addition of a fluorescent substrate may result in a fluorescent reaction. A MicroFluor reader, available from Dynatech Laboratories, Inc., measures the relative fluorescence in the microtiter wells. Fluorescence values above a certain "cutoff" value are considered presumptively positive for *Salmonella*, and these results must be confirmed by the official culture method, **46.115-46.128**.

Thirteen collaborators analyzed one or more of 6 foods (dried whole eggs, nonfat dry milk, soy flour, ground black pepper, milk chocolate, and raw deboned poultry) by the conventional culture method and the EIA. Each of the food types was inoculated with each of 2 levels (high or low) of *Salmonella*. Overall, there was a 98.5% agreement between the 2 methods. False negative rates for the culture and EIA methods were 1.1 and 0.8%, respectively. Thus, the Associate Referee recommended that the Q-TROL EIA be adopted as an interim official first action screening method for detecting *Salmonella* in all foods, and the General Referee concurred.

Yersinia enterocolitica Isolation from Foods.—Co-Associate Referee Georg Kapperud reports that 2 collaborative studies, involving 15 and 19 laboratories, were carried out to evaluate a method for the qualitative determination of *Yersinia enterocolitica* in foods. The method comprises 3 steps: (a) 3 hours resuscitation at room temperature in phosphate-buffered saline (pH 7.6) with 2% sorbitol and 0.15% bile salts (PSB), (b) 8 days preenrichment in PSB at 4°C followed by selective enrichment in modified Rappaport broth (4 days at room temperature), and (c) 3 weeks cold enrichment at 4°C in PSB. After each step, the culture is streaked to a selective agar medium. Each collaborator received samples inoculated with different *Y. enterocolitica* serogroups, as well as unseeded control samples. Between 93.3 and 78.9% of the collaborating laboratories succeeded in demonstrating *Yersinia spp.* in samples to which these bacteria had been added, even though most had little or no previous experience in isolating *Y. enterocolitica*. Correct identification of the bacteria at the species and biotype level proved to be considerably more difficult. He proposes that the pyrazinamidase test be utilized as a rapid screening method for potential pathogenicity in *Yersinia* isolates.

Because these studies were not conducted in conformance with AOAC guidelines, it is recommended that they be repeated.

Associate Referee Reports

Bacillus cereus, Isolation and Enumeration.—Associate Referee Stanley Harmon reports that work was continued

during 1988 to determine the merits of substituting the lipid globule stain of Burdon for biochemical tests specified in **46.106-46.111** for confirming isolates from mannitol-egg yolk-polymyxin (MYP) agar as members of the *Bacillus cereus* group. A total of 173 isolates of *B. cereus,* 18 cultures of *B. cereus* var. *mycoides,* 26 cultures of *B. thuringiensis,* and 56 cultures representing 15 other *Bacillus* species not members of the *B. cereus* group was cultured on MYP agar and tested for lipid globules. Lipid globules were produced on MYP agar by all 273 cultures classified as members of the *B. cereus* group and by 3 other *Bacillus* species (*B. aneurinolyticus, B. sphaericus,* and *B. thiaminolyticus*). *B. megatherium,* which produces lipid globules when grown on nutrient agar, did not grow on MYP agar. It was concluded from this study that the lipid globule stain is useful in confirming isolates from MYP agar as members of the *B. cereus* group. However, because this property is not unique to *B. cereus,* the test for lipid globules must always be used in conjunction with more specific tests (**46.112-46.114**) before isolates can be identified as *B. cereus.*

A paper entitled "Limitations of the Lipid Globule Stain as an Aid in Confirming *Bacillus cereus*" will be presented at the 1988 Annual Meeting. The Associate Referee recommends further study on this topic before any changes are made in the official method for enumeration and confirmation of *B. cereus* in foods.

Enzymatic Methods for E. coli.—Official first action status was given to the alternative method using MUG for the rapid detection of *E. coli* in chilled and frozen foods (excluding shellfish). The method was published in "Changes in Official Methods," **46.D05-46.D08**, and the collaborative study appeared in the May issue of the *Journal* (*J. Assoc. Off. Anal. Chem.* (1988) **71,** 589–602).

Associate Referee Lloyd Moberg is writing a proposal for an additional collaborative study testing the method on shelf stable foods (i.e., dry mixes, chocolate, powdered milk, spices, and cheese). The study is tentatively expected to begin in the fall of 1988.

Fungi in Foods.—Associate Referee Philip Mislivec has completed a study on utilizing the viable yeast and mold count method to determine the efficiency of the reconditioning of moldy capsicums. Results show that in many cases the viable mold count is higher after reconditioning than before. Thus, reconditioning does not remove the mold. Rather, it masks it. These results will be presented at the 1988 Annual Meeting. The Associate Referee has completed studies on the comparison of potato dextrose agar alone with potato dextrose agar amended with 75 g NaCl per liter for determining viable mold counts in foods. The latter medium works well and retards the growth of "spreader" fungi. These results have been accepted for publication. The Associate Referee, with the cooperation of Douglas King of the U.S. Department of Agriculture, is planning a collaborative study on these 2 media, plus a third medium, dichloran rose bengal chloramphenicol agar.

The Associate Referee is presently analyzing fecal pellets from spices for the presence of viable mold species. Preliminary findings show that mold species are present, and often the pellet species differ from the species found in the spices.

Genetic Methods for Detecting Pathogenic Microbes in Foods.—No collaborative studies have been conducted this year, but Associate Referee Walter Hill indicates that consideration is being given to an evaluation of a synthetic DNA probe for *Listeria monocytogenes.*

Hydrophobic Grid Membrane Filter Methods.—Associate Referee Phyllis Entis reports activity in 3 areas. A collaborative study protocol has been approved to evaluate the incorporation of a MUG confirmatory step into the hydrophobic grid membrane filter (HGMF) test for *E. coli.* It is anticipated that the study will be initiated in late summer of 1988.

An in-house evaluation of a new plating medium, EF-18 agar, to replace selective lysine agar in the HGMF test for *Salmonella* organisms is being conducted. If successful, a collaborative study will be conducted to seek approval for the method as well as to expand the scope of the HGMF *Salmonella* method to cover all foods.

Ms. Entis further reports that an in-house validation protocol is being prepared to evaluate an HGMF method for enumerating yeasts and molds in foods.

Redigel Media.—Associate Referee Jonathan Roth completed a collaborative study comparing a temperature-independent pectin gel method for coliform determination in dairy products to the standard violet red bile agar plate count method. (See General Referee Report on Food Microbiology: Dairy.) He reports that other comparative studies with Redigel are being considered in the areas of *Salmonella* and *Listeria* in food and dairy products and of water quality testing.

Staphylococcal Enterotoxins.—Associate Referee Reginald Bennett reports that an interlaboratory study was conducted to evaluate a colorimetric enzyme-linked dipstick immunoassay, a commercial kit, for the rapid, sensitive (0.5 ng/g), and specific identification of *Staphylococcus aureus* enterotoxins A–E. Eight foods were homogenized by blending with distilled water to which 2–24 ng/g of enterotoxins were added. Food slurries were frozen and lyophilized. Dried preparations were mixed in a dry blender to ensure homogeneity. The same foods were also prepared without enterotoxins. The foods and commercial kits were shipped to academic, industrial, federal, and state facilities for method evaluation. Foods were rehydrated, homogenized by blending, pH-adjusted (4.5, then 7.5), treated with $CHCl_3$ if lipids were present, and extracted by centrifugation. Plastic dipsticks coated with antibody specific for the toxins were placed in the agitated extracts for 2 h at room temperature. After the dipsticks were washed, they were treated with antibody-alkaline phosphatase conjugate for 45 min, washed, incubated in substrate for 30 min, dried, and observed for reactivity. A blue color indicated presence of the enterotoxin serotype(s). Correct interpretation varied from 73.8 to 90.0%, with 6.6–60.0% false negatives and 6.2–16.9% false positives.

Recommendations

(1) Adopt as official first action the interim official first action MICRO-ID (General Diagnostics, Division of Organon Teknika, Durham, NC) system (a) as an alternative to conventional biochemicals, **46.121-46.124**, or to other AOAC approved diagnostic kits, **46.133**, for the presumptive generic identification of foodborne *Salmonella* isolates and screening and elimination of non-*Salmonella* isolates; (b) as an alternative to conventional biochemicals, **46.016**, for the identification of foodborne *E. coli* isolates; and (c) for the presumptive generic identification of other *Enterobacteriaceae* isolates from foods.

(2) Adopt as official first action the interim official first action *Salmonella* 1-2 TEST (BioControl Systems, Inc., Bothell, WA) system for screening all foods for the presence of *Salmonella.*

(3) Adopt as official first action the interim official first action TECRA *Salmonella* Visual Immunoassay (Bioenter-

prises Pty, Ltd, Roseville, N.S.W., Australia) system for screening all foods for the presence of *Salmonella.*

(4) Adopt as official first action the interim official first action Q-TROL *Salmonella* Detection Kit (Dynatech Laboratories, Inc., Chantilly, VA) system for screening all foods for the presence of *Salmonella.*

(5) Adopt as official final action the following official first action methods: Frozen, Chilled, Precooked, or Prepared Foods, **46.013-46.015**; Thermophilic Bacterial Spores in Sugars, **46.078-46.082**; Virus in Beef (Ground), **46.188-46.190**; Sporeformers in Low-Acid Canned Foods—Gas Chromatographic Method, **46.A01-46.A05**; Poliovirus 1 in Oysters, **46.A12-46.A22**; *Salmonella* in Foods—DNA Hybridization Method, **46.C07-46.C16**; *Salmonella* in Low-Moisture Foods—Enzyme Immunoassay Screening Method, **46.C17-46.C25**.

(6) In response to the request of Committee Chairperson to redefine Associate Referee topics according to method, analyte, and matrix, the following realignment is recommended:

(a) Redefine the following topics (Associate Referee's name appears in parentheses):

(*1*) Change "*Bacillus cereus* Enterotoxin" to "Microslide Gel Double Diffusion Test for *Bacillus cereus* Enterotoxin" (Reginald Bennett).

(*2*) Change "Bacterial Pathogens—Genetic Methods" to "DNA Hybridization Method for Detecting Enterotoxigenic *Escherichia coli*" (Walter Hill).

(*3*) Change "Enzymatic Methods for *Escherichia coli*" to "MUG Test for *Escherichia coli* in Chilled and Frozen Foods" (Lloyd Moberg).

(*4*) Subdivide "Hydrophobic Grid Membrane Filter Methods" into "Hydrophobic Grid Membrane Filter Method for Aerobic Plate Counts and Coliforms" and "Hydrophobic Grid Membrane Filter Screening Method for *Salmonella*" (Phyllis Entis for both topics).

(*5*) Change "Petrifilm Methods" to "Petrifilm Method for Aerobic Plate Counts and Coliforms in Nondairy Foods" (Vernal Packard).

(*6*) Subdivide "Rapid Methods for the *Enterobacteriaceae*" into "Bio-EnzaBead Enzyme Immunoassay Screening Method for *Salmonella*," "TECRA Enzyme Immunoassay Screening Method for *Salmonella*," "Q-TROL Enzyme Immunossay Screening Method for *Salmonella*," "GENE-TRAK DNA Hybridization Screening Method for *Salmonella*," "ImmunoBand Screening Method for *Salmonella*," and "Micro ID Diagnostic Kit for Identification of *Salmonella, Escherichia coli,* and other *Enterobacteriaceae*" (Russell Flowers for all 6 topics).

(*7*) Change "Redigel Media" to "Redigel Medium for Determining Aerobic Plate Counts of Foods" (Jonathan Roth).

(*8*) Change "Shellfish Microbiology" to "MUG test for *Escherichia coli* in Shellfish" (William Watkins).

(*9*) Change "*Vibrio cholerae*" to "Elevated Temperature Enrichment Method for *Vibrio cholerae* in Oysters" (Angelo DePaola).

(*10*) Change "Yeasts and Molds" to "Mycological Media for Enumeration of Yeasts and Molds" (Philip Mislivec).

(b) Until a specific method can be identified for collaborative study, designation of the following topics remains unchanged: "*Bacillus cereus,* Isolation and Enumeration (Stanley Harmon) and "*Yersinia enterocolitica*" (James Cholensky, Georg Kapperud, and Sallie McLaughlin).

(7) Continue study on all retained Associate Referee topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Microbiology. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411 (A methods); *J. Assoc. Off. Anal. Chem.* (1987) **70**, 385–403 (C methods); *J. Assoc. Off. Anal. Chem.* (1988) **71**, 199–239 (D methods).

GENERAL REFEREE REPORTS: COMMITTEE ON FEEDS, FERTILIZERS, AND RELATED MATERIALS

Antibiotics and Other Products in Feeds

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During the past year (1987–1988), the General Referee recommended changes in antibiotic assays as well as calculation of potencies. The changes do not alter the method of determination but attempt to give the analyst flexibility in preparation of the levels of standard reference solutions as well as in the use of one layer of agar medium instead of two. The analyst may prepare the full range of standard solutions as indicated in the AOAC methods. The analyst may also prepare a narrower range of concentrations (perhaps 4×) with fewer levels and remain within the range specified for each antibiotic as in the current AOAC method.

It was also recommended that the methods of calculation of potencies be changed to permit the use of calculators or computer-aided calculations in addition to the manual method. The least square linear regression or quadratic equations can be used to fit the standard curve whether the concentrations are equally spaced or whether the range of standard (difference between the low and high points) is wide (16×) or narrow (4×). However, if the range of standards used is wide, the errors associated with calculated concentrations are less when the quadratic fit is used as compared to the linear regression method. For manual plotting with evenly spaced standard concentrations, the methods of calculations of fit remain the same as those specified by AOAC for 3 or 4 or 5 dose lines.

A collaborative study using simplified assay design was initiated by S. Brady to compare with the current AOAC design. The new design is based on placing 4 standard concentrations and two unknowns on each plate. If the new design proves to be statistically as good as the current AOAC method, it will save time and material. Currently, results are being evaluated.

M. Coleman of Lilly Research Laboratories recently submitted to AOAC a turbidimetric method for determination of tylosin in feeds. The method eliminates most of the interference by feed components and improves recovery of tylosin, especially at low levels. Future collaborative study will be conducted on this method.

The list of Associate Referees was updated and, for some topics, new Associate Referees were appointed.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Drugs in Feeds

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This report was prepared in conjunction with editing the "Drugs in Feeds" chapter in preparation for publication of the 15th edition of *Official Methods of Analysis*. Therefore, inactive drug topics are considered along with the active topics, especially where it appears that a drug is no longer marketed. In several cases it is recommended that the methods for such drugs be placed in a surplus status in the 15th edition of *Official Methods of Analysis*. Surplus status was recommended if a drug was not marketed in the United States, and not known to be marketed in another country. Absence of the drug from the "Additives and Their Uses" section of the 1988 *Feed Additive Compendium* (Miller Publishing Co., Minnetonka, MN 55343) was used as an indication that a drug was not marketed in the United States.

The inactive topics are listed first.

Nithiazide is not listed in the 1988 *Feed Additive Compendium*, nor is it listed in the Current Drug Listing of the 1988 *Official Publication of the Association of American Feed Control Officials*. No source of standard is listed in the official nithiazide method (**42.122-42.125**). This method should be designated surplus.

Nitrodan is an inactive topic with no Associate Referee. Attempts to contact the supplier for standard listed in **42.126(b)** were not successful. This drug is not listed in the 1988 *Feed Additive Compendium*, or the 1988 *Official Publication of the Association of American Feed Control Officials*. The official nitrodan method (**42.126-42.129**) should be designated surplus.

Reserpine is not listed in the 1988 *Feed Additive Compendium*, or the *Official Publication of Association of American Feed Control Officials*. No source of standard is listed in the official final action reserpine method (**42.148-42.151**). This method should be designated surplus.

Ronnel is also an inactive topic with no Associate Referee. Of the 2 final action methods, one involves GC analysis (**42.152-42.154**) and the other spectrophotometry (**42.155-42.159**) for the determinative step. This material is not listed in the 1988 *Feed Additive Compendium* as a drug, but it is listed in the Current Drug Listing. The 1988 *Farm Chemicals Handbook* (Meister Publishing Co., 37841 Euclid Ave, Willoughby, OH 44094) lists it as "discontinued by The Dow Chemical Co." These methods should be considered for surplus status.

Sulfaguanidine is not listed in the 1988 *Feed Additive Compendium*, or the 1988 *Official Publication of the Association of American Feed Control Officials*. No source of standard is listed in the official nithiazide method (**42.171**). This method should be designated surplus.

Following is a list of the Drugs in Feeds topics for which there is an assigned Associate Referee. Included is a discussion of the status of each subject.

Amprolium.—Elzbieta J. Kentzer, Associate Referee, completed a manuscript on the analysis of amprolium using reverse-phase ion-pair partition chromatography, which has been published [*J. Assoc. Off. Anal. Chem.* (1988) **71**, 251–255]. She plans to send out a questionnaire to potential collaborators in late 1988, and to conduct a collaborative study of the method in 1989.

Instructions in section **42.012(b)** of the official colorimetric method suggest that any alumina that passes the alkalinity test should be satisfactory for sample cleanup. Studies in the GR's laboratory have shown that for different basic aluminas the recovery of amprolium may vary; therefore, it is recommended that users of this method conduct column recovery studies on their alumina. This should be done using a spiked blank extract rather than a standard solution. A note indicating this should be added to the method.

Carbadox.—Virginia Thorpe, Associate Referee. The Committee on Feeds, Fertilizers, and Related Materials has

recommended that further study is needed on the collaborative study for carbadox and pyrantel tartrate conducted in 1985, and reported at the AOAC meeting in San Francisco in 1987. The Associate Referee does not wish to pursue further development in this area and has respectfully submitted her resignation. We thank her for her contributions to this difficult analytical problem.

An interlaboratory study of an LC method for the analysis of carbadox in feeds and premixes by R. M. Aerts and G. A. Werdmuler has been published [*J. Assoc. Off. Anal. Chem.* (1988) **71**, 484–490]. This study, conducted in Europe, includes an examination of the determination of carbadox in feeds pelleted under differing temperature conditions.

Ethopabate.—Joseph Hillebrandt, Associate Referee, is comparing a new ion-pair partition LC method sent to him by Merck & Co. to the reverse-phase LC method he has been studying. His goal is to have a method such that the cleanup is satisfactory for either UV or fluorescence detection. He requests that especially dirty ethopabate samples from different parts of the country be sent to him for testing by the methods.

Furazolidone and Nitrofurazone.—Robert L. Smallidge, Associate Referee. Furazolidone and zoalene in feeds, qualitative tests (**42.077-42.080**), and furazolidone in feeds, liquid chromatographic method (**42.A01-42.A06**), were adopted as official final action ["Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1988) **71**, 222–223].

Lasalocid (LC Method).—Edward Waysek is Associate Referee for Sulfadimethoxine as well as for this topic, and has been actively developing methodology in that area this past year.

Melengestrol Acetate.—Raymond Davis, Associate Referee, reports that he is not carrying out any method development for the analysis of MGA at this time.

J. L. Weigand and D. S. Dille have reported on an LC method for the analysis of melengestrol acetate in feedstuffs [(1988) *J. Assoc. Off. Anal. Chem.* **71**, 707–709]. This analysis involves cleanup by preparative LC prior to determination with a reverse-phase analytical column.

Morantel Tartrate.—Linda Werner, Associate Referee, along with Joyce Konrardy, is currently conducting in-house validation of a method (2.2 & 4.4 g/lb). They are planning a preliminary interlaboratory study prior to a full collaborative study in the future.

Oxytetracycline (LC Method).—Mary Lee Hasselberger, Associate Referee, reports that her work on OTC in feeds by LC analysis shows good correlation with the microbiological method at and above 50 ppm OTC. She is currently investigating different LC columns to improve separations at lower levels of analyte.

Pyrantel Tartrate.—Joyce Konrardy, Associate Referee. Work on this drug has been on hold until the Committee on Feeds, Fertilizers, and Related Materials acted on the recent collaborative study on the LC method for carbadox and pyrantel tartrate. The Associate Referee, along with Linda Werner, hopes to initiate some work in the fall of 1988 to determine if the method can be modified to give satisfactory recovery for pyrantel tartrate. If progress cannot be obtained with this endeavor they will pursue development of a separate method for the analysis of pyrantel tartrate in feeds.

Roxarsone.—Glenn M. George, Associate Referee, reports that no work has been carried out in the area. The official first action, atomic absorption, roxarsone method, **42.B01-42.B10**, has been in print for the required two years with no problems reported to the AR or GR and should be considered for official final action status.

Sulfadimethoxine.—Edward H. Waysek, Associate Referee, presented a method for the normal-phase liquid chromatographic determination of sulfadimethoxine and ormetoprim in animal feeds at the 1988 international meeting of AOAC in Palm Beach.

As reported in the 1987 General Referee's report the ficin (Calbiochem, fig latex) suggested in the official sulfadimethoxine method (**42.168-42.170**) is no longer available [*J. Assoc. Off. Anal. Chem.* (1988) **71**, 106–107]. The official colorimetric method, without the ficin treatment, has been used for several years in the Associate Referee's laboratory; however, this has not been studied collaboratively. The addition of a note to the colorimetric method explaining the problem should be considered.

Sulfa Drug Residues.—Associate Referee Robert K. Munns reports no activity in this area, and because of other commitments has decided to resign from this refereeship.

A reverse-phase LC method, using post-column derivatization, has been published recently for the analysis of sulfamethazine and sulfathizaole residues in feeds [*J. Assoc. Off. Anal. Chem.* (1988) **71**, 710–717].

Sulfamethazine and Sulfathiazole in Feeds and Premixes.—Dwight M. Lowie, Associate Referee, and Samira Gurgis have completed preliminary work on a method for the determination of sulfamethazine and sulfathiazole in feeds. The method involves cleanup of an alkaline acetonitrile extract by partitioning the analytes into chloroform followed by solid-phase extraction on a silica column. Analytes are separated from co-extracted materials by chromatography on a reverse-phase column, and detected at 280 nm. The method has been distributed to several laboratories for their evaluation and input. The Associate Referee hopes that this approach will help him to iron out any remaining problems so that the method can be subjected to a full collaborative study in 1989.

The Associate Referee reports that the official, first action, colorimetric sulfamethazine method (**42.172-42.174**) has been found to give low recovery in those complete feeds made with the Tylan-Sulfa stabilized granules. An editorial note should be added indicating limited applicability to this method. Low recoveries of sulfamethazine have also been reported for the official method on feeds containing cottonseed hulls.

Blanchflower and Rice [*J. Assoc. Off. Anal. Chem.* (1988) **71**, 302–303] have compared various extractants for use in the analysis of sulfamethazine in feeds by liquid chromatography and post-column derivatization. Their results indicate that methanol–water–glacial acetic acid (75 + 23 + 2) extraction at 70°C for 30 min followed by shaking for 30 min gave the best recovery.

General Referee's Note.—Users of the Drugs in Feeds methods in *Official Methods of Analysis* are encouraged to contact the appropriate Associate Referee with any questions concerning methodology. Addresses are listed under the Committee on Feeds, Fertilizers, and Related Materials in the January/February issue of the AOAC *Journal*, or in the AOAC Membership Directory. Where there is no active Associate Referee, the General Referee may be contacted. Anyone willing to be an Associate Referee for any of the drugs in feeds is encouraged to contact AOAC or the General Referee.

Recommendations

(1) Add the following statement to section **42.014** of the official final action method for amprolium, **42.011-42.015**: Column recovery of amprolium may vary among different brands of basic alumina. Test column recovery by spiking extract from nonmedicated feed.

(2) Appoint associate referees for the topics Arsanilic Acid, Carbadox, Ethylenediamine Dihydroiodide, and Sulfa Drug Residues.

(3) Move Lasalocid (LC Method) to General Referee for Drugs in Feeds. Move Lasalocid (Microbiological Assay) to General Referee for Antibiotics in Feeds.

(4) Initiate the topic Oxytetracycline (LC Method) and appoint Mary Lee Hasselberger.

(5) Adopt as official final action the official first action AAS method for roxarsone, **42.B01-42.B10**.

(6) Add the following note to the official first action method for sulfadimethoxine, **42.168-42.170**: The ficin product listed in **42.168**(a) is no longer available. It has been reported that the method is satisfactory without ficin treatment, but users should verify recovery.

(7) Add the following note to the official first action sulfamethazine method, **42.172-42.174**: Not applicable to feed sample made from "granule stabilized" Tylan-Sulfa premix.

(8) Designate the following methods as surplus: **42.122-42.125**, **42.126-42.129**, **42.152-42.154**, **42.155-42.159**, **42.148-42.151**, **42.171**.

(9) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

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Feeds

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Frank Barton, Associate Referee for NIR in Mixed Feeds, has a new method which has been approved and submitted for publication.

Peter Kane, Associate Referee for Crude Protein, has completed a mini-collaborative study on the Technicon TRAACS system. The method is currently being reviewed by the statistician. Rose Sweeney of the University of Missouri has completed her collaborative study on the LECO Nitrogen Analyzer and other similar analyzers. The study has been submitted for review by the Associate Referee, General Referee, and Statistician.

During the past year, the area of Moisture in Feeds was split into two topics to better represent current developments. Roy Schultze of the Ralston Purina Co. was appointed for Moisture in Pet Foods. He is completing a collaborative study of a Karl Fischer method for pet foods containing propylene glycol and other volatile components. Bob Windham, USDA, Russell Research Center, Athens, GA, was appointed Associate Referee for moisture in forages and mixed feeds. He is in the process of completing an NIR method for moisture in forages.

Joel Padmore, Associate Referee for Minerals in Feed, responded to numerous questions concerning the applicability of methods to mixed feeds and premixes. Attempts to locate replacement associate referees for mixed feeds and premixes during the past year have not been successful. Betty Pendleton of the National Feed Ingredients Association (NFIA) has agreed to assist in the search for associate referees and new methods for study.

Patricia Ramsey, Associate Referee for Feed Miscroscopy, has begun a study on a quantitative flotation method for limestone and other minerals. She hopes to complete method development and to begin a collaborative study on her method in the next few months. She also reported responding to a number of telephone inquiries about feed miscroscopy methods from laboratories in the U.S. and Canada during the past year.

Darrel Sharpe, Associate Referee for Feed Sampling, reports initiating studies for sampling feeds with large pellets and extrusions.

Wayne Stockland, Associate Referee for Amino Acids in Feeds, has had a change of job assignment preventing his continuation as AR.

George Latimer, Texas State Chemists Office, was appointed as Associate Referee for Iodine and EDDI in Feeds.

Paul Guenther, Associate Referee for Fat, has had a change of job assignment and has resigned as Associate Referee.

No reports of activity in other areas were received. During the two month period following the annual meeting, each of the inactive associate referees will be contacted to determine their interest in continuing and their plans for their areas of study in the year.

Recommendations

(1) Continue study in all areas.

(2) Replace inactive associate referees.

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Fertilizers and Agricultural Liming Materials

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In reviewing chapters 1 and 2 for the 15th Edition of *Official Methods of Analysis*, some suggested changes are worthy of note. These editorial changes are:

*(1) Urea in Fertilizer, Urease Method (**2.080–2.081**).*—Change sample size to be weighed from 10 g to 1–10 g with restriction to limit urea content to 1 g. This is necessary to make the method consistent with the collaborative study reported in *J. Assoc. Off. Agric. Chem.* (1958) **41**, 637.

*(2) Potassium in Fertilizers, Flame Photometric Method (**2.108–2.113**) and Automated Flame Photometric Method (**2.114–2.118**).*—Declare as surplus and only reference the methods.

*(3) Calcium (Acid-Soluble) in Fertilizer (**2.140**).*—The following statement should be included: "The presence of other analytes pptd by oxalate, such as Ba and Sr, will cause a positive bias in results."

Activities in the subject areas include:

Boron.—A revised collaborative study report on the ICP method has been submitted for consideration.

Dicyandiamide.—A new Associate Referee is being appointed.

Free and Total Water.—Associate Referee R. D. Duncan recently retired.

Melamine.—The first action method for triamino-*S*-triazine will be included in the 15th Edition of *Official Methods of Analysis*.

Nitrogen.—A new Associate Referee has agreed to serve, and the appointment is being finalized.

Phosphorus.—Associate Referee Joe R. Trimm and colleagues are studying new techniques for citrate extraction, comparing AOAC and EC methods, and evaluating bone meal in the laboratory and agronomically. Several contributed papers will be presented at the 101st AOAC annual meeting.

Sampling.—Equipment and procedures are needed to sample super-bags and buckets. The Associate Referee, Douglas Caine, would welcome suggestions.

Study should continue on the topics Biuret, Iron, Potash,

Sample Preparation, Slow-Release Mixed Fertilizer, Sodium, Soil and Plant Amendment Ingredients, Sulfur, Water-Soluble Methylene Ureas, and Zinc.

Recommendations

(1) Adopt as official first action the ICP method for boron as re-submitted.

(2) Appoint new Associate Referees for Dicyandiamide, Free and Total Water, Nitrogen, and others as needed.

(3) Continue official first action status of the following: water elution method for slow-release mixed fertilizers, **2.073-2.074**; flame photometric method for sodium, **2.173-2.176**; atomic absorption spectrophotometric method for sodium, **2.177-1.181**; atomic absorption spectrophotometric method for aluminum in aluminum sulfate-type soil acidifiers, **2.194-2.197**.

(4) Continue study on all other topics.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed.

Plants

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The following methods are recommended for surplus status: **3.005**, Sand and Silica—Gravimetric; **3.039**, Magnesium—Gravimetric; **3.052**, Sodium—Uranyl Acetate; **3.069-3.070**, Chloride-Gravimetric. Continue study on all other topics.

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Section numbers refer to *Official Methods of Analysis* (1984) 14th ed.

Veterinary Analytical Toxicology

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The past year has shown significant activity in several areas of study. Collaborative studies have been completed for 4 methods with another to be finished soon. An extensive interlaboratory study of Se methods, with emphasis on low levels, has been completed with considerable information gathered about method variability at low concentrations.

Several topic changes have taken place since this report was last written. The topic of cholinesterase has been divided into 2 different topics: Cholinesterase, Colorimetric Method and Cholinesterase, pH Method. This change was effected to allow for a more timely completion of collaborative studies of the 2 methods and to allow the Associate Referees to concentrate specifically on a single technique. For similar reasons the topic of vitamin analysis has also been divided into 2 different topics: Vitamins A and E and Vitamins D and K. Two new topics, GC/MS Methods and Cyanide were created to study these 2 widely used techniques.

The Fifth Annual Workshop on Veterinary Analytical Toxicology was held at the Midwest Regional AOAC Meeting, June 21, 1988, Columbia, Missouri. The program was the most comprehensive yet and was well received. Participants came from all areas of the United States and were very busy answering questions and demonstrating techniques. The workshop topics included a GC/MS demonstration, a report on the above-mentioned Se study, a presentation on microwave digestion techniques, a demonstration of serum nitrate/nitrite determination by LC, a demonstration on the LC determination of vitamin E in serum, a poster on the determination of ethylene glycol by GC, posters on the automated digestion of Se samples and Se hydride determination, a demonstration of cholinesterase determination in whole blood, and a demonstration on ELISA procedures for the determination of zearalenone. The sixth annual workshop is planned for the Midwest Regional AOAC Meeting, June 1989, Madison, Wisconsin.

The Association of Veterinary Laboratory Diagnosticians (AAVLD)/American College of Veterinary and Comparative Toxicologists (AAVCT)/AOAC Advisory Committee on Veterinary Analytical Toxicology met at the annual meeting of the AAVLD, October 1987, Salt Lake City, Utah, and its recommendations have been reported (1). At that same meeting, the annual session on Veterinary Analytical Toxicology was held with topic discussions on GC/MS and ICP techniques.

Interlaboratory cooperation and communication has remained strong and continues to improve as witnessed by the strong support and involvement with the above-mentioned workshop and the willingness of everyone to participate in collaborative and interlaboratory studies. The continued support of the AOAC and diagnostic laboratory directors is encouraged to improve further the quality of results. The following summaries of Associate Referee reports reflect some of the past year's activities:

Antibiotic Screening Methods.—Wynne Landgraf (National Veterinary Services Laboratories, Ames, IA) and Stephen C. Ross (Animal Disease Laboratory, Centralia, IL) report a continuing need for the study of antibiotic screening methods. The number of confirmed diagnostic cases where monensin concentrations are not consistent with the label has increased in addition to those cases where monensin was detected in feeds that were guaranteed to be antibiotic-free.

Imported feeds also pose a potential diagnostic problem. Other national feed laws are different than those of the United States and the potential for confusion is high. A recent episode of salinomycin poisoning in rabbits from South American feed is a case in point (2). The Associate Referees also report on the continued successful use of the bioautographic screening method (3) with good agreement with the colorimetric method (4) for monensin.

Arsenic in Animal Tissues.—John Reagor (Veterinary Medical Diagnostic Laboratory, College Station, TX) reports on the continued successful use of the method for As in animal tissue (**49.B01-49.B05**). He recommends that it be adopted official final action.

Cholinesterase, Colorimetric Method.—Karen Harlan (University of Illinois, Urbana, IL) reports that a collaborative study on the determination of cholinesterase (CHE) activity in whole blood has been completed. The method (5, 6) studied involves the measurement of the rate of acetylthiocholine hydrolysis at room temperature. Activity is measured in the linear region over a 3–5 min period. In the study, 10 laboratories analyzed 4 pairs of blind duplicate cattle blood samples with varying degrees of CHE activity. Some samples were inhibited in vitro by the Associate Referee with paraoxon. The collaborators performed the determinations within 48 h after sample receipt to prevent any possible

reversal of the inhibited samples. The following is a summary of the results:

Sample pair	Mean activity	S(r)	CV(r), %	S(R)	CV(R), %
1 & 4	2.06	0.183	8.9	0.218	10.6
2 & 8	1.16	0.122	10.5	0.155	13.4
3 & 6	1.57	0.107	6.8	0.173	11.0
5 & 7	0.26	0.41	15.7	0.052	20.0

Activity expressed as micromoles/mL/min

The Associate Referee recommends that the method be adopted official first action.

Cholinesterase, pH Method.—Paula Martin (Iowa State University, Ames, IA) reports that a collaborative study on the determination of cholinesterase activity by the pH method (7) is currently being conducted. Results from the study will be reported when the study is completed and recommendations will be made.

GC/MS Methods.—Allen Ray (Veterinary Medical Diagnostic Laboratory, College Station, TX) reports that he has conducted an interlaboratory study on the GC/MS confirmation of cantharadin (blister beetle toxin). Ten laboratories were provided with an authentic cantharadin standard and asked to run it on their GC/MS system and provide an EI spectrum. The results from this study are currently being analyzed and will be reported. Comparison of fragment ions and relative yield will provide a basis to determine what type of variation can be expected.

Lead in Animal Tissues.—Robert J. Everson (Purdue University, West Lafayette, IN) reports on the completion of a collaborative study on the determination of Pb in whole blood by furnace/AA (8). The study involved the analysis of 4 pairs of blind duplicate porcine blood samples (concentrations from 0.01 to 0.73 μg/mL blood) by 8 laboratories. Each laboratory was provided with appropriate standards and reagents. Results of the study are summarized as follows:

Sample pair	Mean concn, μg/mL blood	Actual concn, μg/mL blood	S(r), %	S(R), %
1 & 4	0.22	0.31	10.3	34.0
2 & 7	ND*	ND	–	–
3 & 6	0.57	0.73	20.5	24.3
5 & 8	0.39	0.49	18.8	45.7

*ND indicates none detected, less than 0.01 μg/mL blood

Results from the study showed intra- and interlaboratory variances were greater than expected, higher than acceptable, and that accuracy was poor. A previous interlaboratory study (9) where laboratories used furnace/AA methods of choice yielded much lower variances.

The written instructions contained specifications on the furnace ramp conditions that were too rigid. Participants were not provided an opportunity to optimize their instruments and instrument performance instructions were incorrectly given, both of which contributed to the high variances and poor recovery.

The Associate Referee recommends that the study be repeated with different instructions on instrument performance allowing each laboratory to optimize furnace conditions and response.

Multielement Analysis by ICP.—W. Emmett Braselton (Michigan State University, East Lansing, MI) reports on continued work on the assimilation of database information on the frequency distribution of element concentrations in serum, liver, and kidney of animal species. The most recent work (10) was multielemental assays of perinatal lamb livers paralleling previous work with other species (11–14). In light of the increasing number of diagnostic laboratories that are acquiring ICP instrumentation, the Associate Referee recommends continued study of the use of ICP as a diagnostic tool in veterinary toxicology.

Nitrates and Nitrites.—Mike Carlson (University of Nebraska, Lincoln, NE) reports on study of a modified LC/UV ion chromatographic procedure (15) for determining nitrate and nitrite in biological fluids. The adaptation of specimen cleanup by using ultrafiltration to separate proteins from the aqueous fraction has made detection of 1 ppm nitrate, and 0.5 ppm nitrate possible. Lower detection limits are likely with optimization of the detector. Initial recovery with spiked serum was greater than 95% for 10 ppm nitrate and 10 ppm nitrite spikes. Interferences in some ocular fluid specimens indicate this matrix may be more difficult to analyze.

The ultrafiltration system that the Associate Referee has used is the Centrifree micropartition system (Amicon Division, W. R. Grace & Co., Danvers, MA 01923). Samples were prepared (0.125 to 1.00 mL) by centrifuging in the ultrafiltration apparatus at 2000*g* for 15 min. From 0.30 to 0.40 mL filtrate was recovered from 1 mL serum, ocular fluid, and plasma samples after centrifugation.

The ultrafiltration cleanup has also been applied to the Cd reduction detection system (16) as well as the nitrate test strip system (EM Science, Cherry Hill, NJ 08034) with good results in both systems. This new cleanup offers time saving, cost saving, and better reproducibility advantages over the classical cleanup techniques. Work is currently under way on evaluating the reproducibility of the various detection systems. The data from this evaluation will provide information on which technique or techniques are most suitable for collaboration.

The Associate Referee also reports on continued success with the forage nitrate method (**49.B06–49.B13**) and recommends that it be adopted final action. Numerous laboratories worldwide are currently using the method on a routine basis and report no problems.

Selenium in Animal Tissues.—James Roof (Bureau of Animal Industry, Harrisburg, PA) reports that Morrie Craig, Oregon State University, Corvallis, OR, has completed a comprehensive interlaboratory/check sample study of Se in cattle blood. Thirty-five laboratories were provided 2 blood samples every 2 weeks for a 6-month period. The samples used for the study were in the low concentration range in order to accurately assess how various methods perform at the deficient and/or subclinical range (less than 0.1 ppm). The sample protocol included blind duplicates within and between days so that within-day and between-day intralaboratory variances could be calculated in addition to the interlaboratory variation. Additionally, variances on digestion and detection systems will be determined. At the time of this writing, the complete results were being summarized. A detailed analysis will be presented at the upcoming AAVLD Annual Meeting, October 16, 1988, Little Rock, AR.

Vitamins A and E.—Roy Smith (Alberta Agriculture, Edmonton, Alberta, Canada) reports on the preliminary stages of a collaborative study on the determination of vitamins A and E in serum/plasma. The method involves extraction with petroleum ether after precipitation of the serum proteins with ethanol followed by reverse-phase LC/UV and quantitation by internal standard (retinol acetate). Preliminary studies by the Associate Referee have shown recovery of vitamin A at 78% and vitamin E at 77%. Work is continuing to improve this performance.

The Associate Referee proposes to study the method performance for vitamin A in the range of 10–100 μg/100 mL serum and vitamin E in the range of 15–100 μg/100 mL serum. Pools of normal animal serum are being collected from several sites around North America to serve as the

material for the collaborative study. If samples are kept frozen, stability is not a problem.

Zinc in Animal Tissues.—Dana Perry (University of Arizona, Tucson, AZ) reports on the results of a collaborative study of Zn in animal serum. The study involved the analysis by flame/AA of 8 serum samples by 8 laboratories. Each participant was provided with standards and necessary reagents. The results are summarized as follows:

Sample(s)	Mean concn	S(r)	CV(r), %	S(R)	CV(R), %
64 & 66	6.36	0.077	1.2	0.39	6.1
26 & 20	0.58	0.033	5.7	0.095	16.0
31 & 11	0.63	0.072	12.0	0.081	13.0
74	1.00	–	–	0.072	7.2
41	1.48	–	–	0.11	7.2

Recovery was 108%
Concentration expressed as μg/mL

Few problems were encountered with the study. Collaborators generally favored the method, which is simply a direct dilution followed by AA/flame determination.

The Associate Referee also reports on the completion of a collaborative study on the determination of Zn in liver by the method described here in 1986 (9). As of this writing, results were being summarized and will be reported.

The Associate Referee recommends that the Zn serum method be adopted official first action.

Recommendations

(1) Adopt as official final action the official first action method for arsenic in liver (**49.B01-49.B05**).

(2) Adopt as official final action the official first action for forage nitrate (**49.B06-49.B13**).

(3) Adopt as official first action the serum zinc method as recommended by the Associate Referee.

(4) Continue study on all other topics.

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This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Section numbers refer to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69,** 349–390 (B methods).

GENERAL REFEREE REPORTS: COMMITTEE ON HAZARDOUS SUBSTANCES IN WATER AND THE ENVIRONMENT

Air

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This general refereeship had been vacant for some time until my appointment in January 1988. No associate refereeships have been designated. This broad topic can best be described in terms of subtopics to which Associate Referees should be appointed.

Referee Topics

The criteria that have been applied to decide on recommendations for study are general usefulness of the subtopic as evidenced by large numbers of requests for analysis or much litigation, which indicates a need for analyses, and extensive dispute or diversity of opinion about methods. Several topics are obvious almost immediately.

Organics in Outdoor Air.—To include volatile organics–fuel residues, light halocarbons (Freons, etc.), and formaldehyde; semivolatile organics—degenerate fuel residues, polynuclear aromatics, and pesticides (in general) and PCBs; and particle-borne organics.

Inorganics in Air.—This topic will be considered further during the coming year. Modern, reputable consensus methods for determination of O_3, SO_x, and NO_x need to be established because these compounds presently impact public policy.

Particulate Matter in Air.—This topic will be restricted to issues in which the analyte is not a particle-borne substance, but rather a particle. Asbestos, alumina, carbon, and silica should be primary targets for the coming year, with consideration of other topics as they arise. This should be handled as one or more Associate Referee topics, and the need for Associate Referees should be advertised in "The Referee."

Subtopics of Importance

Under the topic organics in outdoor air, methods for formaldehyde are urgently needed because of the considerable litigation pending in U.S. courts since realization by the public that some polyurethane polymers give off formaldehyde as a gas. The present analytical methods lack specificity, except where an adequate history of the sample is available, and therefore the methods need improvement. The method should be applicable primarily to indoor air, but modifications of sampling technique should permit its general application. The method for formaldehyde should prove useful for Freons as well, so these compounds do not require a separate topic. Physical and Chemical Method 101 (NIOSH) is the present NIOSH (National Institute for Occupational Safety and Health) standard method for determination of formaldehyde. The methods for Freons are a more complex issue because they involve several agencies. The authorities for formaldehyde methods are NIOSH and AIHA. I plan to study this topic.

Under the topic particulate matter in air, asbestos, carbon (especially carbon black), and silica and alumina represent continuing health risks (at least according to some authorities), but there seems to be considerable disagreement on the magnitude of individual risk. The disagreement seems to stem from the plethora of methods invoked for determining these materials. I am searching for an expert on each of the several methods, but have not yet obtained copies of all of the methods. These topics merit urgent consideration, in view of the extensive and continuing litigation surrounding them.

The analytes O_3, SO_x, and NO_x merit consideration because of the international concern over their presence as inorganics in air. An international method developed under AOAC guidelines is urgently needed. An Associate Referee should be appointed as soon as possible.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Hazardous Substances in Water and the Environment. See the report of the committee, this issue.

Inorganics in Drinking and Ground Water

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Two Associate Referees were appointed: J. Wilson Hershey (Lancaster Laboratories, Inc.) on Arsenic and Selenium Methods in Ground Water, and Bruce E. Warden (WMI Environmental Monitoring Laboratories, Inc.) on Inductively Coupled Plasma and Ion Chromatographic Methods in Ground Water.

Arsenic and Selenium in Ground Water.—The goals for the past year included evaluation of a number of methods for arsenic and selenium, including both graphite furnace and hydride generation methods. One method of each type was to be selected, modified as appropriate, and a collaborative study was to be designed. The hydride generation methods were evaluated and 2 methods, ASTM 3859 and Standard Method 303-E, are under continued study, with the intent of using one or both in a collaborative study.

ICP and IC Methods in Ground Water.—The goals for this topic included an evaluation of EPA Method 200.7, Method Study 27 (Trace Metals by ICP, conducted during 1982) to determine whether that method has the ruggedness, accuracy, and precision to become an AOAC method. A collaborative study would be conducted if that method did not meet AOAC criteria.

Method 200.7 was evaluated as to design and statistical treatment of data, and additional statistical evaluations were conducted to verify certain conclusions. The high outlier rejection observed in this study was probably caused by the newness of the technology and the inexperience of some of the laboratories. Subsequent work conducted by EPA and other laboratories on Performance Evaluation samples shows this method to have better precision and accuracy than shown in the original collaborative study. Because of the high outlier rejection rate and limited precision and accuracy data, a collaborative study is being designed jointly with EPA to re-evaluate this method. In the meantime, it is recommended that EPA method 200.7 be accepted interim official first action.

The Associate Referee also participated in the evaluation of EPA Method 6010, an SW846 method for trace metals by ICP analysis. This method is well documented and the collaborative study meets AOAC requirements. It is recommended that EPA method 6010 be accepted interim official first action.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Hazardous Substances in Water and the Environment. See the report of the committee, this issue.

Joint Studies

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In March 1988, the U.S. Environmental Protection Agency (EPA) and AOAC agreed to undertake joint collaborative testing of EPA pesticide methodology. Raymond Wesselman (EPA Quality Assurance Branch, Cincinnati, OH) and Viorica Lopez-Avila (Acurex Corp., Mountain View, CA, and AOAC General Referee on Organics in Surface and Waste Water) were named Co-Associate Referees for the first study, Method 2—Determination of chlorinated pesticides in ground water by gas chromatography with an electron capture detector.

The Environmental Monitoring and Support Laboratory (EPA-Cincinnati) is preparing all materials and has completed all the ruggedness testing of Method 2, and AOAC is coordinating the volunteer collaborating laboratories. To date, all preliminary method work is complete. Dr. Wesselman has prepared a protocol for review by the Committee Statistical Consultant, the General Referee, and the Committee as a whole. Dr. Lopez-Avila has organized over 25 laboratories to participate in the study. Since the procedure involves analysis of a complex mixture of 29 chlorinated pesticides, a preliminary test sample will be sent to all laboratories interested in participating in order to confirm their analytical abilities. Those laboratories which perform satisfactorily on the test will continue in the collaborative study program. Sample and standards for the collaborative study are scheduled to be shipped by mid-October 1988. The actual collaborative study will include at least 8 laboratories, and probably more than 15–20 laboratories.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Hazardous Substances in Water and the Environment. See the report of the committee, this issue.

Organics in Surface and Waste Water

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Reports were received from the 2 Associate Referees assigned to this refereeship. Additional referees and topics are needed.

Munitions in Waste Water.—Associate Referee Thomas F. Jenkins reports that the water method was adopted by AOAC in 1986 (**33.B01-33.B08**). Since that time, a similar method for determining explosive residues in soil has been developed. This method involves a slightly different reverse phase LC separation than the original water method. The new separation procedure is being considered for incorporation into the water method. The report describing these results is currently in review, and will be published soon. The soil method was subjected to interlaboratory testing.

Chlorinated Solvents in Water.—Associate Referee Douglas Dube reports progress in developing a GC/MS method for detecting ppb levels of chlorinated hydrocarbons in water. A protocol describing the method is in preparation.

This report of the General Referee was presented at the 102nd AOAC Annual International Meeting, August 29–September 1, 1988, at Palm Beach, FL. The recommendations were reviewed by the Committee on Hazardous Substances in Water and the Environment. See the report of the committee, this issue.

Section numbers refer to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

REPORTS AND TRANSACTIONS

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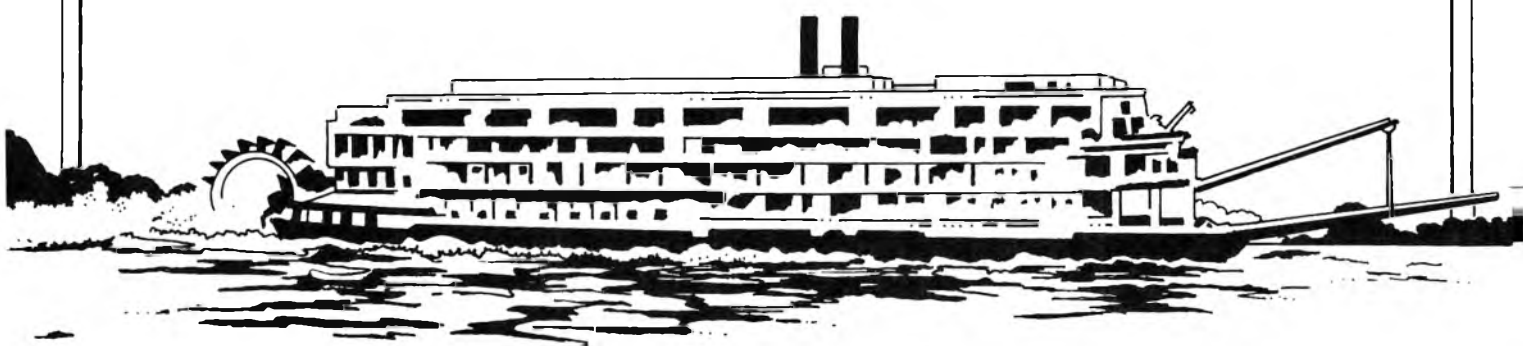

TRANSACTIONS: ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

The one-hundred-second annual international meeting of the Association of Official Analytical Chemists was held at The Breakers, Palm Beach, Florida, on August 29, 30, 31, and September 1, 1988. The following reports, along with the actions of the Association, were given at the business meeting, held Thursday, September 1, 1988, Robert C. Rund, presiding.

Committee on Pesticide Formulations and Disinfectants: Recommendations for Official Methods

THOMAS L. JENSEN (State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502), *Chairman*;
JAMES J. KARR (Pennwalt Technical Center, 900 First Ave, Box C, King of Prussia, PA 19406);
JAMES P. MINYARD, JR (Mississippi State Chemical Laboratory, PO Box CR, Mississippi State, MS 39762);
FRAN PORTER (Florida Department of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301);
H. HEDEGAARD POVLSEN (Kemikaliekontrollen, Morkhoj Bygade 26, DK-2860 Soborg, Denmark);
STEPHEN C. SLAHCK (Mobay Corp., Box 4913, Kansas City, MO 64120);
RICHARD S. WAYNE (American Cyanamid Co., Agricultural Division, Box 400, Princeton, NJ 08540);
RICHARD H. COLLIER (Department of Entomology, Purdue University, West Lafayette, IN 47907), *Secretary*

The Committee on Pesticide Formulations and Disinfectants met on August 28 and 31, 1988, in Palm Beach, FL. Portions of the meeting were attended by several General Referees and Associate Referees, whose topics are assigned to the Committee, and by several visitors.

The Committee wishes to thank James E. Launer for his many years of meritorious service to AOAC. Launer most recently served as General Referee for CIPAC Methods. Previously, he served as General Referee for Organohalogen Insecticides and for Other Insecticides, Synergists, and Repellants. Launer's work was characterized by tireless efforts on behalf of the Association. We owe him particular thanks for his translations of CIPAC methods into AOAC form. Launer resigned as General Referee in 1987 following his retirement from the Oregon Department of Agriculture.

The Committee discussed the many needs for new methods for pesticide formulations. Concerns were expressed that emphasis on analysis of inert ingredients could overshadow the more critical need for methods for active ingredients. A priority list was developed by the Committee which will be distributed to the Associate Referees and General Referees for pesticide formulations and guide their methods development activities. The Committee determined that the priorities for developing new AOAC methods for pesticide formulations should be as follows: (*1*) active ingredients in end use products; (*2*) active ingredients in manufacturing use products; (*3*) inert ingredients and contaminants of toxicological significance; (*4*) other inert ingredients and contaminants.

The Committee discussed the need for AOAC standards for column performance in chromatographic systems. General Referee Arthur H. Hofberg volunteered to investigate the current status of related activities in ASTM and in other AOAC committees and report his findings to the Committee.

The Committee discussed progress being made on harmonization of statistical procedures. Requests for a computer program for use on microcomputers have been numerous. The Committee urges the Official Methods Board and the Statistics Committee to develop and distribute such a program as soon as possible.

The Committee discussed topics raised by the Official Methods Board, including distinctions among interim first action, first action, and final action methods and the methods approval process. From the perspective of the Committee, the current methods approval process is working reasonably well. There is some lack of understanding in the community at large that interim methods have official status. Efforts to widely communicate the distinctions among the various types of official methods should be undertaken.

The Committee is concerned that the emphasis on precision of methods too often obscures the equally important characteristic of accuracy. The Committee recommends that the Official Methods Board consider ways to emphasize the importance of determining the accuracy as well as the precision of official methods.

The Committee discussed the General Referee reports and recommendations. These reports show that the continuing trend toward merger and acquisition in the agrichemicals industry and the large number of recent data requests from EPA has placed an increasing work load on the analytical divisions of those companies. That increase is largely responsible for the low output in new AOAC methodology for pesticide formulations. Increased efforts by the Committee, as well as the General Referees and Associate Referees will be necessary to improve progress in developing and performing interlaboratory studies in this area.

Recommendations concerning the Use-Dilution Test for disinfectants were considered in detail by the Committee. In 1986, an applicability statement was added to indicate that the methods must be conducted with distilled water only, i.e., without a soil challenge. Last year, the Committee recommended, and the Association approved, the first action to repeal the final action methods using *Staphylococcus aureus* and *Salmonella choleraesuis* as the test organisms. Although the method with *Pseudomonas aeruginosa* as the test organism is even more variable than when the other organisms are used, that method was not repealed last year, because it is a first action method requiring only one action of the Association for final repeal. The Committee felt that a year's notice of this potential action was necessary for due process.

At this year's meeting, Eugene C. Cole, co-Associate Referee for the Use-Dilution Test, discussed the results of his investigations of the existing method, the identification of 32 modifications to the method, and interlaboratory studies of the existing method (conducted in 1985) and the modified method (conducted in 1986). These studies were conducted in conjunction with a Task Force with representation from regulatory agencies, industry, academia, and AOAC. Both of

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

The recommendations submitted by the Committee on Pesticide Formulations and Disinfectants were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411 (A methods); *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods); *J. Assoc. Off. Anal. Chem.* (1987) **70**, 385–403 (C methods); *J. Assoc. Off. Anal. Chem.* (1988) **71**, 199–239 (D methods).

these studies produced highly variable results. The original collaborative study of 1953, which used 2 unidentified phenolic disinfectant samples, also produced highly variable results that were not subjected to statistical analysis. The Associate Referee plans to develop a suspension method and a quantitative carrier method. The latter may be a suitable replacement for the existing Use-Dilution Test. Both co-Associate Referees have recommended that the current methods be repealed final action for all 3 test organisms. The General Referee has recommended continued study of the topic. Other interested parties spoke to both sides of the issue.

Betsy Woodward and Fran C. Porter of the Florida Department of Agriculture and Consumer Services submitted evidence that the existing Use-Dilution Test for *Staphylococcus aureus* and *Salmonella choleraesuis* can be reproducible when a number of critial parameters, not specifically delineated in the existing method and not sufficiently addressed in the 1986 study, are carefully controlled. A group of editorial changes were submitted to the Committee as an alternative to repeal. These changes were intended to identify critical control points in the method and to specify procedures to improve performance at those points.

An independent group, representing state and federal regulatory agencies and industry, plans to conduct an interlaboratory study on a modified Use-Dilution Test in the next several months. This study will incorporate a glass carrier, a more uniform inoculum, and tighter controls on selected critical parameters. The group hopes that these changes will produce a more precise method.

The Committee considered whether to allow the methods to remain in their current status, i.e., make no recommendation this year, to recommend adoption of editorial changes to one or more of the methods, or to recommend that the Association take the final action to repeal the methods. In making its determination, the Committee considered the following to be major factors:

(*1*) The methods in their official form allow wide latitude in performance at several critical control points;
(*2*) None of the formal studies (1953, 1985, 1986) have shown acceptable accuracy or precision by current standards;
(*3*) The original collaborative study (1953) included only 2 samples, both unidentified phenolics;
(*4*) The methods are widely used in industry and in regulatory agencies;
(*5*) The methods address a vital public health arena, the disinfection of critical hospital equipment and surfaces;
(*6*) Each user laboratory now determines its own guidelines for control of critical points in performance of the method;
(*7*) Several laboratories report excellent agreement when split samples of products found to be deficient are exchanged;
(*8*) Several laboratory supervisors report that the method can be consistently performed in the proper manner only by well trained, experienced analysts;
(*9*) Another interlaboratory study is expected to begin within the next few months.

A great deal of effort has been put toward modification and validation of the Use-Dilution Test by the co-Associate Referee, the Use-Dilution Task Force, and individual laboratories. Substantial study remains to be done. We urge all concerned parties to continue working to develop validated, collaboratively studied methods in this area of vital importance.

Recommendations for the Tuberculocidal (TB) Tests methods were also considered by the Committee. In 1986, an applicability statement was added which indicated that the method has not been validated for glutaraldehyde-based disinfectants. At last year's meeting, Joseph Ascenzi, the Associate Referee for Tuberculocidal (TB) Tests, discussed the results of a series of studies on the sources of variability in the current official method. These studies point to variations in the number of organisms attached to the porcelain carrier and to carrier/organism surface interactions as major sources of variation in the method. This method was adopted as an official method in 1956 without benefit of collaborative study. The Associate Referee is preparing a collaborative study on a modified method utilizing a suspension of organisms rather than organisms attached to a carrier. The Associate Referee has recommended that the current method (**4.036-4.041**) be repealed, but the General Referee has recommended continued study of the topic.

The Committee also recommends that Peter D. Bland be reappointed as General Referee for Herbicides I and for Fungicides and Disinfectants and that Richard H. Collier be appointed as General Referee for Microbial Pesticides. The Committee further recommends that Arthur Hofberg be appointed as a new member of the Committee.

DISINFECTANTS

(1) *Antimicrobial Agents in Laundry Products:* Continue study.
(2) *Preservatives (Antibacterials) in Textiles:* Continue study.
(3) *Sporicidal Tests:* Continue study.
*(4) *Tuberculocidal Tests:* (a) Repeal final action the official first action methods (**4.036-4.041**). (b) Continue study.
*(5) *Use-Dilution Test:* (a) Make the following editorial changes in the methods for *Staphylococcus aureus, Salmonella choleraesuis,* and *Pseudomonas aeroginosa* (**4.007-4.011**): (*1*) Add the following to the applicability statement: "These microbiological methods are technique-sensitive methods in which extreme adherence to the method with identified critical control points, good microbiological techniques, and quality controls is required for proficiency and validity of results." (*2*) Add the following to the beginning of section **4.007(b)**: "Obtain annually directly from ATCC." (*3*) Add the following at the end of section **4.008(a)**: "Use 25 × 150 mm straight side tubes for disinfectant solution. (Smaller tubes can give a high percentage of false positives when sides are touched.)" (*4*) Add the following new section **4.008(f)**: "Use disposable pipets. (Reusable pipets may have residues or chips.)" (*5*) Add the following at the end of section **4.008(d)**: "Discard cylinders that are visibly damaged (dull, chipped, dented, or gouged). Biologically screen remaining cylinders by performing the Use-Dilution Test with *Staphylococcus aureus* ATCC 6538 and 500 ppm alkyldimethylammonium chloride with alkyl chain distribution C14, 50%; C12, 40%; C16, 10% (e.g., BTC-835, Onyx Chemical Co., Jersey City, NJ 07302). Discard those cylinders giving positive results in screening procedure. In subsequent testing of samples, cylinders in tubes showing growth must be rescreened and may not be reused unless the screen test results in no growth." (*6*) Add the following to section **4.009**, paragraph 1, after sentence 1: "Vortex the nutrient broth test culture for 3–4 s and let stand 10 min at room temperature before continuing." (*7*) Add the following to section **4.009**, paragraph 1, after sentence

2: "One or 2 additional carriers may be added at the same inoculum rate to serve as reserves. Carriers which fall over in the petri dishes cannot be used in the test." (*8*) Add the following to section **4.009**, paragraph 1, sentence 3, after the word, "hook:" ". . . shake carrier vigorously against side of tube to remove excess culture." (*9*) Add the following to section **4.009**, paragraph 1, at the end of sentence 3: ". . . making sure that carriers do not touch to prevent improper drying." (*10*) In section **4.009**, paragraph 1, sentence 4, replace "≥20 min but ≤60 min" with "40 min." (*11*) Add the following to section **4.009**, paragraph 2, after sentence 5: "Dilution of sample should be made using ≥1.0 mL of sample. Use v/v dilutions for liquid products and w/v dilutions for solids. Round to 2 decimal places toward a stronger product. Solutions should be prepared ≤3 h prior to use to ensure a stable product. Place tubes in 20° water bath at least 10 min." (*12*) Add the following to section **4.009**, paragraph 4, to the beginning of sentence 1: "Without touching sides of tube with contaminated carrier or hook either when placing carrier in tube or when withdrawing hook . . ." (*13*) Add the following to section **4.009**, paragraph 4, after sentence 1: "(Note: Proper execution of the above step is one of the most critical, technique-sensitive areas of the method. False positives will result if the sides of the tube are touched.)" (*14*) Add the following to section **4.009**, paragraph 4, at the end of sentence 4: ". . . by shaking carrier against side of tube. Shorter intervals may be used in adding and removing carriers if 2 alternately flamed and cooled hooks are used. Individual manipulation of carriers is required; use of a semi-automated ring carrier is prohibited. (Note: The above step is one of the most critical, technique-sensitive areas of the method. False positives can result from transfer of live organisms to the sides of the tube due to aerosol formation.)" (*15*) Add the following to section **4.009**, at the end of paragraph 4: "Growth in tubes should be checked by gram stain to ensure that no contamination is present. Check at least 20% of the positive tubes. Confirm all positive results by duplicate testing to assure against false positives." (*16*) Add the following to section **4.010**, after sentence 2: "Obtain organism annually, directly from ATCC. Prior to beginning Use-Dilution Test, vortex nutrient broth culture as in **4.009**." (*17*) Add the following at the end of section **4.011**: "Proceed with vortexing as in **4.007** prior to the use of culture. Alternatively, pellicle may be carefully suctioned off, and culture can be poured into a clean, sterile tube before the vortex step. Any disruption of the pellicle resulting in the dropping, breaking up, or stringing of the pellicle in the culture before or during its removal renders that culture unusable in the Use-Dilution Test. This is extremely critical since any pellicle fragments remaining will result in uneven clumping and layering of the organism on the cylinder, allowing unfair exposure to the disinfectant and causing false positive results." (b) Continue study.

(6) *Virucide Tests:* Continue study.

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

(1) *Anilazine (Dyrene):* (a) Continue official first action status of the liquid chromatographic method (**6.D13-6.D18**). (b) Continue study.

(2) *Benomyl:* (a) Continue official first action status of the liquid chromatographic method (**6.522-6.527**). (b) Continue study.

(3) *Carboxin and Oxycarboxin:* Continue study.

(4) *Chlorothalonil:* Continue study.

(5) *Copper Naphthenate:* Open topic.

(6) *Dinocap:* Open topic.

(7) *Dioxins in Pentachlorophenol:* Open topic.

(8) *Dithiocarbamate Fungicides:* Continue study.

*(9) *Oxythioquinox (Morestan):* (a) Adopt as official final action the official first action liquid chromatographic method (**6.B34-6.B39**). (b) Continue study.

(10) *o-Phenylphenol:* Open topic.

(11) *Quaternary Ammonium Compounds:* Open topic.

(12) *Tebucanizole:* Establish new topic and initiate study.

(13) *Thiram:* Open topic.

(14) *Triadimefon (Bayleton):* Continue study.

(15) *Triphenyltin (Fentin):* Continue study.

(16) *Water-Soluble Copper in Water-Insoluble Copper Fungicides:* Continue official first action status of the CIPAC-AOAC atomic absorption and bathocuproine methods (**6.066-6.074**).

PESTICIDE FORMULATIONS: HERBICIDES I

(1) *Alachlor, Butachlor, and Propachlor:* Continue study.

(2) *Alachlor/Atrazine Mixtures:* Continue study.

(3) *Amitrole:* Open tropic.

(4) *Bentazon:* Continue study.

(5) *Bromoxynil:* Continue study.

(6) *Cacodylic Acid, MSMA, and DSMA:* Open topic.

(7) *Cyanazine (Bladex):* Continue study.

(8) *Dichlobenil:* Continue study.

(9) *Fomesafen:* Continue study.

(10) *Metolachlor:* Continue study.

(11) *Metribuzin (Lexone or Sencor):* Continue study.

(12) *Pesticides in Fertilizers:* Continue study.

(13) *Propanil (3',4'-Dichloropropionanilide):* Continue study.

(14) *Sodium Chlorate:* Open topic.

PESTICIDE FORMULATIONS: HERBICIDES II

(1) *Benefin, Trifluralin, Pendimethalin, and Ethafluralin:* Continue study.

(2) *Bensulide (Betasan):* Open topic.

(3) *Benzoylprop-ethyl:* Continue study.

(4) *Bromacil:* Continue study.

(5) *Chlorophenoxy Herbicides:* (a) Continue official first action status of the following liquid chromatographic methods: Combinations of 2,4-D,dicamba, and MCPP amine salts (**6.321-6.327**); 2-methyl-4-chlorophenoxyacetic acid (**6.327-6.377**); 2,4,5-trichlorophenoxyacetic acid (**6.391-6.395**); 2,4-dichlorophenoxyacetic acid esters and amine salts (**6.288-6.292**). (b) Continue study.

(6) *Chlorsulfuron (Glean):* Continue study.

(7) *Dicamba:* Continue study.

(8) *Dimethyl Tetrachloroterephthalate:* Continue study.

(9) *Dinoseb:* Continue study.

(10) *Fluometuron:* Continue study.

(11) *Metsulfuron-methyl (Ally):* Continue study.

(12) *Methazole:* Continue study.

(13) *Naptalam (Alanap):* Open topic.

(14) *Oryzalin (Surflan):* Continue study.

(15) *Pentachlorophenol:* Continue study.

(16) *Plant Growth Regulators:* Continue study.
(17) *Substituted Urea Herbicides:* Continue study.
(18) *Sulfometuron-methyl (Oust):* Continue study.

PESTICIDE FORMULATIONS: CARBAMATE INSECTICIDES AND SUBSTITUTED UREA INSECTICIDES

(1) *Aldicarb:* Continue study.
(2) *Bendiocarb:* (a) Continue official first action status of the liquid chromatographic method (**6.B40-6.B44**). (b) Continue study.
(3) *Carbaryl:* Continue study.
(4) *Carbofuran and Carbosulfan:* (a) Continue official first action status of the liquid chromatographic method for carbofuran (**6.B45-6.B48**). (b) Continue study.
(5) *Methomyl:* Continue study.
(6) *Mexacarbate (Zectran):* Open topic.
(7) *Oxamyl:* Continue study.
(8) *Pirimicarb:* (a) Continue official first action status of the gas chromatographic method (**6.560-6.564**). (b) Continue study.
(9) *3,4,5- and 2,3,5-Triphenylmethyl Carbamate Isomers:* Continue study.

PESTICIDE FORMULATIONS: ORGANOHALOGEN INSECTICIDES

(1) *Benzene Hexachloride and Lindane:* Continue study.
(2) *Chlordane:* Open topic.
(3) *Dicofol (Kelthane):* (a) Continue official first action status of the liquid chromatographic method (**6.B22-6.B27**) and the hydrolyzable chloride method (**6.332-6.337**). (b) Continue study.
(4) *Ethylan:* Continue study.
(5) *Fenvalerate:* Continue study.
(6) *Heptachlor:* Open topic.
(7) *Methoxychlor:* Continue study.
(8) *Methyl Bromide:* Open topic.
(9) *Toxaphene:* Continue study.
(10) *Trichlorfon (Dylox):* Continue study.

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHORUS INSECTICIDES

(1) *Acephate (Orthene):* Continue study.
*(2) *Azinphos-methyl (Guthion):* (a) Adopt as official first action the interim first action liquid chromatographic method described by the Associate Referee. (b) Continue study.
(3) *Coumaphos:* Continue study.
(4) *Dimethoate:* Continue study.
(5) *Dioxathion:* Continue study.
(6) *Encapsulated Organophosphorus Pesticides:* (a) Continue official first action status of the gas chromatographic method for encapsulated diazinon (**6.426-6.429**). (b) Continue study.
(7) *EPN:* Continue study.
(8) *Ethoprop:* Continue study.
*(9) *Fenitrothion:* (a) Adopt as official first action the interim first action gas chromatographic method for water-dispersible powder and emulsifiable concentrate formulations described by the Associate Referee. (b) Continue official first action status of the gas chromatographic method (**6.A19-6.A24**). (c) Continue study.
(10) *Fensulfothion:* (a) Continue official first action status of the gas chromatographic method (**6.B28-6.B33**). (c) Continue study.
(11) *Fenthion:* Continue study.
(12) *Fonofos:* Continue study.
(13) *Isofenphos:* (a) Continue official first action status of the gas chromatographic method (**6.C01-6.C06**). (b) Continue study.
(14) *Malathion:* Continue study.
(15) *Methamidophos (Monitor):* Continue study.
(16) *Methidathion (Supracide):* Continue study.
(17) *Oxydemeton-methyl (Metasystox-R):* Continue study.
(18) *Parathion and Methyl Parathion:* (a) Continue official first action status of the surplus volumetric (**6.472-6.478**), surplus colorimetric (**6.479-6.483**), gas chromatographic (**6.463-6.467**), and liquid chromatographic (**6.468-6.471**) methods for parathion and the gas chromatographic (**6.484-6.488**) and liquid chromatographic (**6.489-6.492**) methods for methyl parathion. (b) Continue study.
(19) *Phorate:* Continue study.
(20) *Pirimiphos-methyl:* Continue study.
(21) *Temephos:* (a) Continue official first action status of the CIPAC-AOAC liquid chromatographic method (**6.509-6.515**). (b) Continue study.
(22) *S,S,S-Tributylphosphorotrithioate:* Continue study.

PESTICIDE FORMULATIONS: OTHER ORGANOPHOSPHORUS INSECTICIDES

(1) *Crotoxyphos:* Continue study.
(2) *Dichlorvos (2,2-Dichlorovinyl Dimethyl Phosphate):* (a) Continue official first action status of the infrared methods (**6.417-6.420** and **6.421-6.424**). (b) Continue study.
(3) *Fenamiphos (Nemacur):* Continue study.
(4) *Mevinphos:* Continue study.
(5) *Monocrotophos:* Continue study.
(6) *Naled:* Continue study.
(7) *Tetrachlorvinphos (Gardona, Rabon):* Continue study.

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND INSECT REPELLANTS

(1) *Allethrin:* (a) Continue official first action status of the titrimetric chromatographic method for technical allethrin (**6.165-6.170**). (b) Continue study.
(2) *Aluminum Phosphide:* Continue study.
(3) *2,3,4,5-Bis(2-butylene)tetrahydro-2-furfural (MGK Repellant 11):* Continue study.
(4) *Cyhalothrin:* Establish new topic and initiate study.
(5) *Cyhexatin (Plictran):* (a) Continue official first action status of the CIPAC-AOAC liquid chromatographic method (**6.D01-6.D06**). (b) Continue study.
(6) *Cypermethrin:* (a) Continue official first action status of the CIPAC-AOAC gas chromatographic method (**6.B01-6.B05**). (b) Continue study.
(7) *Cyromazine (Larvadex):* Continue study.
(8) *Dipropyl Isocinchomeronate (MGK Repellant 326):* Continue study.
(9) *Fumigants:* Continue study.
(10) *Nicotine:* Continue study.
(11) *Permethrin:* Open topic.
(12) *Piperonyl Butoxide and Pyrethrins:* Continue study, especially of low levels and mixed formulations.
(13) *Resmethrin:* Continue study.
(14) *Rotenone and Other Rotenoids:* (a) Continue official first action status of the liquid chromatographic method (**6.182-6.186**) and the infrared method (**6.179-6.180**). (b) Continue study.

PESTICIDE FORMULATIONS: RODENTICIDES AND MISCELLANEOUS PESTICIDES

*(1) *Brodifacoum (Talon):* (a) Adopt as official final action the official first action liquid chromatographic method (**6.597-6.601**) and add the following applicability statement: "Not applicable to wax bait formulations." (b) Continue study.
(2) *Chlorophacinone:* Continue study.
(3) *Diphacinone:* Continue study.
(4) *α-Naphthylthiourea:* (a) Continue official first action status of the surplus method (**6.157**). (b) Continue study.
(5) *Sampling:* (a) Continue official first action status of the sampling procedures for fertilizers (**6.002**) as applied to pesticide-fertilizer mixtures. (b) Continue study.
(6) *Strychnine:* Continue study.
(7) *Warfarin:* Continue study.

PESTICIDE FORMULATIONS: CIPAC STUDIES

(1) *Deltamethrin:* Submit information about the potential interference of a liquid chromatographic method for this compound.
(2) *Maneb plus Fentin (Triphenyltin Acetate):* Submit results of a study on 5 samples of maneb, 4 of which also contain fentinacetate.
(3) *Isoprocarb (MICP):* Submit results of a study on an LC method applied to a solid formulation and a 75% wettable powder.
(4) *Vinclozolin:* Submit results of an LC study.
(5) *Sethoxydim (Poast):* Submit results of a study on a normal-phase LC method.
(6) *Pencycuron:* Submit results of a study on 3 different formulations determined by an LC method.

Committee on Drugs and Related Topics: Recommendations for Official Methods

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The Committee met August 12, 1988, in Rockville, MD. The Committee discussed the need for new General Referees for Drugs I, and Diagnostics and Test Kits and the plan for including all drug methods under one chapter in the 15th edition of *Official Methods of Analysis*. Also discussed were the following: new guidelines, forms, and checklists; generic descriptions of chromatographic columns; stability-indicating assays; number of laboratories for a valid study; methods agreements with other organizations; recognition of collaborators; a volunteer training manual; and use of the AOAC talent file.

COSMETICS

(1) *Aloe:* Continue study.
(2) *Essential Oils and Fragrance Materials, Composition:* Continue study.
(3) *Nitrosamines:* Continue study.
(4) *Preservatives:* Continue study.
(5) *Water and Alcohol:* Continue official first action status of the method for water and alcohol, **35.001-35.006**. Continue study.
(6) *Zirconium:* Continue official first action status of the method for soluble zirconium, **35.020-35.024**. Continue study.

DIAGNOSTICS AND TEST KITS

(1) *Automated Microbial Identification Systems—VITEK:* Continue study.
(2) *Automated Microbial Identification Systems—HP5898A:* Continue study.
(3) *Immunological and Diagnostic Assay of Peptides, Hormones, and Enzymes:* Continue study.
(4) *Multicomponent Analysis of Clinical Specimens:* Continue study.
(5) *Tuberculosis and Enteric Infections by Gene Probe:* Continue study.

DRUGS I

(1) *Acetaminophen in Drug Mixtures:* Continue official first action status of the liquid chromatographic meth-

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."
The recommendations submitted by the Committee on Drugs and Related Topics were adopted by the Association.
Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods); *J. Assoc. Off. Anal. Chem.* (1987) **70**, 385–403 (C methods); *J. Assoc. Off. Anal. Chem.* (1988) **71**, 199–239 (D methods).

od for acetaminophen in tablets, **37.C01-37.C06**. Continue study.

(2) *Acetaminophen with Codeine Phosphate:* Continue study.

(3) *p-Aminobenzoic Acid and Salicylic Acids in Pharmaceuticals:* Continue study.

(4) *Diethylpropion Hydrochloride:* Continue official first action status of the method for determination of diethylpropion hydrochloride in drug substance and tablets, **37.D01-37.D09**. Continue study.

(5) *Phenothiazine and Related Drugs:* Continue study.

DRUGS II

(1) *Aminacrine:* Continue study.

(2) *Belladona Alkaloids:* Appoint Associate Referee. Continue study.

(3) *Colchicine in Tablets:* Continue study.

(4) *Curare Alkaloids:* Continue study.

(5) *Dicyclomine Capsules:* Continue study.

(6) *Epinephrine and Related Compounds by LC-Electrochemical Detectors:* Continue study.

(7) *Ergot Alkaloids:* Continue study.

*(8) *Morphine Sulfate in Morphine Injection:* Adopt as official first action the interim first action liquid chromatographic method for determination of morphine sulfate in drug substance and injections, Bello, A.C., & Jhangiani, R. K. (1988) *J. Assoc. Off. Anal. Chem.* **71**, 1046–1048. Continue study.

(9) *Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine:* Appoint Associate Referee. Continue study.

(10) *Physostigmine and Its Salts:* Continue study.

(11) *Pilocarpine:* Discontinue topic.

(12) *Rauwolfia Alkaloids:* Appoint Associate Referee. Continue study.

(13) *Rauwolfia serpentina:* Continue study.

DRUGS III

(1) *Coumarin Anticoagulants:* Continue official first action status of the liquid chromatographic determination of the courmarin anticoagulants dicoumarol, phenprocoumon, and warfarin sodium in tablets, **37.D10-37.D15**. Continue study.

(2) *Flucytosine:* Continue official first action status of the liquid chromatographic determination of flucytosine in capsules, **36.D01-36.D06**. Continue study.

(3) *Halogenated Hydroxyquinoline Drugs:* Continue study.

(4) *Haloperidol:* Continue study.

*(5) *Hydralazine:* Adopt as official first action the interim first action method for the ultraviolet spectrophotometric determination of hydralazine hydrochloride in tablets, Mopper, B. (1988) *J. Assoc. off. Anal. Chem.* **71**, 1121–1122. Continue study.

(6) *Levodopa:* Continue official first action status of the liquid chromatographic determination of levodopa and levodopa-carbidopa in solid dosage forms, **36.D07-36.D12**. Continue study.

(7) *Medicinal Gases:* Continue study.

(8) *Metals in Drug Bulk Powders:* Continue study.

(9) *Penicillins:* Continue study.

(10) *Salts of Organic Nitrogenous Bases:* Continue study.

DRUGS IV

(1) *D- and L-Amphetamines—LC Separations:* Continue official first action status of the liquid chromatographic determination of enantiomers of amphetamine, **40.D01-40.D06**. Continue study.

(2) *Benzodiazepines:* Continue study.

(3) *Diazepam:* Continue study.

(4) *Heroin:* Continue study.

DRUGS V

*(1) *Antihistamines, Selected Drug Combinations, Ion Exchange Chromatography:* Declare as surplus the official final action method, **36.108-36.114**.

(2) *Betamethasone:* Continue study.

(3) *Chlorpropamide:* Continue official first action status of the liquid chromatographic determination of chlorpropamide in tablets, **37.B01-37.B06**. Continue study.

(4) *Conjugated Estrogens by LC:* Continue study.

(5) *Pentaerythritol Tetranitrate:* Continue study.

*(6) *Prednisolone:* Adopt as official final action the official first action method for liquid chromatographic determination of prednisolone in drug substance and tablets, **39.B01-39.B06**. Discontinue topic.

(7) *Progestins:* Continue study.

(8) *Steroid Acetates:* Continue official first action status of the liquid chromatographic determination of cortisone acetate in drug substance and dosage forms, **39.D01-39.D08**. Continue official first action status of the liquid chromatographic determination of dexamethasone acetate in drug substance and suspensions, **39.D09-39.D16**. Continue study.

(9) *Steroid Phosphates:* Continue official first action status of the liquid chromatographic determination of dexamethasone in drug substance and elixirs, **39.D17-39.D22**, the identification of dexamethasone in drug substance by thin-layer chromatography, infrared spectroscopy, and relative retention times, **39.D23-39.D26**, and the gas chromatographic determination of alcohol in elixirs, **39.D27-39.D30**. Continue study.

DRUG RESIDUES IN ANIMAL TISSUES

(1) *Benzimidazoles in Cattle Tissues:* Continue study.

(2) *Chloramphenicol in Animal Tissues:* Continue study.

(3) *Chloramphenicol in Milk:* Continue study.

(4) *Clopidol in Chicken Tissues:* Continue study.

(5) *Enzyme Immunoassays for Antimicrobial Compounds:* Continue study.

(6) *Ipronidazole in Turkey and Swine:* Continue study.

(7) *Novobiocin in Animal Tissues:* Continue study.

(8) *Screening Methods:* Discontinue topic.

(9) *Sulfa Drug Residues:* Continue study.

(10) *Sulfonamides in Milk (Chromatographic Methods):* Continue study.

(11) *Tetracyclines in Tissues (Chromatographic Methods):* Continue study.

(12) *Tetracyclines in Tissues (Microbiological Methods):* Continue study.

FORENSIC SCIENCES

(1) *Blood:* Continue study.

(2) *Chromatographic Methods for Forensic Characterization of Paints and Other Polymeric Materials:* Discontinue topic.

(3) *Electrophoretic Methods:* Discontinue topic.

(4) *Enzyme-Linked Immunosorbent Assays for Forensic Characterization of Body Fluid Stains:* Discontinue topic.

(5) *Explosives and Explosives Residues:* Discontinue topic.

(6) *Grouping Tests for Blood and Other Body Fluids:* Continue study.
(7) *Gunshot Residues:* Continue study.
(8) *Isoelectric Focusing Methods for Body Fluid Stains:* Continue study.
(9) *Screening and Confirmatory Tests for Dried Blood Stains:* Continue study.
(10) *Soils, Geological Analysis:* Continue study.

IMMUNOCHEMISTRY

(1) *Heparin by Non-RIA Procedures:* Discontinue topic.
(2) *Hybridoma-Monoclonal Antibodies:* Continue study.

Committee on Foods I: Recommendations for Official Methods

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The Committee has instituted new systems for increasing communications between Committee members and General Referees and tracking collaborative studies. Individual Committee members have been assigned liaison responsibilities with General Referees having common areas of professional interests. The Committee stressed the importance that General and Associate Referees review their respective responsibilities. Associate Referees are reminded that their collaborative study design and protocols must be reviewed and approved by the General Referee and Committee statistical consultant prior to commencing the study. The tracking system instituted by the Committee will follow each study from design/protocol approval through review of completed study by the General Referee, statistical consultant, Committee, and Official Methods Board.

COFFEE AND TEA

(1) *Ash in Instant Tea:* Continue study.
(2) *Caffeine in Coffee:* Continue study.
(3) *Methyl Xanthenes in Coffee and Tea:* Continue study.
(4) *Moisture in Coffee and Tea:* Continue study.
(5) *Solvent Residues in Decaffeinated Coffee and Tea:* Continue study.
(6) *Water Extract in Tea:* Discontinue topic.

DAIRY CHEMISTRY

(1) *Adulteration of Dairy Products with Vegetable Fat:* Continue study.
(2) *Babcock Test and Babcock Glassware:* Continue study.
*(3) *Babcock, Mojonnier, and Kjeldahl Tests:* Adopt as official first action the interim official first action revised Babcock method for fat in raw milk and the ether extraction method for fat in milk; appoint Associate Referee; continue study.
(4) *Calcium, Phosphorus, and Magnesium in Cheese:* Review collaborative study results of IDF methods for phosphorus in cheese; continue study.
(5) *Chloride Meters:* Continue study.
(6) *Cryoscopy of Milk:* Review collaborative study results of IDF thermistor method for determination of freezing point of milk; continue study.
(7) *Fat in Dairy Products (Udy Turbidity Test):* Continue study.
(8) *Fat in Milk:* Continue study.
*(9) *Mid-Infrared Instrumentation:* Add calibration procedures and statistical parameters for infrared analysis of milk (**16.083-16.092**); continue study.
(10) *Iodine:* Continue study.
(11) *Lactose in Dairy Products (Chromatographic Determination):* Continue study.
(12) *Lactose in Dairy Products (Enzymatic Method):* Continue study.
(13) *Moisture in Cheese:* Continue study.
(14) *Moisture in Cheese (Microwave):* Review collaborative study results for determination of solids in cottage cheese and cheddar cheese; continue study.
(15) *Mojonnier Method (Robotic):* Continue study.
(16) *Nitrates in Cheese:* Continue study.
(17) *Nonfat Milk Solids:* Discontinue study.
(18) *Phosphatase (Rapid Method):* Continue study.
(19) *Phosphatase (Reactivated):* Continue study.
(20) *Phosphorus:* Continue study.
(21) *Protein Constituents in Processed Dairy Products:* Continue study.
(22) *Protein in Milk (Rapid Tests):* Continue study.
(23) *Protein Reducing Substance Tests:* Continue study.
(24) *Total Solids and Moisture:* Review collaborative study

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* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."
The recommendations submitted by the Committee on Foods I were adopted by the Association.
Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

results for determination of solids content of raw milk; continue study.
(25) *Tyramine:* Continue study.
(26) *Whey Proteins in Nonfat Dairy Milk:* continue study.
*(27) *Other Topics:* Repeal to official first action the official final action direct method for fat in butter (**16.233**).

FOOD ADDITIVES

(1) *Anticaking Agents:* Appoint Associate Referee; continue study.
(2) *Antioxidants:* Continue study.
(3) *Brominated Vegetable Oils:* Continue study.
(4) *Chloride Titrator:* Continue study.
(5) *Diauryl Thiodipropionate:* Appoint Associate Referee; continue study.
(6) *Dressings:* Continue study.
(7) *EDTA in Food Products:* Continue study.
(8) *Gums:* Continue study.
(9) *Indirect Additives from Food Packages:* Continue study.
(10) *Monier-Williams Modification:* Review collaborative study results of method for sulfites in food; continue study.
(11) *Nitrates and Nitrites:* Continue study.
(12) *Nitrates (Selective Ion Electrode Titration):* Continue study.
(13) *Nitrosamines:* Continue study.
(14) *Nitrosamines in Food Contact Items:* Continue study.
(15) *Polycyclic Aromatic Hydrocarbons in Foods:* Continue study.
(16) *Polydimethyl Siloxane (PDMS):* Continue study.
(17) *Polysorbates:* Continue study.
(18) *Propylene Chlorohydrin:* Appoint Associate Referee; continue study.
(19) *Quinine in Soft Drinks:* Continue study.
(20) *Sodium Lauryl Sulfate:* Continue study.
(21) *Sulfiting Agents in Foods:* Continue study.

MEAT, POULTRY, AND MEAT AND POULTRY PRODUCTS

(1) *Automated Methods:* Discontinue topic.
(2) *Chemical Antibiotic Methods:* Transfer topic to Committee on Drugs and Related Topics.
(3) *Fat in Meat Products:* Discontinue topic.
(4) *Identification of Meats, Serological Tests:* Continue study.
(5) *Immunochemical Identification of Additives:* Discontinue topic.
(6) *Immunological Methods for Meat and Poultry Products:* Appoint Associate Referee; continue study.
(7) *LC Methods for Meat and Poultry Products:* Continue study.
(8) *3-Methyl Histidine:* Continue study.
(9) *Microwave Digestion of Meat Samples for Total Protein Analysis:* Continue study.
(10) *Nitrates and Nitrites:* Continue study.
(11) *Nitrosamines in Bacon:* Conduct collaborative study; continue study.
(12) *Nonmeat Protein in Meat:* Continue study.
(13) *Protein (Crude):* Continue study.
(14) *Proteins in Meat and Meat Products:* Review collaborative study results; continue study.
(15) *Species Identification Methods:* Discontinue topic.
(16) *Specific Ion Electrode Applications:* Review collaborative study results; continue study.
(17) *Temperature, Minimum Processing:* Continue study.
(18) *Total Fat:* Continue study.
(19) *Total Solids and Moisture by Microwave Drying:* Review collaborative study results; continue study.
(20) *Other Topic:* Initiate the new topics Robotic Methods for Meat and Poultry Products; Hydroxyproline in Meat; Gluten in Meat; and Bouillons and Consommes. Appoint Associate Referees.

MYCOTOXINS

(1) *Aflatoxin M:* Continue study.
*(2) *Aflatoxin Methods:* Adopt as official first action the interim official first action ELISA screening method for aflatoxin B_1 in cottonseed products and mixed feed; review collaborative study results of ELISA (microliter well method) screening method for aflatoxin B_1 in corn and roasted peanuts, liquid chromatographic method for aflatoxins B_1, B_2, G_1, and G_2 in corn and peanut butter, and ELISA (cup method) screening method for corn, cottonseed, peanuts, and peanut butter; continue study.
(3) *Alternaria Toxins:* Continue study.
(4) *Citrinin:* Continue study.
(5) *Cyclopiazonic Acids:* Continue study.
(6) *Emodin and Related Anthoquinones:* Discontinue topic.
(7) *Ergot Alkaloids:* Continue study.
(8) *Immunochemical Methods:* Continue study.
(9) *Ochratoxins:* Continue study.
(10) *Penicillic Acid:* Continue study.
(11) *Penicillium islandicum Toxins:* Discontinue topic.
(12) *Secalonic Acid:* Discontinue topic.
(13) *Sterigmatocystin:* Continue study.
(14) *Tree Nuts:* Continue study.
(15) *Tricothecenes:* Continue study.
(16) *Xanthomegnin and Related Naphthoquinones:* Continue study.
(17) *Zearalenone:* Review collabortive study results of ELISA screening method for zearalenone in corn, wheat, and feed; continue study.

OILS AND FATS

(1) *Antioxidants:* Continue study.
(2) *Emulsifiers:* Continue study.
(3) *Hydrogenated Fats:* Continue study.
(4) *Lower Fatty Acids:* Review collaborative study results on butyric acid in fat; continue study.
(5) *Marine Oils:* Continue study.
(6) *Olive Oil Adulteration:* Continue study.
(7) *Oxidated Fats:* Continue study.
(8) *Pork Fats in Other Fats:* Discontinue topic.
*(9) *Sterols and Tocopherols:* Adopt as official final action the official first action method for beta-sitosterol in butter oil (**28.104-28.109**); continue study.
*(10) *Other Topics:* Review collaborative study results on volatile hydrocarbon residues in fats and oils; adopt as official final action the official first action methods for cyclopropene fatty acids in oils (**28.120-28.123**), foreign fats containing tristearin in lard (**28.130-28.131**), and chick edema (dioxins) in oils and fats (**28.139-28.141**); revise method **28.B02(b)** to add the following: "Equivalent results may be obtained with use of short capillary column, i.e., 6 m or less."

PLANT TOXINS

(1) *Glucosinolates:* Coordinate collaborative study of LC method with IUPAC; continue study.

(2) *Hydrazines:* Coordinate collaborative study for agaritine; continue study.
(3) *Hypoglycine in Ackee:* Conduct collaborative study of LC method; continue study.
(4) *Phytoestrogens:* Continue study.
(5) *Pyrrolizidine Alkaloids:* Continue study.
(6) *Steroidal Alkaloids:* Conduct collaborative study for solanine and chaconine in potatoes; continue study.

SEAFOOD PRODUCTS

(1) *Ammonia in Seafood:* Continue study.
(2) *Coprostanol:* Appoint Associate Referee; continue study.
(3) *Crabmeat:* Continue study.
(4) *Determination of Fish Content in Coated Products:* Continue study.
(5) *Drained Weight of Block Frozen, Raw Peeled Shrimp:* Conduct collaborative study; continue study.
(6) *Ethanol in Seafoods:* Discontinue topic.
(7) *Gas and Liquid Chromatography:* Complete collaborative study on diamines; continue study.
(8) *GC Determination of Volatile Amines:* Continue study.
(9) *Flow Injection Analysis for Decomposition in Seafood Products:* Continue study.
(10) *Minced Fish in Fish Fillet Blocks:* Continue study.
(11) *Nitrites in Smoked Fish:* Appoint Associate Referee; continue study.
(12) *Shellfish Decomposition:* Appoint Associate Referee; continue study.
(13) *TLC Determination of Amines in Fishery Products:* Discontinue topic.

SEAFOOD TOXINS

(1) *Ciguatoxins, Biochemical Methods:* Complete preliminary collaborative study; continue study.
(2) *Cyanobacterial Peptide Toxins:* Continue study.
(3) *Diarrhetic Shellfish Poisons:* Continue study.
(4) *Neurotoxic Shellfish Poisons:* Continue study.
(5) *Paralytic Shellfish Poisons (Electrochemical Methods):* Continue study.
(6) *Paralytic Shellfish Poisons (Immunoassays):* Appoint Associate Referee.
(7) *Paralytic Shellfish Poisons (LC Methods):* Conduct precollaborative study for PSP toxins; continue study.
(8) *Tetrodotoxins:* Continue study.

Committee on Foods II: Recommendations for Official Methods

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ALCOHOLIC BEVERAGES

(1) *Alcohol Content by Oscillating U-Tube Density Meter:* Continue Study.
(2) *Alcohol Content of High Solids Distilled Spirits:* Continue study.
(3) *Carbon Dioxide in Wine:* Continue study.
(4) *Citric Acid in Wine:* Continue study.
(5) *Color Intensity for Distilled Alcoholic Beverage Products:* Continue study.
(6) *Ethanol in Wine by GC:* Continue study.
(7) *Ethyl Carbamate in Wine:* Initiate collaborative study.
(8) *Flavor Compounds in Malt Beverages:* Continue study.
(9) *Glycerol in Wine:* Continue study.
(10) *Hydrogen Cyanide:* Continue study.
(11) *Malic Acid in Wine:* Continue study.
(12) *Malt Beverages and Brewing Materials:* Continue study.
(13) *Sorbic Acid in Wine:* Continue study.
(14) *Sugars in Wine:* Continue study.
(15) *Sugars in Wine by Enzymatic Methods:* Continue study.
(16) *Sugars in Wine by LC:* Continue study.
(17) *Synthetic Colors:* Continue study.
(18) *Sulfur Dioxide in Wine (Ripper Method):* Continue study.
(19) *Tartrates in Wine:* Continue study.
(20) *Thujone in Alcoholic Beverages:* Continue study.
(21) *Vanillin and Ethyl Vanillin:* Continue study.

CEREAL AND CEREAL PRODUCTS

(1) *Fat Acidity in Flour:* Continue study.
(2) *Iron in Flour:* Initiate collaborative study of iron in cereals by atomic absorption.
(3) *Phytates:* Continue study.
*(4) *Other Topics:* Adopt the following nonsubstantive changes in methods **14.075-14.079**: In section **14.076(a)**, revise to read ". . .include automatic injectors, 410 differential refractometer (Waters Associates, Inc., WISP 710B, R1 410, or equiv.), 100 × 4.6 mm (id) Spheri-5 amino cartridge column (Brownlee Laboratories or equiv.) and use specific. . .". In

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section **14.076(c)**, revise to read ". . .100 × 2 (id) mm (Waters Associates, Inc.), or 15 × 3.2 mm (id) 7 μm, amino guard cartridge (Brownlee Laboratories), or equiv. . .". In section **14.077(a)**, revise to read ". . .vac. Dissolve in alcohol–H_2O (1 + 1) to obtain. . .". In section **14.077(b)**, revise to read "CH_3CN (LC grade) and H_2O (Milli-Q purified or equiv.) (80 + 20). Filter thru Whatman GF/F 0.7 μm glass fiber filter or thru 0.45 μm Pall Nylon 66 filter. Optionally. . .". In section **14.078(b)**, revise to read ". . .to original wt. Filter portion of ext thru 0.45 μm Nylon syringe filter (Nalge or equiv.). If cloudy, centrf. 10 min at ≥ 2000 rpm. If still cloudy, recentrf. portion of ext 5 min at ≥ 3500 rpm and filter thru 0.45 μm Nylon syringe filter. If guard. . .". In section **14.078(c)**, add "Filter thru 0.45 μm Nylon syringe filter if necessary."

CHOCOLATE AND CACAO PRODUCTS

(1) *Carbohydrates in Chocolate Products:* Continue study.
(2) *Moisture in Cacao Products:* Continue study.
(3) *Nonfat Dry Cocoa Solids:* Continue study.
(4) *Shell in Cacao Products, Micro Methods:* Continue study.
(5) *Total and Solid Fat Content in Chocolate Products by NMR:* Continue study.
(6) *Triglyceride Composition in Cocoa Butter and Fat from Chocolate:* Continue study.
*(7) *Other Topics:* In the method for fat, Knorr tube extraction (**13.031**), place precautionary warning concerning use of asbestos (carcinogen) as a filtering medium. Appoint a new General Referee or delete this topic from the Committee.
*(8) *Theobromine in Cacao Products:* Surplus due to successful expanded usage of LC method, **13.066-13.069**.

COLOR ADDITIVES

(1) *Arsenic, Barium, and Heavy Metals:* Continue study.
(2) *Color in Candy and Beverages:* Continue study.
(3) *Color in Cosmetics:* Continue study.
(4) *Color in Nonfrozen Dairy Desserts:* Continue study.
(5) *Color in Other Foods and Drugs:* Continue study.
(6) *Inorganic Salts:* Continue study.
(7) *Uncombined Intermediates in Water-Soluble Azo Colors:* Combine with the topic Uncombined Intermediates in Other (Non-Azo) Certifiable Colors to form a single topic, Uncombined Intermediates in Certifiable Colors.
(8) *Liquid Chromatography:* Eliminate as a separate topic, since LC is now being applied to many of the Associate Referee topic areas.
(9) *Subsidiary Colors in Certifiable Color Additives:* Continue study.
(10) *X-Ray Fluorescence Spectroscopy:* Continue study.
(11) *Determination of Color Additives Exempt from Certification:* Divide into 2 new topics, Anthocyanin Color Additives Exempt from Certification, and Carotenoid Color Additives Exempt from Certification (appoint Associate Referee).
(12) *Other Topics:* Initiate the new topic, Trace Organic Constituents of Certifiable Color Additives.

FLAVORS

(1) *Additives in Vanilla Flavorings:* Continue study.
(2) *Citral:* Continue study.
(3) *Essential Oils:* Continue study.
(4) *Glycyrrhizic Acid and Glycyrrhizic Acid Salts:* Continue study.
(5) *Imitation Maple Flavors, Identification and Characterization:* Continue study.
(6) *Organic Solvent Residues in Flavors:* Continue study.
(7) *Vanillin and Ethyl Vanillin in Foods:* Initiate collaborative study.
(8) *Other Topics:* Establish the new topic, Carbon-14 in Flavoring Materials and appoint an Associate Referee.

FRUIT AND FRUIT PRODUCTS

(1) *Adulteration of Apple Juice:* Continue study.
(2) *Adulteration of Orange Juice by Pulpwash and Dilution:* Continue study.
*(3) *Fruit Acids:* Adopt as official final action the official first action LC method, **22.B01-22.B05,** for determining quinic, malic, and citric acids in cranberry juice cocktail and apple juice.
(4) *Fruit Juices, Identification and Characterization:* Continue study.
(5) *Limonin in Grapefruit Juice:* Continue study.
(6) *Moisture in Dried Fruits:* Continue study.
(7) *Orange Juice Content:* Continue study.
(8) *Sodium Benzoate in Orange Juice:* Continue study.
(9) *Other Topics:* Initiate the new topics, Inductively Coupled Plasma (ICP) Methods, and Carbon Stable Isotope Ratio for Analysis of Fruit Products.

NONALCOHOLIC BEVERAGES

(1) *Caffeine and Methyl Xanthines:* Continue study.
(2) *Caloric Content:* Continue study.
(3) *Glycyrrhizic Acid Salts in Licorice-Derived Products:* Continue study.
(4) *Lasiocarpine and Pyrrolizidines in Herbal Beverages:* Continue study.
(5) *Quinine:* Complete statistical review of collaborative study. Continue study.
(6) *Safrole in Sassafras:* Continue study.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) *Aspartame, Benzoates, Saccharin, and Caffeine by LC:* Continue study.
(2) *Organic Preservatives:* Continue study.
(3) *Sulfites (Flow Injection Methods):* Continue study.
(4) *Sulfites (Polarographic Methods):* Continue study.
(5) *Sulfites in Shrimp (Screening Methods):* Continue study.
(6) *Sulfites (Ion Chromatographic Methods):* Continue study.

PROCESSED VEGETABLE PRODUCTS

(1) *pH Determination:* Continue study.
(2) *Sodium Chloride:* Renew efforts to identify current needs; appoint Associate Referee.
(3) *LC Determination of Sugar in Processed Vegetables:* Appoint Associate Referee.
(4) *Total Solids by Microwave Moisture Methods:* Continue study.
(5) *Water Activity in Foods:* Continue study.

SPICES AND OTHER CONDIMENTS

(1) *Ash and Pungent Principles in Mustard:* Continue study.
(2) *Bulk Index of Spices:* Continue study.

(3) *Ethylene Oxide and Ethylene Chlorohydrin Residues:* Continue study.
(4) *Extractable Color in Capsicum Spices and Oleoresins:* Continue study.
(5) *Moisture in Dried Spices:* Continue study.
(6) *Monosodium Glutamate:* Continue study.
(7) *Piperine in Black Pepper:* Continue study.
(8) *Pungency of Capsicums and Oleoresins:* Continue study.
(9) *Vinegar:* Continue study.
*(10) *Other Topics:* Initiate the new topic, Curcumin in Turmeric. Designate as surplus the following methods: **30.010**, Nitrogen in Spices; **30.011**, Nitrogen in Nonvolatile Ether Extract of Pepper; **30.013**, Alcohol Extract; **30.015**, Starch in Spices; **13.018**, Tannin in Cloves and Allspice.

SUGARS AND SUGAR PRODUCTS

(1) *Chromatographic Methods:* Continue study.
(2) *Color, Turbidity, and Reflectance: Visual Appearance:* Continue study.
(3) *Corn Syrup and Sugars:* Continue study.
(4) *Enzymatic Methods:* Continue study.
(5) *Gas Chromatographic Methods:* Continue study.
(6) *Honey:* Continue study.
(7) *Lactose:* Continue study.
(8) *Liquid Chromatographic Methods:* Continue study.
*(9) *Maple Sap, Maple Syrup, and Maple Syrup Products:* (a) Adopt Berliner maple syrup grading kit as suitable alternative for use in method **31.170**, Color Classification of Maple Products. (b) Adopt a nonsubstantive change in section **31.169**(a)(*1*) (Preparation of Sample), from "filter sample through cotton wool" to "filter sample through cotton wool *or* centrifuge at 1000–3000 rpm for 20 min." (c) Add the following correction to method **31.188** (Corn Syrup and Cane Sugar in Maple Syrup): Results obtained by the alternative preparation method are biased approx. 1.0/mL relative to section **31.187**. All results obtained by this alternative preparative method must be corrected for this bias by adding an appropriate correction value (C). The correction may be determined by analyzing reference sample using both procedures.

$$\delta^{13}C \text{ (corrected)} = \delta^{13}C \text{ (measured)} + C$$

(10) *Oligosaccharides in Sugars and Sugar Products:* Continue study.
(11) *Polarographic Methods for Measurement of Sugars:* Continue study.
(12) *Stable Carbon Isotope Ratio Analysis:* Continue study.
(13) *Standardization of Methods of Sugar Analysis:* Continue study.
(14) *Sugars in Cereals:* Continue study.
(15) *Sugars in Syrups:* Continue study.
(16) *Sulfites in Sugars and Syrups:* Continue study.
(17) *Weighing, Taring, and Sampling:* Continue study.
*(18) *Other Topics:* Add a note to method **31.203-31.208**, Formaldehyde in Maple Syrup, to state: Not suitable for beet or cane sugars. Designate as surplus the following methods: **31.007**, **31.012**, **31.013**, **31.015-31.018**, **31.029**, **31.037-31.044**.

VITAMINS AND OTHER NUTRIENTS

(1) *Amino Acids:* Continue study.
(2) *Automated Nutrient Analysis:* Continue study.
(3) *Biotin:* Continue study.
(4) *Carotenoids:* Continue study.
(5) *Dietary Fiber:* Continue study.
(6) *Fat in Food by Chloroform-Methanol Extraction:* Continue study.
(7) *Folic Acid:* Continue study.
(8) *Iodine:* Continue study.
(9) *Nutrient Assay of Infant Formula:* Continue study.
(10) *Panthothenic Acid, Total Acidity:* Continue study.
(11) *Protein Quality, Evaluation:* Continue study.
(12) *Sodium:* Continue study.
(13) *Thiamine Assay, Enzyme and Column Packing Reagents:* Continue study.
(14) *Vitamin A:* Continue study.
(15) *Vitamins A, D, E, and K by Gel Permeation and LC:* Continue study.
(16) *Vitamin D:* Continue study.
(17) *Vitamin E:* Continue study.
(18) *Vitamin E in Pharmaceuticals (Gas Chromatography):* Continue study.
(19) *Vitamin K:* Continue study.
*(20) *Other Topics:* Initiate the new topic, Cholesterol in Foods. Change the calculation for niacin in section **43.050** to: mg Niacin/g sample = $C/(10 \times g \text{ sample})$.

Committee on Residues: Recommendations for Official Methods

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METALS AND OTHER ELEMENTS

(1) *Atomic Absorption Spectrophotometry (AAS):* Continue the development of biological reference materials with known concentrations of selected elements for use in evaluating performance of AAS methods; continue effort to consolidate present AOAC official AAS methods for individual elements into unified AAS scheme for multielement analysis of foods and other biological substrates.

(2) *Cadmium and Lead in Earthenware:* Continue study of the use of inductively coupled plasma (ICP) emission spectrometry to determine lead, cadmium, and other elements leached from earthenware with AOAC method for leaching earthenware, **25.024-25.027**. Determine if collaboratively studied method is required for elements other than cadmium and lead.

(3) *Emission Spectrochemical Methods:* Prepare protocol for collaborative study of method combining wet acid digestion of plant or animal matrices with inductively coupled plasma (ICP) emission spectroscopy for multielement analysis of foods; upon approval of protocol by General Referee and Statistical Consultant to Committee on Residues, initiate study using collaborators who demonstrate proficiency with the ICP determinative step in pre-study test with trial sample solutions.

(4) *Fluorine:* Continue study to improve microdiffusion and fluoride-specific electrode method for determining fluoride in foods (*J. Assoc. Off. Anal. Chem.* (1979) **62,** 1065–1069) as specified in Associate Referee's report on the collaborative study for fluoride in infant foods (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1021–1026).

(5) *Graphite Furnace-Atomic Absorption Spectrophotometry (GF-AAS):* Continue study to resolve problems found for levels below 20 ppb lead and 1 ppb cadmium in interlaboratory trial of coprecipitation GF-AAS method for lead and cadmium in foods (*Can. J. Spectrosc.* (1986) **31,** 44–52); if problems are resolved, prepare protocol for collaborative study of this method for approval by General Referee and Statistical Consultant to Committee on Residues; conduct interlaboratory trial of improved version of coprecipitation GF-AAS method for arsenic in foods (*Can. J. Spectrosc.* (1985) **30,** 154–157).

(6) *Hydride Generating Techniques:* Continue study of continuous flow hydride generation procedure of Panaro and Kroll (*Anal. Lett.* (1984) **17,** 157–172 for determining arsenic and selenium in foods.

(7) *Lead in Calcium Supplements:* Draft, in AOAC official method format, the method developed for determination of lead in calcium supplements that was adapted from *J. Assoc. Off. Anal. Chem.* (1979) **62,** 1054–1061 for review and comment by General Referee. Recovery and method performance data must be included with proposed method. If satisfactory, carry out interlaboratory trial, prepare protocol for collaborative study for approval by the General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study.

(8) *Methyl Mercury in Fish and Shellfish:* Continue official first action status of rapid gas chromatographic method for methyl mercury in fish and shellfish, **25.D01-25.D06**.

(9) *Neutron Activation Analysis:* Prepare protocol for collaborative study of method for determination of sodium in foods for approval by the General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study.

(10) *Organometallics in Fish:* Committee on Residues will evaluate the collaborative study on the method for determination of methyl mercury in seafood by LC and atomic absorption spectrophotometric detection and make recommendation to Official Methods Board for possible interim first action status.

(11) *Organotin Compounds:* Continue development of multiresidue method for organotin compounds in foods.

(12) *Polarography:* Study the application of square wave voltammetry in place of differential pulse anodic stripping voltammetry for determination of lead and cadmium, **25.008-25.015**.

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* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

The recommendations submitted by the Committee on Residues were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods." *J. Assoc. Off. Anal. Chem.* (1985) **68,** 369–411 (A methods); *J. Assoc. Off. Anal. Chem.* (1986) **69,** 349–390 (B methods); *J. Assoc. Off. Anal. Chem.* (1988) **71,** 199–239 (D methods).

MULTIRESIDUE METHODS

(1) *Comprehensive Multiresidue Methodology:* Carry out interlaboratory evaluation of California Department of Food and Agriculture (CDFA) Multiresidue Method for residues of organochlorine, organophosphorus, and some *N*-methylcarbamate pesticides. If data are satisfactory, do performance comparisons between proposed CDFA Method and AOAC method **29.A01-29.A04**. Prepare report for review and comment by General Referee; if data are satisfactory, prepare protocol for collaborative study of CDFA Method and submit for approval to General Referee and Statistical Consultant to Committee on Residues.

(2) *Extraction of Low Moisture-High Fat Samples:* Change title of topic to Low Moisture-High Fat Samples; appoint Associate Referee to evaluate chromatographic cleanup techniques such as Unitrex, semipreparative LC, GPC, and solid-phase extraction columns as alternatives to acetonitrile partitioning, **29.014**, for removing lipids from extracts of oil seeds and other low moisture-high fat foods for determination of pesticide residues.

(3) *Fumigants:* Draft, in AOAC official method format, a specific method, applicable to representative fumigants in whole grains and milled products and based on the approach published in *J. Assoc. Off. Anal. Chem.* (1983) **66,** 228–233, for review and comment by General Referee. Recovery and method performance data concerning all chemicals tested must be included with proposed method. If satisfactory, carry out interlaboratory trial and prepare protocol for collaborative study for approval by General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study.

(4) *Miniaturization of Multiresidue Methodology:* Draft, in AOAC official method format, a miniaturized method for nonfatty foods based on applicable sections of **29.011-29.018** and **29.034** for review and comment by General Referee. If satisfactory, carry out interlaboratory trial, prepare protocol for collaborative study for approval by General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study.

(5) *Organophosphorus Pesticide Residues:* Continue studies on recovery of organophosphorus (parent and metabolite) chemicals through **29.A01-29.A04** followed by oxidation to "total sulfones" (*Analyst* (1984) **109,** 483–487).

(6) *Sweep Codistillation:* Draft, in AOAC official method format, a method applicable to commercially available Unitrex system for review and comment by General Referee. Carry out interlaboratory study using representative organochlorine pesticides and beef fat. If satisfactory, prepare protocol for collaborative study with input from Australian Quarantine Inspection Services (AQIS) and AOAC (General Referee and Statistical Consultant to Committee on Residues) to assure that the requirements of both the AQIS laboratory accreditation program and collaborative study criteria of AOAC are met; initiate collaborative study.

(7) *Synthetic Pyrethroids:* Establish topic to evaluate and collaboratively study multiresidue methods for residues of synthetic pyrethroids in foods. Continue investigation of nonstability of cypermethrin and root crops on freezer storage. Draft, in AOAC official method format, a method for synthetic pyrethroids based on **29.A01-29.A04** and **29.046** for comment and review by General Referee. Recovery and method performance data concerning chemicals tested must be included with proposed method. If satisfactory, carry out interlaboratory trial, prepare protocol for collaborative study for approval by General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study.

ORGANOHALOGEN PESTICIDES

(1) *Chlordane:* Perform method comparison studies on several methods available for chlordane residues in fatty products: (a) extraction, **29.011-29.012**, acetonitrile partitioning cleanup, **29.014**, Florisil column chromatographic cleanup and residue separation, **29.046-29.048**; (b) extraction, **29.011-29.012**, and gel permeation chromatography (GPC) cleanup, **29.037-29.043**; and (c) extraction, **29.011-29.012** and Unitrex system cleanup (Luke et al. (1984) *J. Assoc. Off. Anal. Chem.* **67,** 295–298). Chlordane residues (*cis*-and *trans*-chlordane, *cis*-and *trans*-nonachlor, octachlor epoxide (oxychlordane) and heptachlor epoxide in butter, eggs, fish, and poultry fat can be determined by Associate Referee's electron capture (EC) capillary column GC system. Based on these studies, the most practical method can be chosen for collaborative study.

(2) *Chlorinated Dioxins:* Continue study to evaluate methods for determining 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and hexachloro-, heptachloro-, and octachloro-substituted dibenzo-*p*-dioxins and dibenzofurans in foods and environmental samples, with ultimate goal of establishing through AOAC collaborative procedures an official method or methods for determining residues of these compounds at parts-per-trillion levels in fish, milk, and other foods.

(3) *Chlorophenoxy Alkyl Acids:* Draft in AOAC official method format, a specific method for chlorophenoxy alkyl acids based on methodology of Hopper (*J. Agric. Food Chem.* (1987) **35,** 265–269) for review and comment by General Referee and Committee on Residues. Recovery and method performance data concerning all chemicals tested must be included with the proposed method. If satisfactory, carry our efficiency studies of extracting field-incurred residues from samples. If satisfactory, carry out interlaboratory trial and prepare protocol for collaborative study on commodities likeliest to be contaminated with residues for approval by General Referee and Statistical Consultant to Committee on Residues; initiate collaborative study.

(4) *Ethylene Oxide and Its Chlorohydrin:* Continue study to evaluate GC method of Scudamore and Heuser (*Pestic. Sci.* (1971) **2,** 80–91) and alternative methods for determining ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin in foods.

(5) *Methyl Bromide:* Draft in AOAC official method format a specific method for methyl bromide for review and comment by General Referee and Committee on Residues. Principle of method, procedure for correcting for recovery, calculation procedures, and recovery and method performance data must be included with proposed method. If satisfactory, develop procedures for handling and transporting food samples containing methyl bromide and test reliability of

procedures by interlaboratory study. If results are satisfactory, prepare protocol for collaborative study on commodities likeliest to retain methyl bromide from fumigation (e.g., nuts, dried fruits, spices) and submit protocol for approval by General Referee and Statistical Consultant to Committee on Residues; initiate collaborative study.

(6) *Pentachlorophenol:* Continue as official first action the GC method for pentachlorophenol in gelatin, **29.A14-29.A18**; continue study to improve GC determination of underivatized pentachlorophenol in this method or to convert pentachlorophenol to derivative amenable to GC quantitation; discontinue topic and combine with topic chlorophenoxy alkyl acids.

(7) *Pentachlorophenol in Animal and Poultry Tissue:* Defer consideration of official status for electron-capture GC method for determining pentachlorophenol in animal livers until collaborative study report is revised to include data supporting use of internal standard and blank liver samples in method and other information specified in Committee on Residues' recommendation for this topic in 1985 (*J. Assoc. Off. Anal. Chem.* (1986) **69,** 299).

(8) *Polychlorinated Biphenyls (PCBs):* Continue study of methods for separating PCBs from organochlorine pesticide residues that remain with them in extraction/cleanup steps of multiresidue method, **29.001-29.018**; continue study to compare results produced by GC quantitation techniques based on analysis for individual congeners of PCBs and those produced by quantitation techniques described in the official method, **29.018** and Table **29:02.**

(9) *Polychlorinated Biphenyl (PCB) Determination by Measurement of Specific Congeners:* Establish topic to collaboratively study a method of quantitating PCBs using specific individual congeners. Draft, in AOAC official method format, a method for PCB determination by measurement of specific individual congeners, including extraction and cleanup steps for review and comment by the General Referee. Recovery and method performance data must be included with proposed method. If satisfactory, carry out interlaboratory trial, prepare protocol for collaborative study acceptable to both AOAC and Nordic Committee on Methods of Food Analysis for approval by General Referee and Statistical Consultant to Committee or Residues, and initiate collaborative study.

(10) *Polychlorinated Biphenyls (PCBs) in Blood:* (a) Continue review of the method for PCBs in blood serum, in preparation for approval as interim first action. (b) Continue study to make method suitable for both PCBs and pesticides in blood serum.

ORGANONITROGEN PESTICIDES

(1) *Anilazine:* Appoint Associate Referee to evaluate LC method of Lawrence and Panopio (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1300–1303) and other LC or GC methods for determining anilazine residues in food crops and to collaboratively study method selected.

(2) *Benzimidazole-Type Fungicides:* Appoint Associate Referee to study method for simultaneous determination of benomyl, thiophanate methyl, and hydrolysis product methyl 2-benzimidazolecarbamate (MBC, also known as the fungicide carbendazim) in fruits and vegetables.

(3) *Captan and Related Fungicides:* Complete interlaboratory trial of Associate Referee's modified method for captan, captafol, and folpet, and submit data to General Referee for review and comment. If data are satisfactory, prepare protocol for collaborative study for approval by General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study.

(4) *Carbamate Herbicides:* Appoint Associate Referee to select and collaboratively study method for determining residues of carbamate herbicides in crops.

(5) *Carbamate Insecticides:* (a) Continue study of official final action LC method for determining aldicarb, aldicarb sulfone, bufencarb, carbaryl, carbofuran, 3-hydroxycarbofuran, methiocarb, methomyl, and oxamyl in grapes and potatoes, **29.A05-29.A13** (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 726–733), to extend applicability of this method to additional *N*-methylcarbamate insecticides and metabolites and to additional fruits and vegetables; (b) Conduct interlaboratory trial on method developed by Associate Referee (*J. Chromatogr.* (1988) **442,** 333–343) for confirmation of phenolic carbamate residues, and submit data to General Referee for review and comment. If data are satisfactory, prepare protocol for collaborative study and submit for approval to General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study.

(6) *Carbofuran:* Appoint Associate Referee to investigate and collaboratively study methods for determining carbofuran and its carbamate and phenolic metabolites in milk and meat and for determining 3-hydroxycarbofuran glucoside and phenolic carbofuran metabolites in crops.

(7) *Chlorothalonil:* Appoint Associate Referee to evaluate existing GC multiresidue methods, such as **29.044-29.049**, as well as methods specifically designed for determination of chlorothalonil residues in foods and subject method selected to collaborative study.

(8) *Daminozide and 1,1-Dimethylhydrazine (UDMH):* (a) Provide details of method for UDMH to General Referee for review and comment. If satisfactory, carry out interlaboratory trial, prepare protocol for collaborative study for approval by General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study. (b) Provide details of alkaline hydrolysis-GC-MS method for daminozide to General Referee for review and comment. If satisfactory, carry out interlaboratory trial, prepare protocol for collaborative study for approval by General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study. (c) If practical, the collaborative studies for UDMH and daminozide be carried out simultaneously.

(9) *Diquat and Paraquat:* Complete collaborative study of method for diquat and paraquat in potatoes, and submit report and recommendation to General Referee and Committee on Residues for possible interim action.

(10) *Dithiocarbamate Fungicides:* Appoint Associate Referee to develop methods for distinguishing dimethyldithiocarbamates from ethylenebisdithiocarbamates and for determining parent fungicides and their metabolites in foods.

(11) *Maleic Hydrazide:* Appoint Associate Referee to de-

velop and collaboratively study GC or LC method for determining maleic hydrazide in crops.

(12) *Organonitro Pesticides:* Continue study of Florisil cleanup of nitro- and dinitro-substituted pesticides and investigate the effect of coextractives on LC-electrochemical detection system.

(13) *Sodium o-Phenylphenate:* Appoint Associate Referee to develop and collaboratively study GC or LC method for determining *o*-phenylphenol in foods.

(14) *Substituted Ureas:* Conduct interlaboratory trial of Associate Referee's LC method for urea herbicides and submit data to General Referee for review and comment. If satisfactory, prepare protocol for collaborative study for approval by General Referee and Statistical Consultant for Committee on Residues, and initiate collaborative study.

(15) *Thiolcarbamate Herbicides:* Appoint Associate Referee to evaluate and collaboratively study methods for determining residues of thiolcarbamate herbicides in crops.

(16) *s-Triazines:* Appoint Associate Referee to evaluate methods for determining residues of atrazine, simazine, and cyanazine in agricultural products and to collaboratively study method selected.

(18) *Trifluralin:* Discontinue topic and transfer study to Organonitrogen Pesticides.

ORGANOPHOSPHORUS PESTICIDES

(1) *Disulfoton:* Conduct interlaboratory study of Associate Referee's GC method for determining disulfoton and disulfoton metabolite residues in foods (*J. Agric. Food Chem.* (1982) **30,** 1082–1086); if satisfactory, submit protocol for collaborative study of method for approval by General Referee and Statistical Consultant to Committee on Residues, and initiate collaborative study.

(2) *Extraction Procedures:* Appoint Associate Referee to study efficiency of procedures for extracting field-incurred residues of organophosphorus pesticides from crops and to develop improved extraction procedures for incorporation into multiresidue methods.

(3) *Gel Permeation Chromatography (GPC) Cleanup:* Continue study and develop plan for collaborative study to extend official final action GPC method for organochlorine pesticide residues in poultry fat, **29.037-29.043**, to determination of polychlorinated biphenyl residues in fish, meat, and poultry fats and to determination of organophosphorus pesticide residues in high fat samples.

(4) *Phorate:* Appoint Associate Referee to evaluate and collaboratively test analytical methods for determining phorate and its metabolites in foods.

(5) *Phosphine:* Continue study to evaluate methods for determining residual phosphine in grains, including solvent soaking procedure for extraction of fumigants in grains, **29.072**.

(6) *Terbufos:* Appoint Associate Referee to evaluate and collaboratively study methods for determining residues of terbufos and its metabolites in foods.

RADIOACTIVITY

(1) *Cesium-137:* (a) Continue as official first action the extension of official final action gamma-ray spectroscopic method for I-131, Ba-140, and Cs-137 in milk, **48.025-48.029**, as modified in **48.B01-48.B02** to include other foods. (b) Appoint Associate Referee to evaluate and collaboratively study radiochemical methods for determining Cs-137 in foods and other biological matrices at lower levels than determinable with official method, **48.025-48.029** and **48.B01-48.B02**.

(2) *Iodine-131:* (a) Continue as official first action the extension of official final action gamma-ray spectroscopic method for I-131, Ba-140, and Cs-137 in milk, **48.025-48.029**, as modified in **48.B01-48.B02** to include other foods. (b) Evaluate data from study for I-131 in water at 3 levels of activity. If satisfactory, prepare protocol for collaborative study of method recommended by Nuclear Regulatory Commission for determining I-131 in milk, as outlined in *J. Assoc. Off. Anal. Chem.* (1979) **62,** 387–389; if protocol is approved by General Referee and Statistical Consultant to Committee on Residues, initiate collaborative study.

(3) *Plutonium:* Appoint Associate Referee to study Department of Energy method for determining plutonium in foods, biological materials, and water (HASL-300-Ed 25, *Energy Monitoring Laboratory Procedures Manual* (1982) pp. E-Pu-01-01) and related procedures; design and conduct collaborative study of selected method.

(4) *Radium-228:* Complete evaluation of collaborative study, and submit report and recommendation to General Referee and Committee on Residues for possible interim first action status.

(5) *Strontium-89 and -90:* Prepare protocol for collaborative study of method of Baratta and Reavey (*J. Agric. Food Chem.* (1969) **17,** 1337–1339) for determining strontium -89 and -90 in foods for approval by General Referee and Statistical Consultant to Committee on Residues; initiate collaborative study.

(6) *Tritium:* Appoint Associate Referee to evaluate and collaboratively study methods for determining tritium in foods and biological materials.

Committee on Microbiology and Extraneous Materials: Recommendations for Official Methods

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The Committee recommends the following item for action to the Official Methods Board:

Evaluate formalization of the process of elevating a method from official first action to official final action.

The Committee took the following action:

Decided that minor changes to methods can be made after in-house study and be adopted without changing the status of the method. Substantive changes must be validated by collaborative study and therefore relegate the method to the status of a new method.

ANALYTICAL MYCOLOGY AND MICROSCOPY

(1) *Chemical Methods for Detecting Mold:* Continue study.
(2) *Geotrichum Mold in Canned and Frozen Fruits, Vegetables, and Fruit Juices:* Continue study.
(3) *Standardization of Plant Tissue Concentrations for Mold Counting:* Continue study.
(4) *Vegetable Substitutes in Horseradish:* Continue study.
*(5) *Other Topics:* (a) In the following methods make changes as described by the General Referee: (*1*) Mold in Tomato Powder (Dehydrated), Howard Mold Count, **44.211.** Add blending speed and procedure for identification of powder as spray-dried product. (*2*) *Geotrichum* Mold in Ground Spices, **44.214-44.216**, and in Vegetables, Fruits, and Juices, **44.219**. Clarify calculations. (*3*) Mold in Comminuted Fruits and Vegetables, *Geotrichum* Mold Count, **44.220-44.222.** Clarify calculation. (*4*) Rot in Tomato Products (Comminuted), **44.224** as revised in "Changes in Official Methods," A supplement. Add additional rinse with water and magnification for reading results. (b) Surplus revised method **44.224**, Rot in Tomato Products (Comminuted).

FILTH AND EXTRANEOUS MATERIALS IN FOODS AND DRUGS

(1) *Baked Goods with Fruit and Nut Tissues, Light Filth in, Flotation Method:* Continue study.
(2) *Basil (Ground), Light Filth in, Flotation Method:* Add topic.
(3) *Botanical Drugs, Adulteration by Foreign Plant Materials:* Discontinue topic.
(4) *Botanicals, Light Filth in, Flotation Method:* Continue study.
(5) *Capsicum Products, Filth in, Brine Extraction Techniques:* Discontinue topic.
(6) *Cheese, Filth and Mite Contamination:* Redefine topic as *Cheese, Filth in, Pancreatin Digestion Method.* Continue study.
(7) *Chocolate Products, Filth in, and Cocoa Powder and Press Cake, Filth in:* Redefine combined topics as *Chocolate and Chocolate Products, Light Filth in, Flotation Method.* Continue study.
(8) *Coffee (Ground), Light Filth in, Flotation Method:* Add topic.
(9) *Crabmeat, Shrimp, and Tuna (Canned), Light Filth in, Brine Flotation Method:* Continue study.
(10 *Fish (Canned) and Fish Products, Light Filth in, Flotation Method:* Continue study.
(11) *Fish Paste and Sauces, Light Filth in, Flotation Method:* Add topic.
(12) *Grain Products, Light Filth in, Flotation Method:* Add topic.
(13) *Grains (Whole), Internal Insect Infestation, Cracking Flotation Method:* Continue study.
(14) *Meats (Processed), Filth in:* Discontinue topic.
(15) *Mite Contamination Profiles and Characterization of Damage to Foods:* Discontinue topic.
(16) *Mites in Stored Foods:* Discontinue topic.
(17) *Mushroom Products (Dried), Filth in:* Discontinue topic.
(18) *Onion and Garlic (Powdered and Granulated), Filth in, Sedimentation Method:* Add topic.
(19) *Performance Evaluation of Methods for Filth:* Discontinue topic.
(20) *Popcorn (Popped), Light Filth in, Flotation Method:* Add topic.
(21) *Popcorn (unpopped), Light Filth in, Sieving Method:* Add topic.
(22) *Rodent Gnawing of Packaging Materials and Food, Salivary Amylase Test:* Add topic.
(23) *Rye Bread, Filth in:* Discontinue topic.
(24) *Soluble Insect and Other Animal Filth:* Discontinue topic.
(25) *Soups (Canned and Dried), Light Filth in, Flotation Method:* Continue study.
(26) *Soybean Curd, Light Filth in, Flotation Method:* Continue study.
(27) *Spices, Mammalian Feces in, Chemical Detection Method:* Add topic.
(28) *Spirulina, Light Filth in, Flotation Method:* Continue study.

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

The recommendations submitted by the Committee on Microbiology and Extraneous Materials were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411 (A methods); *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods); *J. Assoc. Off. Anal. Chem.* (1987) **70**, 385–403 (C methods).

(29) *Tomatoes and Mushrooms (Canned), Filth in, Brine Extraction Technique:* Discontinue study on mushrooms (canned). Redefine topic as *Tomatoes, Light Filth in, Brine Extraction Method.* Continue study.

(30) *Urine, Methods for Detection:* Redefine topic as *Urine Stains on Foods and Containers, Chemical Methods:* Continue study.

(31) *Vegetable Products (Dehydrated). Light Filth in, Flotation Method:* Continue study.

(32) *Vertebrate Excreta, Chemical Identification Tests:* Redefine topic as *Grain Products, Mammalian Feces in, Alkaline Phosphatase Detection Method.* Continue study.

*(33) *Other Topics:* Adopt as official final action the following official first action methods: filth in corn flour by pancreatin digestion method, **44.058(a)**, and by acid hydrolysis method, **44.059**; filth in eggs and egg products, **44.074-44.076**; filth in mushrooms (canned, fresh, frozen, freeze-dried, and dehydrated), **44.115**; light filth in ground allspice by flotation method, **44.127-44.128**; excrement (bird and insect) on food and containers by TLC method for uric acid, **44.B07-44.B09**.

COSMETIC MICROBIOLOGY

Establish Associate Refereeships and begin studies.

DAIRY MICROBIOLOGY

*(1) *Aerobic Plate Counts and Coliforms, Dry Rehydratable Film Methods:* Adopt as official first action the interim official first action dry-rehydratable film method for enumeration of coliforms and aerobic bacteria in dairy products. Continue study.

(2) *Bactoscan Methods:* Continue study.

(3) *Listeria monocytogenes in Dairy Products, Detection by Cultural Methods:* Continue study.

(4) *Listeria monocytogenes in Dairy Products, Detection by DNA Probe:* Continue study.

(5) *Raw Milk in Cheese, Detection by Alkaline Phosphatase Test:* Continue study.

*(6) *Somatic Cells, Automated Optical Counting Method:* In optical somatic cell counting method III, **46.171-46.175**, include in **46.172** use of the Fossomatic-90 Somatic Cell Counters. Continue study.

(7) *Somatic Cells, Fossomatic Method:* Continue study.

*(8) *Other Topics:* (a) Adopt as official first action the temperature-independent pectin gel method for coliform determination in dairy products. (b) Declare as surplus the following methods for somatic cells in milk: optical somatic cell counting (OSCC) method I, **46.152-46.160**, and method II, **46.161-46.170**; and membrane filter-deoxyribonucleic acid method, **46.176-46.180**.

DRUG AND DEVICE RELATED MICROBIOLOGY

(1) *Biological Indicator Testing and Standardization:* Continue study.

(2) *Biological Sterility Indicators:* Continue study.

(3) *Chemical Indicators:* Continue study.

(4) *Endotoxins by Limulus Amebocyte Lysate:* Continue study.

(5) *Medical Devices–Packaging Integrity:* Continue study.

(6) *Medical Devices–Sterility Testing:* Continue study.

(7) *Sporicidal Testing of Disinfectants/Sterilants:* Continue study.

FOOD MICROBIOLOGY (NONDAIRY)

(1) *Bacillus cereus Enterotoxin:* Redefine topic as *Bacillus cereus Enterotoxin, Microslide Gel Double Diffusion Test.* Continue study.

(2) *Bacillus cereus, Isolation and Enumeration:* Continue study.

(3) *Clostridium perfringens, Recovery from Marine Environment by Iron Milk Test:* Continue study.

*(4) *Enterobacteriaceae:* (a) Adopt as official first action the following interim official first action methods: (*1*) MICRO-ID system as an alternative to conventional biochemicals, **46.121-46.124**, or to other AOAC approved diagnostic kits, **46.133**; for presumptive generic identification of foodborne *Salmonella* isolates and screening and elimination of non-*Salmonella* isolates, as an alternative to conventional biochemicals, **46.016**; for identification of foodborne *E. coli* isolates; and for presumptive generic identification of other Enterobacteriaceae isolates from foods. (*2*) *Salmonella* 1-2 TEST for screening all foods for the presence of motile *Salmonella.* (*3*) TECRA *Salmonella* Visual Immunoassay system for screening all foods for the presence of *Salmonella.* (*4*) Q-TROL *Salmonella* Detection Kit system for screening all foods for the presence of *Salmonella.* (b) Adopt as official final action the official first action enzyme immunoassay screening method for *Salmonella* in low-moisture foods, **46.C17-46.C25**. (c) Subdivide topic as *Salmonella, Bio-EnzaBead Enzyme Immunoassay Screening Method; Salmonella, GENE-TRAK DNA Hybridization Screening Method; Salmonella, ImmunoBand Screening Method; Salmonella, Q-TROL Enzyme Immunoassay Screening Method; Salmonella, TECRA Enzyme Immunoassay Screening Method;* and *Salmonella, Escherichia coli, and Other Enterobacteriaceae, Identification by Micro ID Diagnostic Kit.* Continue study.

(5) *Escherichia coli, Enzymatic Methods:* Redefine topic as *Escherichia coli in Chilled and Frozen Food, MUG Test.* Continue study.

*(6) *Genetic Methods for Detection of Bacterial Pathogens:* Adopt as official final action the official first action DNA hybridization method for *Salmonella* in foods, **46.C07-46.C16**. Redefine topic as *Enterotoxigenic Escherichia coli, Detection by DNA Hybridization Method.* Continue study.

(7) *Hydrophobic Grid Membrane Filter Methods:* Subdivide topic as *Aerobic Plate Counts and Coliforms, Hydrophobic Grid Membrane Filter Method;* and *Salmonella, Hydrophobic Grid Membrane Screening Method.* Continue study.

(8) *Listeria, Listeria-Tek Assay:* Continue study.

(9) *Petrifilm Methods:* Redefine topic as *Aerobic Plate Counts and Coliforms in Nondairy Foods, Petrifilm Methods.* Continue study.

(10) *Redigel Media:* Redefine topic as *Aerobic Plate Counts of Foods, Determination Using Redigel Medium.* Continue study.

(11) *Salmonella in Chocolate:* Continue study.

(12) *Shellfish:* Redefine as *Escherichia coli in Shellfish, MUG Test.* Continue study.

(13) *Vibrio cholerae and Its Toxins:* Redefine as *Vibrio cholerae in Oysters.*
Elevated Temperature Enrichment Method. Continue study.

(14) *Yeasts, Molds, and Actinomycetes:* Redefine topic as *Yeasts and Molds, Mycological Media for Enumeration.* Continue study.

(15) *Yersinia enterocolitica:* Continue study.

*(16) *Other Topics:* Adopt as official final action the following official first action methods: microbiological methods for frozen, chilled, precooked, or prepared foods, **46.013-46.015**; thermophilic bacterial spores in sugars, **46.078-46.082**; virus in ground beef, **46.188-46.190**; gas chromatographic method for sporeformers in low-acid canned foods, **46.A01-46.A05**; and poliovirus 1 in oysters, **46.A12-46.A22**.

Committee on Feeds, Fertilizers, and Related Materials: Recommendations for Official Methods

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ANTIBIOTICS IN FEEDS

(1) *Apramycin:* Continue study.
(2) *Bacitracin:* Continue study.
(3) *Bacitracin, Chemical Method:* Continue study.
(4) *Bambermycins:* Continue study.
(5) *Chlortetracycline:* Continue study.
(6) *Coban:* Combine with topics Monensin and Rumensin.
(7) *Cup Plate System for Antibiotic Analysis:* Continue study.
(8) *Direct-Fed Microbiological Products and Silage Inoculants*: Continue study.
(9) *Design and Computerization of Microbiological Tests:* Continue study.
(10) *Erythromycins:* Continue study.
(11) *Lasalocid, Microbiological Assay:* Continue study.
(12) *Lincomycin:* Continue study.
(13) *Microbiological Assays*: Continue study.
(14) *Monensin:* Combine with topics *Coban* and *Rumensin.*
(15) *Narasin:* Continue study.
(16) *Neomycin:* Continue study.
(17) *Oxytetracycline:* Continue study.
(18) *Rumensin:* Combine with topics Coban and Monensin.
(19) *Screening Procedures for Antibiotics:* Continue study.
(20) *Statistics of Microbiological Assay:* Discontinue topic.
(21) *Tylosin:* Continue study.
(22) *Virginiamycin, Diffusion Assay:* Combine with next topic.
(23) *Virginiamycin, Turbidimetric Assay:* Combine with previous topic.
*(24) *Other Topics:* Initiate the topics Microbiological Assay Design and Statistics; Microbiological Plate Count for Silage Inoculants. Adopt the following changes in the calculations for **42.207-42.208**: Prep. concns of ref std as described for each antibiotic. In general, it is preferable to use shorter 4-fold range between lowest and highest doses of std line. Use indicated concn as ref concn. (Values of std or ref. concn could slightly vary from those indicated for each antibiotic without affecting validity of assay.)

Prep. plates with appropriate base agar layer and/or appropriate seed agar layer; one layer of media can be substituted for 2 layers of media if ref. concn gives adequate zone size as described for each antibiotic. Distribute agar evenly by tilting plates from side to side with circular motion and let harden. Use plates same day prepd.

Place 6 cylinders on each plate at ca 60° intervals on 2.8 cm radius. Fill 3 alternate cylinders with ref. concn and other 3 cylinders with one of other concns of std. Use 3 plates for each concn required for std response line, except ref. concn. Incubate plates overnight at appropriate temp., and measure diams of zones of inhibition as accurately as possible. (In most cases, it is possible to est. zone diams to nearest 0.1 mm.) Values given in each method for zones of inhibition to be obtained with ref. concns of antibiotics are for guidance only, but it is important that lowest concns on std response line give measurable zone and that slope of response line be adequate. In each set of 3 plates, average the 9 readings of ref concn and the 9 readings of concn being tested. Av. of all 36 readings of ref. concn from 12 plates is correction point for response line. Correct av. value obtained for each concn to appropriate figure if ref. concn reading on that set of 3 plates was same as correction point.

For example, if in correcting second concn of std response line, av. of 36 readings of ref. concn is 20.0 mm, and av. of 9 readings of ref concn of this set of 3 plates is 19.8 mm, correction is +0.2 mm. If av. reading of second concn on same 3 plates is 17.0 mm, corrected valued is 17.2 mm. Plot corrected values, including correction point, on semilog graph paper, using logarithmic scale for concn and arithmetic scale for av. zone diams. Manual plotting of std lines is possible but could be subject to large variation. Response lines would be more accurate if calcd. When std doses are equally spaced, i.e., interval between successive doses is the same, calc. L and H (calcd zone diams for low and high concn, resp., of std response line) as follows:

For method specifying 5 doses of std,

$$L = (3a + 2b + c - e)/5$$
$$H = (3e + 2d + c - a)/5$$

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

The recommendations submitted by the Committee on Feeds, Fertilizers, and Related Materials were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th ed., and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

where a, b, c, d, and e = corrected av. zone diams. for each concn of std.

For methods specifying 4 doses of std,

$$L = (7a + 4b + c - 2d)/10$$
$$H = (7d + 4c + b - 2a)/10$$

For methods specifying 3 doses of std,

$$L = (5a + 2b - c)/6$$
$$H = (5c + 2b - a)/6$$

Plot values for L and H and connect with straight line. Ref. point is zone size intercept on arithmetic scale. This corrected ref. point is to be used for sample calcns. (If corrected ref. point diam. varies significantly from ref. diam., error in prepn of std solns is indicated and validity of assay is in question.) For more accuracy in calcn, det. slope of std response line $B = (H - L)/(\text{Log } h - \text{Log } l)$ where l and h are high and low std concns, resp., and B is increase in zone for each 10× increase in drug concn.

Computer or calculator can be used to calc. std lines whether std concns are equally spaced or not. Least square fitting using linear or polynomial equations may be performed based on best fit (polynomial fitting is most appropriate, especially for long range 8× or 16× range).

42.208 Determination of Potency

Use 3 plates of each assay soln. On each plate, fill 3 alternate cylinders with ref. concn and fill other 3 cylinders with assay soln. Incubate plates overnight at appropriate temp. and measure diam. of zones of inhibition. Average the 9 readings of ref. concn and the 9 readings of assay soln. If assay soln gives larger av. than ref. concn, add difference between them to ref. point on std response line. If assay soln gives smaller value than ref. concn, subtract difference between them from ref. point on std response line. Using corrected values of assay soln, det. amt of antibiotic by reading concn from std response line. Alternatively, instead of manual reading of unknown, det. log relative potency, $M' = (Yu - Ys)/B$, where Yu and Ys are av. of 9 readings of assay soln and ref concn, resp., and b is slope of std response line. Antilog M′ = potency of assay soln relative to std; and (Antilog M′) × 100 = potency of assay soln as % of std ref. concn.

For calcn of sample potency by computer or calculator, enter sample data and calc. antibiotic potency based on least square linear or polynomial lines.

For calcns, 1 ton = 908 000 g; 1 lb = 454 g.

DRUGS IN FEEDS

*(1) *Amprolium:* Add the following statement to section **42.014** of the official final action amprolium method (**42.011-42.015**): "Column recovery of amprolium may vary among different brands of basic alumina. Test column recovery by spiking an extract from a nonmedicated feed."

(2) *Arsanilic Acid:* Appoint Associate Referee.

(3) *Bacitracin:* Continue study.

(4) *Carbadox:* Appoint Associate Referee.

(5) *Ethopabate:* Continue study.

(6) *Ethylenediamine Dihydroiodide:* Appoint Associate Referee.

(7) *Furazolidone and Nitrofurazone:* Continue study.

(8) *Lasalocid (LC Method):* Move General Referee responsibility from "Antibiotics in Feeds" to "Drugs in Feeds." Continue study.

(9) *Melengestrol Acetate:* Continue study.

(10) *Morantel tantrate:* Continue study.

(11) *Oxytetracycline (LC Method):* Initiate topic under General Referee responsibility for Drugs in Feeds and appoint Mary Lee Hasselberger as Associate Referee.

*(12) *Nithiazide:* Declare as surplus the official final action nithiazide method, **42.122-42.125**.

*(13) *Nitrodan:* Declare as surplus the official final action nitrodan method, **42.126-42.129**.

(14) *Pyrantel Tartrate:* Continue study.

*(15) *Reserpine:* Declare as surplus the official final action reserpine method, **42.148-42.151**.

*(16) *Ronnel:* Declare as surplus the official final action reserpine methods, **42.152-42.154** and **42.155-42.159**.

*(17) *Roxarsone:* Adopt as official final action the official first action roxarsone atomic absorption method, **42.B01-42.B.10**.

(18) *Sampling:* Continue study.

*(19) *Sulfadimethoxine:* Add the following note to the official first action sulfadimethoxine method (**42.168-42.170**): "The ficin product listed in **42.168**(a) is no longer available."

(20) *Sulfa Drug Residues:* Appoint Associate Referee.

*(21) *Sulfaguanidine:* Declare as surplus the official first action sulfaguanidine method, **42.171**.

*(22) *Sulfamethazine and Sulfathiazole:* Add "Not applicable to feed samples made from "granule stabilized" Tylan-Sulfa premix." to the official first action sulfamethazine method, **42.172-42.174**.

FEEDS

(1) *Amino Acid:* Continue study.

(2) *Calcium Salts of Isobutyric and Mixed 5-Carbon Volatile Fatty Acids:* Continue study.

(3) *Carotinoids:* Continue study.

(4) *Enzymes and Microbiological Additives:* Continue study.

(5) *Fat:* Continue study.

(6) *Fiber, Crude:* Continue study.

*(7) *Infrared Reflectance Techniques in Mixed Feeds:* Adopt as official first action the interim first action near infrared reflectance spectroscopy method for analysis of acid detergent fiber in crude protein in forages.

(8) *Iodine and EDDI:* Continue study.

(9) *Microscopy:* Continue study.

(10) *Minerals:* Continue study.

(11) *Moisture in Mixed Feeds and Forages:* Continue study.

(12) *Moisture in Pet Foods:* Continue study.

(13) *Non-Nutritive Residues:* Continue study.

(14) *Protein, Crude:* Continue study.

(15) *Sampling:* Continue study.

(16) *Vitamins:* Adopt as official first action the interim first action method for supplemental vitamin E acetate in feed concentrates.

(17) *Water by Karl Fisher Method:* Continue study.

*(18) *Other Topics:* Add the following applicability statement to **7.125**: "Dry ashing procedure is not applicable for feeds or mineral mixes containing monobasic calcium phosphate."

FERTILIZERS

(1) *Biuret:* Continue study.
(2) *Boron:* Continue study.
(3) *Dicyclodiamide:* Appoint new Associate Referee.
(4) *Free and Total Water:* Appoint new Associate Referee.
(6) *Melamine:* Continue study.
(7) *Nitrogen:* Continue study.
(8) *Phosphorus:* Continue study.
(9) *Potash:* Continue study.
(10) *Sampling:* Continue study.
(11) *Sample Preparation:* Continue study.
(12) *Slow Release Mixed Fertilizers:* Continue study.
(13) *Sodium:* Continue study.
(14) *Soil and Plant Amendment Ingredients:* Continue study.
(15) *Sulfur:* Continue study.
(16) *Water-Soluble Methylene Ureas:* Continue study.
(17) *Zinc:* Continue study.
*(18) *Other Topics:* In the urease method, **2.080-2.081**, change sample size to be weighed from 10 g to 1–10 g with restriction to limit urea content to 1 g.

PLANTS

(1) *Ashing Methods:* Continue study.
(2) *Atomic Absorption Methods:* Continue study.
(3) *Boron:* Continue study.
(4) *Chromium:* Continue study.
(5) *Emission Spectroscopy:* Continue study.
(6) *Fluoride:* Continue study.
(7) *Nitrate and Nitrite:* Continue study.
(8) *Selenium:* Continue study.
(9) *Starch:* Continue study.
(10) *Sulfur:* Continue study.
*(11) *Other Topics:* Declare the following methods surplus: Sand and Silica–Gravimetric (**3.005**); Magnesium–Gravimetric (**3.039**); Sodium–Uranyl Acetate (**3.052-3.053**); and Chloride–Gravimetric (**3.069-3.070**).

TOBACCO

(1) *Alkaloids:* Continue study.
(2) *Polyphenols:* Continue study.
(3) *Tar and Nicotine in Cigarette Smoke:* Continue study.

VETERINARY ANALYTICAL TOXICOLOGY

(1) *Animal Serum Thyroxine:* Continue study.
(2) *Antibiotic Screening Methods:* Continue study.
*(3) *Arsenic in Animal Tissue:* Adopt as official final action the official first action method for arsenic in liver, **49.B01-49.B05**.
(4) *Atomic Absorption Spectrophotometry:* Continue study.
(5) *Clorinated Phenols in Animal Tissue:* Continue study.
(6) *Cholinesterase (Colorimetric method):* Continue study.
(7) *Cholinesterase (pH Method):* Continue study.
(8) *Copper in Animal Tissue:* Continue study.
(9) *Cyanide:* Continue study.
(10) *Fluoride in Animal Tissue:* Continue study.
(11) *GC/MS Methods:* Continue study.
(12) *Lead in Animal Tissue:* Continue study.
(13) *Lipid-Soluble Vitamins:* Continue study.
(14) *Multielement Analysis by ICP:* Continue study.
(15) *Multiple Anticoagulate Screening:* Continue study.
(16) *Natural Products:* Continue study.
*(17) *Nitrates and Nitrites:* Adopt as official final action the official first action method for nitrate in forage, **49.B06-49.B13**.
(18) *Pesticides in Toxicological Samples:* Continue study.
(19) *Selenium in Animal Tissue:* Continue study.
(20) *Sodium Monofluoroacetate:* Continue study.
(21) *Vitamins D and K:* Continue study.
(22) *Zinc in Animal Tissue:* Continue study.

Committee on Hazardous Substances in Water and the Environment: Recommendations for Official Methods

ERIKA E. HARGESHEIMER (City of Calgary, Glenmore Waterworks Laboratory (35), PO Box 2100, Calgary, Alberta T2P 2M5, Canada), *Chairman*;
NILE FRAWLEY (Dow Chemical Co., 1897 Bldg, Midland, MI 48640);
DAVID FRIEDMAN (U.S. Environmental Protection Agency, Office of Solid Waste, 401 M St SW, Washington, DC 20460);
ROBERT GRAVES (U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, 26 W Martin Luther King Dr, Cincinnati, OH 45268);
ANN B. STRONG (U.S. Army Corps of Engineers, Waterways Experiment Station, PO Box 631, Vicksburg, MS 39180);
MARK F. MARCUS (Chemical Waste Management, Inc., 150 W 137th St, Riverdale, IL 60627), *Secretary*;
FOREST C. GARNER (Lockheed Industries, 3877 Platis Circle, Las Vegas, NV 89103), *Statistical Consultant*

The Committee accepted the resignation of Mark Marcus and acknowledged his many contributions. Douglas Dube, past chairman, and Nile Frawley also ended their terms of service on the committee. The Committee decided to communicate quarterly by conference calls to be arranged by Robert Graves.

The Committee continues to review studies completed by the U.S. Environmental Protection Agency (EPA), which meet AOAC criteria for collaborative studies. Currently under review are ICP method 6010 and digestion method 3050; EPA Method 602, Purgeable Aromatics; and Method 200.7, Trace Metals by ICP. The Committee asked that the AOAC Methods Coordinator circulate methods for review to General Referee Viorica Lopez-Avila and Statistical Consultant Forest Garner (over the next 3 months at monthly intervals): EPA Method 624, Volatiles by GC-MS; EPA Method 625, Acids/Base-Neutral by GC-MS; EPA Method 601, Trihalomethanes by GC-Hall Electroconductivity Detection.

The Committee recommends consolidating its general and associate refereeships by discontinuing 2 general referee topics and combining others to form 5 more active refereeships. After topics under Soils and Sediments and Waste Materials have been combined as a new topic Wastes and Solid Materials, the Committee members will review Associate Referee progress until a new General Referee is appointed.

AIR

Continue study.

BIOTA

Discontinue general refereeship.

HAZARDOUS SUBSTANCES

(1) *Benzene in Consumer Products:* Discontinue topic.
(2) *Nitrosamines in Infant Pacifiers:* Discontinue topic.
(3) *Pentachlorophenol in Toy Paints:* Discontinue topic.
(4) *Toxic Metals in Paints:* Discontinue topic.

INORGANICS IN DRINKING AND GROUND WATER

Combine with the topic Inorganics in Surface and Waste Water (general refereeship vacant) as a new topic Inorganics in Water.

(1) *Arsenic and Selenium Methods:* Continue study.
(2) *Inductively Coupled Plasma and Ion Chromatography Methods:* Continue study.

JOINT STUDIES

This special general referee topic was established to review EPA methods for pesticides in well water. Co-Associate Referees have been appointed and are preparing a collaborative study on Method 2. Determination of chlorinated pesticides in ground water by gas chromatography with an electron capture detector. Two other methods will be studied as well. Study progress is described in the report of the General Referee on Joint Studies.

ORGANICS IN DRINKING AND GROUND WATER

(1) *Environmental Protection Agency Methods:* Continue study.
(2) *Phenols:* Continue study.
(3) *Polychlorinated Biphenyls, Quantitation:* Transfer topic to the new general refereeship Wastes and Solid Materials. Submit protocol for a collaborative study.
(4) *Polynuclear Aromatic Compounds, Screening Methods:* Continue study.
(5) *Total Organic Halogen:* Continue study.
(6) *Volatile Organics, Capillary Analysis:* Continue study.

ORGANICS IN SURFACE AND WASTE WATER

(1) *Chemical Pollutants:* Discontinue topic.
(2) *Chlorinated Pesticides in Groundwater, GC/EC Method:* Continue study.
(3) *Chlorinated Solvents:* Continue study.
(4) *Munitions:* (a) Continue study on method for munitions in soils and sediments (protocol has been submitted to AOAC and accepted by the Committee). (b) Evaluate need for minicollaborative study to update LC conditions in official first action method **33.B01-33.B08**, TNT, RDX, HMX, and 2,4-DNT in wastewater and groundwater. Continue **33.B01-33.B08** in official first action status.
(5) *Organohalogen Pesticides:* Discontinue topic.
(6) *Other Topics:* (a) Revise manuscript on LC determination of glyphosate and aminomethyl phosphonic acid in environmental water as requested by the General Referee and Committee Statistician. The study was conducted outside AOAC and was previously published. (b) Appoint an Associate Referee on herbicide residues in environmental waters. Prepare and submit a study protocol. (c) Submit protocols for 2 new LC methods: diquat and paraquat in water and

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."
The recommendations submitted by the committee on Hazardous Substances in Water and the Environment were adopted by the Association.
Section numbers refer to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349-390 (B methods).

formaldehyde in waste water. Appoint Associate Referees on these topics.

SOILS AND SEDIMENTS

Combine with topics now under Waste Materials to form a new topic Wastes and Solid Materials. Appoint a General Referee.

(1) *Chlordane in Soils:* Continue study.
(2) *Explosives Residues:* Continue study.
(3) *Nonvolatile Organics, Distribution Coefficients:* Continue study.
(4) *Soils, Distribution Coefficients:* Continue study.
(5) *Termiticides: Continue study.*
(6) *Volatile Organics, Adsorption Isotherms:* Continue study.
(7) *Volatile Organics, Distribution Coefficients:* Continue study.

WASTE MATERIALS

Combine with topics now under Soils and Sediments to form a new topic Wastes and Solid Materials. Appoint a General Referee.

(1) *Bioassay Methods:* Continue study.
(2) *Inorganic Analytes:* Continue study.
(3) *Organic Analytes:* Continue study.
(4) *Physical/Chemical Properties:* Discontinue topic.
(5) *Prescreening:* Continue study.

Executive Director's Report

RONALD R. CHRISTENSEN
Association of Official Analytical Chemists, 1111 N. 19th St, Suite 210, Arlington, VA 22209

This has been a very difficult talk for me to prepare. Unlike President Rund, in preparing his opening remarks to the convention, I made the grave mistake of referring to previous reports of the executive director, which went into great detail on the accomplishments of the Association over the previous year. I really did not feel very comfortable standing before you today and reading a report of things I had little involvement in or responsibility for. I also noted there were some 25 speakers after me on the agenda, and I assume they are going to tell us a great deal about the work of the Association over the past year and what we need to do in the future.

What I have decided to do, instead, is to select just a few topics which are very important to AOAC, to give you a very brief status on each, and to offer a few observations. These observations are, of course, from one who is very new to your ranks, one who admittedly has a lot to learn (but wants to learn), and one who may really miss the mark with what he is about to say.

There seem to be certain fundamental elements to most every scientific association: members, the science involved (methods, in this case), publications, and meetings. These are the basic qualities of a scientific association, and they seem to be the basic ingredients of AOAC. So, those are the elements I propose to mention briefly this afternoon: membership, methods, publications, and meetings.

Membership

This has not been a bad year for membership. The "equalization" of the membership requirements went into effect the first of the year, and current individual memberships (as of the end of July) stand at 3289 for a net gain of 226 over the past year. Sustaining members went from 201 to 213, for a net gain of 12, during the same period. That is fairly good.

If I had an observation, however, it is that we can do much better. I cannot help but think there are hundreds and even thousands more individuals, organizations, companies, and agencies out there, around the world, whom we have something to offer and who have something to bring to the Association.

A little-recognized fact in this business is that few individuals and organizations join an association unless they are informed of the benefits and *someone asks them to join.* A survey done a couple of years ago by the American Society of Association Executives' Foundation and a similar one done by my former association and others bear this out. The vast majority of individuals and organizations surveyed who were eligible for membership in a professional or scientific association, but did not belong, responded that no one had ever asked them to join.

We, you and I, have got to make the sale. We have got to ask our friends and colleagues and employers to join AOAC so that we may all benefit from a stronger, healthier, and more broadly representative association.

Methods

Membership leads somewhat naturally to methods—the foundation upon which this association was founded 104 years ago. I was recently attracted to the comments made by Joseph Hile in his banquet address of a couple of years ago:

> I am a member of AOAC because I believe in its basic goal: an association of scientists and science administrators dedicated to bringing order to a world of analytical methods—methods that have been studied in a variety of laboratory settings; methods that are suitable and have the accuracy and precision to be used as standards in a legal setting; methods that have been validated and peer-reviewed; methods so certain in their validity that I am willing, as the Associate Commissioner of Regulatory Affairs, to recommend in a court of law that a person's freedom be restricted or that property be seized on the basis of the result.

AOAC is all about methods and the method process. Again, this year has not been a bad year (by recent, past standard) for AOAC methods development. I am told that we will be offered some 15 methods for approval official first action, and, as of the end of June, there were some 41 collaborative studies under consideration for adoption as interim official first action. Another 41 protocols were under review or awaiting the results of collaborative studies at the end of June.

I must tell you, however, that as a neophyte to the analytical method world, but also as one who keeps himself somewhat up to date on general scientific advances in many fields, those seem like neither very large nor very representative numbers. This rapidly changing world has an ever-increasing need for new and better forms of analysis.

We have done very well for the past 104 years, but we must do even better. My congratulations and sincere thanks go out to all those volunteers who have worked so hard this past year within the collaborative process. The challenge I see ahead—a challenge that is not unique to AOAC, but common to every volunteer, scientific organization—is to continue to bring order to the world of analytical methods while we make the process more responsive to the realities and the needs of the ever-changing world.

I think that can be done. It will take the continuing effort of all of us, but it can be done.

Publications

Just as membership led somewhat naturally to methods, methods lead somewhat naturally to publications. Our science and the methods we produce can do the world no good if we do not publish our results. That fact may be even more basic to me than it is to you because about 50% of AOAC's revenues come from the sale of periodic and non-periodic publications, and, so, about 50% of my salary is dependent upon this fact. It is publish or perish for AOAC and me.

The AOAC *Journal* now goes to some 3850 subscribers in 90 countries. Forty-one percent of those subscribers are in the United States while 59% are outside the United States. It is very much an international journal, and it produced 24% of AOAC's total income last year.

The 14th edition of *Official Methods of Analysis* is now in its 5th year of sales. Some 16 000 copies have been sold worldwide. Work on the 15th edition started in January of this year. That work is progressing well, and the new edition should be available in early 1990. *Official Methods of Analysis,* over its 5-year life cycle, is vitally important to the financial health of AOAC. But, to the ordinary buyer of a new edition, it is only worth the value he or she will find in the new and improved methods contained therein. Therefore, new and improved methods are vitally important not only to the world that uses them but also to the long-term financial health of your Association. We must produce new and improved methods in a timely manner.

Before I leave the topic of publications, I want to tell you we have had good success for an association of our size with a number of the smaller, non-serial publications such as the

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Use of Statistics to Develop and Evaluate Analytical Methods, the *Quality Assurance Manual,* and "*BAM,*" the *Bacteriological Analytical Manual.* We need to do more of these small publications, and we need your help. We need to know what is needed out there in the field, and we need individuals and committees willing to commit the time to outline, organize, and author these texts. Just as methods are of no use to anyone if they are not published, unmet needs are never fulfilled if no one puts pen to paper and publishes their individual or collective knowledge. We need you to do that as a scientific community and as a service to that community.

Meetings

Members led us to methods, which led us to publications. It seems clear, however, that when it comes to meetings, the arrow goes both ways or perhaps the circle is closed and the linear progression I started this talk with was never really a straight line. In any event, associations are all about meetings. It is the tried and true method by which we as scientists and managers exchange ideas. The opportunity to meet our peers and discuss our problems and solutions is what normally brings us to the association in the first place.

I suggest that few of us who have been here the past few days could honestly say this has not been a successful meeting—at least overall. I have no reference for comparison with regard to past AOAC meetings, but I have been involved in organizing and conducting 10 major scientific conventions over the past 10 years, and this is certainly among the best, if not the best. We have been exposed to 5 symposia with 12 program units and 71 papers. We have had 10 poster sessions covering 8 AOAC Methods Committee topics with 266 papers. We have had a regulatory roundtable on OSHA safety regulations, a modern laboratory workshop, and nearly 100 exhibitors present. Last, but surely not least, we have been exposed to the wisdom, knowledge, camaraderie, and sound advice of some 1200 old and new friends; perhaps the best reason to meet.

AOAC's meetings, short courses, and symposia are generally sound and well attended. It is another area in which we can do more. We need to improve and update our current short courses. We need to develop new courses to meet the educational needs of the members and others. We need more symposia outside the umbrella of the international meeting in order to share the latest information that is available in particular topic areas. And, we need you, the volunteer member, to do this. One of our most pressing needs at this time is the identification and development of new topics in the meeting and short course area. And, an equally pressing need is the development of a bigger pool of experts willing to share their knowledge and teach others. There is no time like the present if you have ideas and want to get involved. Just contact Marge Ridgell at the AOAC headquarters. I know she and a lot of others would welcome your involvement.

Conclusion

Although members, meetings, methods, and publications are far from everything that AOAC is about, they are the factors that make up the foundation of the Association. They are our roots, and the success of these elements bears heavily on our credibility and our ability to be successful in other areas.

From where I sit, I see these as exciting times for AOAC—times of rapid change and advancement. Despite its 104 years of history, AOAC, in many respects, is a young and developing organization. I see my job, and the job of all AOAC staff, as doing our utmost to assist you in making AOAC all you want it to be. As a newcomer, but one who has studied many other associations, I think we have lots of room for growth and change, and there are a lot of opportunities out there for AOAC to seize.

In the final analysis, however, the choice of directions and how fast we move will have to be yours. And that is the way it should be. The means and the methods are really in your hands.

Secretary/Treasurer and the Finance Committee

THOMAS G. ALEXANDER, *Secretary/Treasurer*
Food and Drug Administration, Center for Drug Evaluation and Research, Washington, DC 20204

Other Members: E. R. Elkins; J. E. McNeal; A. Munson

Recommendation for the Board of Directors

The Finance Committee recommends that Bisselle, Meade & Company, the Association's present auditing firm, be retained for calendar year 1989. The firm has served as our auditors for the past several years, knows our system, and has produced very informative and helpful reports and critiques.

Discussion

During the 1987–1988 year, the Committee met each quarter and reviewed the quarterly "Report of Examination." Committee comments were forwarded to the Board of Directors. The Association's tangible securities were examined quarterly and found to be in order. In addition, matters of reimbursement policy for employee and volunteer travel, authorization of procurement, and time card documentation were dealt with during this year in response to the Long Range Strategic Plan and the auditor's Management Letter.

STATEMENT OF FINANCIAL CONDITION—SEPTEMBER 30, 1988

Assets

Current Assets:		
Cash, Signet Bank*		$ 4,866
Cash, 1st American Bank*		20,222
Cash, 1st American Bank, payroll*		175
Cash, Office fund		500
Cash, Regional sections		27,493
Accounts Receivable		
Books and publications		44,898
Contracts and support		23,793
Sustaining Members		22,850
Membership		1,777
Contributions—Annual Meeting		4,000
Other		24,654
Accrued interest receivable		4,202
Inventory, books and publications—at cost		135,309
Prepaid expenses		15,059
Deposits		17,104
Total Current Assets		$ 346,902
Investments:		
Securities	$ 65,956	
Certificates of Deposit	1,509,407	$1,575,363

Fixed Assets:		
Office furniture, fixtures, and equipment	$ 66,456	
Computer and computer software	351,421	
	$ 417,877	
Less: accumulated depreciation	−296,791	$ 121,086
Deferred Costs:		
Official Methods of Analysis—15th Edition	$ 95,410	
Annual Meeting—1989	4,666	
Annual Meeting Exhibit Booths—1989	6,738	
Sampling Manual	5,693	
QA Prin Update	1,937	
AOAC Handbook	7,927	
Principles of Food Analysis	83	
Q/A Short Courses	16,240	$ 138,694
Total Assets		$2,182,045

* Interest bearing accounts

Liabilities and Fund Balances

Current Liabilities:		
Accounts payable		$ 59,768
Accrued and withheld payroll taxes		−0−
Total Current Liabilities		$ 59,768
Deferred Income:		
Journal subscriptions	$ 117,667	
Sustaining Members	50,321	
Membership	57,623	
Short courses	14,725	
Annual Meeting Exhibits—1989	12,818	$ 253,154
Reserve for Publications		$ 200,000
Restricted Reserve for FAAM		$ 4,043
Restricted Reserve for MAM		$ 2,325
Restricted Reserve for ADAM		$ 4,430
Restricted Fund—Harvey Wiley		$ 47,374
Restricted Fund—15th Edition		$ 618,139
Fund Balance:		
Balance, October 1, 1987	$ 895,668	
Add: adjustment for regional cash funds (net)	694	
restricted fund—liability insurance	4,016	
	900,378	
Add: excess of income over expenses for the twelve months ended September 30, 1988	96,432	
	996,810	
Less: adjustment for Restricted Reserves	−3,998	
Balance, September 30, 1988		$ 992,812
Total Liabilities and Fund Balances		$2,182,045

SUSTAINING MEMBERS AND GOVERNMENT SUPPORTING AGENCIES OF AOAC

September 29, 1988 (211)

ABC Research Corp.
Agriculture Canada
Agway, Inc.
Alabama Dept of Agriculture and Industries
Alberta Agriculture
Alberta Dairymen's Association Research Unit (ADARU)
Alcon Laboratories, Inc.
American Council of Independent Laboratories, Inc.
American Crystal Sugar Co.
American Cyanamid Co.
American Maize Products
Anheuser-Busch, Inc.
Archer-Daniels-Midland
Arizona State Agriculture Laboratory
Arkansas State Plant Board
Association of American Feed Control Officials, Inc.
Association of Public Analysts
Avon Products, Inc.
BASF Corp.
Chemicals Division
Beckman Instruments, Inc.
Biochem Laboratorium BV
Bio-Rad Laboratories
Blue Diamond Growers
Boehringer Mannheim
Borden, Inc.
Bran & Luebbe/Technicon
Bristol-Myers Co.
Bristol-Myers U.S. Pharmaceutical & Nutritional Group
Burdick & Jackson
Division of Baxter Healthcare Corp.
Cacao De Zaan BV
California Dept of Food and Agriculture
Calreco, Inc.
CAMBRIDGE-NAREMCO
CAMCO
Campbell Institute for Research & Technology
Campbell Taggart, Inc.
Cargill, Inc.
Carrington Laboratories, Inc.
Castleton Beverage Corp.
CEM Corp.
Chemical Waste Management Inc.
Chesebrough-Pond's, Inc.
Chevron Chemical Co.
CIBA-GEIGY Corp.
Agricultural Division
The Coca-Cola Co.
Colorado Dept of Agriculture
Comibassal International
Compu-Chem Laboratories, Inc.
ConAgra Consumer Frozen Foods Co.
Continental Baking Co.
Corn Refiners Association, Inc.
CPC International, Inc.
DFA of California

Delaware Dept of Agriculture
Dionex Corp.
Dow Chemical Co.
Duphar BV
E.I. DuPont de Nemours & Co.
DuPont Pharmaceuticals
Dynatech Laboratories, Inc.
Eastman Chemical Products, Inc.
Eli Lilly and Co.
  Elanco Products Company Division
Environmental Testing & Certification Corp.
FMC Corp.
  Agricultural Chemical Group
FMC Corp. Food & Pharmaceutical Products
  Division
The Fertilizer Institute
Fisher Scientific Co.
Florida Dept of Agriculture
  and Consumer Services
A/S N. Foss Electric
Foss Food Technology Corp.
GAF Corp.
Galbraith Laboratories, Inc.
E & J Gallo Winery
General Foods Corp.
General Mills, Inc.
GENE-TRAK Systems
Georgia Dept of Agriculture
Gerber Products Co.
Gist Brocades USA
Good Housekeeping Institute
Haarman & Reimer Corp.
Hawaii Dept of Agriculture
Hawaii Dept of Health
Hazleton Laboratories America, Inc.
Health and Welfare Canada
  Health Protection Branch
Heinz USA
Hershey Foods Corp.
Heublein Wines
Hill Top Biolabs, Inc.
Hoechst-Roussel Pharmaceuticals, Inc.
Hoffmann-La Roche, Inc.
Holly Sugar Corp.
ICI Americas, Inc.
IMC Fertilizer, Inc.
Illinois Dept of Agriculture
Indiana Office of the State Chemist
Indiana State Board of Health
Instituto di Tecnica e Sperimentazione Lattiero-Caseari
  di Thiene
Iowa Dept of Agriculture
Iowa State Veterinary Diagnostic Laboratory
S. C. Johnson & Son, Inc.
Kansas State Board of Agriculture
Kellogg Co.
Kemira Oy
Kentucky Agricultural Experiment Station
  Division of Regulatory Services
Kentucky Dept of Agriculture
Kraft, Inc.
The Kroger Co.
Laboratio di Chimica Analitica Applicata SNC
Laboratory of the Government Chemist
Laboratory Specialists, Inc.
Lancaster Laboratories, Inc.
Land O'Lakes, Inc.
Lehn & Fink Products Co.
Thomas J. Lipton, Inc.
Loma Linda Foods, Inc.
Marion Laboratories, Inc.
Maryland Dept of Agriculture
Massachusetts Dept of Food and Agriculture
McCain Foods Western, Inc.
McCormick & Company, Inc.
McKee Baking Co.
MCLAS Technologies, Inc.
McLaughlin Gormley King Co.
McNeil Consumer Products Co.
Merck Sharp & Dohme Research Laboratories
Mettler Instrument Corp.
Michigan Dept of Agriculture
Miles Laboratories, Inc.
Ministry of Agriculture, Fisheries and Food
Minnesota Dept of Agriculture
3M Co.
Mississippi State Chemical Laboratory
Missouri Experiment Station
  Chemical Laboratory
Mobay Corp.
Monsanto Agricultural Company
Montana Dept of Agriculture
Moorman Manufacturing Co.
Nabisco Brands, Inc.
National Food Processors Association
National Laboratory for Agricultural Chemistry
National Starch & Chemical Corp.
Nebraska Dept of Agriculture
Nestec Ltd
New Jersey Dept of Agriculture
New Mexico Dept of Agriculture
New York Dept of Agriculture and Markets
New Zealand Milk Products, Inc.
Nicolet Instrument Corp.
North Carolina Dept of Agriculture
North Dakota State Laboratories Dept
Novo Biochemicals
O.M. Scott & Sons Co.
Ocean Spray Cranberries, Inc.
Oklahoma Dept of Agriculture
Ontario Ministry of Agriculture and Food
Oregon Dept of Agriculture
Organon Teknika Corp.
Orion Research Inc.
Ortho Pharmaceutical Corp.
Overseas Merchandise Inspection Co., Ltd.
Oxoid USA, Inc.
Penicillin Assays Inc.
Pennsylvania Dept of Agriculture
Pennsylvania Liquor Control Board
Pennwalt Corp.
Pepsico
Pfizer Inc.
Pharmacia AB
Philip Morris USA
The Pillsbury Co.
Pitman-Moore, Inc.
The Procter & Gamble Company
Quaker Oats Co.
Quebec Dept of Agriculture
Ralston-Purina
Rhone-Poulenc Ag Co.

R. J. Reynolds Industries, Inc.
R. J. Reynolds Tobacco Co.
Ross Laboratories
Sandoz Crop Protection Corp.
Schenley Distillers
Joseph E. Seagram & Sons, Inc.
Shaklee Corp.
Shasta Beverages, Inc.
ShriRam Institute for Industrial Research
Silliker Laboratories, Inc.
SmithKline Beckman Animal Health Products
South Carolina Dept of Agriculture
South Dakota State Chemical Laboratories
E. R. Squibb & Sons, Inc.
State Laboratory (Ireland)
Swift-Eckrich, Inc.
Technological Institute
Tennessee Dept of Agriculture
Texas Agriculture Experiment Station
UFI, Inc.
Unilever Research Laboratory Vlaardingen
United States Dept of Agriculture
  Agricultural Research Service
U.S. Environmental Protection Agency
  Office of Pesticide Programs
U.S. Food and Drug Administration
University of Vermont
  Agriculture Testing Laboratory
The Upjohn Co.
Utah State Dept of Agriculture
Virginia Division of Consolidated Laboratory Services
Vitek Systems
Wallace Laboratories
  Division of Carter-Wallace, Inc.
Warner Lambert Co.
Waters Division of Millipore
Welch Foods, Inc.
Westinghouse Bio-Analytic Systems Co.
Wisconsin Dept of Agriculture
  Trade/Consumer Affairs
Wyoming Dept. of Agriculture
Zoecon Corp.

Official Methods Board

ALAN R. HANKS, *Chairman*
Office of the Indiana State Chemist,
Purdue University, Biochemistry Department,
West Lafayette, IN 47907

Other Members: M. H. Brodsky; R. L. Ellis; E. E. Hargesheimer; T. L. Jensen; R. J. Noel; R. Schmitt; E. Sheinin; L. L. Zaika

Recommendations for the Board of Directors

The Official Methods Board recommends the following for adoption as AOAC official policy by the Board of Directors:

A. Add reader response cards to AOAC publications, especially *Official Methods of Analysis* and supplements, to allow method users to respond with problems encountered in using AOAC approved methods.

B. Establish under the Official Method Board a task force to develop recommendations, with options, for a policy/strategy to manage problems or potential problems associated with adopting official methods which depend on proprietary reagents, commercial test kits, rapid tests based on the new biotechnologies, etc.

Basis for Recommendations

A. If AOAC is truly interested in being responsive to the users of its primary product, validated methods, then user feedback must be encouraged and, if possible, made easy/simple.

B. Test kits and other rapid methods that employ proprietary compounds, usually derived from recent developments in biotechnology, present a number of issues. For example, manufacturers can change kits without notice. Such issues must be the subject of a focused AOAC policy. A policy would assure that AOAC can adopt these methods without distracting from the internationally recognized value/reliability of its methods.

Meetings

The Official Methods Board met 4 times during the year to fulfill its primary responsibility of administering the AOAC collaborative study and approval process: September 17 in San Francisco, CA; December 10-12 in Clearwater Beach, FL; April 14-16 in Indianapolis, IN; and August 27 in Palm Beach, FL, at the AOAC annual meeting. During the fall and spring meetings, the Official Methods Board reviews the proposed content of its 2 informational letters. This year, the letters were sent in January and July to all method volunteers. The Official Methods Board hopes that sharing the information contained in the letters among all volunteers will promote a better understanding of the roles of others in the various method areas.

Awards

Outstanding performance by Associate and General Referees is recognized by annual awards coordinated through the Official Methods Board.

A. In 1988, the Official Methods Committee nominees for General Referee of the Year were: Committee on Drugs and Related Topics—Thomas G. Alexander, Food and Drug Administration, Washington, DC; Committee on Foods I—Jon E. McNeal, U.S. Department of Agriculture, Washington, DC; Committee on Foods II—Sandra Bell, Food and Drug Administration, Washington, DC; and Committee on Microbiology and Extraneous Materials—Wallace H. Andrews, Food and Drug Administration, Washington, DC. During its April meeting in Indianapolis, IN, the Official Methods Board voted to select Thomas G. Alexander as the General Referee of the Year for 1988.

B. One of the Associate Referee awards recognizes the single best collaborative study, approved interim official first action prior to the Annual International Meeting, as the Collaborative Study of the Year, based on scientific innovation and collaborative study design, implementation, and reporting. The nominees for this award were: Committee on Pesticide Formulations and Disinfectants—"Liquid Chromatographic Method for the Determination of Azinphos-Methyl in Formulated Products," by Stephen C. Slahck, Mobay Corp.; Committee on Drugs and Related Topics—"Liquid Chromatographic Determination of Morphine Sulfate and Some Contaminants in Injections and Bulk Drug Materials: A Collaborative Study," by Ada C. Bello and Rita K. Jhangiani, Food and Drug Administration; Committee on Foods I—"Collaborative Comparison of the Babcock and Ether Extraction Methods for Determination of Fat Content of Raw Milk," by David M. Barbano and Jenny L. Clark, Cornell University, and Chapman E. Dunham, Texas Milk

Market; Committee on Microbiology and Extraneous Materials—"Use of Dry-Rehydratable Films to Enumerate Coliforms and Aerobic Bacteria in Dairy Products," by Michael S. Curiale and Paul Fahey, Siliker Laboratories, and T. L. Fox and J. Sue McAllister, 3M Co.; and Committee on Feeds, Fertilizers, and Related Topics—"The Use of Near Infrared Reflectance Spectroscopy (NIRS) for the Analysis of Acid Detergent Fiber and Crude Protein in Forages: A Collaborative Study," by Franklin E. Barton, II and William R. Windham, U.S. Department of Agriculture. The studies conducted by Ada C. Bello and Franklin E. Bartin, II were selected as the Collaborative Studies of the Year for 1988.

C. The recipients of the methods committee Associate Referee of the Year awards for 1988 were: Committee on Pesticides and Disinfectants—Frederick C. Churchill, Centers for Disease Control, Atlanta, GA; Committee on Drugs and Related Topics—Barry Mopper, Food and Drug Administration, Brooklyn, NY; Committee on Foods I—Douglas L. Park, University of Arizona, Tucson, AZ; Committee on Foods II—Alan J. Sheppard, Food and Drug Administration, Washington, DC; Committee on Pesticide Residues and Related Topics—Virlyn Burse, Center for Environmental Health, Atlanta, GA; Committee on Microbiology and Extraneous Materials—Angelo DePaolo, Gainesville, FL; Committee on Feeds, Fertilizers, and Related Topics—Dana Perry, Veterinary Diagnostic Laboratory, Tucson, AZ; Committee on Hazardous Substances and Environment—Thomas F. Jenkins, USA CRREL, Hanover, NH. The Methods Committee Associate Referee awards recognize outstanding work and service by Associate Referees in their topic areas.

Task Forces

Several task forces have been appointed in the last few years. Their status and progress on specific tasks follow:

A. The Methods Outreach Task Force under the leadership of Michael H. Wehr has completed a report with several prioritized recommendations:

1. Crisis management is a most important area requiring a plan or strategy which includes rapid validation of an analytical method. The Official Methods Board will make recommendations to AOAC in this area once the Crisis Methods Task Force (see below) submits its final report. This report will cover AOAC's role in crisis situations demanding method validation.
2. The image of AOAC suffers from the negative time factor associated with the conduct, review, and study approval for some methods. An effective marketing strategy is needed to combat this problem and to demonstrate that the AOAC method validation process is not always slow and can, indeed, be quite rapid. AOAC staff will take the lead in this area.
3. General Referees are not always as effective as might be expected. The Official Methods Board will search for ways to improve the effectiveness of General Referees while also monitoring their performance. One suggestion is a one-to-one assignment of General Referees to specific methods committee members for coordination and reporting—the "adopt a GR plan."
4. Identification of new analytical technologies and their incorporation/acceptance is critical to the progress of the AOAC method validation process. General Referees play key roles in the understanding and acceptance of new technology, and they need to be adequately informed/oriented in new technical areas.
5. Methods validation by less than a full collaborative study is needed to save time and satisfy demand for methods in emergency situations. The Official Methods Board will defer action in this area until after receiving the Crisis Methods Task Force report.

B. The Crisis Methods Task Force was established last year under the leadership of Henry B. S. Conacher. There appears to be a tremendous interest in obtaining an AOAC position relative to methods needed in crisis situations when there is very little time available for validation. A draft document on crisis management has been developed and is under review by this task force.

C. The Sampling Task Force headed by Douglas L. Park will be evaluating existing information on sampling and developing recommendations on future areas for AOAC involvement.

D. Two new task forces were recently created to address testing/evaluation of instruments to be used in AOAC methods and in generic performance descriptions for methods.

1. The Instrument Test Methods Task Force will provide written procedures, including quantitative limits or ranges of acceptable results, that will allow a laboratory to determine whether an instrument passes or fails AOAC requirements. This approach to instrument evaluation should eventually allow reference to instruments meeting requirements of a test method rather than naming specific instruments in an AOAC method.
2. The Generic Performance Description of Methods Task Force will develop examples of different types of analytical methods written in performance style. The examples will be useful to authors and should result in a summary document to help guide the writing of methods in performance terms.

When combined, the products of these 2 Task Forces should help eliminate/decrease the need to reference specific instruments by manufacturer and model number.

Other Actions, Activities, and Decisions

Many actions and activities of the Official Methods Board may be of interest to different segments of the membership. During this year, these included but were not limited to the following:

1. Finalized and initiated the use of a collaborative study manuscript review guide for use by General Referees, Statistical Consultants, and official methods committee members.

2. Responded to IUPAC for AOAC on a questionnaire covering "The Drafting of Standard Methods of Analysis."

3. Obtained opinions of the Statistical Consultants and recommended an approach to incorporate performance parameters in *Official Methods of Analysis.*

4. Participated in the development of AOAC/EPA cooperative efforts in the validation of methods for the determination of pesticides in groundwater.

5. Responded to the Board of Directors request for comments on the Long Range Planning Committee's Scientific Activities Report.

6. Completed a "Historical Compilation of the Policies and Procedures of the Official Methods Board."

7. Reviewed the need to coordinate efforts with organizations which have cooperative agreements with AOAC and initiated actions to remind appropriate method volunteers of obligations in this area.

8. Responded to a survey on the revision of the "Handbook of the AOAC."

9. Moved the following topics from the general refereeship on "Dairy Microbiology" under the Committee on Microbiology and Extraneous Materials to the general refereeship on "Dairy Chemistry" under the Committee on Foods I: penicillin in milk by affinity quantitation; β–lactam residues in milk by qualitative methods; β–lactam residues in milk by quantitative methods; and β–lactam residues in milk by Delvotest.

The Official Methods Board concludes this report by first thanking all of the volunteers who have contributed to the methods validations process this year. Special thanks are due for jobs well done by 3 Methods Committee Chairmen who concluded their terms of service: Laura L. Zaika, Chairman of the Methods Committee on Foods II; Michael H. Brodsky, Chairman of the Methods Committee on Microbiology and Extraneous Materials; and Rodney J. Noel, Chairman of the Methods Committee on Feeds, Fertilizers, and Related Topics.

Our many thanks are also due to Rita Bahner and her assistants, Michelle Glass and Corry Retzke, whose staff support make the work of the Official Methods Board possible.

Editorial Board

RAFFAELE BERNETTI, *Chairman*
CPC International, Inc., Moffett Technical Center, Box 345, Argo, IL 60501

Other Members: P. R. Beljaars; K. W. Boyer; J. A. Burke; W. Y. Cobb; C. Franklin; T. M. Hopes; S. W. Klein, C. H. Van Middelem, I. H. Pomerantz; J. K. Taylor; A. W. Tiedemann

The Editorial Board directs the attention of the Board of Directors to the final report of the Ad Hoc Committee for the Review and Evaluation of the *Journal* of the AOAC, chaired by I. H. Pomerantz. The report will be made available shortly after the annual meeting. Its conclusions and recommendations are a strong contribution to improve on the *Journal's* role in the scientific community. The Editorial Board also wishes to inform the Board of Directors of its recommendation that *Official Methods of Analysis* continue to be sold as a unit, whether bound in one volume or in individual sections of more than one volume.

The following 1988 summary covers activities and accomplishments of the Editorial Board through spring and fall meetings.

In 1989, "E" changes to the 14th edition of *Official Methods of Analysis,* will be published in the AOAC *Journal* in full. Typically, only notices of changes would be published, but the 15th edition will not be published until 1990. Beginning in 1991, only notices of changes will, again, be published in the *Journal* and "A" changes to the 15th edition of the *Official Methods of Analysis* will be published in the appropriate supplement.

In response to a proposal to offer individual sections of the *Official Methods of Analysis* separately, the Editorial Board feels that the book should only be purchased in its entirety.

The use of nonstandard abbreviations in *Official Methods of Analysis* will be discontinued, beginning with methods approved next year.

Beginning this year, official methods that have been defined as "surplus methods" will be deleted, without the customary waiting period of one edition of *Official Methods of Analysis.*

A proposal by an ad hoc committee chaired by T. M. Hopes to improve the policy on publication of symposia has been approved by the Editorial Board. The new procedure emphasizes strengthened peer review and advance planning. A symposium organizer must submit a pre-publication plan by the deadline of submission of symposium abstracts and complete manuscripts must be provided within 30 days after the symposium. Symposia consisting of approximately 10 papers or less may be considered for publication in one issue of the *Journal* as in the past; however, these symposia are subject to the *Journal's* policy of acceptance of scientific papers, including section editor's responsibility, peer review, and page charges. Larger symposia may be published as individual monographs if they form strong technical units. In both cases, the organizer of a symposium may not be the only reviewer of the scientific content. A detailed description of the new procedure is being finalized and will be distributed by the end of this year.

The Editorial Board also modified the *Journal's* policy on invited review papers by specifying the nature of an acceptable review paper and the roles of the section editors and editorial office in the acceptance process.

The new subscription rates for the AOAC *Journal* for 1989 will be $84.50 and $125.00 in the United States for AOAC members and non-members, respectively; and $94.50 and $145.00 outside the United States for AOAC members and nonmembers, respectively. These changes were approved before mailing costs were recently increased by the U.S. Postal Service.

Archives Committee

CHARLOTTE A. BRUNNER, *Chairman*
Food and Drug Administration, Division of Cardio-Renal Drug Products, Rockville, MD 20857

Other Members: S. A. Barkan; W. V. Eisenberg; W. Landgraf; R. Pierce; H. L. Reynolds; E. Sarnoff; H. M. Stahr

During the past year, the Archives Committee continued to send archival material to the library at Iowa State University. This material was collected chiefly from the AOAC centennial file. Some discussions were held with Richard Blakely, AOAC comptroller, about the practicality of archiving some of the financial records of the Association. Plans have been made for the Committee to examine these files shortly before AOAC moves to its new headquarters in Arlington, VA. At that time, the Committee will also consider the possibility of archiving some of the past records of the Editorial Board and standing committees.

One of the Committee's projects for 1988 was to obtain interviews with long-term AOAC members who could provide some historical perspective on the Association. The first such interview was obtained by W. V. Eisenberg from Joseph Levine, retired from the Food and Drug Administration, who was active in the drug area. Another interview was obtained at the 1988 meeting from D. Earle Coffin of the Canadian Food Directorate; Coffin is a past president of AOAC. Members of the Committee hope to travel to the home of Rosalind Pierce, former AOAC business manager, to obtain an interview.

This year's Committee meeting on August 31 was attended by C. A. Brunner, H. L. Reynolds, and H. M. Stahr. Cecilia Cassidy of the AOAC *Referee* attended part of the meeting

to discuss how she could work with the Archives Committee and obtain material for brief historical highlights to appear in various issues of the AOAC *Referee*. In return for the Committee's help, Cassidy would remind the membership about providing archival material.

The Committee had a table in the exhibit area of the 1988 meeting to display old photographs, answer questions, and increase interest in depositing archival material. A number of attendees showed interest, and several picked up copies of the question sheet used to assist in the interviews. The committee hopes to obtain several interviews as a result.

For next year's annual meeting in St. Louis, the Committee is considering a display with a double theme: the Canadian contribution to AOAC and memorabilia from the FDA St. Louis laboratory. Coffin prepared a scrapbook of the Canadian participation in AOAC which he kindly agreed to let the Committee use. Thomas P. Layloff, director of the FDA St. Louis laboratory, has discussed the St. Louis participation and identified materials that would make an excellent display.

Interlaboratory Studies Committee

WILLIAM HORWITZ, *Chairman*
Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC 20204

Other Members: R. H. Albert; P. W. Britton; P. Caudill; T. Dols; R. L. Ellis; R. L. Epstein; D. W. Fink; J. Gallagher; R. Grappin; M. Ihnat; S. E. Katz; P. A. Kelly; C. J. Kirchmer; M. Margosis; K. A. McCully; H. S. Ragheb; J. O'Rangers; R. C. Rund; S. Sherken; E. Smith; J. A. Springer; W. Steller; L. Stoloff; B. K. Thompson; M. Thompson; L. Williams; J. S. Winbush; E. S. Windham; J. Winter; R. Wood

Harmonized Protocol

There has been only a limited amount of experience with the application of the IUPAC harmonized procedures for collaborative studies [*J. Assoc. Off. Anal. Chem.* **71**, 161–172 (1988)]. Only one study utilizing this protocol was known to the attendees; no problems were encountered with the procedures in that study. It was pointed out that the International Dairy Federation and IUPAC have accepted the protocol and ISO has changed their outlier treatment to correspond with the recommendation.

The major change has been the way of identifying outliers. The Grubbs test is now the test of choice for identifying outliers, replacing the Dixon test.

Definitions for Interlaboratory Studies

The Committee reviewed the definitions and purposes of interlaboratory studies. Several changes were recommended by the Committee and noted by the chairman. The Committee agreed to present this document, "Appendix I," to the IUPAC Harmonization meeting April 1989.

Presenting Results of Collaborative Studies

The Committee considered the question of how the results of collaborative studies should be presented. It was concluded that the most important information is the actual values and that different ways of summarizing results (such as graphical presentations) should be considered as alternatives to the standard deviations now presented. It is recommended that the chair of the Statistics Committee meet with the chairs of other interested committees (including the Methods Committees) to determine and recommend ways of expressing results of collaborative studies which will be easy for the practical analytical scientist to interpret and use. The present system of expressing statistical parameters has proven confusing and misleading to chemists.

Internal Method Quality Control

The Official Methods Board should recommend that Associate Referees include specific in-process quality controls (e.g., absorptivity) which are characteristic of the analytical operations in the descriptions of the methods. This is recommended as one way of reducing analytical errors and the likelihood of outliers in the results of collaborative tests.

Yes/No Methods

The problem of assessing the results of collaborative studies of qualitative methods (i.e., methods which give a "yes/no," "go/no go," "either/or" answers) is a difficult problem that requires considerable further study. In particular, the number of laboratories needed for such studies is very high (30–40). The Official Methods Board should ensure uniformity in review of qualitative tests by the different methods committees.

APPENDIX I

Definitions Involved in Interlaboratory Studies

By definition, interlaboratory studies in analytical chemistry require active participation by more than one laboratory, although the statistical conclusions are not very sound unless data are provided by at least 5 laboratories. Interlaboratory studies of methods of analysis differ greatly in design and interpretation depending on the purpose of the study. The failure to recognize that there are a number of different purposes can result in confusion in the interpretation of the acceptability of the results.

Types of Interlaboratory Studies

Five different purposes for interlaboratory studies can be identified, each requiring its own statistical assumptions (model), statistical analysis, and outlier treatment. The phrase "interlaboratory study" is the general term for any study requiring the participation of more than one laboratory to attain the desired information. The different types of interlaboratory studies are:

(1) A *Collaborative study* determines the performance characteristics of a method of analysis;
(2) A *Comparative study* compares the performance of several methods of analysis;
(3) A *Proficiency study* determines the performance of analysts or laboratories;
(4) A *Consensus study* determines a value for use as an estimate of the "true value";
(5) A *Certification study* assigns the "true value" of a material with a stated uncertainty.

All interlaboratory studies require the distribution of one or more homogeneous materials to avoid confounding the results with a between-laboratory sampling error. The different types of studies require holding certain factors constant while permitting free choice of other factors.

A collaborative study focuses on the method. It requires the use of the same method by all participants, and the lab-

oratories used should be representative of those with the necessary skills and who will use the method in practice.

A comparative study is an expanded collaborative study involving several methods, each of which should be followed exactly. It is preferred that all participating laboratories use all the methods under study to avoid introducing uncontrolled laboratory-method interactions, but sometimes this cannot be arranged. For example, in comparisons of automated methods with manual methods, some laboratories with automated instrumentation may lack the experience required for performance of the manual method, and some laboratories can perform the manual method but do not have the automated instrumentation. Where possible, comparison with a standard method should be conducted.

A proficiency study focuses on the laboratory. It usually permits free choice of method by *any* laboratory wanting to know how its own results compare with those from other participating laboratories. It should be noted that although the term "laboratory" is used throughout, this term really refers to the analyst together with the physical facilities and environment of the laboratory. In addition, the term "analyst" also includes those situations where a team of analysts is used, with different analysts performing separate steps of the entire method. If the proficiency of individual analysts within a laboratory is desired, provision for independent analyses by analysts within a laboratory must be provided in the design.

Certification and consensus studies focus on the material, not the method(s). A consensus study is intended to provide an average value for a material as given by representative laboratories. The material may then be used as a reference (comparative) standard for other interlaboratory studies or for quality control by individual laboratories.

A certification study is usually conducted by an organization with legal or professional responsibility to prepare and distribute reference standards. The certified value is usually obtained by selected laboratories, using methods of analysis with low or correctable systematic errors. The final result is usually also accompanied by a statement of uncertainty. Values with a lesser degree of reliability may be provided "for information."

Organization of Accumulated Data from Interlaboratory Studies

The primary design factors of an interlaboratory study are usually the number of materials (colloquially termed "samples" by chemists and "levels" by statisticians) with similar or different compositions and/or concentrations (levels) of analyte, and blanks, where necessary; the number of participating laboratories; and the number of replicates. The accumulated results from interlaboratory studies may be tabulated or reported in many different ways: by complete studies (e.g., publications or reports), materials, assays (the set of results by all laboratories on a single material by a single method), number of laboratories, and number of determinations (laboratories × replicates). For consistency in counting, a few rules are necessary:

Replicates/assays.—The study design determines the assignment of replicates to assays. Replication may be blind (unknown to the analyst) or known (deliberately conducted by the analyst). The results from a series of known replicates are always considered as constituent parts of the same assay. When the set of values are obtained as blind (unknown) replicates from the same material or obtained from a pair of materials (of a split level design), the values should be combined statistically so as to extract the within-laboratory (repeatability) standard deviation component as well as the between-laboratory (reproducibility) standard deviation. Therefore, results from blind or known replicates on a single material and results from the pair of split levels over all laboratories constitute only a single assay. However, although unknown and known replicates may have the same statistical significance, they have considerably different practical significance. Parallel, simultaneous, or open replicates determinations cannot be considered as independent, and repeatability estimated from them is usually smaller than the corresponding attribute estimated from unknown replicates or split levels. A split level design consists of 2 materials whose concentrations (levels) are quite close to each other, a fact that is unknown to the analyst in interlaboratory studies.

Laboratories.—Special consideration must be given to the statistical analysis when, contrary to instructions, a laboratory provides more results than were requested. This is not a problem if a computer program for the calculation of precision parameters from an unbalanced design is available. Otherwise, discard the excess results by a randomization technique (e.g., table of random numbers, tossing a coin, etc.). By providing enough test portions for only the specified number of determinations within a laboratory, the problem of too many results from the same laboratory can be avoided.

Laboratories of the same organization in different locations, operating independently (different supervision, instruments, reagents, analysts, etc.) are considered as separate laboratories.

Determinations.—The general term "determination" applies to a reported value from any analyst or laboratory on a single material. "Replicate" applies to multiple determinations by the same analyst on the same test sample. Replicates may be known or unknown and each replicate is considered as a single determination.

Some protocols require several instrument readings from the same test solution, as for example, 2 injections from the same extract for gas chromatography. When these are averaged, they constitute a single determination or analytical result. If they are reported separately, they will provide an estimate only of instrument repeatability, not analyst repeatability. Instructions and reports should be very clear with respect to readings that are reported separately or are combined and whether the replicate values apply to the complete analytical procedure or to only a portion of it.

Definitions

Interlaboratory Study.—A study consisting of a series of analyses conducted by more than one laboratory under specified conditions, the results of which are distributed in a single report.

Collaborative Study.—An interlaboratory study in which each laboratory uses the same method on a series of *identical materials* to perform analyses by the same protocol. The protocol and materials analyzed are selected so as to provide results from which the performance characteristics of the method, usually the within- and between-laboratory precision and systematic error, can be derived.

Comparison Study.—An interlaboratory study in which each laboratory uses more than one specified method on a series of identical materials to perform analyses by the same protocol. The protocol and materials analyzed are selected as in a collaborative study for the purpose of comparing the

performance of the methods. Although it is preferred that all laboratories use all the methods under consideration, each laboratory may conduct analyses by only those methods for which it has the capabilities.

Proficiency Study.—An interlaboratory study consists of one or more assays conducted by a group of laboratories on one or more identical materials, *by whatever method is in use* in each laboratory, for the purpose of comparing the results of each laboratory with those of the other laboratories, usually with the objective of evaluating or improving laboratory performance. Such studies are also referred to as performance evaluation studies.

Consensus Study.—An interlaboratory study utilizing a group of laboratories to analyze a material(s) for the purpose of providing an average value and its uncertainty to characterize the material for further interlaboratory studies or for use in quality control.

Certification Study.—An interlaboratory study utilizing a group of selected laboratories to analyze a candidate reference material by methods judged most likely to provide the least biased estimates of concentration (or of a property) and the smallest associated uncertainty for the purpose of providing a reference value of the analyte concentration (or property) in the material.

Material.—The combination of analyte at a given concentration (level) in a specific matrix. Practically, it is the substance that is homogenized and subdivided into the identical portions distributed to the laboratories participating in an interlaboratory study.

Matrix.—Everything in the substance, sample, or test portion other than the analyte; the carrier of the analyte.

Blank.—The material without the analyte. ("Matrix" involves the concept of the material without the analyte, whereas the blank is physically a material without the analyte or with as low a concentration of the analyte as is possible to obtain.)

Assay.—The set of concentration estimates or decisions (yes/no) obtained from the group of participating laboratories for a specific material by a single method in an interlaboratory study. [A less ambiguous term is needed. One possibility is "collaborative study data set."]

Laboratory.—The environment in which analysts operate to provide results for an interlaboratory study. When only a single analyst (or team of analysts) provides results from each laboratory, the laboratory contribution to an interlaboratory study is the same as that of the analyst.

Determination.—Each single analytical result or property estimate reported by a participating laboratory.

Replicate.—Each individual result obtained from the application of the analytical operations to identical *test portions* by a single analyst (or team of analysts). The replication may be known to the analyst and the analyses conducted either simultaneously (open replication) or at different times (parallel replication), or the fact of replication of materials may be unknown to the analyst (blind or unknown replication). When the replication is conducted at the same time, the values can provide an estimate of within-run precision; if the replication is conducted at different times, the values can provide an estimate of between-run precision. (Note: Replications beginning with the test portion provide an estimate of repeatability. Replication beginning at any later stage does not provide a measure of repeatability since the entire method is not included, e.g., presenting the test solution repeatedly to the instrument provides only an estimate of instrument precision.)

Study Protocol.—The detailed set of instructions for the conduct of an interlaboratory study.

International Coordination Committee

BEN BORSJE, *Chairman*
P. H. van Rijnstraat 44, 3904 HJ Veenendaal, The Netherlands

Other Members: C. Y. Ang; R. Battaglia; W. R. Bontoyan; L.-H. Chen; R. F. Coleman; L. F. Corominas; G. Henniger; E. Hopkin; W. Horwitz; M. Ihnat; J. R. Iturbe; P. Martin; G. G. Moy; K. Naguib; D. L. Park; H. Povlsen; M. Rogers; R. C. Rund; I. Santich; M. Smith; A. Williams; R. Wood

Recommendations for Board of Directors

The International Coordination Committee recommends that the Board of Directors authorize sending an AOAC representative to the open meeting of the Executive Committee of the International Organisation of Sciences in Development (IOCD) on October 25, 1988, in London.

Basis for Recommendation

Robert H. Maybury, Executive Director of IOCD and visiting guest of ICC, reported that IOCD tries to address the isolation of scientists in developing countries by providing organization and funding for short courses and development of methods. ICC wants to study ways of cooperating with AOAC and using AOAC expertise and suggests that an AOAC representative be present at the next executive meeting in London.

Other Discussion

Reports of the status of joint actions with other bodies indicated a high level of cooperation. These international organizations included: International Standards Organisation (ISO), Analytical Methods Committee (AMC) of the Royal Society of Chemistry (UK), Collaborative International Pesticide Analytical Council (CIPAC), Food and Agricultural Organization (FAO) of the United Nations, International Dairy Federation (IDF), World Health Organization (WHO), European Economic Community (EEC), American Association of Cereal Chemists (AACC), Association of Public Analysts (APA-UK), International Union of Pure and Applied Chemistry (IUPAC).

The Committee was pleased to note that a regional section, "AOAC Europe," will be formed in March 1989 and indicated that from that moment the regional sections in the United States have to be renamed, as, for example, US Mid-West, etc. The Committee was pleased to note that countries such as Israel and Egypt want to be associated with AOAC Europe. The Committee further noted with pleasure that the representative from Taiwan intends to work on forming an AOAC-Asia, following AOAC Europe's experiences.

The Committee was pleased to receive guests from Brazil, Mexico, and Canada; members from Mexico, Egypt, Denmark, United Kingdom, Taiwan, Holland, and the United States were also present.

Ileana Santich, the 1988 chairperson of ICC, could not continue; I was requested by the President and the President-Elect in March 1988 to extend my chairmanship for one more year. The new Chairman will be Roger Wood from the Ministry of Agriculture, Fisheries and Food of the UK. I thank AOAC for its confidence in and the opportunities given to me. I wish you all good luck.

Laboratory Quality Assurance Committee

JON E. McNEAL, *Chairman*
U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Other Members: R. Alvarez; J. Ault; R. Baldwin; C. Brannon; M. Brodsky; A.S.Y. Chau; J. Dux; L. L. Gough; J. Guira; G. Hirsch; E. Klesta; E. Meier; T. Mekaru; T. Stijve

Recommendation for Board of Directors

It is recommended that the Board of Directors continue the task force to further investigate a role for AOAC in laboratory accreditation.

Basis for Recommendation

On Monday, August 29, 1988, a task force appointed by President Robert Rund convened to discuss the possible role AOAC could take in accreditation of laboratories. Many issues were proposed at that time, and again on Wednesday, August 31, 1988, at the Laboratory Quality Assurance Committee Meeting, that require further discussion, more information, and possible resolution where conflicts are perceived to exist. A list of these issues, not all-inclusive and not presented in any priority, is as follows:

Why is laboratory accreditation needed?
What laboratories would participate in an AOAC accreditation program?
What would be the mechanism for application for AOAC accreditation?
What would be the scope, "across the board" or specific analyte, family of analytes, analytical technique, or commodity?
What performance standards (acceptability criteria) would have to be met by the applicant laboratory?
Would it be necessary to accredit analysts?
Should the accreditation be based on paper credentials or on proficiency testing or a combination of both?
If proficiency samples are required, at what frequency and with what reporting procedures, to AOAC and to the participants? Does the Association supply these samples or accept an already existing check sample program?
What would be the on-site auditing requirements, of AOAC and of participating laboratories?
What would be the cost to AOAC and to a laboratory?
What credentials in experience and education would be required of the laboratory management, supervisors, and analysts?
Should such a program be national or international?
What legal and liability issues are involved?
Should AOAC fill a vacant need of laboratories, compete with existing accreditation programs, or work toward some unity of accreditation with organizations already doing so by providing the expertise of its members as a service as auditors for those programs?
How would AOAC produce a defensibility of data it would generate under its own program?
What acceptance of such a program would come from other organizations and state or federal agencies?
Should both microbiology and chemical analyses be included?
Should a pilot trial be conducted first?
Could AOAC put a stamp of approval on other accrediting bodies?

After all these discussions, the bottom line from those attending was that AOAC could define a role in accreditation of laboratories and that the role could be implemented as these and other issues are resolved.

Other Discussion

AOAC Short Courses.—Since the last annual meeting, short courses on Quality Assurance for Analytical Laboratories were held on October 20–21, 1987, in Winnipeg, Manitoba, sponsored by the Mid-Canada Regional Section; on April 13–14 and July 12–13, 1988, in Arlington, VA, and on August 27–28, 1988, at the 102nd Annual Meeting. All courses contained a full complement of attendees and interest appears to remain high for the course. A similar course for microbiological testing laboratory personnel was held in Canada in June 1988, but one originally scheduled for last April had to be cancelled because of a lack of a sufficient number of participants. Additional QA courses are scheduled for July 11–12 and November 13–14, 1989, in Arlington, VA, and on September 28–29, 1989, in St. Louis, MO.

A new short course on Field and Laboratory Sampling of Food, Drugs, and Agricultural Commodities will be held in Arlington, on November 30 and December 1, 1988. It is also scheduled for April 11–12, 1989, in Arlington, and on September 23–24, 1989, in St. Louis, MO.

Fred Garfield was not successful in obtaining cosponsorship with FDA's Center for Food Safety and Applied Nutrition, in Washington, DC, or the Center for Drug Analysis in St. Louis, for a course on Quality Control for Bench Analysts. He hopes to approach instrument manufacturers for their cooperation on such a course, to include planning, statistics, and specific analytical instrument QA with actual demonstrations.

Harmonization.—During the week of April 17, 1989, the National Academy of Sciences will hold a meeting in Washington, DC, with IUPAC on the topic of harmonization of quality assurance/control schemes. Members of the Association are invited to attend and talk on what is being done in this area at their places of employment. Such talks cannot be construed as being an AOAC official position. Further information on this meeting will be presented in the *Referee* around the first of the year.

Check Samples.—During the past year, the subcommittee for determining areas in which the Association could service its membership by providing check samples for needs not covered by existing programs sent survey questionnaires on this subject to approximately one-fourth (randomly selected—863 total) of the membership. Much useful information was obtained. Highlights revealed that, for those surveyed, check samples are most needed in elemental, microbiological, and vitamin analyses. Respondents were willing to pay, on the average, from $25 to $75. A list of current suppliers of check samples was also generated along with the numbers of samples needed per year for each type of analysis. A copy of this survey and its results may be obtained by writing to Eugene J. Klesta, Manager QA/QC, Chemical Waste Management, Inc. 150 W 137th St, Riverdale, IL 60627. (Also, as stated in the *Referee,* a list of The Association of Food and Drug Official's Proficiency-Round Robin and/or Check Sample Programs may be obtained by writing to William George Fong, Bureau of Chemical Residue Laboratory, Florida Dept of Agriculture and Consumer Services, 3125 Conner Blvd, Tallahassee, FL 32399-1650).

This subcommittee and its work will continue for the coming year.

Standard Reference Materials.—At last year's meeting, this committee was asked to investigate the feasibility of AOAC becoming the manager/marketer for pesticide standards now

supplied by EPA through the contract with Northrup Industries. On October 30, 1987, the AOAC Executive Director and the Laboratory QA Committee Chairman met with EPA officials to discuss EPA plans to externalize the program. The conclusion of that meeting was that although the program is buffeted by internal politics, the administrators had no desire to let the program be operated by an outside organization. They were opposed to recovering costs through fees, opposed to turning over quality control to any other body, and opposed to auditing laboratories through some other body. Since that time, the contract with Northrup has ended and a new RFP has been posted for award by December 1988. The new contractor will have to provide a complete overhaul of the program and facilities, with Superfund providing most of the money. EPA will no longer provide free pesticide standards except to government laboratories or those doing work for the government. All others will have to purchase their standards from the contractor or some other source.

The National Bureau of Standards has changed its name to the National Institute of Standards and Technology (NIST). Among the new standard reference materials that are now or soon to be available are coconut oil for cholesterol and fat-soluble virtamins, Florida phosphate rock, marijuana and cocaine and metabolites in urine, a variety of standard solutions, freeze-dried cholesterol, bovine serum with 11 elements including aluminum and selenium, cod liver oil with PAHs, PCBs, and alpha-tocopherol, PCBs in sediment, some new high purity pesticides, and cholesterol in powdered eggs. Suggestions for future reference materials are fatty acids and quality of protein. These new standard reference materials do not all appear in the latest catalog, but a list can be obtained by writing to NIST. The EEC is also developing certified reference materials.

Eugene Klesta, QA/QC Manager for Chemical Waste Management, Inc., has been appointed as the new Chairman of the Laboratory Quality Assurance Committee. I thank the staff and membership for their efforts and support of the activities of this Committee the past three years during my tenure as Chairman.

Long Range Planning Committee

RICHARD L. ELLIS, *Chairman*
U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Other Members: R. A. Baldwin; B. Borsje; E. Braselton; K. A. Carlberg; W. P. Cochrane; F. J. Farrell; C. W. Gehrke; E. T. Haeberer; R. Matulis; R. C. Rund; J. L. Schermerhorn; W. J. Spain; A. Shroff; G. R. Tichelaar; L. Whalen

Highlights and major accomplishments for 1988 include review and update of areas for AOAC involvement in methods needs and new possible outreach areas. These were submitted for consideration to the Official Methods Board. The Committee discussed with the comptroller the computer capacity for AOAC. Also discussed were areas for improvement in the AOAC Liaison Officers programs. This has been addressed by strengthening the reporting requirements, formalizing the appointments, and generally using the General Referee or Associate Referee that is appropriate for the area of liaison. Recommendations have been made for consideration by the Membership Committee on recruiting new members, for example, supplementing travelships to annual meetings, sectional meetings, and training courses. Recommendations to the Finance Committee or Ways and Means Committee include increasing dues to levels more comparable to other scientific organizations. Recommendations to the Meetings, Symposia, and Education Programs Committee include investigating the possibility of AOAC awarding Continuing Education Units (CEUs) for participation in short courses, symposia, workshops, and the like.

A report of the Committee discussion on the issue of AOAC adopting test kits, in which the method being adopted is a proprietary product, will be prepared and returned to the Official Methods Board. Several other areas identified by the Committee for further discussion were methods for identification of protein sources, methods for food safety and food processing, long-term fund-raising, direct support for conducting collaborative studies, upgrading public relations, and methods communications.

Committee on Meetings, Symposia, and Educational Programs

JAMES J. KARR, *Chairman*
Pennwalt Corp., Pennwalt Technical Center, Box C, King of Prussia, PA 19406

Other Members: R. Bianchi; P. D. Bland; M. H. Brodsky; J. P. Cherry; T. L. Jensen; E. Meier; J. E. Michelson; S. W. Page; A. Romaro; R. D. Stubblefield

Recommendations for the Board of Directors

A. Continue to move the annual meeting around the country.

B. Consolidate several AOAC short courses into one educational week.

C. Continue to foster development of short courses, especially in statistics, chemometrics, and safety.

D. Attempt to obtain continuing education credits for AOAC short courses.

E. Consider restructuring the opening day of the annual meeting by having a keynote speaker address the meeting theme. Well known speakers will draw attention to AOAC and popularize the meeting.

F. Obtain professional training for short courses instructors and consider hiring paid instructors to help develop and deliver the courses.

Basis for Recommendations

A. Attendance and membership comments endorse continuing this practice; recruiting speakers for symposia is enforced, and an obvious increase in enthusiasm has been noticed in membership contacts.

B. More programming flexibility would be allowed by consolidating several AOAC short courses. An educational week would be cost effective, probably easier to market, and economical in staff requirements.

C. Continuing to foster development of short courses improves membership interest as well as participation by committee members who have expressed interest in undertaking the project.

D. Obtaining continuing education credit for AOAC short courses would be a service to membership, and help to attract new membership, improve relations with other professional societies, and make programming easier for annual meetings.

E. If a well known speaker emphasizes the theme of a meeting, a meeting identity would be developed and the message of AOAC would be brought to the general public.

F. Paid instructors would add depth and breadth to a course, as well as improve spontaneity, keep paying membership abreast of current technologies and development in the laboratory, and add professionalism to the course.

Other Discussions

In response to the Board of Directors' request, the Committee has agreed to include at least one session on microbiology in the annual meeting program.

The 1988 Annual International Meeting Program included the following symposia: "Biotechnology: A Critical Review of Methods and Applications," Co-Chairmen: Dennis M. Hinton and Michael Brodsky; "Screening and Confirmatory Methods for Detecting Drug Residues in Food Products of Animal Origin," Co-Chairmen: Badir Shaikh and William Moats; "Laboratory Information Management Systems," Co-Chairmen: James J. Karr and Hershel Morris; "Pesticides in Foods: Coping with the Issue—Programs and Analytical Methods," Chairman: Paul Corneliussen; "Fertilizers: Phosphate Evaluation and Analysis," Chairman: Frank J. Johnson. The Regulatory Roundtable focused on the new OSHA Safety Regulations for Laboratories, Moderator: Kenneth Helrich, Organizer: Eugene Cole.

The meeting program included 266 contributed papers organized into 10 poster sessions. A new feature this year was the "Modern Laboratory Workshop" featuring products and technology of 16 1988 exhibitors attended by 250 scientists.

Symposia scheduled for the 1989 Annual International Meeting include: "Detection of Environmental Contaminants and Natural Toxins in Food Products of Animal Origin," Co-Chairmen: Badir Shaikh and William Moats; "Laboratory Information Management Systems, Part II," Co-Chairmen: James J. Karr and Hershel Morris; "Enantiomeric Separations," Chairman: Irvin Wainer; "Laboratory Waste Disposal Technology," Chairman: Mark Marcus; "Analysis of Illegal Drugs in Body Fluids" and "Pharmaceutical Methods Development," Co-Chairmen: Albert W. Tiedemann and another organizer yet to be named.

In addition to the above symposia, the program for 1989 will also include a "Workshop on Antibiotics and Drugs in Animal Feeds: Methodology Update," Organizers: William Cobb and Mary Lee Hasselberger, Co-Sponsor: AAFCO.

The AOAC exhibitors are planning an expanded "Modern Laboratory Workshop" in response to the success of this year's effort. Poster Sessions will again be organized to correspond with the AOAC methods topic areas. The theme of the 1989 meeting is Environmental Concerns in the Laboratory and the Community—Who's Taking the Risks? The theme is reflected in the choice of symposia and will be emphasized throughout the meeting as well as in publicity and marketing. The committee hopes to develop and emphasize the theme,concept more extensively in future meetings.

Symposia for 1990 will continue the emphasis on risks and concerns: "Risk Management in the Laboratory, Part II"; "Laboratory Waste Disposal Technology, Part II"; "Laboratory Information Management Systems, Part III"; "Immunoassays (Mycotoxins and Animal Toxins)" which will include a hands-on computer session; "Microbial Contamination of Groundwater."

A new meeting feature will debut in 1990. Titled, "Old Friends, New Enemies," it will deal with the microbiology area and will be offered annually. Future programming ideas which the Committee plans to discuss further include Food Irradiation, and Safety in Laboratory Design and Operation.

The Committee will continue to work with the Editorial Board to finalize the policy recommendations on publication of symposia and is redrafting the proposed policy on cosponsorship of meetings and other programs with other organizations, which was requested by the Board of Directors.

Committee on Membership

JAMES F. LAWRENCE, *Chairman*
Food Research Division, Health Protection Branch, Banting Research Center, Ottawa, Ontario K1A 0L2, Canada

Other Members: C. T. Bell; R. H.. Bowers; W. F. Carey; A. S. Carman; H. Casper; W. P. Cochrane; R. D. Fischbeck; A. R. Hanks; K. R. Hill; D. Kassera; V. A. Thorpe; J. Williams

Recommendations for the Board of Directors

A. The Committee recommends that the requirement of sponsorship for new members be studied.

B. The Committee recommends that a new class of membership be established for retired members.

C. The Committee recommends that waivers of membership fees be phased out as soon as possible.

Basis for Recommendations

A. The pros and cons of new member sponsorship should be studied. This could be a means of ensuring that the high quality of the membership is maintained. However, its administration, implementation, and effect on membership must be studied.

B. Retired members have much to offer the Association in terms of experience, leadership, and knowledge. They should continue to be eligible for membership in some form. In order to retain retired members in any capacity, with the exception of fellows, the bylaws will have to be changed.

C. For committee members, General Referees, Associate Referees, etc., payment of fees is optional but encouraged. It was the general opinion of the Membership Committee that this option be removed and all members be required to pay the membership fee, at the risk of losing some active members.

Other Discussion

Much discussion was spent on Key Issues 4.E.1.C. of the Long Range Plan and 1.P.4., concerning an employment service for members and member products and services, respectively. It was recognized that information from the membership itself was required, thus appropriate questions will be proposed for a membership survey.

Committee on Regional Sections

DAVID OSHEIM, *Chairman*
U.S. Department of Agriculture, PO Box 844, Ames, IA 50010

Other Members: G. H. Boone; M. L. Foster; A. V. Jain; K. A. McCully; L. L. Murray; S. M. Walters; G. Willkens; J. Wiskerchen

The Committee met on Wednesday, August 31, 1988, at the AOAC annual meeting in Palm Beach, FL.

The Committee distributed copies of the "Guidelines for Regional Sections" to all section chairs. In the future, these

guidelines may be amended by the Committee or individual sections.

The Committee reviewed members' comments on the implementation protocol for the AOAC Long Range Plan. A final draft will be completed for examination by the Committee and submission to the AOAC Board of Directors.

Regional section promotion of AOAC membership and training at regional section meetings were also discussed.

Committee on Safety

EUGENE C. COLE, *Chairman*
University of North Carolina, Department of Medicine, Division of Infectious Diseases, Chapel Hill, NC 27599

Other Members: F. G. Burton; R. J. Everson; N. W. Henry; G. Markakis; R. Nelson; G. L. Roach; D. Root; G. Schwartzman; D. Shoemaker; M. G. Torchia

The Terms of Reference were reviewed and finalized.

The Committee agreed to publish a list of recommended laboratory safety training videos. Included will be those viewed by the membership and critiqued by the Committee during the 1987 and 1988 annual meetings.

The Committee agreed to submit a summary report of the Committee-sponsored 1988 Regulatory Roundtable on Laboratory Safety to the AOAC Referee.

The Committee agreed to investigate the possibility of organizing a workshop on laboratory design, construction, and renovation, and/or a short course on laboratory safety.

The Committee will finalize a rewrite of Section 13 (Safety), p. 49, of the *Handbook for AOAC Members.*

The Committee will survey the Association membership for interest regarding audio/visual training materials pertaining to AIDS.

Committee on State and Provincial Participation

MAX L. FOSTER, *Chairman*
Kansas State Board of Agriculture, Division of Laboratories, 2524 W 6th St, Topeka, KS 66606

Other Members: P. J. Brignac; S. Cannon; D. Harder; T. L. Jensen; R. H. Lane; H. F. Morris; L. L. Murray; R. L. Schwarberg; O. L. Shotwell; G. R. Tichelaar

The Committee spent a considerable amount of time reviewing its Terms of Reference. In particular, each of the objectives and goals was discussed in detail, and the Committee concluded that these goals have either been accomplished or are a duplication of goals of other AOAC committees (particularly, the Regional Section Committee and the Membership Committee). Therefore, the Committee asks the Board of Directors for an additional charge or possibly a new purpose. If the Board feels that the Committee has "served its purpose," then it should be dissolved before the 1989 annual meeting. If this Committee is eventually dissolved, the Committee members offer their services to AOAC on future boards or committees. If a new purpose for the Committee is found, then the Committee will work toward accomplishing these new goals.

Committee on Statistics

MOLLIE A. READY, *Chairman*
Alcon Laboratories, Inc., Conner Research Center, 6201 S Freeway, Ft. Worth, TX 76134

Other Members: R. H. Albert; J. L. Cawley; P. C. Kelly; H. Marks; D. Mowrey; T. Peeler; J. G. Phillips

Recommendations for the Board of Directors

A. The Statistics Committee recommends that the Board of Directors not adopt the NMKL "Statistical Evaluation of Results from Quantitative Microbiological Examinations," until after October 15, 1988.

B. The Statistics Committee recommends a closer working relationship between the Official Methods Board and the Statistics Committee, with the Chairman of the Statistics Committee as a member of the Official Methods Board, if possible.

Basis for Recommendations

A. The basis for this recommendation is to allow individual members and committee statisticians time to evaluate the document.

B. Communications between the Official Methods Board and the Statistics Committee have been improved; however, a more rapid interchange is important.

Other Discussions

The Committee requests further information on the needs and requirements of the Magruder check sample program for statistical service.

A training film has been prepared and distributed.

A computer program, entitled Precision Statement, is undergoing final debugging. An outlier program will be added.

Precision Statement forms are in final draft and have been approved by the Committee.

A symposium on statistics is proposed for the 1989 annual meeting.

Committee on Ways and Means

JOHN B. BOURKE, *Chairman*
New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

Other Members: W. P. Cochrane; J. Goleb; C. Opp; L. Perlman; M. R. Ready

Recommendations for the Board of Directors

A. The Committee recommends that the Board of Directors appoint a task force to study the possibility of AOAC coordinating outside audits on a contract basis for laboratories operating under good laboratory practice (GLP) programs and supplying the program and training aids necessary for the maintenance of a GLP program.

B. The Committee recommends the publication of an "AOAC Buyer's Guide" for equipment and materials used in its approved methods.

C. The Committee recommends that AOAC request donations from exhibitors, advertisers, and members for items which may be auctioned at an evening affair of the next annual meeting.

D. The Committee recommends that the Board of Directors consider the incorporation into the headquarters oper-

ations of a venture assistance program that will make available regulatory, scientific, and good manufacturing assistance to newly emerging agricultural and food establishments.

Basis for Recommendations

The Ways and Means Committee in attempting to suggest new and novel ways to assist in financing the Association's activities tried to keep in mind both service to its members and programs which would broaden its membership base. The 4 recommendations listed meet these criteria.

A. The first recommendation assists the membership in meeting both statutory and corporate requirements for effective QA/QC programs utilizing the FDA/EPA good laboratory practices (GLP). Those requirements include outside audits, in-house and external training, as well as defined standard operating procedures. The Association could make services available both through its staff and cooperating members for these functions and could coordinate audits of laboratories on a contract basis. The Committee recommends that a task force be formed to study this program, which supports the Board's interest in potential certification programs. The current FDA/EPA requirements, if implemented by a laboratory, essentially constitute self-certification. The external audits measure the laboratories compliance to its self-certification program. Making these audits available could be a service to members and could result in expanded membership.

B. The second recommendation needs to be considered both with respect to its income-producing potential and the possible effects it could have on the AOAC *Journal.* The Committee thinks that as the diversity of methods continues to expand, the diversity of supplies and materials also grows. While many suppliers are identified in the methods themselves, many are not, or products are available from several suppliers. The Committee feels that the distribution of a buyers guide will result in significant potential for income. Wide distribution to AOAC *Journal* subscribers, all members, state and federal regulatory laboratories, and targeted private contract laboratories should make the guide attractive to advertisers.

C. The Committee feels that fund-raising should not only assist the operations of the Association but also result in entertainment for its members. With that thought in mind, the Committee suggests to the Board that an auction be held in conjunction with the annual meeting, possibly on the all-sports night. Materials for the auction should be solicited from members, advertisers, exhibitors, and others interested in the functions of AOAC. Although this will not be a big money project, it will raise funds and be an enjoyable interlude to an otherwise busy meeting.

D. A venture assistance program would probably be the most difficult recommendation to implement. It also is the one which in the long run could bring the Association the greatest income. Many small and emerging companies and public programs need assistance with regulatory, quality assurance, scientific expertise, and laboratory services but do not know where to go for that information. The Committee feels that the Association could coordinate such a referral service while collecting a percentage of the consultation fees levied. All consultants listed must be members of the Association and should include retired state, federal, university, and corporate scientists as well as professional consultants. The Committee recommends that the Board appoint a task force to work on the details by identifying the clientele and consultants and by devising methods of advertising the service.

Other Discussion

The Committee recommends that its membership should be made of 6 members including the Chairman and the term of the members should be limited to 2 or 3 years.

Committee on the Constitution

AUDREY V. GARDNER, *Chairman*
New York State Agricultural Experiment Station, Geneva, NY 14456

Other Members: S. A. Barnett; R. H. Bowers; E. J. DeVries; C. A. Geisler; N. Hardin; J. P. Minyard; A. E. Pohland; R. J. Ronk; D. L. Terry; V. A. Thorpe; B. Woodward

The Committee met on August 29, 1988, during the AOAC Annual Meeting in Palm Beach, FL. The Committee's primary activity during the past year has been to formulate 2 bylaw amendments. The purpose of the first amendment was to eliminate the conflict in time frames between Article XIV and Amendment I. The second amendment served to incorporate all references to voting and all references to the Official Methods Board, Official Methods Committees, Referees, and Referee Committees, into separate articles. These amendments were submitted to the membership for vote by mail ballot, and received the three-fourths majority necessary for adoption. The Committee, having received no direct charge from the Board of Directors, respectfully requests clarification and direction relative to possible bylaw amendments to accomplish the following: *(1)* Provide a formal mechanism for liaison between the Board of Directors and its junior boards, the Official Methods Board and the Editorial Board; and *(2)* Provide a mechanism for the Board of Directors to establish, as a standing committee of the Association, a Committee on Elections to serve as official representative to the Board and have responsibility for balloting and certification of elections.

BYLAWS

Revised September 1, 1988

ARTICLE I

Name

The name by which this Association shall be known is the "Association of Official Analytical Chemists" (hereinafter referred to as the "Association").[1]

ARTICLE II

Purpose

This Association is organized and operated exclusively for scientific and educational purposes within the meaning of section 501(c)(3) of the Internal Revenue Code of 1954. Its primary purpose is to provide government agencies and other interested parties, particularly regulated industries, with analytical methods (chemical, biological, or physical) which have undergone interlaboratory tests, have been found to be satisfactory, and have been adopted by the Association for use as follows: by government agencies for enforcement of, and by industry for compliance with, legal or voluntary requirements or recommendations; for monitoring; for research; and for any other pertinent applications. To accomplish its purpose, the Association will carry out the following activities:

A. Obtain, improve, develop, test, and adopt uniform, precise, and accurate methods for the analysis of foods, vitamins, food additives, pesticides, drugs, cosmetics, plants, feeds, fertilizers, hazardous substances, air, water, and any other products, substances, or phenomena affecting the public health and safety, the economic protection of the consumer, or the protection of the quality of the environment;
B. Publish: (*1*) a compendium of official analytical methods; (*2*) a journal for reporting research in analytical chemistry and related areas, for reporting the results of tests of analytical methods, and for reporting actions of the Association, particularly those with respect to the adoption of methods; and (*3*) special monographs, books, and informational or instructional material to fulfill needs of analysts in the areas of the Association's concern;
C. Provide a forum for discussion of all aspects of analytical methods within the areas of the Association's concern;
D. Promote wider use of official methods of the Association by all sectors in order to obtain the benefits of uniform, practicable, and reliable methods for regulatory, compliance, and forensic purposes; for monitoring, for research, for quality control, and for other pertinent purposes;
E. Maintain cooperative arrangements with other national and international associations and societies for achieving insofar as possible uniformity in analytical methods for the products within the areas of concern to the Association;
F. Carry out such other activities which, in the opinion of the Board of Directors of the Association, assist in accomplishing its purpose.

ARTICLE III

Membership

Section 1. Classes of Membership

There shall be three (3) classes of membership in the Association: Individual Members, Sustaining Member Organizations, and Honorary Members.

Section 2. Qualifications for Membership

A. *Individual Members*

There shall be one (1) class of individual membership. Qualifications for individual membership shall be a degree in Science, or equivalent if approved by the Board of Directors, and interest in the purpose and goals of the Association. Scientists shall be eligible for membership provided they are engaged, directly or indirectly, in analysis or research with respect to commodities or other substances named in Article II, Section A, and pay the annual Individual Member dues. Individual Members shall be eligible to hold office, serve on AOAC Boards and Committees, and vote on amendments to the bylaws, election of officers, methods, dues, and all matters not otherwise excluded.

B. *Sustaining Member Organizations*

There shall be one (1) class of sustaining membership. A Sustaining Member Organization shall be any agency of a local, state, provincial, or national government, a college or university, or any firm, business, or organization with an interest in the development and interlaboratory evaluation of analytical methodology, provided it is engaged, directly or indirectly, in analysis or research with respect to commodities or other substances named in Article II, Section A.

Section 3. Application for Membership

Application for Membership shall be submitted to the Association. Election to membership shall become effective upon payment of dues, or as otherwise provided in these bylaws.

Section 4. Honorary Members

Honorary Members shall be persons recognized for their substantial contribution toward the achievement of the objectives of the Association. They shall be nominated by the Board of Directors and may be elected by a two-thirds vote of the Members voting. An Honorary Member shall have all the rights and privileges of a Member.

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[1]The Association of Official Analytical Chemists was incorporated in the District of Columbia on January 16, 1932, as the Association of Official Agricultural Chemists.

ARTICLE IV
Elected Officers

Section 1. Officers

The officers of the Association shall be Members and shall consist of a President, President-Elect, Secretary-Treasurer, and any other officers designated by the Board of Directors. The President-Elect and Secretary-Treasurer and five (5) Directors shall be elected by the majority of Members voting, and shall serve for the year beginning with the adjournment of the annual meeting at which they are elected, and ending with the adjournment of the next annual meeting. The President-Elect shall become President upon adjournment of the next succeeding annual meeting.

Section 2. President

The President shall be the principal executive officer of the Association, and shall be responsible for all the business and affairs of the Association between meetings of the Board of Directors and in accordance with its policies. He shall preside at all meetings of the members and the Board of Directors. He shall call regular or special meetings or authorize mail ballots by the Board of Directors to handle necessary matters, or instruct the appropriate appointed officer to do so.

The President shall appoint the following for lengths of terms designated by the Board of Directors: the Chairman of the Official Methods Board; Committees on Official Methods; an Editorial Board; a Nominating Committee; a Committee on the Wiley Award; a Committee on Fellows; a Long-Range Planning Committee; any other Committees established by the Board; and Liaison Officers.

Section 3. President-Elect

In the absence of the President, or in the event of his inability or refusal to act, the President-Elect shall perform the duties of the President, and when so acting, shall have all the powers of, and be subject to all the restrictions upon the President. The President-Elect shall perform such other duties as from time to time may be assigned to him by the President or by the Board of Directors.

Section 4. Secretary-Treasurer

The Secretary-Treasurer shall be responsible for the funds and securities of the Association, and in general, perform all the duties incident to the office of Treasurer and such other duties as may be assigned to him by the President or by the Board of Directors.

ARTICLE V
Nominations and Elections

Section 1. Nominating Committee

The Nominating Committee shall recommend Members as candidates for elected offices. It shall consist of three (3) members who shall be the previous three Past Presidents of the Association, provided that, if Past Presidents are not available to serve, other Members shall be appointed to the extent necessary to constitute the three member committee.

Section 2. Vacancies: Office of President

If the office of the President shall become vacant, the President-Elect shall thereupon become President of the Association for the unexpired term. Such service will not affect such person becoming President of the Association upon adjournment of the next annual meeting. In the event that the office of President becomes vacant at the time when the office of President-Elect is also vacant, such vacancy shall be filled by the action of the other members of the Board of Directors. If any office other than that of President shall become vacant, the office shall be filled for the remainder of the year by action of the Board.

Section 3. Vacancies: Board of Directors

If a vacancy of one of the elected positions of the Board of Directors should occur, any Past President may be named by other members of the Board to temporarily fill such vacancy until an election is held at the next annual meeting.

ARTICLE VI
Board of Directors

Section 1. Composition

The Board of Directors shall consist of nine (9) Members to include the President, President-Elect, Secretary-Treasurer, Immediate Past President, and five (5) Directors, all of whom shall be Individual Members of the Association. The majority of the Board shall be representatives from one or more of the following: a national, state, provincial, or municipal government, a regulatory agency, or academia. No member of the Board of Directors may be elected for more than six (6) consecutive years.

Section 2. Powers and Duties

The Board of Directors shall:

—establish general policies, and shall manage the affairs of the Association between meetings of the membership
—determine all activities of the Association
—fix the annual dues for membership, subject to approval by the members
—determine the membership year and the delinquency date
—approve procedures for election to membership and requests for dues waivers, and may nominate Honorary Members
—act on the subscription rates and prices submitted for approval by the Editorial Board

—be responsible for the employment and appointment of individuals necessary for the efficient operation of the Association, and may assign them appropriate titles
—further define, when necessary, official duties of the employees, officers, and committees of the Association, and may assign additional duties to the President-Elect, Secretary-Treasurer, Directors, or other officers, except that of President, in accordance with these bylaws
—fill vacancies in the offices of President-Elect, Secretary-Treasurer, or other offices, except that of President, in accordance with these bylaws
—determine the number and tenure of members of the Editorial Board, Standing Committees, and Liaison Officers
—establish and terminate committees
—determine the time and place of the annual meeting, and may call a special meeting of the Association membership
—set geographic limits, authorize establishment and dissolution of regional sections, and approve bylaws adopted by the regions
—consider recommendations of the Standing and Special Committees
—review all proposed amendments to the bylaws which are received by the Board at least 90 days in advance of a meeting of the Association, and submit the proposals, with Board recommendations, to the membership in accordance with procedures in these bylaws
—upon dissolution of the Association, distribute remaining assets in accordance with these bylaws and other applicable authorities

Section 3. Meetings

A. Regular meetings shall be held at the call of the President, or as ordered by the Board.
B. Special meetings of the Board may be called by the President.
C. The President shall preside at all meetings except as otherwise provided in these bylaws.

Section 4. Voting

A mail ballot may be authorized by the President.

ARTICLE VII
Appointed Officers

Section 1. Appointed Officers

The Board of Directors may appoint such individuals as are necessary to carry out the following functions: execute the directives and policies of the Board; supervise the Business Office and perform any duties necessary in the day-to-day management of the Association; keep the minutes of the meetings of the Members and the Board of Directors; see that all notices are duly given in accordance with the provisions of the bylaws; keep a register of membership of the Association and their addresses; edit and produce the publications of the Association.

An appointed officer with concurrence of the President shall appoint General Referees from the Members of the Association, one for each of the general subjects designated by the Official Methods Board.

An appointed officer with concurrence of the President shall appoint Associate Referees on subjects complementary to the general subjects assigned to the General Referees. In lieu of an Associate Referee, the appointed officer with concurrence of the President may appoint an Associate Referee Committee of two or more persons, designating one as Chairman. Appointment of General Referees, Associate Referees, and members of an Associate Referee Committee must have the approval of each appointee's appropriate supervisor.

Section 2. Liaison Officers

The Liaison Officers shall coordinate the development and adoption of uniform analytical methods between the Association and related interested organizations.

ARTICLE VIII
Editorial Board and Standing Committees

Section 1. Editorial Board

The Editorial Board shall be responsible for developing, editing, and publishing the publications of the Association. The appointed officer responsible for editing and producing the publications of the Association shall be a member *ex officio*.

Section 2. Committee on the Wiley Award

The Committee shall consist of six (6) members with three (3) appointed each year to a two-year term. They shall decide on the winner of the Harvey W. Wiley Award from those nominated for the award. The President shall chair the Committee.

Section 3. Committee on Fellows

The immediate Past President of the Association shall act as chairman of the Committee.

The Committee on Fellows shall select candidates from those nominated and from the general membership for recommendation to the Board of Directors. Any Member who has rendered ten years or more of meritorious service to the Association as a General Referee, Associate Referee, Committee Member, or in any other official capacity shall be eligible for nomination as a Fellow of the Association.

Section 4. Long-Range Planning Committee

The Committee shall make recommendations to the Board of Directors on improving the functions of the Association.

ARTICLE IX

Official Methods Board, Referees, and Committees

Section 1. Official Methods Board and Committees on Official Methods

A. *Official Methods Board (OMB).* The Official Methods Board shall be composed of the Chairmen of the Committees on Official Methods plus a Chairman, the majority of whom shall be from one or more of the following: governmental agencies, regulatory agencies or academic institutions. The OMB shall promote uniform policy in the consideration, adoption and repeal of Official Methods, Changes in Methods, and procedures. The OMB shall determine the number, tenure, and duties of General Referees, Associate Referees, and Statistical Consultants.

B. *Committee Assignments.* Each Committee shall consist of at least seven (7) members including a chairman and secretary. The Chairman of the Official Methods Board shall assign each Committee the subject categories and reports which it shall consider. Each Committee shall prepare a written report on the status of its subjects and make recommendations for adoption, for deletion, and for changes in official methods and procedures.

C. *Committee Composition.* Not more than half the members of each Committee shall be from a single agency. The Chairman shall be a Member. The Chairman shall rule on procedural matters. Unresolved disputes on adoption of methods shall be referred to the voting membership at the business meeting.

Section 2. General Referees

It shall be the duty of a General Referee to:

A. Review the assigned subject area on a continuing basis.
B. Recommend scientists for appointment as Associate Referees.
C. Serve as a guide and consultant to the Associate Referees.
D. Check on the progress of Associate Referees.
E. Review the reports and recommendations of the Associate Referees.
F. Prepare reports and make appropriate recommendations.

Section 3. Associate Referees and Associate Referee Committees

It shall be the duty of an Associate Referee or Associate Referee Committee to:

A. Review the assignment and pertinent literature for methods.
B. Devise or choose and test methods that will be practicable and reliable for regulatory use.
C. Prepare instructions for distribution to participants.
D. Select a group of interlaboratory participants.
E. Prepare and distribute samples or instruct participants how to prepare or obtain suitable samples.
F. Direct and conduct collaborative, interlaboratory studies.
G. Evaluate the participants' results.
H. Prepare reports and make appropriate recommendations.

Section 4. Reports by Committees of the Official Methods Board

Each committee of the Official Methods Board shall present a written report of the status of its subjects, and read in full its specific recommendations on all new methods or changes in methods prior to the vote thereon at the business meeting. A two-thirds vote of members present and voting at the annual business meeting shall be required for adoption or repeal of methods, changes in methods, and procedures.

Methods of analysis shall be adopted as "official first action" and "official final action." No method shall be adopted as "official first action" until it has undergone interlaboratory study and has been recommended by the appropriate General Referee and Committee on Official Methods at the annual meeting. In case a General Referee does not concur in the recommendation of the Associate Referee, the appropriate Committee on Official Methods may consider such a recommendation upon written request from the Associate Referee. Appeals of recommendations on methods are processed by the Official Methods Board in accordance with procedures established by the Board of Directors. A method shall be adopted as "official final action" only after the adoption of such method as "official first action." No method shall be adopted as "official final action," except on recommendation by the appropriate Committee on Official Methods at an annual meeting. An analytical method which has undergone interlaboratory study between annual meetings and has been approved by the appropriate General Referee, the appropriate Committee on Official Methods, and the Chairman of the Official Methods Board is designated "interim official first action" until the Association votes on full acceptance at the first subsequent annual meeting.

No basic change in an official method shall be adopted until after the changed method has undergone interlaboratory study and has been recommended by the appropriate Committee. No official method shall be repealed until such action has been recommended by the appropriate Committee at an annual meeting.

A sampling or sample preparation or other type of procedure for which an interlaboratory study is impractical may be adopted as official when accumulated data or statistically planned study indicates that the procedure is reliable. Upon recommendation of the appropriate General Referee and Committee on Official Methods, the Association may adopt as procedures, well established methods of examination or treatment of a mechanical, microscopic, physical, chemical, or other nature.

Section 5. Effective Date of Methods and Procedures

Adoption, change, or repeal of methods and procedures shall become effective on the 30th day after publication of the record of such action in the *Journal* of the Association.

ARTICLE X

Meetings

Section 1. Annual Meeting

The annual meeting of the Association shall be held at the time and place decided by the Board of Directors. A special meeting of the entire Association may be called by the Board of Directors. Announcement thereof shall be made at least two months prior to the time of said meeting.

Section 2. Quorum

Those Members present in person or by proxy shall constitute a quorum at any meeting of the Association which is duly called pursuant to the provisions of these bylaws.

ARTICLE XI

Voting

Section 1. Voting by Mail Ballot

All substantive matters requiring a vote of the membership shall be presented to the Members at least sixty (60) days in advance of the Annual International Meeting to allow for mail balloting. Deposit in the United States mail shall serve as verification of this requirement. Such matters shall include amendments to the AOAC bylaws, election of officers, and dues.

Section 2. Voting at the Annual Meeting

Voting on matters relating to the analytical methods and other business transactions of the Association shall be conducted at the business meeting held during the annual meeting. A two-thirds vote of Members present and voting at the annual business meeting shall be required for adoption or repeal of methods, changes in methods, and procedures, and for election of Honorary Members.

Section 3. Voting by Proxy

At any meeting of members, a Member entitled to vote may vote by proxy executed in writing by the member or his duly authorized attorney-in-fact. No proxy shall be valid for more than eleven (11) months after the date of its execution unless otherwise provided in the proxy.

ARTICLE XII

Dues and Subscriptions

Section 1. Membership Dues

A. Annual dues for membership in the Association shall be fixed by the Board of Directors, subject to approval by the majority of the members voting by mail ballot.
B. The membership year and the delinquency date shall be determined by the Board of Directors.
C. Membership dues may be waived upon written request of the Member for any membership year in which he is serving the Association in an official capacity, such as General Referee, Associate Referee, Committee Member, or other appointed assignment considered by the President to be of similar importance.
D. Honorary Members and retired Fellows of the Association shall be exempt from payment of dues and annual meeting registration fees.

Section 2. Publications

Subscription rates and prices for any publication of the Association shall be determined by the Editorial Board, subject to approval of the Board of Directors.

ARTICLE XIII

Earnings and Assets

Section 1. Non-Profit Status

A. Regardless of any provision of the bylaws which may be construed otherwise:
[1] No part of the net earnings of the Association shall under any circumstances inure to the benefit of any member or individual.
[2] The Association shall not be operated for a profit.
B. On lawful dissolution of the Association and after settlement of all just obligations of the Association, the Board of Directors shall distribute all remaining assets of the Association to one or more organizations selected by the Board of Directors which have been held exempt from Federal Income tax as organizations described in section 501(c)(3) of the Internal Revenue Code of 1954.

Section 2. Political Activities

A. No substantial part of the Association's activities shall consist of carrying on propaganda or otherwise attempting to influence local, state, or national legislation. All activities of the Association shall be determined by the Board of Directors.

B. The Association shall not participate or intervene in any manner in any campaign on behalf of any candidate for a political office.

ARTICLE XIV

Subsidiary Organizations

Section 1. Regional Sections

The Board of Directors shall set geographic limits and grant authority to groups of Members of the Association residing or working in the same geographical regions for the establishment of regional sections.

Section 2. Purpose of Regional Sections

The purpose of regional sections shall be to promote and sponsor the purpose of the Association.

Section 3. Membership in Regional Sections

Membership in a regional section shall consist of Members and Honorary Members of the Association residing and working within the geographical boundaries of the section. Other individuals interested in the purpose of the regional section and residing and working within the boundaries of the section shall also be eligible for section membership but shall not be eligible for election to the Executive Committee for the section. No person shall be a member of more than one regional section.

Section 4. Bylaws of Regional Sections

Each regional section shall adopt for its own government, subject to approval of the Board of Directors, bylaws not inconsistent with these bylaws.

Section 5. Dissolution of Regional Sections

When any regional section shall cease to function as a section for a period of more than one year, or if its membership shall be less than 10 Members of the Association for a period of one year, the Board of Directors may terminate the existence of such regional section.

ARTICLE XV

Parliamentary Authority

The rules contained in the current edition of *Robert's Rules of Order Newly Revised* shall govern the Association in all cases in which they are applicable and in which they are not inconsistent with these bylaws or any special rules of order the Association may adopt.

ARTICLE XVI

Amendments to the Bylaws

All proposed amendments of these bylaws shall be presented in writing to the Board of Directors. The Board shall present the proposals to the Association membership, with recommendations. A three-fourths vote of the Members voting by mail ballot is required for adoption.

Joint AOAC-AOCS-AACC-IUPAC Mycotoxin Committee

PETER M. SCOTT, *Chairman*
Health and Welfare Canada,
Health Protection Branch,
Ottawa, Ontario K1A 0L2, Canada

Other Members: R. Bernetti (AACC); H. Casper (AOAC); J. C. Henderson (*Secretary*) (AOCS); D. L. Park (AOAC); A. E. Pohland (IUPAC); O. L. Shotwell (AACC); R. D. Stubblefield (AOCS); S. N. Tanner (AACC); A. E. Waltking (AOCS)

The annual meeting of the Joint Mycotoxin Committee was held on August 30, 1988, in Palm Beach, FL.

(A) The meeting opened with the sad announcement that a long time colleague and pioneer researcher, Leo Goldblatt, passed away the previous week. Discussion among all present led to the commitment to dedicate the mycotoxin portion of the 1989 AOCS meeting in Cincinnati and the 1990 AOAC mycotoxin symposium in New Orleans in Dr. Goldblatt's name.

(B) Steven Tanner was introduced as a new committee member representing AACC.

(C) The minutes of the Joint Mycotoxin Committee meeting of September 16, 1987, were approved.

(D) The report of the IUPAC Commission on Food Chemistry was given by Douglas Park for Albert Pohland. The commission has several projects on mycotoxins that are completed or under way. These were listed in last year's Joint Mycotoxin Committee report.

(E) Peter Scott highlighted the AOAC General Referee report on mycotoxins. (a) The new Associate Referee on immunochemical methods for mycotoxins is James J. Pestka of Michigan State University. (b) Mary Trucksess of FDA has been appointed as the new Associate Referee on aflatoxin methods. (c) The following methods have been recommended to the Committee on Foods I for adoption as official first action: the ELISA screening method for aflatoxin B_1 in corn and roasted peanuts; the LC method for determination of aflatoxins B_1, B_2, G_1, and G_2 in corn and peanut butter; the ELISA cup method for screening for aflatoxins in corn, cottonseed, peanuts, and peanut butter; and the ELISA screening method for zearalenone in corn, wheat, and feed. (d) A recommendation was made to drop the topics on emodin and related anthraquinones, *Penicillium islandicum* toxins, and secalonic acids.

(F) The chairman requested that all members and interested persons submit any questions they may have pertaining to the long range plans of AOAC as they affect the Joint Mycotoxin Committee. These will be included in a survey of the AOAC membership.

(G) Arthur Waltking gave an overview of the AOCS Mycotoxin Committee meeting of May 10, 1988, in Phoenix, AZ. (a) The report of the Smalley Committee on aflatoxins was reviewed. (b) Field tests will be conducted in 1988–89 using 5 aflatoxin test kits. (c) Proposed Committee activities during 1988 and beyond include more work on affinity columns and liquid chromatography, including post-column iodine derivatization.

(H) Odette Shotwell gave the report for the AACC Mycotoxin Committee. (a) The method "Aflatoxin in Corn: Presumptive Test" (Black light) was moved to First Approval status because of its wide usage. (b) Steven Tanner has been appointed chairman of the AACC Mycotoxin Committee. (c) It was announced that G. Garcia-Aguirre has written grain methods in Spanish.

(I) The report of the International Dairy Federation Group E33-Mycotoxins was summarized by Peter Scott. (a) The revised Stubblefield method for aflatoxin M_1 in milk has been recommended for publication as a final IDF standard. (b) The Group will not at present embark on standardization of an immunochemical method for aflatoxin M_1. (c) No action was taken on aflatoxin M_4.

(J) Peter Scott summarized 2 international conferences pertaining to mycotoxins that had taken place during the last year: Journées d'Étude, Paris, France, held November 25–27, 1987, and the 7th IUPAC International Symposium on Mycotoxins and Phycotoxins, Tokyo, Japan, August 16–19, 1988.

(K) The current status of ammoniation was reviewed by Douglas Park, drawing attention to the article "Review of the Decontamination of Aflatoxins by Ammoniation: Current Status and Regulation" published in the July/August 1988 issue of this journal. FDA is still reviewing its position on ammoniation. Arizona is continuing to study the question through a trout-feeding program whereby milk from animals fed aflatoxin-containing feed and animals fed aflatoxin-free feed is dried and added to trout feed rations. Ammoniation is still restricted to only a few states (California, Arizona, Georgia, Alabama, North Carolina) but is in use in several countries.

(L) M. Stack informed the Committee that Stanley Neshheim (FDA) will continue to work on his method for ochratoxin A and will initiate the joint AOAC–IUPAC collaborative study.

(M) S. W. Page (FDA) presented an overview of FDA's position on action levels in light of the U.S. Supreme Court decision. Each case will be examined on an individual basis because there are now no action levels for aflatoxins. There are no plans to establish tolerances.

(N) S. W. Page spoke on the National Toxicology Program (NTP) study on ochratoxin A. Results clearly show carcinogenicity in male and female rats. The report is entitled "Technical Report on the Toxicology and Carcinogenicity Studies of Ochratoxin A." A copy may be obtained by writing to: National Toxicology Program, PO Box 12233, Research Triangle Park, NC 27709.

(O) ELISA and immunoaffinity column test kits evoked a broad discussion. Topics of concern raised by Mary Trucksess included lack of AOAC guidelines for collaborative studies, uncertainty as the level of study for an aflatoxin kit, and differing method instructions in collaborative studies and manufacturer's test kits. There were further technical concerns about such points as antibody affinity, cross reactivity, sensitivity, stability, lot-to-lot variation and temperature requirements, substrate information, and pass/fail vs quantitation. The AOAC Official Methods Board has appointed a Task Force to cover all immunoassays. The goal will be to write guidelines for such tests. James J. Pestka was mentioned as the logical choice to represent the Joint Mycotoxin committee on the Task Force.

Donald Koeltzow presented a report on a Collaborative Field Test of Aflatoxin Test Methods conducted by the Federal Grain Inspection Service. Six rapid methods were compared with TLC methodology.

(P) The possibility of contracting out collaborative studies was discussed. This was considered too expensive but contract laboratories may wish to participate as collaborators.

(Q) Future meetings on mycotoxins are (a) Cellular and Molecular Mode of Action of Selected Microbial Toxins in Foods and Feeds, October 31–November 2, 1988, Washington, DC; (b) American Oil Chemists' Society 80th Annual Meeting, May 3–7, 1989, Cincinnati, OH; (c) Gordon Research Conference on Mycotoxins and Phycotoxins in Human and Animal Health, June 26–30, 1989, Plymouth State College, North Plymouth, NH; (d) Second Latin American Congress on Food Microbiology and Mycotoxins, November 5–10, 1989, Caracas, Venezuela; (e) Symposium on immunoassays as applied to animal toxins and mycotoxins at the 104th AOAC Annual International Meeting, September 10–13, 1990, New Orleans, LA (postponed from 1989).

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Dried Milk, Heat (E-17): John W. Sherbon (Cornell University, Dept of Dairy and Food Science, Ithaca, NY 14853)

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Microbiology (ISO/TC 34/SC 9): Wallace H. Andrews (Food and Drug Administration, Washington, DC 20204)

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International Union of Pure and Applied Chemistry: Philip C. Kearney (U.S. Dept of Agriculture, Beltsville, MD 20705)

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Office International du Cacao et du Chocolat: Henk J. Vos (Populierenlaan, NL 3735LG Bosch en Duin, The Netherlands)

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PESTICIDE FORMULATIONS: CARBAMATE AND SUBSTITUTED UREA INSECTICIDES

Referee: Marshall Gentry, Florida Dept of Agriculture and Consumer Services, Division of Chemistry, 3125 Conner Blvd, Tallahassee, FL 32399-1650

Aldicarb
Karin A. Mede, Rhone Poulenc Ag Co., Box 12014, Research Triangle Park, NC 27709

Bendiocarb
Peter L. Carter, CAMCO, Hauxton, Cambridge, UK CB2 5HU

Carbaryl
Karin A. Mede, Rhone Poulenc Ag Co., Box 12014, Research Triangle Park, NC 27709

Carbofuran and Carbosulfan
Edward J. Kikta, FMC Corp., Agricultural Chemical Group, PO Box 8, Princeton, NJ 08540

Methomyl
James E. Conaway, Jr, E. I. du Pont de Nemours & Co., Agricultural Products Dept, Wilmington, DE 19898

Mexacarbate (Zectran)
Karin A. Mede, Rhone Poulenc Ag Co., Box 12014, Research Triangle Park, NC 27709

Oxamyl
Glenn A. Sherwood, Jr, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Pirimicarb

Trimethylphenyl Carbamate Isomers
Karin A. Mede, Rhone Poulenc Ag Co., Box 12014, Research Triangle Park, NC 27709

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

Referee: Peter D. Bland, ICI Americas, Inc., PO Box 4023, Richmond, CA 94804

Anilazine (Dyrene)
Stephen C. Slahck, Mobay Corp., Box 4913, Kansas City, MO 64120

Benomyl
Mikio Chiba, Agriculture Canada, Research Station, Vineland Station ON L0R 2E0, Canada

Carboxin and Oxycarboxin
Milton Parkins, Uniroyal Chemical Co., Crop Protection Analytical Div., Naugatuck, CT 06770

Chlorothalonil
Brian H. Korsch, Ricerca, Inc., PO Box 1000, Painesville, OH 44079

Copper Naphthenate

Cyfluthrin
Stephen C. Slahck, Mobay Corp., Box 4913, Kansas City, MO 64120

Dinocap

Dioxins in Pentachlorophenol

Dithiocarbamate Fungicides
Warren R. Bontoyan, Environmental Protection Agency, Office of Pesticide Programs, ARC East, Bldg 402, Beltsville, MD 20705

Oxythioquinox (Morestan)
Stephen C. Slahck, Mobay Corp., Box 4913, Kansas City, MO 64120

***o*-Phenylphenol**

Quaternary Ammonium Compounds

Tebucanizole

Thiram

Triademefon (Bayleton)
Stephen C. Slahck, Mobay Corp., Box 4913, Kansas City, MO 64120

Triphenyltin (Fentin)
P. Pasma, M&T Chemicals BV, PO Box 70, 4380 AB Vlissingen, Netherlands

Water-Soluble Copper in Water-Insoluble Copper Fungicides

PESTICIDE FORMULATIONS: HERBICIDES I

Referee: Peter D. Bland, ICI Americas, Inc., PO Box 4023, Richmond, CA 94804

Alachlor, Butachlor, and Propachlor
David F. Tomkins, Monsanto Co., PO Box 473, Muscatine, IA 52761

Amitrole

Atrazine/Alachlor Mixtures
David F. Tomkins, Monsanto Co., PO Box 473, Muscatine, IA 52761

Bentazon
Thomas M. Schmitt, BASF Corp., 1419 Biddle Ave, Wyandotte, MI 48192-3736

Bromoxynil
Laurence J. Helfant, Rhone Poulenc Ag Co., PO Box 12014, Research Triangle Park, NC 27709

Cacodylic Acid, MSMA, and DSMA

Cyanazine

Dichlobenyl
A. De Reyke, Duphar BV, C. J. Van Hourehlann 36, 1381 CP Weesp, The Netherlands

Fomesafen
Stephen J. Eitelman, ICI Americas, Inc., PO Box 4023, Richmond, CA 94804

Metolachlor
Arthur H. Hofberg, Ciba Geigy Corp., PO Box 18300, Greensboro, NC 27419

Metribuzin
William Betker, Mobay Corp., Agricultural Chemicals Division, PO Box 4913, Kansas City, MO 64120

Pesticides in Fertilizers
Paul D. Korger, Wisconsin Dept of Agriculture, Trade, and Consumer Protection, Bureau of Laboratory Services, 4702 University Ave, Madison, WI 53707

Propanil
Steve Gazaway, Rohm and Haas Tennessee Inc., PO Box 591, Knoxville, TN 37901

Sodium Chlorate

PESTICIDE FORMULATIONS: HERBICIDES II

Referee: Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

Benefin, Trifluralin, Ethafluralin, and Pendimethalin
Rodger Stringham, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Bensulide

Benzoylprop-ethyl

Bromacil
Paul K. Tseng, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Chlorophenoxy Herbicides
Richard D. Larson, South Dakota State University, Chemistry Dept, Brookings, SD 57007

Chlorosuifuron
Glenn A. Sherwood, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Dicamba
Benjamin A. Belkind, Sandoz Crop Protection Corp., 1300 E Touhy Ave, Des Plaines, IL 60018

Dimethyl Tetrachloroterephthalate
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Dinoseb

Fluometuron
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

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Glenn A. Sherwood, Jr, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Methazole
Benjamin A. Beikind, Sandoz Crop Protection Corp., 1300 E Touhy Ave, Des Plaines, IL 60018

Naptalam (Alanap)

Oryzalin

Pentachlorophenol

Plant Growth Regulators

Substituted Urea Herbicides

Sulfometuron-methyl
Glenn A. Sherwood, Jr, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

PESTICIDE FORMULATIONS: ORGANOHALOGEN INSECTICIDES

Referee: David F. Tomkins, Monsanto Co., PO Box 473, Muscatine, IA 52761

Benzene Hexachloride and Lindane
Charles F. Harper, Quality Consultants Services, Inc., Box 1372, Ennis, TX 75119

Chlordane

DDT
Frederick C. Churchill, CTB DPD CID Bldg 23, Centers for Disease Control, Atlanta, GA 30333

Dicofol (Kelthane)
Alan M. Rothman, Rohm and Haas Co., Research Laboratories, 727 Norristown Rd, Spring House, PA 19477

Ethylan (Perthane)
Dianne Bradway, Environmental Protection Agency, Denver Federal Center, Denver, CO 80225

Fenvalerate

Heptachlor

Methoxychlor
Juan F. Muniz, Oregon Dept of Agriculture, Laboratory Services, 635 Capitol St NE, Salem, OR 97310

Methyl Bromide

Toxaphene
William H. Clark, Hercules, Inc., Analytical Div., Research Center, Wilmington, DE 19808

Trichlorfon (Dylox)
Dianne Bradway, Environmental Protection Agency, Denver Federal Center, Denver, CO 80225

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHORUS INSECTICIDES

Referee: ———

Acephate
Robert H. Iwamoto, Chevron Chemical Co., 15049 San Pablo Ave, Richmond, CA 94804

Azinphos-methyl
Stephen C. Slahck, Mobay Corp., Box 4913, Kansas City, MO 64120

Coumaphos

Dimethoate
Richard S. Wayne, American Cyanamid Co., Agriculture Div., Box 400, Princeton, NJ 08540

Dioxathion
William H. Clark, Hercules, Inc., Analytical Div., Wilmington, DE 19808

Encapsulated Organophosphorus Pesticides
James J. Karr, Pennwalt Technological Center, 900 First Ave, King of Prussia, PA 19406

EPN
Benjamin A. Belkind, Sandoz Crop Protection Corp., 1300 E Touhy Ave, Des Plaines, IL 60018

Ethoprop
Richard W. Smith, Rhône-Poulenc, Inc., Box 352, Mt Pleasant, TN 38474

Fenitrothion
Dwight L. Mount, Centers for Disease Control, 1600 Clifton Rd, Atlanta, GA 30333

Fensulfothion
Margie Owen, State Chemical Laboratory, Box 329, Auburn, AL 36830
William Betker, Mobay Corp., PO Box 4913, Kansas City, MO 64120

Fenthion
Willard G. Boyd, Jr, State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Fonofos
William Y. Ja, Stauffer Chemical Co., 1200 S 47th St, Richmond, CA 94804

Isofenfos (Oftanol)
Daniel Terry, Mobay Corp., Box 4913, Kansas City, MO 64120

Malathion
Richard S. Wayne, American Cyanamid Co., Agriculture Div., Box 400, Princeton, NJ 08540

Methamidophos
James W. Baird, Mobay Corp., PO Box 4913, Kansas City, MO 64120

Methidathion (Supracide)
G. Thomas Gale, Ciba-Geigy Corp., Box 18300, Greensboro, NC 27409

Oxydemeton-methyl (Metasystox-R)
Stephen C. Slahck, Mobay Corp., Box 4913, Kansas City, MO 64120

Parathion and Methyl Parathion
Edwin R. Jackson, Mississippi State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Phorate

Pirimiphos-methyl
Stephen J. Eitelman, 4224 Walnut Blvd, Walnut Creek, CA 94596

Temephos

***S,S,S*-Tributyl Phosphorotrithioate**
William Betker, Mobay Corp., Box 4913, Kansas City, MO 64120

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND REPELLANTS

Referee: David F. Tomkins, Monsanto Co., PO Box 473, Muscatine, IA 52761

Allethrin
Dean Kassera, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Aluminum Phosphide

2,3,4,5-Bis(2-butylene)tetrahydro-2-furaldehyde
Vernon Meinen, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Cyhalothrin

Cyhexatin

Cypermethrin
Stephen J. Eitelman, 4224 Walnut Blvd, Walnut Creek, CA 94596

Cypromazine (Larvadex)
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

Dipropyl Isocinchomeronate
Dave Carlson, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Fumigants

Glyphosate
Lynn W. Morlier, Monsanto Co., PO Box 174, Luling, LA 70070

Nicotine
Rodney J. Bushway, University of Maine, Dept of Food Science, Orono, ME 04469

Permethrin

Piperonyl Butoxide and Pyrethrins
Dean Kassera, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Resmethrin
C. P. Sharda, Shri Ram Institute for Industrial Research, 19 University Rd, 110007 Delhi, India
Binod Maitin, Shri Ram Institute for Industrial

Research, 19 University Rd, 110007 Delhi, India

Rotenone and Other Rotenoids
Rodney J. Bushway, University of Maine, Dept of Food Science, Orono, ME 04469

PESTICIDE FORMULATIONS: OTHER ORGANOPHOSPHORUS INSECTICIDES

Referee: Marshall Gentry, Florida Dept of Agriculture and Consumer Services, Division of Chemistry, 3125 Conner Blvd, Tallahassee, FL 32399-1650

Crotoxyphos
Wendy King, Florida Dept of Agriculture and Consumer Services, 3125 Conner Blvd, Tallahassee, FL 32399-1650

Dichlorvos

Fenamiphos (Nemacur)
Carl Gregg, Mobay Corp., Box 4913, Kansas City, MO 64120

Mevinphos

Monocrotophos

Naled
Robert H. Iwamoto, Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804

Tetrachlorvinphos

PESTICIDE FORMULATIONS: RODENTICIDES AND MISCELLANEOUS PESTICIDES

Referee: Marshall Gentry, Florida Dept of Agriculture and Consumer Services, Division of Chemistry, 3125 Conner Blvd, Tallahassee, FL 32399-1650

Brodifacoum
Stephen J. Eitelman, 4224 Walnut Blvd, Walnut Creek, CA 94596

Chlorophacinone

Diphacinone

α-Naphthylthiourea

Sampling

Strychnine

Warfarin

CIPAC STUDIES

Referee: Alan R. Hanks, IN Office of The State Chemist, Purdue University, West Lafayette, IN 47907

MICROBIAL PESTICIDES

Referee: Richard H. Collier, Purdue University, Entomology Hall, West Lafayette, IN 47907

DISINFECTANTS

Referee: Aram Beloian (TS-768-C), Environmental Protection Agency, Benefits and Use Division, Washington, DC 20460

Antimicrobial Agents in Laundry Products
Jamie McGee, Dow Corning Corp., Midland, MI 48640

Sporicidal Tests
Ted Wendt, Surgikos, Inc., 2500 Arbrook Dr, Arlington, TX 76010

Textile Antibacterial Preservatives

Tuberculocidal Tests
Joseph Ascenzi, Surgikos, Inc., 2500 Arbrook Dr, Arlington, TX 76010

Use-Dilution Test, Variation and Amendments
Gayle Mulberry, Hill Top Research, Inc., Box 42501, Cincinnati, OH 45242
Eugene C. Cole, University of North Carolina, School of Medicine, Chapel Hill, NC 27514

Virucide Tests
Dale Fredell, Economics Laboratory, Inc., 840 Sibley Memorial Hwy, St. Paul, MN 55118

Committee on Drugs and Related Topics

Eric Sheinin (Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857), *Chairman;* Gaylord Anthony (Warner Lambert Co., Parke Davis Division, 170 Tabor Rd, Morris Plains, NJ 07950); Ronald C. Backer (Office of Medical Investigator, University of New Mexico, Albuquerque, NM 87131); Kenneth Manning (The Upjohn Co., Kalamazoo, MI 49001); Ted M. Hopes (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232); Richard M. Montgomery (Avon Products, Inc., 1 Division St, Suffern, NY 10901); John O'Rangers (Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857); Charles C. Clark (Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166); Thomas D. Doyle (Food and Drug Administration, 200 C St, SW, Washington, DC 20204); William W. Wright (U.S. Pharmacopeial Convention, 12601 Twinbrook Pkwy, Rockville, MD 20852), *Secretary*

DRUGS I

Referee: ———

Acetaminophen in Drug Mixtures
David J. Krieger, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Acetaminophen with Codeine Phosphate
Norlin W. Tymes, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

***p*-Aminobenzoic Acid and Salicylic Acid**
Richard D. Thompson, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Diethylpropion Hydrochloride
Walter E. Dunbar, Food and Drug Administration, 1560 E Jefferson Ave, Detroit, MI 48207

Phenothiazine and Related Drugs
Kurt Steinbrecher, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

Salicylic Acid in Acetylsalicylic Acid Preparations
Maria Santoro, Universidade de Sao Paulo, Cidade Universitaria, Conj Quim B13 CP 30786, 01000 Sao Paulo SP, Brazil

DRUGS II

Referee: Edward Smith, Food and Drug Administration, Biopharmaceutics Research Branch, Washington, DC 20204

Aminacrine
Elaine A. Bunch, Food and Drug Administration, 5003 Federal Office Bldg, Seattle, WA 98174

Belladonna Alkaloids

———

Colchicine in Tablets
Richard D. Thompson, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Curare Alkaloids
John R. Hohmann, Food and Drug Administration, Division of Drug Biology, Washington, DC 20204

Dicyclomine Capsules
Henry S. Tan, University of Cincinnati, College of Pharmacy, Cincinnati, OH 45267

Epinephrine and Related Compounds by LC-Electrochemical Detectors
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Ergot Alkaloids
Thomas C. Knott, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Morphine Sulfate
Ada C. Bello, Food and Drug Administration, 2nd & Chestnut Sts, Philadelphia, PA 19106

Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine

———

Physostigmine and Its Salts
Norlin W. Tymes, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Rauwolfia Alkaloids

———

Rauwolfia serpentina
Ugo R. Cieri, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

DRUGS III

Referee: Martin Finkelson, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Coumarin Anticoagulants
Ella Walker, Food and Drug Administration, New York Regional Laboratory, 850 Third Ave, Brooklyn, NY 11232

Flucytosine
Donald Shostak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Halogenated Hydroxyquinoline Drugs
Edward J. Wojtowicz, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202

Haloperidol
Ella Walker, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Hydralazine
Barry Mopper, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Levodopa
Susan Ting, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Medicinal Gases
Martin Woodhouse, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Metals in Drug Bulk Powders
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Microchemical Tests
Marshall Rabkin, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Penicillins
Barry Mopper, Food and Drug Administration, New York Regional Laboratory, 850 Third Ave, Brooklyn, NY 11232

Salts of Organic Nitrogenous Bases
Samuel Walker, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

DRUGS IV

Referee: Linda Ng, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486

D- and L-Amphetamines
Irving Wainer, St. Jude's Children's Research Hospital, 332 N Lauderdale, PO Box 318, Memphis, TN 38101

Benzodiazepines
Eileen Bargo, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Diazepam
Michael Tsougros, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Heroin
Charles C. Clark, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

DRUGS V

Referee: Thomas G. Alexander, Food and Drug Administration, Center for Drug Evaluation and Research, Washington, DC 20204

Betamethasone
David J. Krieger, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Chlorpropamide
Richard L. Everett, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Conjugated Estrogens by LC
Robert W. Roos, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Pentaerythritol Tetramitrate
Marvin Carlson, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Progestins
Larry K. Thornton, Food and Drug Administration, Division of Drug Analysis, 1114 Market St, St. Louis, MO 63101

Steroid Acetates
Linda Ng, Merck, Sharpe & Dohme, Research Laboratories, West Point, PA 19486

Steroid Phosphates
Elaine A. Bunch, Food and Drug Administration, 5003 Federal Office Bldg, Seattle, WA 98174

DIAGNOSTICS AND TEST KITS

Referee: ———

Analytrelease Rate of Drugs from Transdermal Patches
Norlin W. Tymes, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Automated Microbial Identification Systems—Vitek
Joseph Tardio, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Automated Microbial Identification Systems—HP5898A
Linda English, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Immunological and Diagnostic Assay of Peptides, Hormones and Enzymes
John W. Dyminski, Cooper Laboratories, Inc., 455 E Middlefield Rd, Mountain View, CA 94039

Multicomponent Analysis of Clinical Specimens
Uday J. Mehta, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Tuberculosis and Enteric Infections by Gene Probe
Harvey George, Commonwealth of Massachusetts Center for Laboratory and Communicable Disease Control, 305 South St, Boston, MA 02130

IMMUNOCHEMISTRY

Referee: Dennis Hinton, Food and Drug Administration, 200 C St SW, Washington, DC 20204

Hybridoma-Monoclonal Antibodies

———

DRUG RESIDUES IN ANIMAL TISSUES

Referee: Charlie J. Barnes, Food and Drug Administration, Office of New Animal Drug Evaluation, Beltsville, MD 20705

Benzimidazole
Leon LeVan, Hazleton Raltech, 3301 Kinsman Blvd, Madison, WI 53706

Chemical Antibiotic Methods
William Moats, U.S. Dept of Agriculture, Meat Science Research Laboratory, Beltsville, MD 20705

Chloramphenicol in Animal Tissues
Edward H. Allen, Food and Drug Administration, Bureau of Veterinary Medicine, Beltsville, MD 20705

Chloramphenicol in Milk
Dieter Arnold, Robert Koch Institute, Nordufer 20, 1000 Berlin 65, West Germany

Clopidol in Chicken Tissues
Claire Simmons, Food and Drug Administration, Agricultural Research Service, Beltsville, MD 20705

Enzyme Immunoassays for Antimicrobial Compounds

———

Ipronidazole in Turkey and Swine
Raymond B. Ashworth, U.S. Dept of Agri-

culture, Food Safety and Inspection Service, Washington, DC 20250

Novobiocin in Animal Tissues
William A. Moats, U.S. Dept of Agriculture, Meat Science Research Laboratory, Beltsville, MD 20705

Sulfa Drug Residues

Sulfonamides in Milk (Chromatographic Methods)
John D. Weber, 7204 7th St, NW, Washington, DC 20012

Tetracyclines in Tissues (Chromatographic Methods)

Tetracyclines in Tissues (Microbiological Methods)
Stanley E. Katz, Rutgers University, Dept of Biochemistry and Microbiology, New Brunswick, NJ 08903

COSMETICS

Referee: Ronald L. Yates, Food and Drug Administration, Division of Colors and Cosmetics, Washington, DC 20204

Aloe
Alexis Eberendu, Carrington Laboratories, 3137 Irving Blvd, Dallas, TX 75247

Essential Oils and Fragrance Materials, Components
Harris H. Wisneski, Food and Drug Administration, Division of Colors and Cosmetics, Washington, DC 20204

Nitrosamines
Hardy J. Chou, Food and Drug Administration, Division of Colors and Cosmetics, Washington, DC 20204

Preservatives

Water and Alcohol
Mohinder Singh, Blistex, Inc., 1800 Swift Dr, Oak Brook, IL 60521

Zirconium

FORENSIC SCIENCES

Referee: Stanley M. Cichowicz, Bureau of Engraving and Printing, Research and Development Division, Rm 20725, 14th & C St, SW, Washington, DC 20228

Blood
Ralph Plankenhorn, Pennsylvania State Police, Box P, Greensburg, PA 15601

Fingerprints
Charles M. Connor, 1025 Fashion Ave, Cincinnati, OH 45238

Grouping Tests for Blood and Other Body Fluids
Henry C. Lee, State Police Forensic Science Laboratory, 294 Colony St, Meriden, CT 06450

Gunshot Residue

Immunoelectrophoresis of Biological Fluids
James D. Hauncher, Michigan State Police, 42145 W Seven Mile Rd, Northville, MI 48167

Isoelectric Focusing Methods for Body Fluid Stains

Screening and Confirmatory Tests for Dried Blood Stains

Soils, Geological Analysis
R. C. Murray, University of Montana, Missoula, MT 89801

Committee on Foods I

Richard L. Ellis (U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250), *Chairman;* Elmer George (State Dept of Agriculture and Markets, Capital Plaza, Albany, NY 12235); Michael E. Knowles (Ministry of Agriculture, Fisheries and Food, Horseferry Rd, London SW1P 2AE, UK); Douglas Engebretsen (Land O'Lakes, Inc., PO Box 116, Minneapolis, MN 55440); James F. Lawrence (Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada); James R. Brooker (National Marine Fisheries Service, 1825 Connecticut Ave NW, Washington, DC 20235); Raymond H. Bowers (General Mills, Inc., James Ford Bell Technical Center, Minneapolis, MN 55427); Albert E. Pohland (Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204); Douglas L. Park (University of Arizona, Dept of Nutrition and Food Science, 309 Schantz Bldg, Tucson, AZ 85721), *Secretary;* Michael W. O'Donnell (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

COFFEE AND TEA

Referee: Robert H. Dick, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Ash in Instant Tea
Francis J. Farrell, Thomas J. Lipton, Inc., 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Caffeine in Coffee
Daniel Zuccarello, 214 Greely St, Hightstown, NJ 08520

Methyl Xanthenes
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Moisture
William P. Clinton, General Foods Corp., 250 North St, White Plains, NY 10625

Solvent Residues in Decaffeinated Coffee and Tea
B. Denis Page, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

DAIRY CHEMISTRY

Referee: Gary H. Richardson, Utah State University, College of Agriculture, Logan, UT 84322

Adulteration of Dairy Products with Vegetable Fat
Graham MacEachern, Agriculture Canada, Plant Products, Plant Products Bldg 22, Ottawa, Ontario K1A 0C5, Canada

Babcock Test and Babcock Glassware
Robert L. Bradley, University of Wisconsin–Madison, Food Science Dept, Madison, WI 53706

Babcock, Mojonnier, and Kjeldahl Tests
David M. Barbano, Cornell University, Dept of Food Science, Ithaca, NY 14853

Calcium, Phosphorus, and Magnesium in Cheese
Roger Pollman, New York State Dept of Agriculture and Markets, Capital Plaza, Albany, NY 12235

Chloride Meter
Bruce Vines, Kraft, Inc., 2211 Sanders Rd NB9, Northbrook, IL 60062

Cryoscopy of Milk

Fat, Automated Methods
W. Frank Shipe, Cornell University, Dept of Dairy and Food Science, Ithaca, NY 14853

Fat, Udy Turbidity Test
Doyle C. Udy, Udy Corp., 201 Rome Ct, Ft. Collins, CO 80524

Fat in Milk
Dick H. Kleyn, Rutgers University, Dept of Food Science, New Brunswick, NJ 08903

Fat, Protein, and Total Solids in Milk
David M. Barbano, Cornell University, Dept of Food Science, Ithaca, NY 14853

Iodine
David C. Sertl, Ross Labs, 625 Cleveland Ave, Columbus, OH 43216

β-Lactam Residues in Milk—Delvo-Test
Wesley N. Kelley, State Dairy Laboratory, South Dakota State University, Dairymicro Bldg, Brookings, SD 57007

β-Lactam Residues in Milk—Qualitative Methods
James Messer, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226

β-Lactam Residues in Milk—Quantitative Methods
Roy E. Ginn, Dairy Quality Control Institute, Inc., 2353 Rice St, St. Paul, MN 55133
Raymond Matulis, Kraft, Inc., 801 Waukegan Rd, Glenview, IL 60025

Lactose (Chromatographic Determination)
Leslie G. West, Kraft Co., 801 Waukegan Rd, Glenview, IL 60025

Lactose (Enzymatic Determination)
Dick H. Kleyn, Rutgers University, Dept of Food Science, New Brunswick, NJ 08903
John W. Sherbon, Cornell University, Dept of Dairy and Food Science, Ithaca, NY 14853

Mid-Infrared Instrumentation
D. A. Biggs, Biggs Consulting, 3050 Keighly Rd, Nanaimo, British Columbia, Canada

Moisture in Cheese
Raymond Matulis, Kraft, Inc., 801 Waukegan Rd, Glenview IL 60025

Moisture in Cheese (Microwave)

Mojonnier Method (Robotic)
Robert L. Bradley, University of Wisconsin–Madison, Food Science Dept, Madison, WI 53706

Nitrates in Cheese
James E. Hamilton, Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857

Penicillins in Milk—Affinity Quantitation
Stanley E. Charm, Penicillin Assays, Inc., 36 Franklin St, Malden, MA 02148

Phosphatase, Rapid Method
Dick H. Kleyn, Rutgers University, Dept of Food Science, New Brunswick, NJ 08903

Phosphatase, Reactivated
Gopala K. Murthy, Food and Drug Administration, Division of Microbiology, 1090 Tusculum Ave, Cincinnati, OH 45226

Phosphorus
Wallace S. Brammell, 12423 Chalford Ln, Bowie, MD 20715

Protein Constituents in Processed Dairy Products
Frederick W. Douglas, Jr, U.S. Dept of Agriculture, Eastern Regional Research Center, Philadelphia, PA 19118

Protein in Milk, Rapid Tests
John W. Sherbon, Cornell University, Dept of Dairy and Food Science, Ithaca, NY 14853

Protein Reducing Substance Tests
Joseph T. Cardwell, Mississippi State University, Dairy Science Dept, Mississippi State, MS 39762

Total Solids and Moisture by Microwave Drying

Tyramine
Thea A. B. Reuvers, Centro Nacional de Alimentation y Nutricion, Carretera Majahouda-Pozuelo, Majaharda, Madrid, Spain

Whey Proteins in Nonfat Dry Milk
C. Olieman, Netherlands Institute for Dairy Research, 6710 BA Ede, The Netherlands

FOOD ADDITIVES

Referee: Thomas Fazio, Food and Drug Administration, Office of Physical Sciences, Washington, DC 20204

Anticaking Agents

Antioxidants
B. Denis Page, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Brominated Oils
James F. Lawrence, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Chloride Titrator
Alfred H. Free, Ames Co., Technical Services, Elkhart, IN 46514

Dilauryl Thiodipropionate

Dressings
Charles R. Warner, Food and Drug Administration, Division of Food Chemistry and Technology, Washington, DC 20204

EDTA in Food Products
Gracia A. Perfetti, Food and Drug Administration, Division of Food Chemistry and Technology, Washington, DC 20204

Gums
John K. Baird, Kelco Co., 8225 Aero Dr, San Diego, CA 92123

Indirect Additives from Food Packages
Charles V. Breder, Keller and Heckman, 1150 17th St NW, Washington, DC 20036
Henry Hollifield, Food and Drug Administration, Division of Food Chemistry and Technology, Washington, DC 20204

Monier-Williams Modification
Charles R. Warner, Food and Drug Administration, Division of Food Chemistry and Technology, Washington, DC 20204

Nitrates and Nitrites
Jay Fox, U.S. Dept of Agriculture, Eastern Regional Research Center, Philadelphia, PA 19118

Nitrates (Selective Ion Electrode Titration)

Nitrosamines
Nisu P. Sen, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Nitrosamines in Food Contact Items
J. T. Gray, Michigan State University, Dept of Food Science and Human Nutrition, East Lansing, MI 48824

Nitrosoamino Acids
John W. Pensabene, U.S. Dept of Agriculture, Eastern Regional Research Center, Philadelphia, PA 19118

Polycyclic Aromatic Hydrocarbons
Frank L. Joe, Food and Drug Administration, Division of Food Chemistry and Technology, Washington, DC 20204

Polydimethylsiloxane
R. Firmin, Dow Corning Europe, Chaussee de la Hulpe 154, B-1170, Brussels, Belgium

Polysorbates
Charles F. Smullin, 2402 Heather Rd, Wilmington, DE 19803

Propylene Chlorohydrin

Quinine in Soft Drinks

Sodium Lauryl Sulfate

Sulfiting Agents

MEAT, POULTRY, AND MEAT AND POULTRY PRODUCTS

Referee: Jon E. McNeal, U.S. Dept of Agriculture, Agricultural Marketing Service, Washington, DC 20250

Bouillons and Consommes
Serge Vinogradoff, AIIBP, SA Liebig, II, 2120 Scoten, Belgium

Glutamic Acid and Monosodium Glutamate
Tapani Hattula, Valtion Teknillinen Tutkimuskeskus, Statens Tekniska Forskningscentral, Food Research Laboratory, Vuorimiehentie 5, SF-02150 Espoo 15, Finland

Gluten in Meat
John H. Skerritt, Csiro Wheat Research Unit, Div. of Plant Industry, PO Box 7, North Ryde, NSW, Australia

Hydroxyproline in Meat
Kurt Kolar, Kottforskingsinstitutet, Box 504, 24400 Kavlinge, Sweden

Immunological Methods for Meat and Poultry Products

LC Methods for Meat and Poultry Products
Sher Ali, U.S. Dept of Agriculture, PO Box 6085, Athens, GA 30604

3-Methyl Histidine
Roger Wood, Ministry of Agriculture, Fisheries and Food, 65 Romney St, London, SW1P 3RD, UK

Nitrates and Nitrites
Juan F. Muniz, Oregon Dept of Agriculture, 635 Capitol St NE, Salem, OR 97310

Nitrosamines in Bacon
Walter Fiddler, U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118

Nonmeat Protein in Meat and Poultry Products
Christopher Hitchcock, Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK

Protein, Crude
David Christians, Hach Co., PO Box 907, Ames, IA 50010

Proteins in Meat
Carolyn Henry, U.S. Dept of Agriculture, Food Safety and Inspection Service, PO Box 5080, St. Louis, MO 63115

Robotic Methods
Randy Simpson, U.S. Dept. of Agriculture, PO Box 6085, Athens, GA 30604

Sample Preparation Techniques
Sylvan Eisenberg, Anresco, Inc., 1370 Van Dyke Ave, San Francisco, CA 94124

Serological Identification of Meats
Arthur P. Marin, State Dept of Agriculture and Markets, State Campus, Albany, NY 12235

Specific Ion Electrode Applications
Frank P. McGovern, Omnion, Inc., PO Box 0, Rockland, MA 02370

Sulfamethazine in Plasma and Serum
Douglas E. Rife, Idetek, Inc., 1057 Sneath Ln, San Bruno, CA 94066

Temperature, Minimum Processing
Grover Pickel, U.S. Dept of Agriculture, Food Safety and Inspection Service, PO Box 6085, Athens, GA 30604

Total Fat
Max L. Foster, Kansas State Board of Agriculture, 2524 W 6th St, Topeka, KS 66606

Total Protein by Microwave Digestion
David L. Fish, CEM Corp., PO Box 200, Matthews, NC 28105

Total Solids, Protein, and Moisture by Microwave Drying and Digestion
David L. Fish, CEM Corp., PO Box 200, Matthews, NC 28105

MYCOTOXINS

Referee: Peter M. Scott, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Aflatoxin M
Robert D. Stubblefield, U.S. Dept of Agriculture, Northern Regional Research Center, Peoria, IL 61604

Aflatoxin Methods
Mary W. Trucksess, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Alternaria Toxins
Edgar E. Stinson, U.S. Dept of Agriculture, Eastern Regional Research Center, Philadelphia, PA 19118

Citrinin
David Wilson, University of Georgia, Dept of Plant Pathology, Tifton, GA 31793

Cyclopiazonic Acids
John A. Landsen, U.S. Dept of Agriculture, National Peanut Research Laboratory, Dawson, GA 31742

Ergot Alkaloids
George Ware, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Immunochemical Methods
James J. Pestka, Michigan State University, Dept of Food Science and Human Nutrition, East Lansing, MI 48824

Ochratoxins
Stanley Nesheim, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Penicillic Acid
Charles W. Thorpe, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Sterigmatocystin
Octave J. Francis, Jr, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Tree Nuts
Vincent P. DiProssimo, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Trichothecenes
Robert M. Eppley, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Xanthomegnin and Related Naphthoquinones
Allen S. Carman, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Zearalenone
Glenn A. Bennett, U.S. Dept of Agriculture, Northern Regional Research Center, Peoria, IL 61604

OILS AND FATS

Referee: David Firestone, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Antioxidants
B. Denis Page, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Emulsifiers
H. Bruschweiler, Laboratoire Federal d'Essat des Materiaux, Industrie Genie Civil Arts et Metiers, Unterstrasse 11, CH-9001 St. Gallen, Switzerland

Hydrogenated Fats
Richard A. De Palma, Procter & Gamble Co., Winton Hill Technical Center, 6071 Center Hill Rd, Cincinnati, OH 45224

Lower Fatty Acids
Giovanni Bigalli, Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033

Marine Oils
Robert G. Ackman, Nova Scotia Technical College, Box 1000, Halifax, Nova Scotia B3J 2X4, Canada

Olive Oil Adulteration
Enzo Fedeli, Experimental Station for Oils and Fats, Via Giuseppe Colombo 79, 20133 Milano, Italy

Oxidized Fats
Michael Blumenthal, Libra Laboratories, Inc., 44 Stelton Rd, Piscataway, NJ 08854

Sterols and Tocopherols
Robert J. Reina, Food and Drug Administration, 109 Holton St, Winchester, MA 01890

PLANT TOXINS

Referee: Samuel W. Page, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Glucosinolates
Douglas I. McGregor, Agriculture Canada, 107 Science Crescent, Saskatoon, Saskatchewan S7N 0X2, Canada

Hydrazines
Joseph M. Betz, FDA, Division of Contaminants Chemistry, Washington, DC 20204

Hypoglycine in Ackee
G. William Chase, FDA, Center for Nutrient Analysis, Atlanta, GA 30309

Phytoestrogens
Shia S. Kuan, FDA, Natural Toxins Research Center, New Orleans, LA 70122

Pyrrolizidine Alkaloids
Robert M. Eppley, FDA, Division of Contaminants Chemistry, Washington, DC 20204

Steroidal Alkaloids
Allen S. Carman, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

SEAFOOD PRODUCTS

Referee: Marleen M. Wekell, Food and Drug Administration, 22201 23rd Dr SE, Bothell, WA 98021-4421

Ammonia in Seafood
Beverly A. Hunter, National Marine Fisheries Service, PO Drawer 1207, Pascagoula, MS 39567

Coprostanol

Decomposition of Crabmeat
Kurt Steinbrecher, Food and Drug Administration, 22201 23rd Dr SE, Bothell, WA 98021-4421

Decomposition by Flow Injection Analysis
James Hungerford, Food and Drug Administration, 22201 23rd Dr SE, Bothell, WA 98021-4421

Decomposition by Gas and Liquid Chromatography
Walter F. Staruszkiewicz, Jr, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Drained Weight of Block Frozen, Raw, Peeled Shrimp
Michael F. Blattner, Central Analytical Labs, Inc., 2600 Marietta Ave, Kenner, LA 70062

Fish Content in Coated Products (Breaded or in Batter)
Frederick J. King, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930
H. Houwing, TNO, Division of Nutrition and Food Research, Box 183, 1970 AD, Ijmuiden, The Netherlands

Minced Fish in Fish Fillet Blocks
Frederick J. King, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Nitrites in Smoked Fish

Shellfish Decomposition

Volatile Amines—TMA and DMA by GC
Ronald C. Lundstrom, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

SEAFOOD TOXINS

Referee: ———

Ciguatoxins, Biochemical Methods
Yoshitsugi Hokama, University of Hawaii at Manoa, School of Medicine, Honolulu, HI 96844

Cyanobacterial Peptide Toxins
Harold W. Siegelman, Associated Universities, Inc., Biology Dept, Upton, NY 11973

Diarrhetic Shellfish Poisons
Takeshi Yasumoto, Tokoku University, Dept of Food Chemistry, Tsutsumidori, Sendai 980, Japan

Neurotoxic Shellfish Poisons
Daniel G. Baden, Rosenstiel School of Marine and Atmospheric Sciences, Div. of Biology & Living Research, Miami, FL 33149

Paralytic Shellfish Poisons (Electrochemical Methods)

Paralytic Shellfish Poisons (Immunoassays)
Patrick Guire, BioMetric Systems, Inc., 9932 W 74th St, Eden Prairie, MN 55346

Paralytic Shellfish Poisons (LC Methods)

Shellfish Poisons
William L. Childress, Food and Drug Administration, 109 Holton St, Winchester, MA 01890

Tetrodotoxins
Yururu Shimizu, University of Rhode Island, College of Pharmacy, Kingston, RI 02881

Committee on Foods II

Robert Martin (Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033), *Chairman;* Roger Wood (Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, 65 Romney St, London SW1P 3RD, UK); Adeline A. Peake (3711-37 St NW, Calgary, Alberta T2L 2J2, Canada); Earl F. Richter (Hazleton Labs America, Box 7545, Madison, WI 53707); Patricia Bulhack (Food and Drug Administration, Division of Color Technology, Washington, DC 20204); Laura L. Zaika (U.S. Dept of Agriculture, 600 E Mermaid Ln, Philadelphia, PA 19118); Jon Devries (General Mills, Inc., 900 Plymouth Ave, Minneapolis, MN 55427), *Secretary;* John G. Phillips (U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118), *Statistical Consultant*

ALCOHOLIC BEVERAGES

Referee: Randolph H. Dyer, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Alcohol Content
Samuel I. Blittman, Bureau of Alcohol, Tobacco and Firearms, Treasure Island Naval Station, San Francisco, CA 94130

Carbon Dioxide in Wine
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Citric Acid in Wine
Leonard Mascaro, Boehringer Mannheim Biochemicals, Box 50816, Indianapolis, IN 46250

Ethanol in Wine by GC
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Ethyl Carbamate in Alcoholic Beverages
Randolph H. Dyer, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Flavor Compounds in Malt Beverages
George Charalambous, Anheuser-Busch Inc., Technical Center, St. Louis, MO 63118

Glycerol in Wine
Eric N. Christensen, E & J Gallo Winery, Box 1130, Modesto, CA 95353

Hydrogen Cyanide

Malic Acid in Wine
David T. Chia, Letterman Medical Center, Dept of Pathology, San Francisco, CA 94129

Malt Beverages and Brewing Materials
Peter Gales, Anheuser-Busch, Inc., One Bush Pl, St. Louis, MO 63118

Sorbic Acid in Wine
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Sugars in Wine by Enzymatic Methods
Guenther Henniger, Boehringer Mannheim

GmbH, Bahnhofstrasse 5, D-8132, Tutsing/Obb. Postfach 120, GFR

Sugars in Wine by LC
Jeffrey Kasavan, Taylor California Cellars, Box 780, Gonzales, CA 93926
Thomas Peffer, Beringer Vineyards, 1000 Pratt Ave, St. Helena, CA 94574

Sulfur Dioxide in Wine
Barry Gump, California State University, Dept of Food Science and Enology, Fresno, CA 93740

Synthetic Colors
John A. Steele, 13305 Burkhart St, Silver Spring, MD 20904

Tartrates in Wine
Masao Ueda, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Thujone
Willie Thurman, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Vanillin and Ethyl Vanillin
Felipe Alfonso, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

CEREALS AND CEREAL PRODUCTS

Referee: Ralph H. Lane, University of Alabama, Dept of Human Nutrition and Hospitality Management, Tuscaloosa, AL 35487-0158

Fat Acidity in Flour
Ralph H. Lane, University of Alabama, Dept of Food, Nutrition, and Institute Management, University, AL 35486

Iron
James I. Martin, Food and Drug Administration, 60 8th St, NE, Atlanta, GA 30309

Phytates
Barbara F. Harland, Howard University, Dept of Human Nutrition/Food, Washington, DC 20259

CHOCOLATE AND CACAO PRODUCTS

Referee: ———

Carbohydrates in Chocolate Products
W. Jeffrey Hurst and John C. Robbins, Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033

Moisture in Cacao Products
Robert A. Martin Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033

Nonfat Dry Cocoa Solids

———

Shell in Cacao Products, Micro Methods
W. Jeffrey Hurst and John C. Robbins, Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033

Triglycerides Composition in Cocoa Butter and Fat from Chocolate

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Total and Solid Fat Content in Chocolate Products by NMR

———

COLOR ADDITIVES

Referee: Sandra Bell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Anthocyanin Color Additives Exempt From Certification

———

Arsenic, Barium, and Heavy Metals
William J. McShane, Kraft, Inc., Research and Development Dept, 801 Waukegan Rd, Glenview, IL 60025

Carotenoid Color Additives Exempt from Certification

———

Color in Candy and Beverages
Mary Young, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Color in Cosmetics
Sandra Bell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Color in Nonfrozen Dairy Desserts
Desire L. Massart, Vrije Universiteit Brussel, Pharmaceutical Institute, Laarbeeklaan 103, B-1090 Brussels, Belgium

Color in Other Foods and Drugs
Nicholas Adamo, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Inorganic Salts
Wallace S. Brammell, 12423 Chalford Ln, Bowie, MD 20715

Subsidiary Colors in Certifiable Color Additives
John Bailey, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Trace Organic Constituents of Certifiable Color Additives

———

Uncombined Intermediates in Certifiable Colors
Alan Scher, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

X-Ray Fluorescence Spectroscopy
Catherine Bailey, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

FLAVORS

Referee: Dana A. Krueger, Krueger Food Laboratories, Inc., 24 Blackstone St, Cambridge, MA 02139

Additives in Vanilla Flavorings
Sidney Kahan, Kahansultants, Inc., 66 Peachtree Ln, Roslyn Heights, NY 11577

Carbon-14 in Flavoring Materials
Dana A. Krueger, Krueger Food Laboratories, Inc., 24 Blackstone St, Cambridge, MA 02139

Citral

———

Essential Oils

———

Glycyrrhizic Acid and Glycyrrhizic Acid Salts
Peter S. Vora, McAndrews & Forbes Co., 3rd St and Jefferson Ave, Camden, NJ 08104

Imitation Maple Flavors, Identification and Characterization

———

Organic Solvent Residues in Flavorings

———

Vanillin and Ethyl Vanillin in Food
Sidney Kahan, Kahansultants, Inc., 66 Peachtree Ln, Roslyn Heights, NY 11577

FRUITS AND FRUIT PRODUCTS

Referee: Frederick E. Boland, Food and Drug Administration, Division of Food Chemistry and Technology, Washington, DC 20204

Adulteration of Apple Juice
John Heuser, National Food Processors, 1401 New York Ave, NW, Washington, DC 20005

Adulteration of Orange Juice by Pulpwash and Dilution
Donald R. Petrus, Florida Dept of Citrus, Box 1088 AREC, Lake Alfred, FL 33850

Carbon Stable Isotope Ratio Analysis of Fruit Products
Rae Gabrielle Krueger, Krueger Food Laboratory, Inc., 24 Blackstone St, Cambridge, MA 02139

Determination of Geographic Origin of Orange Juice—Computer Analysis of Trace Elements
Seifollah Nikdel, Florida Dept of Citrus, 700 Experiment Station Rd, Lake Alfred, FL 33850

Fruit Acids
Elia D. Coppola, Ocean Spray Cranberries, Inc., 225 Water St, Plymouth, MA 02360

Fruit Juices, Identification and Characterization
Ronald E. Wrolstad, Oregon State University, Dept of Food Science and Technology, Corvallis, OR 97331

Inductively Coupled Plasma (ICD)
John Heuser, National Food Processors, 1401 New York Ave, NW, Washington, DC 20005

Limonin in Citrus Juices
Russell Rouseff, Florida Dept of Citrus, 700 Experiment Station Rd, Lake Alfred, FL 33850

Moisture in Dried Fruits
Wayne Stevenson, Dried Fruit Association, Box 86 Fresno, CA 93707

Orange Juice Content

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Sodium Benzoate in Orange Juice
Hyong S. Lee, Florida Dept of Citrus, 700 Experiment Station Rd, Lake Alfred, FL 33850

NONALCOHOLIC BEVERAGES

Referee: John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Caffeine and Methyl Xanthines in Nonalcoholic Beverages
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Caloric Content

Glycyrrhizic Acid Salts in Licorice-Derived Products

Lasiocarpine and Pyrrolizidines in Herbal Beverages

Quinine
Leonard Valenti, Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857

Safrole in Sassafras

PRESERVATIVES AND ARTIFICIAL SWEETENERS

Referee: Benjamin Krinitz, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Aspartame, Benzoates, Saccharin, and Caffeine, Liquid Chromatography

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Organic Preservatives (Thin Layer Chromatography)

Sulfites (Flow Injection Methods)
Marleen M. Wekell, Food and Drug Administration, 909 1st Ave, 5009 Federal Office Bldg, Seattle, WA 98174

Sulfites (Ion Chromatographic Methods)
Darryl M. Sullivan, Hazleton Labs, P.O. Box 7545, Madison, WI 53707
Hie Joon Kim, U.S. Army Natick Research and Development Center, Natick, MA 01760

Sulfites (Polarographic Methods)
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Sulfites in Shrimp (Screening Methods)
Martha Hudak-Roos, National Marine Fisheries Service, 3209 Frederick St, Pascagoula, MS 39568-1207

PROCESSED VEGETABLE PRODUCTS

Referee: Thomas R. Mulvaney, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

pH Determination
Frederick E. Boland, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Sodium Chloride

Sugars in Processed Vegetables by LC

Total Solids by Microwave Moisture Analyzer
Henry B. Chin, National Food Processors Association, 1950 Sixth St, Berkeley, CA 94710

Water Activity in Foods
William H. Stroup, Food and Drug Administration, Food Engineering Branch, 1090 Tusculum Ave, Cincinnati, OH 45226

SPICES AND OTHER CONDIMENTS

Referee: James E. Woodbury, Cal-Compack Foods, Inc., PO Box 265, Santa Ana, CA 92702

Ash and Pungent Principles in Mustard

Bulk Index Methods
Gary C. Oetting, Basic American Foods, Inc., 700 Airport Dr, King City, LA 93930

Curcumin in Turmeric
Ted Lupina, Kalsec, Inc., PO Box 511, Kalamazoo, MI 49005

Ethylene Oxide and Ethylene Chlorohydrin Residues
Lynn Theiss, Durkee-French, Inc., PO Box 2460, Fairfield, NJ 07007

Extractable Color in Capsicum Spices and Oleoresins
James E. Woodbury, Cal-Compack Foods, Inc., PO Box 265, Santa Ana, CA 92702

Moisture in Dried Spices
Louis A. Sanna, Santa Maria Chili, Inc., Box 1028, Santa Maria, CA 93456

Monosodium Glutamate in Foods

Piperine in Black Pepper
Ted Lupina, Kalsec, Inc., PO Box 511, Kalamazoo, MI 49005

Pungency of Capsicums and Oleoresins
James E. Woodbury, Cal-Compack Foods Inc., PO Box 265, Santa Ana, CA 92702

Vinegar
Dana A. Krueger, Krueger Food Labs, 24 Blackstone St, Cambridge, MA 02139

SUGARS AND SUGAR PRODUCTS

Referee: Margaret A. Clarke, Sugar Processing Research, Inc., Box 19687, New Orleans, LA 70179

Color, Turbidity, and Reflectance-Visual Appearance
Margaret A. Clarke, Sugar Processing Research, Inc., Box 19687, New Orleans, LA 70179

Corn Syrup and Corn Sugars
Raffaele Bernetti, CPC International, Box 345, Argo, IL 60501

Enzymatic Methods
Marc Mason, Yellow Springs Instrument Co., Box 279, Yellow Springs, OH 45387

Gas Chromatographic Methods
Mary A. Godshall, Sugar Processing Research, Inc., PO Box 19687, New Orleans, LA 70179

Honey
Jonathan W. White, 217 Hillside Dr, Navasota, TX 77868

Lactose Purity Testing
Janice R. Saucerman, Bristol-Myers, 2404 Pennsylvania Ave, Evansville, IN 47721

Liquid Chromatographic Methods
W. S. Charles Tsang, Sugar Processing Research Inc., Box 19687, New Orleans, LA 70179

Maple Sap and Syrups
Lynn Whalen, University of Vermont, Dept of Botany, 225 Marsh Life Science Bldg, Burlington, VT 05405-0086

Oligosaccharides
George Steinle, Suddeutsche Zucker AG, Obrigheim 5, Postfach 1127, D-6718, Gruenstadt 1, FRG

Polarimetric Methods
Ronald Plews, Tate and Lyle Refineries, Thames Refinery, Silvertown, London, UK E16 2EW

Stable Carbon Isotope Ratio Analysis
Landis W. Doner, U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118

Standardization of Methods of Sugar Analysis
Mary A. Godshall, Sugar Processing Research, Inc., Box 19687, New Orleans, LA 70179

Sugars in Cereals
L. Zygmunt, Quaker Oats Co., 617 W Main St, Barrington, IL 60010

Sugars in Syrups
Rose E. Goff, Food and Drug Administration, 60 Eighth St NE, Atlanta, GA 30309

Sulfites
Richard Riffer, C&H Sugar, Crockett, CA 94525

Weighing, Taring, and Sampling

Michael Steele, R. Markey & Sons, 5 Hanover Square, New York, NY 10004

VITAMINS AND OTHER NUTRIENTS

Referee: Mike J. Deutsch, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Amino Acids
John P. Cherry, U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118
Robert Zumwalt, University of Missouri–Columbia, Agriculture Bldg, Columbia, MO 65211

Automated Nutrient Analysis
Jonathan DeVries, General Mills Inc., 9000 Plymouth Ave, Minneapolis, MN 55427

Biotin
Jacob M. Scheiner, Hoffmann-La Roche, 340 Kingsland St, Nutley, NJ 07110

Carotenoids
Forrest W. Quackenbush, 2911 Browning St, West Lafayette, IN 47906

Cholesterol in Foods

Dietary Fiber
Leon Prosky, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Fat in Food by Chloroform-Methanol Extraction

Folic Acid
Lynn Hoepfinger, Henkel Corp., Box 191, Kankakee, IL 60901

Iodine
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Nutrient Assay of Infant Formula
James T. Tanner, Food and Drug Administration, Division of Nutrition, Washington, DC 20204
Stephen A. Barnett, Bristol-Meyers, 2404 Pennsylvania Ave, Evansville, IN 47721

Pantothenic Acid, Total Activity

Protein Quality, Evaluation
Phillip H. Derse, DS Associates, 979 Jonathan Dr, Madison, WI 53713

Sodium
Edgar R. Elkins, National Food Processors Association, 1401 New York Ave, NW, Washington, DC 20005

Thiamine Assay, Enzyme and Column Packing Reagents
Wayne Ellefson, Hazleton Laboratories America, Box 7545, Madison, WI 53707

Vitamin A
J. Neville Thompson, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario K1A 0L2, Canada

Vitamin D
Ellen J. de Vries, Duphar B.V., Research Dept 30, PO Box 2, Weesp, The Netherlands

Vitamin E
Edward Waysek, Hoffmann-La Roche, Inc., 340 Kingsland St, Nutley, NJ 07110

Vitamin E in Pharmaceuticals (Gas Chromatography)
Alan J. Sheppard, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Vitamin K
Stephen A. Barnett, Bristol-Meyers, 2404 Pennsylvania Ave, Evansville, IN 47721

Vitamins A, D, E, and K by Gel Permeation and LC
Abdel-Gawad Soliman, Food and Drug Administration, 60 Eighth St, Atlanta, GA 30309

Committee on Residues

Richard Schmitt (Environmental Protection Agency, Office of Pesticide Programs, Washington, DC 20460), *Chairman;* Kenneth W. Boyer (Southern Testing and Research Laboratories, Inc., PO Box 1849, Wilson, NC 27893); Paul E. Corneliussen (Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204); W. George Fong (State Dept of Agriculture, Division of Chemistry, Tallahassee, FL 32301); Ronald F. Cook (FMC Corp., Agricultural Chemicals Group, PO Box 8, Princeton, NJ 08543); Brian D. Ripley (Ontario Ministry of Agriculture and Food, Providence Pesticide Residue Testing, Guelph, Ontario N1G 2W1, Canada); Henry B. Chin (National Food Processors Association, 6363 Clark Ave, Dublin, CA 94568); Keith A. McCully (Health and Welfare Canada, Field Operations Directorate, Tunney's Pasture, Ottawa, Ontario K1A 1B7, Canada), *Secretary;* Richard H. Albert (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

METALS AND OTHER ELEMENTS

Referee: Stephen G. Capar, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Atomic Absorption Spectrophotometry
Milan Ihnat, Agriculture Canada, Land Resource Research Centre, Ottawa, Ontario K1A 0C6, Canada

Cadmium and Lead in Earthenware
Benjamin Krinitz, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Emission Spectrochemical Methods
Fred L. Fricke, Food and Drug Administration, 1141 Central Pkwy, Cincinnati, OH 45202

Fluorine
Robert W. Dabeka, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario K1A 0L2, Canada

Graphite Furnace—Atomic Absorption Spectrophotometry
Robert W. Dabeka, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario K1A 0L2, Canada

Hydride Generating Techniques
Stephen G. Capar, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Lead in Calcium Supplements
Patricia Maroney Benassi, Food and Drug Administration, IITRI, 10 W 35th St, Chicago, IL 60616

Methyl Mercury in Fish and Shellfish
Susan Hight, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Neutron Activation Analysis
William C. Cunningham, Food and Drug Administration, Division of Contaminants Chemistry, NIST, Bldg 235, Rm B108, Gaithersburg, MD 20899

Organometallics in Fish
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Organotin Compounds
Allen D. Uhler, Battelle Ocean Sciences, 397 Washington St, Duxbury, MA 02332

Polarography
Susan Hight, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

MULTIRESIDUE METHODS

Referee: Leon D. Sawyer, Food and Drug Administration, 200 C St SW, Washington, DC 20204

Comprehensive Multiresidue Methodology
S. Mark Lee, California Dept of Food and Agriculture, Div of Inspection Services, 3292 Meadowview Rd, Sacramento, CA 95832

Extraction of Low Moisture-High Fat Samples

Fumigants
James L. Daft, Food and Drug Administration, 1009 Cherry St., Kansas City, MO 64106

Miniaturization
D. Ronald Erney, Food and Drug Administration, 1560 Jefferson Ave, Detroit, MI 48207

Organophosphorus Pesticide Residues
Ronald R. Laski, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202

Sweep Codistillation
Barry Luke, State Chemistry Laboratory, 5 MacArthur St, East Melbourne, Victoria 3002, Australia

Synthetic Pyrethroids
Darryl E. Johnson, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

ORGANOHALOGEN PESTICIDES

Referee: Bernadette McMahon, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Chlordane
Wilbur Saxton, Food and Drug Administration, 22201 23rd Dr SE, Bothell, WA 98021-4421

Chlorinated Dioxins
David Firestone, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Chlorophenoxy Alkyl Acids
Marvin L. Hopper, Food and Drug Administration, 1009 Cherry St, Kansas City, MO 64106

Ethylene Oxide and Its Chlorohydrin
Ronald D. Ross, University of Kentucky, Dept

of Preventative Medicine, Lexington, KY 40536

Methyl Bromide
Joseph H. Ford, U.S. Dept of Agriculture, NMRAL, PO Box 3209, Gulfport, MS 39503
Richard A. DePalma, Procter and Gamble, 6071 Center Hill Rd, Cincinnati, OH 45224

Polychlorinated Biphenyls in Blood
Virlyn Burse, Center for Environmental Health, Clinical Chemistry Division, 1600 Clifton Rd, Atlanta, GA 30333

Polychlorinated Biphenyls (PCBs)
Leon D. Sawyer, Food and Drug Administration, Division of Contaminants Chemistry, 200 C St SW, Washington, DC 20204

Polychlorinated Biphenyls (PCBs) Determination by Measurement of Specific Congeners
Kimmo K. Himberg, Technical Research Center of Finland, Food Research Laboratory, Biologinkuja 1, ESP00 SF-02150, Finland

ORGANONITROGEN PESTICIDES

Referee: W. Harvey Newsome, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada

Anilazine

Benzimidazole-Type Fungicides

Captan and Related Fungicides
Dalia Gilvydis, Food anc Drug Administration, 1560 E Jefferson Ave, Detroit, MI 48207

Carbamate Herbicides

Carbamate Insecticides (Liquid Chromatography)
Richard Krause, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Carbofuran

Chlorothalonil

Daminozide and 1,1-Dimethylhydrazine
Milton Parkins, Uniroyal Chemical Co., Crop Protection Research and Development, Naugatuck, CT 06770

Diquat and Paraquat
Brian Worobey, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Dithiocarbamate Fungicides

Maleic Hydrazide

Organonitro Pesticides
Richard Krause, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Sodium *o*-Phenylphenate

Substituted Ureas
Ronald Luchtefeld, Food and Drug Administration, 1009 Cherry St, Kansas City, MO 64106

Thiolcarbamate Herbicides

s-Triazines

ORGANOPHOSPHORUS PESTICIDES

Referee: Gail Abbott Parker, Florida Dept of Agriculture and Consumer Services, 3125 Conner Blvd, Tallahassee, FL 32399-1650

Disulfoton
Sunny Y. Szeto, Agriculture Canada, 6660 NW Marine Dr, Vancouver, British Columbia V6T 1X2, Canada

Extraction Procedures

Gel Permeation Chromatography Cleanup
W. Frank McCullough, Analytical Biochemistry Laboratories, PO Box 1097, Columbia, MO 65205

Phorate

Phosphine
Bartholomew Puma, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Terbufos

RADIOACTIVITY

Referee: Edmond J. Baratta, Food and Drug Administration, Winchester Engineering and Analytical Center, Winchester, MA 01890

Cesium-137
Edmond J. Baratta, Food and Drug Administration, Winchester Engineering and Analytical Center, Winchester, MA 01890

Iodine-131
Eugene Easterly, Environmental Protection Agency, PO Box 15027, Las Vegas, NV 89114

Plutonium

Radium-228
Jacqueline Michel, Research Planning Institute, 925 Gervais St, Columbia, SC 29201

Strontium-89 and -90

Tritium

Committee on Microbiology and Extraneous Materials

Donald A. Mastrorocco (Hershey Chocolate Co., PO Box 1028, Stuarts Draft, VA 24477), *Chairman;* Khalil Rayman (Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada); Robert M. Twedt (Food and Drug Administration, Division of Microbiology, Washington, DC 20204); Phillip Alioto (State Dept of Agriculture, 4702 University Ave, Madison, WI 53707); Michael H. Brodsky (Ministry of Health, Laboratory Services Branch, PO Box 9000, Terminal A, Toronto, Ontario M5W 1R5, Canada); Virginia N. Scott (National Food Processors Association, 1401 New York Ave, Suite 400, Washington, DC 20005); John S. Gecan (Food and Drug Administration, Division of Microbiology, Washington, DC 20204), *Secretary;* Foster D. McClure (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

ANALYTICAL MYCOLOGY AND MICROSCOPY

Referee: Ruth Bandler, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

***Geotrichum* Mold in Canned and Frozen Fruits, Vegetables, and Fruit Juices**

Mold Counts by Compound Microscope
Don Vail, Food and Drug Administration, 60 Eighth St, NE, Atlanta, GA 30309

Mold Detection by Chemical Methods
Ruth Bandler, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Standardization of Plant Tissue Concentrations for Mold Counting

Vegetable Substitutes in Horseradish
Raymond Galacci, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

FILTH AND EXTRANEOUS MATERIALS IN FOODS AND DRUGS

Referee: Jack L. Boese, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Baked Goods with Fruit and Nut Tissues, Flotation Method
Joseph K. Nagy, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

Basil (Ground), Flotation Method
Beverly Kent, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Botanicals, Flotation Method
Marvin Nakashima, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Canned and Dried Soups, Flotation Method
Richard Klein, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Canned Crabmeat, Shrimp, and Tuna, Brine Flotation Method
James D. Barnett, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Canned Fish and Fish Products, Flotation Method
Wilfred A. Sumner, Food and Drug Administration, 50 United Nations Plaza, San Francisco, CA 94102

Cheese, Pancreatin Digestion Method
Mary-Ann Gardiner, Health and Welfare Canada, Microbiology Research Division, Ottawa, Ontario K2A 0L2, Canada

Chocolate and Chocolate Products, Flotation Method
C. Robert Graham, Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601

Coffee (Ground), Flotation Method
Gerald E. Russell, Food and Drug Administration, 1560 E Jefferson Ave, Detroit, MI 48207

Dehydrated Vegetable Products, Flotation Method
Francis J. Farrell, Thomas J. Lipton, Inc., 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Fish Paste and Sauces, Flotation Method
Larry E. Glaze, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Grain Products, Flotation Method
Larry E. Glaze, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Grain Products, Mammalian Feces Detection by Alkaline Phosphatase Method
Harriet R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Grains (Whole), Internal Insect Infestation by Cracking Flotation Method
Richard Trauba, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Onion and Garlic Powders and Granules, Sedimentation Method
James Buhlert, University of California–Davis, Dept of Food Science and Technology, Davis, CA 95616

Popcorn (Popped), Flotation Method
James Karpus, Food and Drug Administration, 10 W 35th St, Chicago, IL 60616

Popcorn (Unpopped), Sieving Method
Alan Whiteman, Food and Drug Administration, 10 W 35th St, Chicago, IL 60616

Rodent Gnawing of Packaging Materials and Foods, Salivary Amylase Test
Jack L. Boese and Roger L. Heitzman, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Soybean Curd, Flotation Method
Marvin Nakashima, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Spices, Chemical Detection of Mammalian Feces
Harriet R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Spirulina, Flotation Method
Marvin Nakashima, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Tomatoes, Brine Extraction Method
Bernice Beavin, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Urine Stains on Foods and Containers, Chemical Methods
Robert S. Ferrera, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

COSMETIC MICROBIOLOGY

Referee: Anthony D. Hitchins, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

DAIRY MICROBIOLOGY

Referee: James Messer, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226

Aerobic Plate Counts and Coliforms—Petrifilm Method
Michael S. Curiale, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Bactoscan Methods

Listeria—Culture Methods
Joseph Lovett, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226

Raw Milk in Cheese
G. K. Murthy, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226

Somatic Cells, Automated Optical Counting Method
Wesley N. Kelley, State Dairy Laboratory, South Dakota State University, Dairymicro Bldg, Brookings, SD 57007

Somatic Cells, Fossomatic Counting Method
R. D. Mochrie, North Carolina State University, Animal Science Dept, PO Box 7621, Raleigh, NC 27695

DRUG AND DEVICE RELATED MICROBIOLOGY

Referee: Gordon Oxborrow, 3M Co., 3M Center, Bldg 270-5N-01, St. Paul, MN 55144

Chemical Indicators
Marvin L. Hart, 3M Co., 3M Center, Bldg 270-3N-04, St. Paul, MN 55144

Limulus Amebocyte Lysate Tests for Endotoxins
Christine W. Twohy, Food and Drug Administration, Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Packaging Integrity of Medical Devices
Anna M. Placencia, Food and Drug Administration, Sterility Research Center, 100 Union St, Minneapolis, MN 55455

Sporicidal Testing of Disinfectants/Sterilants
James Danielson, Food and Drug Administration, 100 Union St SE, Minneapolis, MN 55455

Testing and Standardization of Biological Indicators
Robert Berube, 3M Co., 3M Center, Bldg 270-5N-03, St. Paul, MN 55144
Gordon Oxborrow, 3M Co., 3M Center, Bldg 270-5N-01, St. Paul, MN 55144

FOOD MICROBIOLOGY (NONDAIRY)

Referee: Wallace H. Andrews, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Aerobic Plate Counts and Coliforms—ISO-GRID Method
Phyllis Entis, QA Laboratories, Ltd, 135 The West Mall, Toronto, Ontario M9C 1C2, Canada

Aerobic Plate Counts and Coliforms—Petrifilm Methods
Vernal S. Packard, University of Minnesota, Dept of Food Science and Nutrition, St. Paul, MN 55108

Aerobic Plate Counts of Foods Using Redigel Medium
Jonathan N. Roth, RCR Scientific, Inc., 206 W Lincoln Ave, Goshen, IN 46526

***Bacillus cereus* Enterotoxin, Microslide Gel Double Diffusion Test**
Reginald W. Bennett, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

***Bacillus cereus*, Isolation and Enumeration**
Stanley M. Harmon, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Bactoscan Methods
J. D. Cunningham, University of Guelph, Environmental Biology, Guelph, Ontario N1G 2W1, Canada

Campylobacter Species

Enterotoxigenic *Escherichia coli*—Detection by DNA Hybridization
Walter Hill, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

***Escherichia coli* in Chilled and Frozen Foods—MUG Test**
Lloyd J. Moberg, General Mills, Inc., 9000 Plymouth Ave N, Minneapolis, MN 55427

***Escherichia coli* in Shellfish—MUG Test**
William Watkins, Food and Drug Administration, Construction Battalion Center, Bldg S 26, North Kingstown, RI 02852

Iron Milk Test for Recovery of *Clostridium perfringens* from Marine Environment
Carlos Abeyta, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

***Listeria*—DNA Probe**

***Listeria*—Listeria-Tek Assay**
Michael S. Curiale, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

***Salmonella*—Bio-EnzaBead Enzyme Immunoassay Screening Method**
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

***Salmonella*—GENE-TRAK DNA Hybridization Screening Method**
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

***Salmonella*—ImmunoBand Screening Method**
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

***Salmonella*—ISO-GRID Screening Method**
Phyllis Entis, QA Laboratories, Ltd, 135 The West Mall, Toronto, Ontario M9C 1C2, Canada

***Salmonella*—Q-TROL Enzyme Immunoassay Screening Method**
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

***Salmonella*—TECRA Enzyme Immunoassay Screening Method**
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

***Salmonella, Escherichia coli,* and Other *Enterobacteriaceae,* Identification by Micro ID Diagnostic Kit**
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

***Salmonella* in Chocolate**
O. Bindschedler, Nestec, Ave Nestle 55, CH 1800 Vevey, Switzerland

***Vibrio cholerae* in Oysters—Elevated Temperature Enrichment Method**
Angelo DePaola, RR 1 Box 980, Coden, AL 36523

Yeasts and Molds—Mycological Media for Isolation
Philip B. Mislivec, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Yersinia enterocolitica
James S. Cholensky and Sallie McLaughlin, Food and Drug Administration, Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401
Georg Kapperud, National Institute of Public Health, Norwegian Defense Microbiology Laboratory, Geitmyrsvein 75, N-0462, Oslo 4, Norway

WATER MICROBIOLOGY

Referee: Al DuFore, Environmental Protection Agency, 1675 Vanquera Pl, Cincinnati, OH 45268

Committee on Feeds, Fertilizers, and Related Materials

Howard Casper (North Dakota State University, Veterinary Diagnostic Laboratory, Fargo, ND 58102), *Chairman;* Lars M. Reimann (Woodson Tenent Laboratories, 345 Adams, Memphis, TN 38103); Rodney J. Noel (Purdue University, Biochemistry Department, West Lafayette, IN 47907); Stanley E. Katz (Rutgers University, Dept of Biochemistry and Microbiology, New Brunswick, NJ 08903); Thomas M. Parham (Triazone Corp., PO Box 307, Geismar, LA 70734); Lori L. Rhodig (Smith Kline Animal Health Products, 4444 S 76th St, Omaha, NE 68127); Carolyn Geisler (Food and Drug Administration, Denver Federal Center, Bldg 20, PO Box 25087, Denver, CO 80225); Billy M. Colvin (University of Georgia, College of Veterinary Medicine, Box 1389, Tifton, GA 31794), *Secretary;* Daniel H. Mowrey (Lilly Research Laboratory, Div. of Eli Lilly Corp., Greenfield, IN 46140), *Statistical Consultant*

ANTIBIOTICS IN FEEDS

Referee: H. S. Ragheb, Purdue University, Biochemistry Department, West Lafayette, IN 47907

Apramycin
John W. Lamb, Elanco Product Co., PO Box 1750, Indianapolis, IN 46285

Bacitracin
Linda L. Knotts, Pittman Moore, PO Box 207, Terre Haute, IN 47808

Bacitracin (Chemical Method)
John Gallagher, Pittman Moore, PO Box 207, Terre Haute, IN 47808

Bambermycins

Chlortetracycline

Cup Plate System for Antibiotic Analysis

Direct-Fed Microbiological Products and Silage Inoculants
Donald L. Wallace, Marschall Products, PO Box 592, Madison, WI 53701

Erythromycins

Lincomycin
Gerald L. Stahl, The Upjohn Co., Agricultural Div., Kalamazoo, MI 49001

Microbiological Assay Design and Statistics
Marietta S. Brady, Rutgers University, Dept of Biochemistry and Microbiology, New Brunswick, NJ 08903

Monensin
Robert E. Scroggs, Elanco Products Co., PO Box 1750, Indianapolis, IN 46206

Narasin
Robert E. Scroggs, Elanco Products Co., PO Box 1750, Indianapolis, IN 46206

Neomycin
Gerald L. Stahl, Upjohn Co., Agricultural Div., Kalamazoo, MI 49001

Oxytetracycline (Microbiological Methods)
Mary Lee Hasselberger, Nebraska Dept of Agriculture, 3703 S 14 St, Lincoln, NE 68502

Tylosin
Mark R. Coleman, Eli Lilly & Co., Box 708, Greenfield, IN 46140

Virginiamycin (Diffusion Assay)
James A. Miller, Smith Kline Animal Health, 1600 Paoli Pike, West Chester, PA 19380

Virginiamycin (Turbidimetric Assay)
Hussein S. Ragheb, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

DRUGS IN FEEDS

Referee: Robert L. Smallidge, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Amprolium
Elzbieta Kentzer, Abbott Laboratories, 1400 Sheridan Rd, North Chicago, IL 60064

Arsanlic Acid

Bacitracin (Chemical Method)
John B. Gallagher, Pitman Moore, Inc., PO Box 207, Terre Haute, IN 47808

Carbadox
Alicia Wren, Pfizer Inc., 1107 South Missouri 291, Lee's Summit, MO 64063

Ethopabate
Joseph Hillebrandt, Agway, Inc., 777 Warren Rd, Ithaca, NY 14850

Ethylenediamine Dihydroiodide

Furazolidone and Nitrofurazone
Robert L. Smallidge, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Lasalocid (Liquid Chromatographic Methods)
Edward Waysek, Hoffmann-La Roche, Inc., 340 Kingsland St, Nutley, NJ 07110

Melengestrol Acetate
Raymond Davis, The Upjohn Co., Henrietta St Labs, Kalamazoo, MI 49001

Morantel Tartrate
Linda D. Werner, Pfizer, Inc., 1107 S Missouri 291, Lee's Summit, MO 64063

Oxytetracycline (Liquid Chromatographic Methods)
Mary Lee Hasselberger, Nebraska Dept of Agriculture, 3703 S 14 St, Lincoln, NE 68502

Pyrantel Tartrate
Joyce Konrardy, Pfizer, Inc., Agriculture Div., 1107 S Missouri St, Lee's Summit, MO 64063

Roxarsone
Glenn M. George, Salsbury Laboratories, 2000 Rockford Rd, Charles City, IA 50616

Sulfadimethoxine
Edward Waysek, Hoffmann-La Roche, 340 Kingsland St, Nutley, NJ 07110

Sulfa Drug Residues
Valerie Reeves, Food and Drug Administration, BARC-East, Bldg 328-A, Beltsville, MD 20705

Sulfamethazine and Sulfathiazole (Premix and Finished Feed Levels)
Dwight M. Lowie, State Dept of Agriculture, 4000 Reedy Creek Rd, Raleigh, NC 27607

FEEDS

Referee: Joel Padmore, North Carolina Dept of Agriculture, 4000 Reedy Creek Rd, Raleigh, NC 27607

Amino Acid Analysis in Mixed Feeds
Wayne Stockland, Supersweet Research Farm, Box 117, Courtland, MN 56021

Calcium Salts of Isobutyric and Mixed 5-Carbon Volatile Fatty Acids
John A. Rogers, Eastman Chemical Division, Animal Nutrition Supplements, Box 1955, Kingsport, TN 37662

Carotenoids
D. E. McNaughton, Ministry of Agriculture and Fisheries, Private Bag, Hamilton, New Zealand

Enzymes and Microbial Additives

Fat
George Wilkens, Agway Technical Center, 77 Warren Rd, Ithaca, NY 14850

Fiber
David R. Mertens, U.S. Dairy Forage Research Center, 1925 Linden Dr W, University of Wisconsin, Madison, WI 53706

Infrared Reflectance Techniques in Mixed Feeds
Franklin E. Barton II, U.S. Dept of Agriculture, Agricultural Research Service, Southern Region, Box 5677, Athens, GA 30604

Iodine and EDDI
George W. Latimer, Office of the Texas State Chemist, PO Box 3160, College Station, TX 77841

Microscopy
Patricia Ramsey, CDFA Chemical Laboratories, 3292 Meadowview Rd, Sacramento, CA 95832

Minerals
Joel Padmore, North Carolina Dept of Agriculture, 4000 Reedy Creek Rd, Raleigh, NC 27607

Moisture in Mixed Feeds and Forages
William R. Windham, U.S. Dept of Agriculture, PO Box 5677, Athens, GA 30677

Moisture in Pet Foods
Roy E. Schulze, Ralston Purina Co., Checkerboard Square, St. Louis, MO 63164

Non-Nutritive Residues
Peter J. Van Soest, Cornell University, Dept of Animal Science, Ithaca, NY 14850

Protein, Crude
Peter F. Kane, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Sampling
Darrel L. Sharpe, State Dept of Agriculture, PO Box 630, Jefferson City, MO 65102

Vitamins

FERTILIZERS

Referee: Frank J. Johnson, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Bluret
Luis F. Corominas, Apartado Postal No. 7, Cuautitlan 54800, Edo de Mexico, Mexico

Boron
James R. Melton, Texas A&M University, Agricultural Analytical Services, College Station, TX 77843

Dicyandiamide

Free and Total Water

Iron
James Silkey, State Dept of Agriculture. Laboratory Services Div., Salem, OR 97310

Melamine
Billy Arcement, Melamine Chemicals, Inc., Box 748, Donaldsonville, LA 70346

Nitrogen
Robert L. Roser, OM Scott & Sons Co., Marysville, OH 43041

Phosphorus
Joe R. Trimm, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Potasssium
Peter F. Kane, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Sample Preparation
Rose A. Sweeney, University of Missouri, Experiment Station, Columbia, MO 65211

Sampling
Douglas Caine, Vigoro Industries, Inc., PO Box 4139, Fairview Heights, IL 62208

Slow-Release Mixed Fertilizers
Stanley E. Katz, Rutgers University, Dept of Biochemistry and Microbiology, New Brunswick, NJ 08903

Sodium
Luis F. Corominas, Apartado Postal No. 7, Cuautitlan 54800, Edo de Mexico, Mexico

Soil and Plant Amendment Ingredients
Clyde E. Jones, State Dept of Agriculture, 2331 W 31st Ave, Denver, CO 80211

Sulfur
Virginia A. Thorpe, State Dept of Agriculture, Laboratory Div., 1615 S Harrison Rd, East Lansing, MI 48823

Water-Soluble Methylene Ureas
Robert L. Roser, OM Scott & Sons, Co., Marysville, OH 43041

Zinc

PLANTS

Referee: Robert A. Isaac, University of Georgia, Agricultural Services Laboratories, Athens, GA 30605

Ashing Methods
J. Benton Jones, University of Georgia, Dept of Horticulture, Athens, GA 30602

Atomic Absorption Methods
W. C. Johnson, University of Georgia, Soil Testing and Plant Analysis Laboratory, Athens, GA 30605

Boron

Chromium
Earle E. Cary, U.S. Dept of Agriculture, Plant, Soil, and Nutritional Laboratory, Tower Rd, Ithaca, NY 14853

Emission Spectroscopy
Robert A. Isaac, University of Georgia, Agricultural Services Laboratories, Athens, GA 30605

Fluoride
Jay S. Jacobson, Boyce Thompson Institute, Tower Rd, Ithaca, NY 14853

Nitrate and Nitrite

Selenium
Ivan S. Palmer, South Dakota State University, Experiment Station, Biochemistry Dept, Brookings, SD 57006

Starch
T. Powell Gaines, University of Georgia, College of Agriculture, Dept of Agronomy, Tifton, GA 31797

Sulfur
Charles W. Gehrke and Rose A. Sweeney, University of Missouri–Columbia, Columbia, MO 65211

TOBACCO

Referee: W. Wesley Weeks, North Carolina State University, Crop Science Dept, PO Box 7620, Raleigh, NC 27695

Alkaloids
Kenneth L. Rush, RJ Reynolds Tobacco Co., Winston Salem, NC 27102

Nicotine in Environmental Tobacco Smoke
Michael W. Ogden, RJ Reynolds Tobacco Co., Winston Salem, NC 27102

Polyphenols
Maurice E. Snook, U.S. Dept of Agriculture, PO Box 5677, Athens, GA 30613

VETERINARY ANALYTICAL TOXICOLOGY

Referee: P. Frank Ross, U.S. Dept of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Animal Serum Thyroxine
Daniel J. Sullivan, Vet-A-Mix, Inc., 604 W Thomas Ave, Shenandoah, IA 51601

Antibiotic Screening Methods
Wynne Landgraf, National Veterinary Services Laboratory, PO Box 844, Ames, IA 50010
Stephen C. Ross, Illinois Dept of Agriculture, Animal Disease Laboratory, Centralia, IL 62801

Arsenic in Animal Tissue
John Reagor, Texas Veterinary Medicine Diagnostic Laboratory, Box 3040, College Station, TX 77840

Atomic Absorption Spectrophotometry
Steve Kasten, Dept of Agriculture, Animal Disease Lab., Shattuc Rd, Centralia, IL 62801

Cholinesterase (Colorimetric Method)
Karen S. Harlin, University of Illinois, UMBSB, Urbana, IL 61807

Cholinesterase (pH Method)
Paula Martin, Iowa State University, Veterinary Diagnostic Laboratory, Ames, IA 50010

Copper in Animal Tissue
David Osheim, U.S. Dept of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Cyanide
Howard Casper, North Dakota State University, Veterinary Diagnostic Laboratory, Fargo, ND 58102

Fluoride in Animal Tissue
David Osheim, U.S. Dept of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

GC/MS Methods
Allen C. Ray, Texas Veterinary Medicine Diagnostic Laboratory, PO Drawer 3040, College Station, TX 77841

Lead in Animal Tissue
Robert J. Everson, Purdue University, School of Veterinary Medicine, West Lafayette, IN 47907

Multielement Analysis by ICP
Emmett Brazelton, Michigan State University, Dept of Pharmacology and Toxicology, East Lansing, MI 48824

Multiple Anticoagulant Screening
John D. Reynolds, Dept of Agriculture, Animal Disease Laboratory, Shattuc Rd, Centralia, IL 62801

Natural Products
George Rottinghaus, University of Missouri, College of Veterinary Medicine, Columbia, MO 65211

Nitrates and Nitrites
Norman R. Schneider and Michael P. Carlson, Veterinary Diagnostic Center, Dept of Veterinary Science, Lincoln, NE 68583

Pesticides in Toxicological Samples
Henry M. Stahr, Iowa State University, College of Veterinary Medicine, Ames, IA 50010

Selenium in Animal Tissue
James E. Roof, State Veterinary Diagnostic Laboratory, PO Box 1430, Harrisburg, PA 17105

Sodium Monofluoroacetate
Henry M. Stahr, Iowa State University, College of Veterinary Medicine, Ames, IA 50010

Vitamins A and E
Roy A. Smith, AB Agriculture, 6909 116th St, Edmonton AB T6H 49L, Canada

Vitamins D and K
Karen S. Harlin, University of Illinois, UMBSB, Urbana, IL 61801

Zinc in Animal Tissues
Dana Perry, Veterinary Diagnostic Laboratory, Dept of Veterinary Science, Tucson, AZ 85721

Committee on Hazardous Substances in Waste and the Environment

Erika E. Hargesheimer (City of Calgary, Glenmore Waterworks Lab., Calgary, Alberta T2P 2M5, Canada), *Chairman;* David Friedman (Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460); Ann B. Strong (U.S. Army Corps of Engineers, Waterways Experiment Station, PO Box 631, Vicksburg, MS 39180); Robert Graves (Environmental Protection Agency, Monitoring and Support Lab., 26 W Martin Luther King Dr, Cincinnati, OH 45268); Gerald Myrdal (Wisconsin Dept of Agriculture, Trade, and Consumer Protection, 4702 University Ave, PO Box 7883, Madison, WI 53707); Larry G. Lane (Mississippi State Chemical Lab., PO Box CR, Mississippi State, MS 39762); Joan K Bartz (Chemical Waste Management, Technical Center, 150 W 157th St, Riverdale, IL 60627); Charles Staples (Monsanto Co., 800 N Lindbergh Blvd, St. Louis, MO 63166); Yvonne Stokker (Environment Canada, 867 Lakeshore Rd, Burlington, Ontario L7R-4A6, Canada); *Secretary;* Forest C. Garner (Lockheed, 3877 Platis Circle, Las Vegas, NV 89103), *Statistical Consultant*

AIR

Referee: Michael P. McCown, Caleb-Brett USA, Houston, TX 77099

BIOMONITORING

Referee: ———

EFFLUENTS

Referee: ———

INORGANICS IN WATER

Referee: Kenneth P. Stoub, Waste Management, Inc., Environmental Monitoring Labs, 2100 Cleanwater Dr, Geneva, IL 60134

Arsenic and Selenium in Groundwater
J. Wilson Hershey, Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601

Inductively Coupled Plasma and Ion Chromatographic Methods for Groundwater
Bruce Warden, Waste Management, Inc., Environmental Monitoring Labs, 2100 Cleanwater Dr, Geneva, IL 60134

ORGANICS IN DRINKING AND GROUND WATER

Referee: Andrew D. Sauter, 2356 Aqua Vista, Henderson, NV 89015

Environmental Protection Agency Methods

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Phenols

———

Polynuclear Aromatic Compounds—Screening Methods

———

Total Organic Halogen

———

Volatile Organics—Capillary Analysis

———

ORGANICS IN SURFACE AND WASTE WATER

Referee: Viorica Lopez-Avila, Acurex Corp., PO Box 7044, Mountain View, CA 94039

Chlorinated Pesticides in Groundwater—GC/EC Method
Viorica Lopez-Avila, Acurex Corp., PO Box 7044, Mountain View, CA 94039
Raymond Wesselman, Environmental Protection Agency, Environmental Monitoring and Support Lab., Cincinnati, OH 45268

Chlorinated Solvents
Douglas J. Dube, State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706

Diquat and Paraquat in Water

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Formaldehyde in Waste Water

———

Herbicide Residues in Environmental Waters

———

Herbicides in Water and Sediment

———

Major Ions and Nutrients in Water

———

Munitions in Wastewater
Thomas F. Jenkins, Cold Regions Research and Engineering Lab., Hanover, NH 03755

Organophosphorus Pesticides in Water

———

Phenols

———

Salt

———

Triazine Herbicides in Water

———

WASTES AND SOLID MATERIALS

Referee: ———

Bioassays
Llewellyn R. Williams, Environmental Protection Agency, Environmental Monitoring Systems Lab., Box 15027, Las Vegas, NV 89114

Chlordane in Soils
Wayne M. Pash, Office of the Indiana State Chemist, Purdue University, West Lafayette, IN 47907

Explosives Residues

———

Inorganic Analytes
Gerald McKee, Environmental Protection Agency, Environmental Monitoring Systems Lab., 26 W St. Clair St, Cincinnati, OH 45268

Nonvolatile Organics—Distribution Coefficients
Danny R. Jackson, Battelle-Columbus Labs, 505 King Ave, Columbus, OH 43201

Organic Analytes
Paul Friedman, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

Polychlorinated Biphenyls—Quantitation
Deborah Lazoski, Chemical Waste Management, 150 W 137th St, Riverdale, IL 60627

Prescreening

———

Sampling

———

Soils—Distribution Coefficients
Danny R. Jackson, Battelle-Columbus Labs, 505 King Ave, Columbus, OH 43201

Termiticides

———

Volatile Organics—Adsorption Isotherms
Leverett R. Smith, 622 Clayton Ave, El Cerrito, CA 94530

Volatile Organics—Distribution Coefficients

———

CHANGES IN OFFICIAL METHODS OF ANALYSIS

The following changes in the methods of the Association become effective, as provided in Article IX, Section 5, of the Bylaws, on the thirtieth day after publication of this *Journal* issue. Section numbers refer to *Official Methods of Analysis,* 14th edition (1984), and its supplements, unless otherwise specified.

Newly adopted methods are numbered in the style of the 14th edition. The first section of the first new method in each chapter is numbered with the chapter number plus **.E01**. Subsequent sections are numbered **.E02**, **.E03**, **.E04**, et seq. The E signifies that the method was adopted at the 1988 Annual International Meeting, and was published and became official in 1989 in the fifth supplement to the 14th edition.

"Changes in Official Methods" is accompanied by an index. Errata and emendations are published under the appropriate chapter, and are indexed. The "Changes in Official Methods" index is cumulative between editions of *Official Methods of Analysis.*

1. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

2. FERTILIZERS

(1) In the final action urease method for urea in fertilizers, **2.080**–**2.081**, make editorial changes in **2.081** so that the sample weighed contains ≤1.0 g urea:

(a) Change the first line to read: "Weigh 1–10 ± 0.01 g sample (≤1.0 g urea) and transfer . . .".

(b) Line 8, delete "(equiv. to 1 g sample)."

(c) Change the equation to read:

$$\%\ \text{Urea} = [(\text{mL } 0.1N\ \text{HCl} - \text{mL } 0.01N\ \text{NaOH}) \times 0.3003]/\text{g sample}$$

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(2) In the final action method for calcium (acid-soluble) in fertilizers, **2.140**–**2.142**, add a qualifying statement: "(Presence of other analytes pptd by oxalate, such as Ba and Sr, will cause pos. bias in results in **2.140** and **2.142**.)."

3. PLANTS

The following final action methods were declared surplus:

(a) Sand and silica in plants, gravimetric method, **3.005**.

(b) Magnesium in plants, gravimetric method, **3.039**.

(c) Sodium in plants, uranyl acetate method, **3.052**–**3.053**.

(d) Chloride in plants, gravimetric method, **3.069**–**3.070**.

4. DISINFECTANTS

(1) The first action use-dilution methods for testing disinfectants against *Salmonella choleraesuis,* **4.007**–**4.009**, *Staphylococcus aureus,* **4.010**, and *Pseudomonas aeruginosa,* **4.011**, were editorially revised. The revisions are shown here. The complete text of the revised methods is printed in full at the end of this supplement. Revise the methods as follows:

(a) Add to the applicability statement preceding **4.007**: "These microbiological methods are technique-sensitive methods in which extreme adherence to the method with identified critical control points, good microbiological techniques, and quality controls is required for proficiency and validity of results."

(b) Add at the beginning of **4.007(b)**: "Obtain annually directly from ATCC."

(c) Add at the end of **4.008**(a): "Use 25 × 150 mm straight side tubes for disinfectant soln. (Smaller tubes can give high percentage of false positives when sides are touched.)."

(d) Add at the end of **4.008(d)**: "Discard cylinders that are visibly damaged (dull, chipped, dented, or gouged). Biologically screen remaining cylinders by performing Use-Dilution Test with *Staphylococcus aureus* ATCC 6538 and 500 ppm alkyldimethylammonium chloride with alkyl chain distribution C14, 50%; C12, 40%; C16, 10% (e.g., BTC-835, Onyx Chemical Co., Jersey City, NJ 07302). Discard those cylinders giving pos. results in screening procedure. In subsequent testing of samples, cylinders in tubes showing growth must be rescreened and may not be reused unless screen test results in no growth."

(e) Add new subsection **4.008(f)**: "Use disposable pipets. (Reusable pipets may have residues or chips.)."

(f) Add to **4.009**, par. 1, after sentence 1: "Vortex-mix nutrient broth test culture 3–4 s and let stand 10 min at room temp. before continuing."

(g) Add to **4.009**, par. 1, after sentence 2: "One or 2 addnl carriers may be added at same inoculum rate to serve as reserves. Carriers that fall over in petri dishes cannot be used in test."

(h) In **4.009**, par. 1, revise sentence 3 to read: ". . . wire hook, shake carrier vigorously against side of tube to remove excess culture, and place on end in vertical position in sterile petri dish matted with filter paper, **4.008(e)**, making sure that carriers do not touch to prevent improper drying."

(i) In **4.009**, par. 1, sentence 4, replace "≥20 min but ≤60 min" with "40 min."

(j) Add to **4.009**, par. 2, after sentence 5: "Diln of sample should be made using ≥1.0 mL of sample. Use v/v dilns for liq. products and w/v dilns for solids. Round to 2 decimal places toward a stronger product. To ensure stable product, solns should be prepd ≤3 hr prior to use. Place tubes in 20° H_2O bath ≥10 min."

(k) In **4.009**, par. 4, revise sentence 1 to read: "Without touching sides of tube with contaminated carrier or hook, either when placing carrier in tube or when withdrawing hook, add 1 contaminated. . .".

(l) Add to **4.009**, par. 4, after sentence 1: "(*Note:* Proper execution of transfer step is one of the most critical, technique-sensitive areas of method. False positives will result if sides of tube are touched.)."

(m) Add to **4.009**, par. 4, at end of sentence 4: ". . . by shaking carrier against side of tube. Shorter intervals may be used in adding and removing carriers if 2 alternately flamed and cooled hooks are used. Individual manipulation of carriers is required; use of semiautomated ring carrier is prohibited. (*Note*: Above step is one of the most critical, technique-sensitive areas of method. False positives can result from transfer of live organisms to sides of tube due to aerosol formation.)."

(n) Add to **4.009** at end of par. 4: "Growth in tubes should be checked by gram stain to ensure that no contamination is present. Check ≥20% of pos. tubes. Confirm all pos. results by duplicate testing to assure against false pos. tests."

(o) Add to **4.010**, after sentence 2: "Obtain organism annually, directly from ATCC. Prior to beginning Use-Dilution Test, vortex-mix nutrient broth culture as in **4.009**."

(p) Add to **4.011** at end of sec.: "Proceed with vortex-mixing as in **4.007** prior to use of culture. Alternatively, pellicle may be carefully suctioned off, and culture can be poured into clean, sterile tube before vortex-mixing. Any disruption of pellicle resulting in dropping, breaking up, or stringing of pellicle in culture before or during its removal renders that culture unusable in Use-Dilution Test. This is extremely critical because any pellicle fragments remaining will result in uneven clumping and layering of organism on cylinder, allowing unfair exposure to disinfectant and causing false pos. results."

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(2) The following first action methods were repealed:

Tuberculocidal activity of disinfectants: I. presumptive in vitro screening test using *Myobacterium smegmatis,* **4.036–4.038**; II. confirmative in vitro test, **4.039–4.041**.

5. HAZARDOUS SUBSTANCES

No additions, deletions, or other changes.

6. PESTICIDE FORMULATIONS

(1) The following first action methods were adopted final action:

(a) Brodifacoum technical and formulations, liquid chromatographic method, **6.597–6.601**. Add an applicability statement: "Not applicable to wax bait formulations."

(b) Oxythioquinox in formulations, AOAC-CIPAC liquid chromatographic method, **6.B34–6.B39**.

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(2) The following interim AOAC-CIPAC liquid chromatographic method for azinphos-methyl (*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)yl)methyl] phosphorodithioate) in formulations was adopted first action:

Azinphos-Methyl in Pesticide Formulations

Liquid Chromatographic Method

First Action

AOAC-CIPAC Method

(Applicable to wettable powder and
liq. formulated products
contg azinphos-methyl as only active ingredient)

6.E01 ***Principle***

Azinphos-methyl is detd by liq. chromatgy. Peak hts of sample and anal. std are compared using *n*-butyrophenone as internal std.

6.E02 ***Apparatus***

(a) *Liquid chromatograph.*—Able to generate >7 MPa (>1000 psi). Equipped with spectrophtr to measure *A* at 285 nm and peak ht integrator or recorder. Operating conditions: column temp. ambient; flow rate ca 1.5 mL/min (ca 1000 psi); recorder speed 0.5 cm/min; recorder range 10 mV; injection vol. ca 10 μL; *A* range 0.16 AUFS. Retention times: azinphos-methyl ca 4.0 min, internal std ca 4.8 min. Pump LC mobile phase thru column until system is equilibrated (flat baseline). After each injection, allow 10 min after internal std for elution of formulation excipients.

(b) *Chromatographic column.*—250 × 4.6 mm id packed with ≤10 μm C_{18} bonded silica gel (Du Pont Zorbax ODS, or equiv.).

(c) *Filters.*—0.45 μm porosity (Gelman Acrodisc-CR, or equiv.).

6.E03 ***Reagents***

(a) *Acetonitrile.*—LC grade or distd in glass (Burdick & Jackson, or equiv.).

(b) *Water.*—LC grade or distd in glass (Burdick & Jackson, or equiv.).

(c) *LC mobile phase.*—CH_3CN–H_2O (65 + 35).

(d) *n-Butyrophenone internal std soln.*—10% *n*-butyrophenone (Aldrich No. 12,433-8, or equiv.) (v/v) in CH_3CN.

(e) *Azinphos-methyl reference std.*—(Mobay Corp., Agricultural Chemicals Div., PO Box 4913, Hawthorne Rd, Kansas City, MO 64120-0013). Store ref. std at refrigeration temp. (4–8°).

(f) *Azinphos-methyl std soln.*—Accurately weigh ca 220 mg azinphos-methyl ref. std into 100 mL vol. flask. Pipet 10.0 mL internal std soln into flask, dil. to vol. with CH_3CN, and mix thoroly. Filter portion of final soln and hold for LC analysis.

6.E04 ***Preparation of Samples***

Accurately weigh amt sample contg ca 220 mg azinphos-methyl into 100 mL vol. flask. Pipet 10.0 mL internal std soln into flask, dil. to vol. with CH_3CN, and mix thoroly. Filter portion of final soln and hold for LC analysis.

6.E05 ***Determination***

Adjust operating parameters so that azinphos-methyl elutes in 3.8–4.2 min. Adjust injection vol. and attenuation to give largest possible on-scale peaks. If peaks cannot be brought on scale at 0.32 AUFS setting with 10 μL injection, further dil. std and sample solns by pipetting 10 mL of each into 100 mL vol. flasks, dilg to vol. with CH_3CN, and mixing thoroly. Readjust injection vol. and attenuation to give largest possible on-scale peaks.

Make repetitive injections of std and calc. response ratios by dividing azinphos-methyl peak ht by internal std peak ht (area measurements are not acceptable). Response ratios must agree within ±1% before continuing. Inject duplicate aliquots of each sample soln (no more than 2 samples [4 injections] between std injections). Response ratios of sample injections must agree within ±1%. If not, repeat detn, starting with std injections. Reinject std soln. Average response ratios of std injections immediately preceding and following sample injections. These must agree within ±1%. If not, repeat detn.

6.E06 ***Calculation***

For each injection:

Response ratio (R)
= (azinphos-methyl peak ht/internal std peak ht)

$$\text{Azinphos-methyl, wt \%} = (R/R') \times (W'/W) \times P$$

where R and R' = av. response ratios for sample and std solns, resp.; W' and W = wt (mg) of azinphos-methyl in std and sample solns, resp.; P = % purity of azinphos-methyl std.

Ref.: JAOAC **71**, 988(1988).

CAS-86-50-0 (azinphos-methyl)

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(3) The following interim gas chromatographic method for fenitrothion (phosphorothioic acid *O,O*-dimethyl *O*-(3-

methyl-4-nitrophenyl) ester) technical and in formulations was adopted first action:

Fenitrothion Technical and Pesticide Formulations
Alternative Gas Chromatographic Method
First Action

(Applicable to fenitrothion tech. and its emulsifiable conc. and H_2O-dispersible powder formulations)

6.E07 ***Principle***

Samples of fenitrothion tech. and formulations are dissolved in $CHCl_3$ with dibutyl sebacate added as internal std. Fenitrothion content is detd by gas chromatgy with flame ionization detection using peak area measurements. Method is alternative to **6.A19** which uses PPE-6R column packing.

6.E08 ***Apparatus and Reagents***

(a) *Gas chromatograph.*—Suitable for on-column injection and equipped with flame ionization detector.

(b) *Chromatographic column.*—2 mm id × 1.83 m glass column packed with 7.5% OV-210 (Alltech Associates, Inc., 2051 Waukegan Rd, Deerfield, IL 60015) on 100–120 mesh Chromosorb W-HP. Operating conditions: temps—injector, 190°; detector, 250°; column, 165°. N carrier gas flow ca 40 mL/min.

Approx. retention times for fenitrothion and internal std are 16.9 and 19.5 min, resp.

(c) *Internal std soln.*—Accurately weigh ca 3.0 g dibutyl sebacate into 500 mL vol. flask, dil. to vol. with $CHCl_3$, and mix.

(d) *Fenitrothion std soln.*—Accurately weigh amt std fenitrothion (Sumitomo Chemical Co., Ltd, Osaka, Japan) contg ca 200 mg active ingredient into 50 mL screw-cap bottle. Add by pipet 25.0 mL internal std soln and mix to dissolve fenitrothion.

6.E09 ***Preparation of Sample Solutions***

(Acute oral LD_{50} of fenitrothion tech. for rats is 250–500 mg/kg.) Accurately weigh samples of fenitrothion tech., emulsifiable conc., and H_2O-dispersible powder, each contg ca 200 mg active ingredient, into sep. 50 mL screw-cap bottles. To each bottle add by pipet 25.0 mL internal std soln and shake 30 s. Filter or centrf. H_2O-dispersible powder to remove particulates.

6.E10 ***Determination***

Inject 2 μL portions of std soln until response ratios (peak area) of fenitrothion to internal std agree ±2%. Verify that small peak just preceding that of fenitrothion [due to isomeric impurity *O,O*-dimethyl-*O*-(4-methyl-3-nitrophenyl) phosphorothionate] is effectively sepd from fenitrothion peak. Make duplicate injections of std soln followed by duplicate injections of sample solns. At end of each run raise column temp. to 230° at 20°/min and hold 5 min to allow rapid elution of late-eluting peaks before next detn. Recalibrate after no more than 4 injections of sample solns.

6.E11 ***Calculation***

For each injection, response ratio (R) = area of fenitrothion peak/area of internal std peak.

$$\text{Fenitrothion, wt \%} = (R/R') \times (W'/W) \times P$$

where R' and R = av. response ratio for std and sample solns, resp.; W' and W = wt (mg) of fenitrothion std and sample, resp.; and P = purity (%) of fenitrothion std.

Ref.: JAOAC **71**, 991(1988).

CAS-122-14-5 (fenitrothion)

7. ANIMAL FEED

(1) In the final action photometric method for phosphorus in animal feed, **7.125–7.128**, add as an applicability statement: "(Dry ashing procedure is not applicable to feeds or mineral mixes contg monobasic calcium phosphate.)".

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(2) The following interim near-infrared reflectance spectroscopic method for acid-detergent fiber and crude protein in forages was adopted first action:

Fiber (Acid Detergent) and Protein (Crude) in Animal Feed and Forages
Near-Infrared Reflectance Spectroscopic Method
First Action

(Generally applicable to detn of acid-detergent fiber and crude protein in any forage or feed sample)

Successful use of method is based on obtaining suitable calibration for instrument by selecting learning set of samples and performing calibration described in *Determination* (**b**). Four rules to be followed in calibration of instrument are stated in USDA Agriculture Handbook No. 643, p. 45 ("Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality." *U.S. Dep. Agric. Handb. 643,* U.S. Government Printing Office, Washington, DC, 96 pp.). In brief, rules are as follows:

(*1*) Be certain calibration samples adequately represent population to be analyzed.

(*2*) Conduct accurate laboratory analyses on calibration samples. This step cannot be overemphasized.

(*3*) Select appropriate data processing technique to extract pertinent information from spectra.

(*4*) Select correct wavelengths.

It may be necessary to periodically update a calibration. Analyst must monitor results of method to ascertain when this is necessary. Test sample is usually run each day and its analytical value is detd periodically by laboratory analysis method. Accuracy of any chemometric procedure is limited only by validity of laboratory method used to measure desired quality parameter for all samples and selection of appropriate learning set.

7.E01 ***Principle***

Random portions of prepd sample are loaded into sample holder of NIR spectrometer. Instrument is part of system that has been calibrated using representative samples from population to be tested. Equations selected from calibration statistics, which have been validated, are used to calc. acid-detergent fiber and crude protein content of feed and forage samples.

7.E02 ***Apparatus***

(**a**) *Wavelength-scanning instrument.*—Model 6100 or 6350 grating monochromator (Pacific Scientific Corp., Gardner/Neotec Instrument Div., Silver Spring, MD 20910), or equiv. Monochromator is described in detail in (*1*) Landa,

I. Rev. Sci. Instrum. **50**, 34–40(1979); and (*2*) Landa, I., & Norris, K. H. Appl. Spectrosc. **23**, 105–107(1979).

(**b**) *Computer.*—PDP 11 Series computer equipped with 64 K bytes of main memory; dual RX02 double-density floppy disks; RL01 5-megabyte or RL02 10-megabyte hard disks. PDP system software RT-11 V5.0 or later (Digital Equipment Corp., Nashua, NH 03061), or equiv.

(**c**) *USDA public software.*—Software is described in detail in USDA Agriculture Handbook No. 643. Software consists of 14 programs written in FORTRAN IV to collect, store, and process NIRS data. Repository for software: U.S. Department of Agriculture, Richard B. Russell Agricultural Research Center, Plant Structure and Composition Research Unit, PO Box 5677, Athens, GA 30613. Commercial software is available from several vendors.

(**d**) *Mill.*—Tecator cyclone sample mill with 1 mm screen (Fisher Scientific Co.), or equiv. Periodically change grinding ring to ensure consistency of particle size over time.

(**e**) *NIRS sample holder.*—Nylon, 2.5 cm diam., 1 cm thick, with IR transmittance quartz window. Sample capacity 0.75–1.75 g. Sample is held in place with sep. back made of rubber or foam core (Pacific Scientific Corp., Gardner/Neotec Instrument Div.), or equiv.

(**f**) *Sample storage container.*—For maintaining const moisture concn in samples. For best results, use Poly Kraft Bags-Mil-B-121 Type II, Grade A, Class I. Place sample in bag and heat-seal (EDCO Supply Corp., Brooklyn, NY 11232, or equiv.).

7.E03 *Instrument Operation*

(**a**) *Start-up.*—For best results, run instrument continuously. If instrument is cold, warm-up time should be >15 min and may require 1 h.

(**b**) *Monochromator diagnostic tests.*—*(1) Instrument noise.*—Scan ceramic ref. to itself. Collect 25 repetitions of 64 scans. Express deviations from zero as av. deviation (bias), and as root mean sq (RMS), expressed as log $(1/R)/10^6$, where R = reflectance. Bias indicates any systematic change in log (1/R) level of scans taken over time. Bias values that are all pos. or all neg. indicate problem with instrument. RMS value can range from low of 10 to high of 50 without affecting analysis. Monochromators should have av. noise level below 30 RMS over 100 scans.

(2) Wavelength accuracy.—Use clear polystyrene petri dish to measure wavelength repeatability and accuracy. Place petri dish in light beam and then pull out sample drawer to expose ceramic std. Ref. this scan to measurements without petri dish. Locate and compare major styrene peaks with known locations at 1680.3, 2164.9, and 2304.2 nm. Repeatability std dev. should be <0.05 nm, and accuracy from known location should be <0.5 nm. Large pos. values for wavelength accuracy and repeatability usually indicate mech. problems in monochromator.

(**c**) *Maintenance.*—Whenever dust accumulates, use vacuum, brush, or soft tissue to clean ceramic std, all parts of drawer assembly, and windows above and below detector. Instrument operation is described in detail in USDA Agriculture Handbook No. 643 and in Shenk, J. S., Westerhaus, M. O., & Hoover, M. R. Proc. Am. Soc. Agric. Eng. (1978), p. 242.

7.E04 *Determination*

(**a**) *Preparation of sample.*—Grind samples for NIRS analysis in cyclone mill through 1 mm screen. Clean mill between samples to minimize cross-contamination. Prior to grinding, dry samples contg >25% moisture in 60° air oven for 24 h. Mix milled samples well, and place random portion in sample holder. Continue to add random portions until NIRS sample holder is ⅔ full. Press back into holder until it is tight and level. As check, invert holder and make certain sample is firmly pressed against window. If any abnormality is apparent, remove back and repeat procedure. Consistency in sample handling and prepn is crucial to successful use of NIRS technique.

(**b**) *Calibration.*—To calibrate system for acid-detergent fiber and crude protein detns, randomly select samples that are representative of population to be analyzed, using either finite or infinite population. Finite population has defined boundaries set by analyst which limit population; infinite population has no such boundaries. Select sufficient number of samples to represent range of acid-detergent fiber and crude protein concns and all other variables that may affect chem. and physical composition of feed or forage (stage of growth, species, preservation method, etc.). In practical terms, min. of 50 samples should be considered.

Collect reflectance (R) measurements (log 1/R) of calibration samples with program SCAN, *Apparatus* (**c**), at 2.0 nm intervals from 1100 to 2500 nm. Develop multiterm calibration equations by multiple linear regression of reflectance measurements to acid-detergent fiber and crude protein concns using program CAL (**c**). Before regression statistics are evaluated, examine differences (residuals) between NIRS data and ref. method data for samples with large *t*-values. Large pos. or neg. *t*-value indicates that residual is 2.5 times std error of difference between NIRS detn and ref. method detn, and that laboratory values from ref. method were inaccurate or did not represent samples at time scan was taken (i.e., subsampling error). Reanalyze these samples by ref. method.

In addn, evaluate output for samples that have large H-values. Large H-statistic (>3) indicates that NIRS spectra used in calibration for that sample differ substantially from NIRS spectra of other samples. Calcn of H was mathematically derived from covariance matrix according to formula: $H = X(s^1x)^{-1}x^1$ [Landa. *Apparatus* (**a**)]. High value on diagonal of H matrix indicates sample that is dissimilar to calibration set at wavelengths used in equation. Rescan such samples. If 2 scans agree and sample belongs in population, then retain sample. If scans disagree, then first scan was mistake and should be discarded.

Examine std error of calibration (SEC) to det. fit of calibration samples: the lower the SEC, the better the fit. Select equation with SEC about 2 times laboratory repeatability std dev. for acid-detergent fiber and crude protein ref. method. Examine coeff. of detn (R^2) to det. proportion of variation in ref. method values among samples explained by NIRS regression equation. Low R^2 values often indicate that laboratory data from ref. method are imprecise. If laboratory repeatability error from ref. method is ¼ of std. dev., select equation with $R^2 \geq 0.75$.

Examine *F*-statistic of regression coeffs. High *F*-values indicate that regression coeff. is significantly different from zero; small *F*-values indicate that coeff. contributes little to equation except to fit random errors. Probability that observed *F*-value was obtained solely through chance does not follow std *F* tables because observed *F* is selected as max. of all wavelength combinations considered. As number of choices increases, large *F*-values are needed to signify coeff. fitting more than just random errors. Reject equations with *F*-values <10.

(**c**) *Validation.*—Conduct NIRS analysis (program PRE) with equations selected from calibration statistics on

population of unknown samples. Examine NIRS data for samples with larger H-values. Large H-value (>3.0) for a few samples indicates that their NIR spectra are different from spectra of calibration population. Large *t*-value (2.5 times SED) for a few samples indicates that laboratory values from ref. method were inaccurate or did not represent samples at time scan was taken. If many validation samples have large *t*- and H-values, over-fitting has occurred, and equation is specific to samples in calibration set. Next, use validation statistics from program STAT to examine std error of analysis (SEA) by NIRS of chem. composition of validation samples. SEA is true indication of performance of equation on unknown samples. Select equation with lowest bias and SEA. Unlike SEC, which must decrease with each addnl term, SEA only decreases with addnl terms until over-fitting becomes important and forces it to increase. Best equation for routine NIRS analysis is based on both superior calibration and validation statistics.

Ref.: JAOAC **71**, 1162(1988).

8. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or other changes.

9. BEVERAGES: DISTILLED LIQUORS

No additions, deletions, or other changes.

10. BEVERAGES: MALT BEVERAGES AND BREWING MATERIALS

No additions, deletions, or other changes.

11. BEVERAGES: WINES

No additions, deletions, or other changes.

12. BEVERAGES: NONALCOHOLIC AND CONCENTRATES

No additions, deletions, or other changes.

13. CACAO BEAN AND ITS PRODUCTS

Add a warning statement concerning use of asbestos in the final action Knorr tube method for fat in cacao products, **13.031**: "**Caution**: Asbestos is a carcinogen." Add **51.086** as an additional cross reference.

14. CEREAL FOODS

Adopt nonsubstantive changes in the final action liquid chromatographic method for glucose, fructose, sucrose, and maltose in presweetened cereals, **14.075–14.079**:

(a) Change **14.076(a)** to read: ". . . include automatic injectors, 410 differential refractometer (Waters Associates, Inc., WISP 710B, R1 410, or equiv.), 100 × 4.6 (id) mm Spheri-5 amino cartridge column (Brownlee Laboratories or equiv.) and use specific . . .".

(b) Change **14.076(c)** to read: ". . . 100 × 2 (id) mm (Waters Associates, Inc.), or 15 × 3.2 (id) mm 7 μm amino guard cartridge (Brownlee Laboratories), or equiv. . .".

(c) Change **14.077(a)** to read: ". . . vac. Dissolve in alcohol–H_2O (1 + 1) to obtain . . .".

(d) Change **14.077(b)** to read: "CH_3CN (LC grade) and H_2O (Milli-Q purified or equiv.) (80 + 20). Filter thru Whatman GF/F 0.7 μm glass fiber filter or thru 0.45 μm Pall Nylon 66 filter. Optionally . . .".

(e) Change **14.078(b)** to read: ". . . to original wt. Filter portion of ext thru 0.45 μm Nylon syringe filter (Nalge or equiv.). If cloudy, centrf. 10 min at ≥2000 rpm. If still cloudy, recentrf. portion of ext 5 min at ≥3500 rpm and filter thru 0.45 μm Nylon syringe filter. If guard . . .".

(f) Change **14.078(c)**, last sentence, to read: "Filter thru 0.45 μm Nylon syringe filter if necessary."

15. COFFEE AND TEA

No additions, deletions, or other changes.

16. DAIRY PRODUCTS

(1) The first action infrared spectroscopic method for fat, lactose, and protein in milk, **16.083–16.092**, was revised to incorporate the use of absorptions at 3.48 μm and to provide procedures for use of a computer for calibrations and quality control. The following revisions are required:

(a) Change the first line of the title to read:

Fat, Protein, Lactose, and Solids in Milk

(b) Change **16.083**, *Principle*, to read:

Analysis of milk by IR is based on absorption of IR energy at specific wavelengths by CH groups in fatty acid chain of fat molecules (3.48 μm), by carbonyl groups in ester linkages of fat molecules (5.723 μm), by peptide linkages between amino acids of protein molecules (6.465 μm), and by OH groups in lactose molecules (9.610 μm). Total solids (TS) or solids-not-fat (SNF) are computed by assigning experimentally detd factor to percentage of all other solid milk components, and by adding this amt to appropriate % fat, protein, and lactose, or by direct multiple regression calcns using instrument signals at combinations of above-mentioned wavelengths. Latter method has been shown to be more accurate method of detg milk solids. Analysis by IR is dependent on calibration against suitable std method. See "Definitions of Terms and Explanatory Notes," item (24), for calcn of regression lines.

(c) Add as a second paragraph in **16.085**:

Instrument must be well maintained and functioning correctly. Malfunctions that influence calibration can cause large errors.

(d) Add as a second calibration procedure in **16.086** and **16.088**:

Alternatively, for better accuracy, use simple linear regression equation that relates instrument ests and std ref. values, using latter as dependent variable, to correct these ests. In earlier instruments, which do not have this capability in instrument software, this calcn must be performed outside instrument. To perform this calcn on calibration data that are collected on successive days or weeks, it is necessary to record instrument ests as well as final results. Purpose of this alternative, preferred procedure is to avoid adjusting instrument slope controls.

(e) Add as a second calibration procedure in **16.090**:

Alternatively, for better accuracy, multiply instrument ests by $\Sigma L'/\Sigma L$ to obtain final results. In earlier instruments, which do not have this capability in instrument software, this calcn must be performed outside instrument. To perform this calcn on calibration data that are collected on successive days or weeks, it is necessary to record instrument ests as well as final results. Purpose of this alternative, preferred procedure is to avoid adjusting instrument slope controls.

(f) Add as a second calibration procedure in **16.092**:

Alternatively, calibrate by multiple regression to calc. equation for estn of either TS or SNF as function of fat, protein, and lactose uncorrected signals. For recalibration, use simple linear regression equation which relates regression ests and std ref. values, using latter as dependent variable, to correct these ests and obtain final result. In earlier instruments, which do not have this capability in instrument

software, this calcn must be performed outside instrument. To perform this calcn on calibration data that are collected on successive days or weeks, it is necessary to record regression ests as well as final result. This alternative, preferred method has been demonstrated to be more accurate.

Note: For products that are fortified or dild, both original multiple regression and simple regression calcns must be based on regression that forces calibration line thru origin. First calcn procedure in this sec. (**16.092**, par. 1) cannot be used successfully with these products.

(g) Add new sections to apply when a computer is used for calibration and quality control:

16.E01 *Computer-Based Calibration and Control*

Program computer to:

(*1*) Calc. $S_2' = S_1 + [(S_2 - S_1)/PF]$, where S_2' = purge-corrected signal; S_2 is signal at any instrument channel; S_1 is same signal for previous sample; and PF is predicted purge fraction = purging efficiency/100 (see **16.085**). Apply correction to all signals received from instrument.

(*2*) Calc. $(\Sigma CS_2')/10$, where CS_2' = purge-corrected control milk signal. Sum control milk signals at each channel for 10 control samples, and average. At time of calibration, store and designate as required control milk signals.

(*3*) Calc. $S = S_2' + CSE$, where S = purge- and drift-corrected signal; CSE is control signal error = (required control signal av.) − (detd control signal av.) for any check on control milk. Apply value to all except control milks.

(*4*) Calc. ests from purge- and drift-corrected signals using calibration signals, and store. Equation type is Est = A + B (main equation). Initially, A = 0 and B = 1.

(*5*) Calc. calibration equations and store.

(*6*) Store random sample signals, ests, and std method ref. results.

(*7*) Calc. and store mean difference, std dev. of difference, limits for mean difference, and population std dev. of difference for statistical quality control.

(*8*) Store results for checks on purging efficiency tests, homogenization efficiency tests, linearity tests, and repeatability tests.

16.E02 *Instrument Controls*

For instrument with secondary slope controls, operate in uncorrected mode. For instruments without secondary slope controls, reduce interference correction coefficients to 0.

16.E03 *Control Milk*

Prep. adequate supply of pasteurized, homogenized, preserved milks with % fat about that of population av. Transfer amts for single tests to sample containers and keep refrigerated. Zero-adjust instrument channels. Heat 11 samples to 40°, and pump each thru instrument, collecting signals. Average last 10 results for each signal to calc. required signal. Record required control milk signals to 4 decimals to prevent rounding errors in continuous control situation.

16.E04 *Setting Primary Slope Controls*

Det. % fat, protein, and lactose on series of ≥8 preanalyzed (e.g., **16.064**, **16.036**, **16.055**, or **16.059**) milks of type to be analyzed by instrument. Warm samples to 40°, mix thoroly, and pump thru instrument, collecting and correcting signals for purge and drift. For each signal, calc. regression equation of type $S = aF + bP + cL$, where S is signal and *F*, *P*, and *L* are results for std. At fat channel, adjust slope amplification by 1/a. At protein and lactose channels, adjust by 1/b and 1/c, resp. Check success of attempt to obtain coefficient of 1 for main components by repeating test. Readjust slope amplifications if necessary. Lock slope controls and do not change settings thereafter. Warm 11 samples of control milk and redet. required control milk values as indicated above.

16.E05 *First Calibration*

Obtain 20 milk samples by random selection from population to be tested, and analyze by std ref. method for components to be estd by IR method. Key std results into computer. Warm and prep. 11 control milk samples and the calibration samples. Pump thru instrument and collect and store signals. Computer applies purge correction, calcs control signal correction values, applies these to calc. milk signals, and stores these values in matrix with std ref. values. Select equation that is applicable to instrument from following main equation types.

Components	*Signals*			
	F_A	F	L	F_B
1. F, P, L, TS, SNF	*	*	*	
2. F, P, L, TS, SNF		*	*	*
3. F	*	*	*	*
4. F	[0.27 × F(1)] + [0.73 × F(2)]			

Note: Except for TS and SNF, all main equation types force regression line thru 0. With TS and SNF, either may be used, but equation with intercept is preferred and gives better results unless product being analyzed has been fortified or dild.

Calc. by multiple linear regression main equations for components for which IR analysis is required. It is not necessary to calc. ests for any component for which ests are not required or to perform std ref. method analysis for that component except when slopes for initial calibration are adjusted. However, all signals must be controlled using required control milk signal values.

16.E06 *Production Analysis*

At beginning of each production period and at definite intervals during production run, check control milk signals and recalc. drift corrections for application to product samples. Interval between checks can be shortened if drift for current interval is excessive. If instrument signals stability is acceptable, 1-h interval is satisfactory. Signals for product samples are corrected for purge and drift and then are used to calc. ests.

16.E07 *Quality Control*

Select production samples at random at predetd intervals and perform std ref. method analyses on these. Store signals, ests, and ref. values for these samples in matrix on accumulative basis. Check control milks and make adjustments to drift correction just prior to these selections. Interval between selection (days) and number selected may be varied to suit laboratory but ≥5 should be selected in 1 day and ≥20 in 1 week. Current population std devs of difference between est. and ref. methods may be calcd from last 50 differences in these accumulated data.

For quality control, calc. statistical limits for daily mean difference as 3 × population std dev./$\sqrt{N}$, where N is number of random samples tested that day. Action situations for changing A intercept in overall equations are:

(*1*) Daily mean difference is greater than limit.

(*2*) Six successive or 7 of 8 successive or 8 of 10 successive mean differences are on same side of 0. To correct this, adjust A in opposite direction to mean of differences. To correct for (*1*), adjust A in opposite direction by excess of difference over limit plus one-third of limit. Suggested addnl statistical calcns are: (a) accumulated monthly mean difference, std dev. of difference, and limits for mean difference; and (b) accumulated annual mean difference, std dev. of difference, and limits for mean difference. Overall objectives are to control current analyses as well as possible and to achieve annual mean difference very close to 0.

16.E08 ***Recalibration***

Use signals and std ref. values for last N samples in accumulated random sample data. Calcn may be made either to redet. coefficients of main equation or by simple regression using ests of main equation as independent variable and std ref. method values as dependent variable. Latter method would det. values for A and B in overall equation.

Whenever control milk is changed, whether at time of recalibration or between calibrations, run 11 samples of both old and new control milks. Use drift errors of old control milk to adjust avs for new control milk signals in calcg required signals for it. Do not use control milk for longer than 1 week.

With random sample data accumulated over long periods of time, number of samples used for calibration and frequency of calibration can be optimized. Signals and std ref. values in data bank can be used to vary choices and det. net effect for different equations generated in this way.

For test population used first with this control method, optimum conditions were $N = 20$ and interval between calibrations = 1 week.

16.E09 ***Precautions***

See **16.085** for normal precautions. This control method uses required control milk signals to control calibration level in preference to maintaining 0 settings at various channels with H_2O. Advantages are that drift errors identify signal subject to drift and that control of level is in range of test analysis. Water zeros may still be checked. Drift away from 0 indicates extraneous absorptions which could be due to excessive moisture in console, buildup of scale on cell windows, or other instrument faults. Continuous drifting in 1 direction for 0 signal should be investigated.

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(2) The final action method for direct determination of fat in butter, **16.233**, was repealed to first action.

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(3) The final action Babcock method, **16.065–16.066**, was revised first action as follows:

Fat in Raw Milk

Babcock Method

First Action

(**Caution**: See **51.005**, **51.030**, **51.065**.)

16.E10 ***Principle***

Known wt of milk is delivered into Babcock bottle, and heat generated by addn of H_2SO_4 releases fat. Centrfg and H_2O addn isolates fat for quantitation in graduated portion of Babcock bottle. Result is expressed as % fat by wt.

16.E11 ***Apparatus***

(**a**) *Std Babcock milk-test bottle.*—8%, 18 g, milk-test bottle, total ht 160–170 mm (6.3–6.7 in.). Bottom of bottle is flat, and axis of neck is vertical when bottle stands on level surface. Quantity of milk for bottle is 18 g. (Kimble Glass Co.).

(1) Bulb.—Capacity of bulb to junction with neck must be ≥45 mL. Shape of bulb may be either cylindrical or conical. If cylindrical, od of base must be between 34 and 36 mm; if conical, od of base must be between 31 and 33 mm, and max. diam. must be between 35 and 37 mm.

(2) Neck.—Cylindrical and of uniform diam. from ≥5 mm below lowest graduation mark to ≥5 mm above highest mark. Top of neck is flared to diam. of ≥10 mm. Graduated portion of neck has length ≥75.0 mm and is graduated in whole %, 0.5%, and 0.1%, resp., from 0.0 to 8.0%. Graduations may be etched, with black or dark pigment annealed to graduation, or may be unetched black or dark lines permanently annealed to glass. Graduation line widths ≤0.2 mm. Tenths % graduations are ≥3 mm long; 0.5% graduations are ≥4 mm long and project 1 mm to left; and whole % graduations extend at least halfway around neck to right but no more than three-quarters of way around and project ≥2 mm to left of tenths % graduations. Each whole % graduation is numbered, with number placed to left of scale. Vertical line may be etched and annealed with black or dark pigment or may be an unetched black or dark line permanently annealed to glass located 1 mm to right of 0.1% graduation marks and extends ≥1 mm above 8% line and ≥1 mm below 0% line. Zero line must be etched and annealed with black or dark pigment, and must be ≤0.2 mm wide. Capacity of neck for each whole % on scale is 0.200 mL. Max. error of total graduation or any part thereof must not exceed 0.008 mL (0.04% fat).

Each bottle must be constructed so as to withstand stress to which it will be subjected in centrf.

(3) Testing.—Accuracy of each bottle shall be detd. Bottle calibration accuracy is detd by placing bottle upside down on Babcock bottle calibration app. (modified Nafis tester) that is capable of delivering known vols of Hg into Babcock bottle neck. Bottle calibration app. delivery is calibrated and vol. of Hg contained between 8 and 4% (0.800 mL), 4 and 0% (0.800 mL), and 8 and 0% (1.600 mL) marks is detd. Accuracy of any bottle can also be detd by calibration with Hg (13.5471 g clean, dry Hg at 20° to be equal to 5% on scale of 18 g milk bottle and 10% on scale of 9 g cream bottle, **16.177(a)**), bottle having been previously filled to 0 mark with Hg. Accuracy should be equal to ≤ ±0.04% on scale of 18 g milk-test bottle. Calibrated bottles are available from Kimble Glass Co.

(**b**) *Pipet.*—Std milk pipet conforms to following specifications:

	mm
Total length	≤330
Od of suction tube	6–8
Length of suction tube	130
Od of delivery tube (must fit into bottle (**a**))	4.5–5.0
Length of delivery tube	100–120
Distance of graduation mark above bulb	15–45

Nozzle parallel with axis of pipet, but slightly constricted so as to discharge in 5–8 s when filled with H_2O.

Graduation, marked to contain 17.6 mL H_2O at 20° when bottom of meniscus coincides with mark on suction tube.

Max. error in graduation, ≤0.05 mL.

To test pipet, place tip of pipet against firm rubber surface, clamp pipet in vertical position, and use buret (Class A—graduations ≤0.05 mL) to fill pipet to graduation mark with H_2O (at 20°).

(c) *Acid measure.*—Device used to measure H_2SO_4 should deliver in range from 10 to 20 mL. Use device that can be set to consistently deliver appropriate amt of acid to obtain desired milk-acid reaction temp. in *Determination.*

(**d**) *Centrifuge or "tester."*—Std centrf., however driven, must be constructed thruout and so mounted as to be capable, when filled to capacity, of rotating at necessary speed with min. vibration and without liability of causing injury or accident. Centrf. must be heated, elec. or otherwise, to temp. of 55–60° during centrfg. It must be provided with speed indicator, permanently attached, if possible. Proper rate of rotation may be detd by ref. to table below. Rotation speed with full centrf. should be checked periodically with tachometer. "Diam. of wheel" means distance between inside bottoms of opposite cups measured thru center of rotation of centrf. wheel while cups are horizontally extended.

Diam. of wheel, in.	*rpm*
14	909
16	848
18	800
20	759
22	724
24	693

(**e**) *Dividers or calipers.*—For measuring fat column.

(**f**) *Water bath for test bottles.*—Provided with thermometer and device to maintain temp. of fat column at 57.5 ± 1°.

(**g**) *Water bath for tempering milk samples prior to pipetting.*—Provided with thermometer and device to maintain temp. of milk at 38 ± 1°.

(**h**) *Water bath for addition of water after 1st and 2nd centrifugations.*—Provided with thermometer, device to maintain temp. of soft H_2O at 59–61°, and device to deliver H_2O into Babcock bottles.

(**i**) *Bottle shaker.*—Variable speed and matched to max. capacity of centrf.

(**j**) *Digital thermometer for measurement of milk-acid reaction temperature.*—To read to nearest degree in range of 100–120°. Use acid-resistant probe, with small diam. (≤0.5 mm) to ensure rapid response time. Length of probe should be such that its tip is ca 1 cm above bottom of bottle when fully inserted.

(**k**) *Reading light.*—As background when measuring fat columns. Light should be diffused (soft white color) and provide illumination from angles above and below level of fat column. Magnification device must be used to aid reading.

16.E12 *Determination*

(**a**) *Sample preparation and temperature adjustment.*—With pipet, *Apparatus* (**b**), transfer 17.60 ± 0.05 mL prepd sample at 38°, **16.020**, to milk-test bottle. Blow out milk in pipet tip ca 30 s after free outflow ceases. Adjust milk in test bottles to 20–22°. Adjust H_2SO_4 (sp gr 1.82–1.83 at 20°) to 20–22°. Pipet some addnl milk samples for use as temp. control samples.

(**b**) *Measurement of milk-acid reaction temperature and determination of amount of H_2SO_4 to use.*—Prior to testing group of samples, det. correct amt of acid to be used by measuring milk-acid reaction temp. Start by adding 17.5 mL 20–22° Babcock H_2SO_4 to bottle contg 18 g milk of same temp.; add 17.5 mL portion of acid in 1 delivery that washes all traces of milk into bulb and cleanly layers acid under milk. Fully insert digital thermometer probe down bottle neck, and immediately shake by hand rotation until all traces of curd disappear. Peak reaction temp. should be 108 ± 2°. Adjust amt of H_2SO_4 added until reaction temp. is within this range and fat column is translucent golden-yellow to amber. Amt of acid required may be different for different technicians and different batches of acid.

(**c**) *Testing milk samples.*—Add appropriate amt of H_2SO_4 (as detd in (**b**)) by delivering acid in 1 addn that washes all traces of milk into bulb and cleanly layers acid under milk. Immediately shake by hand rotation (as in (**b**)) until all traces of curd disappear. Place bottle in Babcock bottle shaker set at medium speed. Continue to add acid to all samples to be tested, and then shake full set 1 addnl min. Temp. of milk plus acid in first bottle should not be less than 60° at time bottles are transferred to centrf. Place bottles in heated centrf., counterbalance, and, after proper speed is reached, centrf. 5 min. Add soft H_2O at 59–61°, until bulb of bottle is filled. Centrf. 2 min. Add soft H_2O at 59–61° until top of fat column approaches 7% mark of bottle calibration. Centrf. 1 min longer at ca 60°. Transfer bottle to warm H_2O bath maintained at 57.5 ± 1°, immerse bottle to level slightly above top of fat column, and leave until column is in equilibrium and lower fat surface assumes final convex form (≥5 min). Remove 1 bottle from bath, wipe it, and, with aid of reading light and magnification (**k**), use dividers or calipers to quickly measure fat column (before it begins to cool and contract). Place caliper points in vertical line on neck of bottle, with 1 point at lowest surface of *lower* meniscus and other point at *top* of upper meniscus. Without changing distance between 2 points on calipers, move calipers down bottle neck until lower point rests in etched horizontal graduation mark at 0%. Place upper point of calipers against bottle graduation and read test in % by wt to nearest 0.05%. Repeat for each bottle.

Fat column, at time of measurement, should be translucent, golden-yellow or amber, and free of visible suspended particles. Reject all tests in which fat column is milky or shows presence of curd or charred matter, or in which meniscus is indistinct or distorted; repeat test, adjusting vol. H_2SO_4 added to obtain proper color and milk-acid reaction temp.

Max. recommended difference between duplicates is 0.1% fat.

At 3.6% fat:

$s_r = 0.029$	$RSD_R = 1.014\%$
$s_R{}^1 = 0.037$	r value = 0.081%
$RSD_r = 0.742\%$	R value[1] = 0.104%

[1] Using regression equations for milk containing 3.6% fat:

$$s_R = (0.0080 \times 3.6\%) + 0.0080$$
$$\text{R value} = (0.0227 \times 3.6\%) + 0.0226$$

Refs.: JAOAC **8**, 4(1924); **8**, 471(1925); **56**, 1401(1973); **58**, 949(1975); **71**, 898(1988).

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(4) The following interim first action modified Mojonnier ether extraction method for determination of fat in milk was adopted first action:

Fat in Milk

Modified Mojonnier Ether Extraction Method

First Action

(Caution: See **51.011**, **51.033**, **51.039**, **51.054**, **51.055**, **51.070**, **51.073**.)

16.E13 ***Principle***

Fat is extd with mixt. of ethers from known wt of milk. Ether ext is decanted into dry weighing dish, and ether is evapd. Extd fat is dried to const wt. Result is expressed as % fat by wt.

16.E14 ***Apparatus***

(a) *Flask.*—Mojonnier-style ether extn flask with vol. of 21–23 mL in lower bulb plus neck at bottom of flask. Flask should have smooth, round opening at top that will seal when closed with cork (Kimble Glass Co.).

(b) *Weighing dishes.*—Metal, 8.5–9.5 cm diam. and 4.5–5.5 cm tall; or 250 mL glass beakers.

(c) *Calibration weights.*—Class S, std calibration wts to verify balance accuracy within wt range to be used for weighing empty flasks and flask plus sample and weighing empty dishes and dish plus fat.

(d) *Analytical balance.*—To read to nearest 0.0001 g. Accuracy on verification within 0.0002 g. Check periodically and whenever balance is moved or cleaned. Keep record of balance calibration checks.

(e) *Desiccator.*—Room temp. for cooling weighing dishes after preliminary and final drying. Use coarse desiccant (mesh size 6–16) that contains min. of fine particles and that changes color when moisture is absorbed.

(f) *Tongs.*—For handling weighing dishes.

(g) *Hot plate.*—Steam bath or other heating device. For evapn of ether at ≤100°. Carry out evapn in hood.

(h) *Corks.*—High quality natural cork stoppers (size 5) for flasks. Soak corks in H_2O several h to improve seal.

(i) *Vacuum or forced air oven.*—Vac. oven capable of maintaining temp. of 70–75° at > 50.8 cm (20 in.) of vac., or forced air oven capable of maintaining temp. of 100 ± 1°.

(j) *Water bath for tempering milk samples prior to weighing.*—With thermometer and device to maintain milk temp. of 38 ± 1°.

16.E15 ***Reagents***

(a) *Ethyl ether.*—ACS grade, peroxide free. No residue on evapn.

(b) *Petroleum ether.*—ACS grade, boiling range 30–60°. No residue on evapn.

(c) *Ammonium hydroxide.*—Concd, ACS grade, sp gr 0.9.

(d) *Ethyl alcohol.*—95%. No residue on evapn.

(e) *Distilled water.*—Free of oil and mineral residue.

(f) *Phenolphthalein indicator.*—0.5% (w/v) in EtOH.

16.E16 ***Determination***

(a) *Sample preparation.*—Prep. by tempering milk to 38° as in **16.020**. Weigh empty flask with clean, dry cork stopper. Remove stopper. Pipet ca 10 g milk into flask. Place stopper in flask. Weigh to nearest 0.1 mg. Check balance zero between samples.

(b) *Weighing dish preparation.*—Number clean weighing dishes and predry under same conditions that will be used for final drying after fat extn. Be sure that all surfaces where weighing dishes will be placed (i.e., hot plate, desiccator, etc.) are clean and free of particulates. At end of oven drying, place pans in room temp. desiccator and cool to room temp. On same day as fat extn (c), weigh dishes to nearest 0.1 mg and record wts. Check balance zero after weighing each pan. Protect weighed pans from contamination with extraneous matter.

(c) *Fat extraction.*—To sample in flask add 1.5 mL NH_4OH and mix thoroly. NH_4OH neutralizes any acid present and dissolves casein. Add 3 drops of phthln indicator to help sharpen visual appearance of interface between ether and aq. layers during extn. Add 10 mL EtOH, stopper with H_2O-soaked cork, and shake flask 15 s. For first extn, add 25 mL ethyl ether, stopper with cork, and shake flask very vigorously 1 min, releasing built-up pressure by loosening stopper as necessary. Add 25 mL pet ether, stopper with cork, and repeat vigorous shaking for 1 min. Centrf. flasks at ca 600 rpm for ≥30 s to obtain clean sepn of aq. (bright pink) and ether phases. Decant ether soln into suitable weighing dish prepd as in (b). When ether soln is decanted into dishes, be careful not to pour over any suspended solids or aq. phase into weighing dish. Ether can be evapd at ≤100° from dishes while conducting second extn.

For second extn, add 5 mL EtOH, stopper with cork, and shake vigorously 15 s. Next, add 15 mL ethyl ether, replace cork, and shake flask vigorously 1 min. Add 15 mL pet ether, stopper with cork, and repeat vigorous shaking for 1 min. Centrf. flasks at ca 600 rpm for ≥30 s to obtain clean sepn of aq. (bright pink) and ether phases. If interface is below neck of flask, add H_2O to bring level ca half way up neck. Add H_2O slowly down inside surface of flask so that there is min. disturbance of sepn. Decant ether soln for second extn into same weighing dish used for first extn.

For third extn, omit addn of EtOH and repeat procedure used for second extn. Completely evap. solvents in hood on hot plate at ≤100° (avoid spattering). Dry extd fat plus weighing dish to const wt in forced oven at 100° ± 1° (≥30 min) or in vac. oven at 70–75° at >50.8 cm (20 in.) of vac. for ≥7 min. Remove weighing dishes from oven and place in desiccator to cool to room temp. Record wt of each weighing dish plus fat.

Run pair of reagent blanks each day tests are conducted. To run reagent blank, replace milk sample with 10 mL water and run test as normal. Record wt of any dry residue collected and use value in calcn. Reagent blank should be <0.0020 g residue. If reagent blanks for set of samples are neg., use neg. number in calcn. *Note*: To subtract neg. number (av. wt blank residue) in equation below, add it to [(wt dish + fat) − wt dish]. Neg. blank usually indicates that dishes were not completely dry at start of detn or that balance calibration shifted between weighing of empty pans and pans plus fat. Cause of neg. blanks should be identified and corrected.

16.E17 ***Calculation***

$$\text{Fat, \%} = 100 \times \frac{[(\text{wt dish} + \text{fat}) - (\text{wt dish})] - (\text{av. wt blank residue})}{(\text{wt milk})}$$

Max. recommended difference between duplicates is <0.03% fat.

At 3.6% fat:

$s_r = 0.015$	$RSD_R = 0.512\%$
$s_R = 0.020$	r value = 0.044%
$RSD_r = 0.396\%$	R value = 0.056%

Ref.: JAOAC **71**, 898(1988).

17. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

18. FISH AND OTHER MARINE PRODUCTS

No additions, deletions, or other changes.

19. FLAVORS

No additions, deletions, or other changes.

20. FOOD ADDITIVES: DIRECT

No additions, deletions, or other changes.

21. FOOD ADDITIVES: INDIRECT

No additions, deletions, or other changes.

22. FRUITS AND FRUIT PRODUCTS

The first action liquid chromatographic method for determination of quinic, malic, and citric acids in cranberry juice cocktail and apple juice, **22.B01–22.B05**, was adopted final action.

23. GELATIN, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or other changes.

24. MEAT AND MEAT PRODUCTS

No additions, deletions, or other changes.

25. METALS AND OTHER ELEMENTS

No additions, deletions, or other changes.

26. NATURAL POISONS

(1) In the final action confirmation method for aflatoxin B_1 identification, **26.A01–26.A08**, make the following editorial changes to add an extraction and column chromatographic cleanup procedure for ginger root (*J. Assoc. Off. Anal. Chem.* (1980) **63**, 1052):

(a) Add as additional apparatus in **26.A02**:

Blender.—Waring, or equiv., with 1 L jar and cover.

Chromatographic column.—Glass, 10.5 mm id × 200 mm, with Teflon stopcock and 200 mL reservoir (K420280, Kontes Glass Co., or equiv.).

(b) Add ACS grade CH_2Cl_2 as a solvent in **26.A03(a)**.

(c) Add as additional reagents in **26.A03**:

NaCl soln.—Satd (ca 40 g NaCl/100 mL H_2O).

Sodium sulfate.—Anhyd., granular.

Granular silica gel.—Silica gel 60 (E. Merck No. 7734), 0.063–0.200 mm (70–230 mesh). Activate by drying 1 h at 105°. Then add 1 mL H_2O/100 g, seal in jar, shake until thoroughly mixed, and store overnight before use.

(d) Change **26.A04** to read as follows:

26.E01 ***Preparation of Extracts***

(**a**) *Roasted peanuts.*—Prep. ext as in **26.026–26.030**.

(**b**) *Cottonseed.*—Prep. ext as in **26.052–26.056**.

(**c**) *Ginger root.*—(*1*) *Dry root.*—Grind sample as in **26.003**. Weigh 50 g ground sample into blender jar; add 25 mL satd NaCl soln and 250 mL CH_2Cl_2. (**Caution**: CH_2Cl_2 boils at low temp. and has low specific heat; apply heat carefully to avoid boilover.) Blend 3 min at high speed. Filter thru high porosity folded paper into 50 mL graduate. Transfer 50 mL filtrate to 250 mL g-s erlenmeyer. Evap. filtrate to near dryness on steam bath and proceed as in (*3*).

(*2*) *Oleoresin.*—Weigh 0.7 g ext into 250 mL g-s erlenmeyer. Proceed as in (*3*).

(*3*) *Partition.*—Add 100 mL MeOH–5% NaCl soln (50 + 50) and 50 mL hexane to 250 mL g-s erlenmeyer; secure stopper with masking tape. Shake 10 min on wrist-action shaker; then transfer to 250 mL separator. Let phases sep. (2–3 min). Drain lower aq. layer into another 250 mL separator. Add 50 mL CCl_4 to aq. layer, shake separator vigorously ca 1 min, and let layers sep. Discard lower layer. Repeat partition with 25 mL CCl_4 and discard lower layer. Add 50 mL CH_2Cl_2 to retained aq. layer; shake 1 min. Drain CH_2Cl_2 layer into 250 mL erlenmeyer and ext aq. layer with addnl 25 mL CH_2Cl_2. Combine CH_2Cl_2 exts. Evap. combined CH_2Cl_2 exts to near dryness on steam bath.

(*4*) *Column cleanup.*—To clean up ginger ext, place ball of glass wool in bottom of chromatgc column and add ca 1 cm anhyd. Na_2SO_4 to give base for silica gel. Add CH_2Cl_2 until tube is ca ½ full; then add 4 mL silica gel. Add small amt CH_2Cl_2 and slurry silica with stainless steel rod. Drain CH_2Cl_2 to settle silica, and rinse sides of column with CH_2Cl_2. Leave 3 cm CH_2Cl_2 above silica. Slowly add 2 cm bed of anhyd. Na_2SO_4. Drain CH_2Cl_2 to top of Na_2SO_4.

Dissolve ext in 5 mL CH_2Cl_2. Add ext soln to column. Wash sides of flask with two 5 mL portions of CH_2Cl_2 and add washings to column. Elute sequentially at max. flow rate with 40 mL CH_2Cl_2, 40 mL benzene–HOAc (9 + 1), 40 mL hexane, and 40 mL anhyd. ether; discard eluates. After each addn of ext or solv., drain column until liq. level meets top of packed bed. Elute aflatoxins with 40 mL $CHCl_3$–acetone (80 + 20). Collect this fraction in 250 mL erlenmeyer from time of solv. addn until flow stops. Evap. eluate to near dryness on steam bath; quant. transfer residue to 4 dram vial, using CH_2Cl_2 for transfer, and evap. to dryness on steam bath under N stream. (**Caution**: CH_2Cl_2 boils easily; avoid overheating.) Reserve dry ext for TLC. Final ext represents 10 g dry, ground ginger.

(e) Add as an additional reference: JAOAC **63**, 1052(1980).

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(2) The following interim first action enzyme-linked immunosorbent screening method for aflatoxin B_1 in cottonseed products and mixed feed was adopted first action:

Aflatoxin B_1 in Cottonseed Products and Mixed Feed

Enzyme-Linked Immunosorbent Screening Method

First Action

AOAC-IUPAC Method

(Applicable to screening aflatoxin B_1 at ≥15 ng/g)

26.E02 ***Principle***

Aflatoxin B_1 is conjugated to enzyme, and conjugate is used as known antigen (enzyme-conjugate). Antibodies specific to aflatoxins are coated onto plastic microtiter wells. Aflatoxins are extd from test portion with solv. Ext is mixed with enzyme-conjugated aflatoxin B_1 and mixt. is placed in well of antibody-coated microtiter plate. Contaminating aflatoxin from test portion, if present, and enzyme-conjugated aflatoxin B_1 compete for binding sites on antibody. Excess unbound aflatoxins and enzyme-conjugated aflatoxin B_1 are rinsed away. Enzyme substrate, added to each well, is catalyzed by bound enzyme and changes from colorless to colored soln. Intensity of color depends on amt enzyme-conjugated aflatoxin B_1 bound to antibodies present: Darker color indicates less aflatoxins from sample portion (free aflatoxins) whereas lighter color indicates presence of more free aflatoxins. After few min, change in color can be evaluated visually or by measurement of *A*. By addn of colored enzyme-stopping soln, color intensity can be used to rate toxin level in product

by comparison with color intensity obtained using aflatoxin B_1 std solns.

Method is screening procedure for qual. detn of aflatoxins in cottonseed products and mixed feed, i.e., detn of presence or absence of aflatoxins (primarily B_1) at given concn. Pos. response can be detd either visually or by using microtiter well (EIA) reader. Results are valid *only* when test sample reactions are compared with (known) std concn ref(s). Assay is *not* confirmatory method because antibodies used in test cross-react with aflatoxins G_1, B_2, and G_2.

26.E03 *Determination of Sensitivity and Specificity of Antibody*

Suitability of ELISA method is influenced strongly by its sensitivity and specificity for analyte. Methods for detn of sensitivity of antibody-based assays are straightforward and may be found in several std texts. Specificity of ELISA methods depends on variety of factors including structure of analyte and protein to which it has been bound to form immunogen for raising antibody.

To det. sensitivity of antibody in use to aflatoxin B_1, add solns of known stds to successive antibody-coated microtiter wells so that concns represent 0, 0.25, 0.5, 1.0, 5, 10, 20, and 30 ng aflatoxin B_1/mL. Using microplate reader (spectrophtr), det. lowest concn yielding *A* difference from 0.0 ng/mL of 0.1; this concn is lowest level of sensitivity. Min. of 2 test series should be conducted. To evaluate cross-reactivity with other aflatoxins, repeat above procedure for aflatoxins B_2, G_1, and G_2 at same concns plus 100, 200, 300, 400, and 500 ng each aflatoxin/mL. Relative cross-reactivity (analog:B_1) is measured from amt toxin necessary to cause 50% inhibition of max. conjugate binding (50% of max. *A* measured at 0.0 ng/mL). For example, cross-reactivity to aflatoxin B_1 for antibody used in collaborative study of this method was 100, 25, 31, and 12% for aflatoxins B_1, B_2, G_1, and G_2, resp.

When microplate reader is available, coefficient of variation (CV) as function of concn level should be detd using any 1 comparable known concn; CV $\leq$20% is desirable. Aflatoxin concns rated on sequential diln of ext of highly contaminated product should agree within 0.5 CV of std curve.

26.E04 *Reagents*

Reagents (**a**)–(**g**) are available in Agri-Screen for Aflatoxins kit (Neogen Corp., 620 Lesher Pl, Lansing, MI 48912; stds may be prepd from purified toxin from Sigma Chemical Co.); alternative sources can be used provided criteria of suitability in above section are met.

(*Note:* Do not use rubber stoppers at any step in method.)

(**a**) *Aflatoxin B_1 enzyme-conjugate.*—Lyophilized horseradish peroxidase-conjugated aflatoxin B_1 at approx. molar ratio of 1:1; equiv., such as liq. conc., may be used. Do not use beyond expiration date.

(**b**) *Enzyme-conjugate hydration soln.*—Distd H_2O.

(**c**) *Antibody-coated solid support.*—12-well microtiter strips, or equiv. Do not use beyond expiration date.

(**d**) *Enzyme substrate.*—500 mg 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonate) (ABTS)/L citrate buffer (pH 4.0). Stopper after use and store under refrigeration (4°).

(**e**) *Hydrogen peroxide.*—1.5 mL 30% H_2O_2/L citrate buffer (pH 4.0). Stopper after use and store under refrigeration (4°).

(**f**) *Color-stopping soln.*—3.5 mg HF, 10.5 g Na citrate, 6 mL 1N NaOH, and 400 mg Na_4EDTA/L H_2O. Stopper after use and store under refrigeration (4°).

(**g**) *Aflatoxin B_1 stds.*—Prep. 3 and 10 ng/g solns by adding aflatoxin B_1 (**26.006**) to ext (see *Extraction*) of aflatoxin-free product to be tested (equiv. to 15 and 50 ng/g actual levels).

(**h**) *Methanol soln.*—Reagent grade MeOH–H_2O (55 + 45).

(**i**) *Hexane.*—Reagent grade.

26.E05 *Apparatus*

(**a**) *High speed blender.*—Explosion-proof, with 1 L (qt) jar.

(**b**) *Filter paper.*—Whatman No. 1, or equiv.

(**c**) *Pipets.*—Calibrated pipet or automatic pipettor with disposable tips, capable of delivering accurate amts in 0.1–1.0 mL range; Eppendorf, or equiv.

(**d**) *Mixing tubes.*—Glass or plastic, 12 × 75 mm, with caps.

(**e**) *Mixing wells.*—12-well microtiter mixing strip, or equiv.

(**f**) *Microtiter well reader.*—Optional. Spectrophtr with 405 nm screening filter that will read thru microtiter wells in strips (microplate reader, or equiv.).

(**g**) *Vortex mixer.*—Capable of vigorous agitation of mixing tubes.

26.E06 *Preparation of Working Solutions*

(**a**) *Enzyme-conjugated aflatoxin B_1 working soln.*—Add 2 mL enzyme-conjugate hydration soln (**b**) to enzyme-conjugated aflatoxin B_1 (**a**). Mix well to completely dissolve pellet. Do not shake hard enough to cause foaming in bottle. (*Note:* If enzyme-conjugated aflatoxin B_1 is prepd sep., concn needs to be optimized. Working soln *must* be at room temp. before use. Refrigerated, it will keep for 5 days.)

(**b**) *ABTS substrate working soln.*—Using different pipets/tips, transfer 1 mL ABTS substrate (**d**) and 1 mL H_2O_2 (**e**) to 12 × 75 mm mixing tube. Cover tube and mix. (*Note:* Working soln *must* be used within 1 h after prepn.)

26.E07 *Preparation of Sample*

(**a**) *Mixed feed.*—Grind using ultracentrifugal, hammer, Wiley, or disk mill to pass No. 20 sieve.

(**b**) *Cottonseed products.*—Grind as in (**a**) to pass thru No. 20 sieve (see **26.003**).

26.E08 *Extraction*

Weigh 5 g test portion into 50 mL screw-cap test tube. Add 25 mL MeOH soln (**h**) and 10 mL hexane; shake vigorously 1–3 min. Gravity-filter ext thru paper into 50 mL test tube. Alternatively, filter before addn of hexane or centrifuge 5 min at 3000 rpm. Let layers sep.; draw off upper hexane layer and discard.

26.E09 *Enzyme Immunoassay*

Note: Different pipet/tip must be used with each of following steps. Always pipet into wells in same order.

(*1*) Set up mixing wells (**e**). *Do not use antibody-coated microtiter wells.* Pipet 0.1 mL enzyme-conjugated aflatoxin B_1 working soln into control well(s) and into 1 well for each test sample. Add 0.1 mL aflatoxin B_1 std soln (15 and 50 ng/g or desired std concns) and test sample filtrate(s) to sep. wells and mix thoroly by drawing fluid back into pipet/tip. Change pipets/tips between each test sample and control. At least 1 aflatoxin B_1 control well (15 or 50 ng/g) should be used with each strip.

(*2*) Set up antibody-coated wells (**c**). Transfer 0.1 mL soln from each mixing well to corresponding antibody-coated well. Change pipets/tips between each transfer. Let stand 10 min at ambient temp.

(*3*) Shake soln out of antibody-coated wells into waste receptacle contg decontaminating soln (see *Safety Notes*). Do not let decontaminating soln enter wells. Rinse wells 10 times

by filling with H_2O and shaking out wash soln. After final wash, invert wells on absorbent paper and tap to remove excess H_2O droplets.

(*4*) Immediately add 0.1 mL ABTS substrate working soln (**b**) to each antibody-coated well. Let stand 10 min at ambient temp. Gently agitate wells several times during assay.

(*5*) After second 10 min incubation, add 0.1 mL enzyme-stopping soln (**f**) to each antibody well, mix gently, and compare color development of control wells with that of test sample wells. Hold microtiter well strips against white background for comparison. When using ELISA reader, neg. control must be prepd by adding 0.1 mL ABTS substrate working soln and 0.1 mL color-stopping soln to empty well as blank detn.

26.E10 ***Interpretation of Results***

(a) *Visual.*—Aflatoxin B_1 control wells should be light gray-green and pink for 15 and 50 ng/g levels, resp. Test samples contg <15 ng aflatoxins/g will be darker green; those contg >15 ng aflatoxins/g will be lighter green to pink with increasing concns of aflatoxins (see *Principle*).

(**b**) *Microplate (EIA) reader.*—Insert 405 nm filter and calibrate on blank well contg only substrate and enzyme-stopping soln. Record % absorption (optical density) for each well.

26.E11 ***Safety Notes***

Soak all used laboratory ware, pipet tips, and kit components in 10% soln of household bleach before discarding. (Household bleach generally contains 5.25% NaOCl.)

Extn vessels must be very clean. Following cleaning procedure between test samples is recommended:

(*1*) Fill contaminated blender jar half full with 10% household bleach soln.

(*2*) Blend 1 min.

(*3*) Pour out bleach soln and repeat steps *1* and *2* with distd H_2O.

(*4*) Pour out distd H_2O and repeat steps *1* and *2* with extn soln.

(*5*) Empty jar and use, or dry jar for storage.

Ref.: JAOAC **72**, March/April issue (1989).

27. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

28. OILS AND FATS

(1) The following first action methods were adopted final action:

(a) beta-Sitosterol in butter oil, gas chromatographic method, **28.104–28.109**.

(b) Cyclopropene fatty acids in oils, Halphen test, **28.120–28.123**.

(c) Foreign fats containing tristearin in lard, melting point method, **28.130–28.131**.

(d) Chick edema factor (dioxins) in oils and fats, gas chromatographic method, **28.139–28.141**.

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(2) The first action IUPAC-AOAC gas chromatographic method for determination of triglycerides in fats and oils, **28.B01–28.B05**, was revised by adding the following sentence in **28.B02(b)**: "Equiv. results may be obtained with use of short (≤6 m) capillary column."

29. PESTICIDE RESIDUES

No additions, deletions, or other changes.

30. SPICES AND OTHER CONDIMENTS

(1) In the final action gravimetric method for ether extract (volatile and nonvolatile) of spices, **30.012**, CH_2Cl_2 has been designated to replace ether as the extraction solvent. Change the title to read Methylene Chloride Extract (Volatile and Nonvolatile) of Spices, and show CH_2Cl_2 as solvent throughout the method.

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(2) The following final action methods were declared surplus:

(a) Nitrogen in spices, improved Kjeldahl method, **30.010**.

(b) Nitrogen in nonvolatile ether extract of pepper, improved Kjeldahl method, **30.011**.

(c) Alcohol extract of spices, gravimetric method, **30.013**.

(d) Starch in spices, method I, **30.015**.

(e) Tannin in cloves and allspice, titrimetric method, **30.018**.

31. SUGARS AND SUGAR PRODUCTS

(1) In the procedure for preparation of maple product samples, **31.169**, make a nonsubstantive change in **31.169(a)** (*1*): "... filter sample thru cotton wool *or* centrf. 20 min at 1000–3000 rpm."

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(2) In the final action method for color classification of maple products, **31.170–31.171**, add the Berliner maple syrup grading kit as a suitable alternative in **31.170**.

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(3) In the final action carbon ratio mass spectrometric method for corn syrup and cane sugar in maple products, **31.185–31.190**, results obtained when the alternative sample preparation procedure is used are biased approx. 1.0/mL relative to results obtained when samples are prepared as described in **31.187**. The bias must be corrected by adding a correction value (C) which may be determined by analyzing reference samples by both procedures.

$$\delta^{13}C(\text{corrected}) = \delta^{13}C(\text{measured}) + C$$

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(4) In the final action spectrophotometric method for formaldehyde in maple syrup, **31.203–31.208**, add an applicability statement: "(Not applicable to beet or cane sugars)."

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(5) The following final action methods were declared surplus:

(a) Moisture in sugars by drying on pumice stone, **31.007**.

(b) Ash of sugars and syrups, method I, **31.012**; method II, **31.013**; soluble and insoluble ash, **31.015**; alkalinity of soluble ash, **31.016**; alkalinity of insoluble ash, **31.017**; mineral adulterants in ash, **31.018** (first action).

(c) Sucrose and raffinose in sugars and syrups, polarimetric

method before and after inversion with hydrochloric acid, **31.029**.

(d) Invert sugar in sugars and syrups, Munson-Walker general method, **31.037–31.044**.

32. VEGETABLE PRODUCTS, PROCESSED

No additions, deletions, or other changes.

33. WATERS; AND SALT

No additions, deletions, or other changes.

34. COLOR ADDITIVES

No additions, deletions, or other changes.

35. COSMETICS

No additions, deletions, or other changes.

36. DRUGS: GENERAL

(1) The final action ion exchange chromatographic method for determination of antihistamines alone or in selected drug combinations, **36.108–36.114**, was declared surplus.

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(2) The following interim spectrophotometric method for determination of hydralazine hydrochloride in tablets was adopted first action:

Hydralazine Hydrochloride in Drug Tablets
Spectrophotometric Method
First Action

36.E01 ***Principle***

Hydralazine HCl is converted to tetrazolo[5,1-α]phthalazine, which is detd by UV detection at 274 nm.

36.E02 ***Apparatus and Reagents***

(a) *Spectrophotometer.*—UV-vis., recording, with matched 1 cm quartz cells, to measure A at 274 nm.

(b) *$NaNO_2$ soln.*—1% w/v $NaNO_2$ (ACS grade or equiv.). Prep. fresh daily.

(c) *Hydralazine HCl std soln.*—Accurately weigh ca 25 mg USP Hydralazine Hydrochloride Ref. Std previously dried under vac. over P_2O_5 for 8 h, and transfer to 250 mL vol. flask. Dil. to vol. with 0.1N HCl and mix well. Pipet 20.0 mL dild soln into 100 mL vol. flask, add 1.0 mL 1% $NaNO_2$ soln, mix, and heat mixt. ca 1 h on steam bath. Cool soln to room temp., dil. to vol. with H_2O, and mix.

36.E03 ***Preparation of Sample***

Weigh and finely powder ≥20 tablets. Accurately weigh portion of powder (or crushed tablet) equiv. to ca 25 mg hydralazine HCl, and transfer to 250 mL vol. flask. Add ca 125 mL 0.1N HCl and mech. shake 20 min. Dil. to vol. with 0.1N HCl, mix, and filter, discarding first 20 mL filtrate. Pipet 20.0 mL filtrate into 100 mL vol. flask, add 1.0 mL 1% $NaNO_2$ soln, mix, and heat ca 1 h on steam bath. Cool soln to room temp., dil. to vol. with H_2O, and mix.

36.E04 ***Determination***

Use suitable spectrophtr (a) to concomitantly det. A of sample and std solns contg ca 20 μg hydralazine HCl/mL.

Calc. mg hydralazine HCl/tablet as follows:

$$\text{Hydralazine HCl, mg/tablet} = (A_u/A_s) \times (W_s/W_u) \times T$$

where A_u and A_s = A of sample and std solns, resp.; W_s and W_u = mg ref. std and sample taken for std and sample solns, resp.; and T = av. tablet wt, mg.

Ref.: JAOAC **71**, 1121(1988).

CAS-86-54-4 (hydralazine)
CAS-304-20-1 (hydralazine monohydrochloride)

37. DRUGS: ACIDIC

No additions, deletions, or other changes.

38. DRUGS: ALKALOIDS AND RELATED BASES

The following interim liquid chromatographic method for determination of morphine sulfate in bulk drug and injections was adopted first action:

Morphine Sulfate in Bulk Drug and Injections
Liquid Chromatographic Method
First Action

38.E01 ***Principle***

Bulk drug and injection samples are prepd by direct diln in modified LC mobile solv. Morphine sulfate is quantitated and preservative phenol is identified by UV detection at 284 nm. Degradation product pseudomorphine and contaminant 2-mercaptobenzothiazole (2-MCBT) are detected and identified at 230 nm.

38.E02 ***Apparatus***

(Equiv. app. may be substituted.)

(a) *Liquid chromatograph.*—Equipped with injection valve with 20 μL sample loop, solv. delivery system, recording integrator, and variable wavelength UV detector capable of monitoring at 230 and 284 nm. Operating conditions: flow rate 1.5–2.0 mL/min (morphine sulfate should be eluted between 5 and 8 min); temp. ambient; injection vol. 20 μL. (System must meet system suitability requirements.)

(b) *LC column.*—Stainless steel, 30 cm × 3.9 mm id, packed with μBondapak C_{18}, 10 μm (Waters Associates, Inc.). Use of different C_{18} column may require addn of amine modifier to meet system suitability requirements.

(c) *Membrane filters.*—Nylon 66, pore size 0.45 μm, 25 and 47 mm diam.

38.E03 ***Reagents***

(Protect all morphine sulfate solns from direct light.)

(a) *Methanol.*—LC grade.

(b) *Acetic acid.*—Anal. grade.

(c) *1-Heptanesulfonic acid Na salt.*—Monohydrate. ≥98% (Eastman Kodak Co., or equiv.).

(d) *Morphine sulfate reference std.*—USP Morphine Sulfate (Pentahydrate) Ref. Std. Do *not* dry before use.

(e) *Phenol.*—Crystals, anal. grade.

(f) *Pseudomorphine.*—Prep. as follows: Add 10 g morphine to hot soln of 2.0 g KOH in 1 L H_2O, and let mixt. cool to room temp. Then add soln of 11.58 g $K_3Fe(CN)_6$ in 400 mL H_2O during 50 min, with stirring, and continue stirring addnl 30 min. Collect solid matter and stir with hot MeOH to remove morphine (1 g). Dissolve MeOH-insol. material (8.4 g) in concd NH_3 soln, dil. with H_2O to 700 mL, and boil mixt. 7.7 g pseudomorphine is deposited as almost colorless rods that decompose at ca 330°. A maxima are at 231 and 261 nm in 0.1N H_2SO_4. (From Bentley, K.W., & Dyke, S.F. J. Chem. Soc. 2574(1959).)

(g) *2-Mercaptobenzothiazole (2-MCBT).*—98% (Aldrich Chemical Co.).

(h) *Mobile solvent.*—Mix 240 mL MeOH with 720 mL 0.005M 1-heptanesulfonic acid Na salt monohydrate in H_2O and add 10 mL HOAc. Pass soln thru 0.45 μm filter and degas before use. Adjust MeOH or 1-heptanesulfonic acid Na salt soln content so that system meets suitability test requirements.

(i) *Dilution solvent.*—Mobile solv. (h) prepd without 1-heptanesulfonic acid Na salt.

38.E04 *Preparation of Standard Solutions*

(a) *Morphine sulfate std soln.*—Dissolve accurately weighed amt USP Morphine Sulfate Ref. Std in diln solv. to prep. soln contg ca 0.24 mg morphine sulfate/mL.

(b) *Morphine sulfate–phenol std soln.*—Dissolve accurately weighed amts USP Morphine Sulfate Ref. Std and phenol in diln solv. to prep. soln contg ca 0.24 mg morphine sulfate and ca 0.15 mg phenol/mL.

(c) *Phenol std solns.*—*Stock soln.*—About 2.0 mg phenol/mL H_2O. *Working soln.*—Add diln solv. to stock soln to prep. soln contg ca 0.15 mg phenol/mL.

(d) *Pseudomorphine std solns.*—*Stock soln.*—0.2–0.3 mg pseudomorphine/mL diln solv. *Working soln.*—Add diln solv. to stock soln to prep. soln contg ca 20 μg pseudomorphine/mL.

e) *2-MCBT std solns.*—About 80 μg 2-MCBT/mL MeOH. *Working soln.*—Add diln solv. to stock soln to prep. soln contg ca 5–6 μg 2-MCBT/mL.

f) *Mixed std soln.*—Prep. soln in diln solv. to contain morphine sulfate, phenol, pseudomorphine, and 2-MCBT at concns approx. equal to individual working std solns.

38.E05 *Preparation of Samples*

(a) *Bulk drug.*—Accurately weigh ca 100 mg bulk drug into 25 mL vol. flask and dil. to vol. with diln solv. Dil. soln with diln solv. to final concn of ca 0.24 mg morphine sulfate/mL.

(b) *Injections.*—Dil. accurately measured vol. morphine sulfate injection with diln solv. to final concn of ca 0.24 mg morphine sulfate/mL.

38.E06 *Determination*

Filter all solns thru 0.45 μm filter before injection.

Let LC system equilibrate 1 h with mobile solv. flow rate ca 1.5 mL/min. Set wavelength at 284 nm. Inject 20 μL morphine sulfate–phenol std soln (b). Coeff. of var. (CV) of morphine sulfate peak area for 5 replicate injections should be <2%. Resolution factor for phenol–morphine sulfate pair should be >2. Tailing factor at 5% peak ht for morphine sulfate peak should be <2. Proceed with analysis by alternating duplicate 20 μL injections of samples and morphine sulfate std soln (a).

If contaminants such as 2-MCBT and pseudomorphine are suspected in sample, det. identity by using stds (c)–(f). Detect phenol and morphine sulfate and quantitate latter at 284 nm. Detect 2-MCBT and pseudomorphine at 230 nm. Approx. retention times are 10–20 min for 2-MCBT and 20–45 min for pseudomorphine.

38.E07 *Calculations*

Calc. results as follows:

$$\text{Morphine sulfate bulk drug, \% purity} = (R/R') \times (W'/W) \times (D'/D) \times 100$$

$$\text{Morphine sulfate, mg/mL injection} = (R/R') \times W' \times (D'/D)$$

where R and R' = av. peak areas for duplicate injections of sample and std, resp.; W' and W = wt of std and sample, resp.; and D' and D = diln of std and sample, resp.

Ref.: JAOAC **71**, 1046(1988).

CAS-6211-15-0 (morphine sulfate, pentahydrate)

39. DRUGS: STEROIDS AND HORMONES

The first action liquid chromatographic method for determination of prednisolone in tablets and bulk drugs, **39.B01–39.B06**, was adopted final action.

40. DRUGS: ILLICIT

No additions, deletions, or other changes.

41. DRUGS AND FEED ADDITIVES IN ANIMAL TISSUES

No additions, deletions, or other changes.

42. DRUGS IN FEEDS

(1) The first action atomic absorption spectrophotometric method for roxarsone in feeds, **42.B01–42.B10**, was adopted final action.

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(2) In the final action spectrophotometric method for amprolium in feeds, **42.011–42.015**, add the following in **42.014**: "*Note:* Column recovery of amprolium may vary among different brands of basic alumina. Test column recovery by spiking ext from nonmedicated feed."

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(3) In the first action colorimetric method for sulfadimethoxine in feeds, **42.168–42.170**, add the following in **42.168(a)**: "Ficin product listed is no longer available."

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(4) In the first action spectrophotometric method for sulfamethazine in feeds, **42.172–42.174**, add to the applicability statement: "Not applicable to feed made from "granule stabilized" Tylan-Sulfa premix."

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(5) In the microbiological methods for antibiotics in feeds, **42.203–42.208**, **42.207** and **42.208** were revised to include changes in the calculations:

42.207 *Design and Plotting of Standard Response Line*

Prep. concns of ref. std as described for each antibiotic. In general, it is preferable to use shorter 4-fold range between lowest and highest doses of std line. Use indicated concn as ref. concn. (Values of std or ref. concn could slightly vary from those indicated for each antibiotic without affecting validity of assay.)

Prep. plates with appropriate base agar layer and/or appropriate seed agar layer; one layer of media can be substituted for 2 layers of media if ref. concn gives adequate zone size as described for each antibiotic. Distribute agar evenly by tilting plates from side to side with circular motion and let harden. Use plates same day prepd.

Place 6 cylinders on each plate at ca 60° intervals on 2.8 cm radius. Fill 3 alternate cylinders with ref. concn and other 3 cylinders with one of other concns of std. Use 3 plates for

each concn required for std response line, except ref. concn. Incubate plates overnight at appropriate temp., and measure diams of zones of inhibition as accurately as possible. (In most cases, it is possible to est. zone diams to nearest 0.1 mm.) Values given in each method for zones of inhibition to be obtained with ref. concns of antibiotics are for guidance only, but it is important that lowest concns on std response line give measurable zone and that slope of response line be adequate. In each set of 3 plates, average the 9 readings of ref. concn and the 9 readings of concn being tested. Av. of all 36 readings of ref. concn from 12 plates is correction point for response line. Correct av. value obtained for each concn to appropriate figure if ref. concn reading on that set of 3 plates was same as correction point.

For example, if in correcting second concn of std response line, av. of 36 readings of ref. concn is 20.0 mm, and av. of 9 readings of ref. concn of this set of 3 plates is 19.8 mm, correction is +0.2 mm. If av. reading of second concn on same 3 plates is 17.0 mm, corrected value is 17.2 mm. Plot corrected values, including correction point, on semilog graph paper, using logarithmic scale for concn and arithmetic scale for av. zone diams. Manual plotting of std lines is possible but could be subject to large variation. Response lines would be more accurate if calcd. When std doses are equally spaced, i.e., interval between successive doses is the same, calc. L and H (calcd zone diams for low and high concns, resp., of std response line) as follows:

For method specifying 5 doses of std,

$$L = (3a + 2b + c - e)/5$$
$$H = (3e + 2d + c - a)/5$$

where a, b, c, d, and e = corrected av. zone diams for each concn of std.

For methods specifying 4 doses of std,

$$L = (7a + 4b + c - 2d)/10$$
$$H = (7d + 4c + b - 2a)/10$$

For methods specifying 3 doses of std,

$$L = (5a + 2b - c)/6$$
$$H = (5c + 2b - a)/6$$

Plot values for L and H and connect with straight line. Ref. point is zone size intercept on arithmetic scale. This corrected ref. point is to be used for sample calcns. (If corrected ref. point diam. varies significantly from av. ref. diam., error in prepn of std solns is indicated and validity of assay is in question.) For more accuracy in calcn, det. slope of std response line $B = (H - L)/(\log h - \log l)$ where l and h are high and low std concns, resp., and B is increase in zone for each 10× increase in drug concn.

Computer or calculator can be used to calc. std lines whether std concns are equally spaced or not. Least square fitting using linear or polynomial equations may be performed based on best fit (polynomial fitting is most appropriate, especially for long range 8× or 16× range).

42.208 ***Determination of Potency***

Use 3 plates of each assay soln. On each plate, fill 3 alternate cylinders with ref. concn and fill other 3 cylinders with assay soln. Incubate plates overnight at appropriate temp. and measure diam. of zones of inhibition. Average the 9 readings of ref. concn and the 9 readings of assay soln. If assay soln gives larger av. than ref. concn, add difference between them to ref. point on std response line. If assay soln gives smaller value than ref. concn, subtract difference between them from ref. point on std response line. Using corrected values of assay soln, det. amt of antibiotic by reading concn from std response line.

Alternatively, instead of manual reading of unknown, det. log relative potency, $M' = (Y_u - Y_s)/B$, where Y_u and Y_s are av. of 9 readings of assay soln and ref. concn, resp., and B is slope of std response line. Antilog M' = potency of assay soln relative to std; and (antilog M') × 100 = potency of assay soln as % of std ref. concn.

For calcn of sample potency by computer or calculator, enter sample data and calc. antibiotic potency based on least square linear or polynomial lines.

For calcns, 1 ton = 908 000 g; 1 lb = 454 g.

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(6) The following final action methods were declared surplus:

(a) Nithiazide in feeds, spectrophotometric method, **42.122–42.125**.

(b) Nitrodan in feeds, colorimetric method, **42.126–42.129**.

(c) Reserpine in feeds, photofluorometric method, **42.148–42.151**.

(d) Ronnel in feeds: gas chromatographic method, **42.152–42.154**; spectrophotometric method, **42.155–42.159**.

(e) Sulfaguanidine in feeds, spectrophotometric method, **42.171** (first action).

43. VITAMINS AND OTHER NUTRIENTS

(1) In the final action colorimetric method for niacin and niacinamide in drugs, foods, and feeds, **43.048–43.050**, correct the calculation, **43.050**, to read:

$$\text{mg Niacin/g sample} = C/(10 \times \text{g sample})$$

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(2) The following interim method for vitamin E in supplemental vitamin E concentrates was adopted first action:

α-Tocopheryl Acetate in Supplemental Vitamin E Concentrates

Gas Chromatographic Method

First Action

(Applicable to oil and powder concs contg 400–1000 IU vitamin E/g)

43.E01 ***Principle***

Vitamin E oil concs are dissolved in *n*-hexane. Soxhlet extn followed by dissolution in *n*-hexane is used for prepn of dry adsorbates. Spray-formulated powders are treated with enzyme dissolved in H_2O, and extd. Prepd samples are analyzed by internal std GC method with H flame ionization detection and quantitation by peak area.

43.E02 ***Apparatus***

a) *Gas chromatograph.*—Equipped with H flame ionization detector; capable of accepting glass column and glass-lined sample introduction system. Operating conditions: temps—oven 265° isothermal, injector 275°, detector 275°; adjust carrier gas flow to obtain hexadecyl palmitate peak at 30–32 min; adjust detector gas flow for max. flame sensitivity; injection vol. 3–5 μL.

(b) *GC column.*—Glass, 6–8 ft (1.8–2.4 m) × 4 mm id, packed with 5% SE-30 on 80–100 mesh Gas-Chrom Q, or equiv.

(c) *Soxhlet extractor and condenser.*
(d) *Soxhlet thimbles.*

43.E03 ***Reagents***

(a) *n-Hexane.*—Reagent grade.
(b) *Cyclohexane.*—Reagent grade.
(c) *Vitamin std.*—Vitamin E acetate. USP Ref. Std No. 6677 (Alpha Tocopheryl Acetate), or equiv., or secondary std for which purity has been established on basis of USP Ref. Std.
(d) *Internal std.*—Hexadecyl hexanoate (hexadecyl palmitate, cetyl palmitate) (Analabs, Inc., No. LMS-067).
(e) *Enzyme.*—Pronase (Protease) type XIV (Sigma Chemical Co. P5147).
(f) *DL-α-tocopherol (vitamin E alcohol).*—For column performance evaluation (No. 25,802-4, Aldrich Chemical Co.).

43.E04 ***Column Preparation and Conditioning***

Insert glass wool plugs (5 mm) into packed GC column (b). Connect column to injector and heat 3 h at 300° without carrier gas flow. Cool to 285°. Condition column overnight at 285° with carrier gas flow at 30–50 mL/min. Cool column and connect to detector. Record baseline to check instrument stability. Baseline drift should be ≤1% in 30 min. (Pretested columns available from Applied Science Laboratories.) Occasionally, after prolonged use, column is rendered unusable for 1–4 h following direct injection. If this happens, either increase column temp. for short time to drive off sample contaminants or wait until baseline stabilizes at column operating temp.

43.E05 ***Column Performance Evaluation***

Prep. soln of 1 mg α-tocopherol and 1 mg α-tocopheryl acetate/mL *n*-hexane. Use ref. or secondary stds. Chromatograph aliquot of soln in GC system. Calc. resolution factor (*R*) from chromatogram:

$$R = [2 \times (t_2 - t_1)]/(W_1 + W_2)$$

where t_2 and t_1 = retention times, mm, of 2 compds, and W_1 and W_2 = corresponding peak widths, mm, at base.

R must be ≥1.0. $R < 1.0$ indicates that column is not suitable. Reevaluate column performance every 6 months.

43.E06 ***Determination of Response Factor***

Prep. response std soln by weighing (to nearest 0.1 mg) 100 mg USP Ref. Std (α-tocopheryl acetate) or secondary std (c) and 100 mg hexadecyl palmitate into 100 mL vol. flask. Dil. to vol. with *n*-hexane. Inject aliquot of soln into GC system. Calc. response factor (*RF*) as follows:

$$RF = (PA_{IS}/PA_{RS}) \times (C_{RS}/C_{IS}) \times P$$

where PA_{RS} and PA_{IS} = peak areas of ref. std and internal std, resp.; C_{RS} and C_{IS} = concns, mg/mL, of ref. std and internal std, resp., in response std soln; and *P* = % purity of ref. std.

Run replicate analyses until ≥3 *RF* values agree within ±1% of median value. Use av. *RF* in equation for calcn of assay result.

43.E07 ***Preparation of Sample***

(Weigh samples and internal std to nearest 0.1 mg.)

(a) *Oil concentrates.*—Into 50 mL vol. flask, weigh 50 mg sample and 50 mg hexadecyl palmitate. Dil. to vol. with *n*-hexane.

(b) *High potency (~50% vitamin E) feed concentrates.*—(*1*) *Adsorbates.*—Weigh 2 g 50% adsorbate (equiv. to 1 g vitamin E acetate) into tared Soxhlet extn thimble. Ext. sample in Soxhlet extractor ≥3 h with 175 mL *n*-hexane–cyclohexane (50 + 50 v/v). Let soln cool and then use *n*-hexane to quant. transfer soln to 200 mL vol. flask. Dil. to vol. with *n*-hexane.

Into sep. 50 mL vol. flask, weigh 48–52 mg hexadecyl palmitate. Into this flask, pipet 10 mL aliquot of extd sample soln and dil. to vol. with *n*-hexane.

(2) Spray-formulated powders.—Weigh into 50 mL erlenmeyer, portion of sample that contains 100 mg vitamin E, e.g., 200 mg powder contg 50% vitamin E. Add to flask 5 mL H_2O and amt on spatula tip (ca 50 mg) of Pronase. Mix until powder is wetted. Heat mixt. 20–30 min in 60° H_2O bath. Cool flask to room temp. and use 25 mL EtOH to transfer contents to 250 mL separatory funnel.

Ext aq. mixt. 5 times with 50 mL portions of *n*-hexane. Filter each *n*-hexane layer thru Na_2SO_4 in funnel plugged with glass wool into 500 mL r-b flask. Rinse Na_2SO_4 filter cake with two 50 mL portions of *n*-hexane and collect rinses in same flask with *n*-hexane exts. Evap. combined *n*-hexane fractions and rinses to near dryness (ca 15 mL) using rotary evaporator with 40° H_2O bath. Using *n*-hexane, rinse residue in flask into 100 mL vol. flask contg 100 mg hexadecyl palmitate. Dil. to vol. with *n*-hexane.

43.E08 ***Determination***

Inject aliquot of prepd sample into GC system. Perform replicate analyses until ≥3 consecutive results agree within ±1% of median value. Report av. of assay results.

Calc. % vitamin E (detd as *d,l*-α-tocopheryl acetate) as follows:

$$\text{Vitamin E, \%} = (PA_U/PA_{IS}) \times (C_{IS}/C_U) \times RF \times 100$$

where PA_U and PA_{IS} = peak areas of sample and internal std, resp.; C_U and C_{IS} = concns, mg/mL, of sample and internal std in sample soln, resp.; and *RF* = response factor.

Ref.: JAOAC **71**, 1168(1988).

CAS-7695-91-2 (α-tocopheryl acetate)

44. EXTRANEOUS MATERIALS: ISOLATION

(1) The following first action methods were adopted final action:

(a) Filth in corn flour: pancreatin digestion method, **44.058(a)**; acid hydrolysis method, **44.059**.
(b) Filth in eggs and egg products, **44.074–44.076**.
(c) Filth in mushrooms (canned, fresh, frozen, freeze-dried, and dehydrated), **44.115**.
(d) Light filth in ground allspice, **44.127–44.128**.
(e) Excrement (bird and insect) on food and containers, thin layer chromatographic method for uric acid, **44.B07–44.B09**.

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(2) In the final action method for Howard mold count of dehydrated tomato powder, **44.211**, make the following changes:

(a) Add "ca 3200 rpm" as the blending speed. Add as references, JAOAC **55**, 73(1972); **59**, 352(1976); **61**, 475, 992(1978).

(b) Add a procedure for identification of the powder as a spray-dried product. To precede **44.211**, *Determination*, add:

(Tomato powder is produced by dehydrating concd tomato pulp. In prepg powder for mold counting, moisture content

is disregarded and diln with H_2O is made to give mixt. with approx. tomato solids content of stdzd prepn for mold count of tomato puree or paste, i.e., 8.5%.)

44.E01 *Microscopic Identification as Spray-Dried Product*

Mold counts of spray-dried tomato powder show significantly higher counts than paste from which it is made because of breakage of mold hyphae aggregates. Use following procedure to det. whether powder represents spray-dried product.

Suitably mount a small portion of product on microscope slide in mineral oil or other nonaqueous mounting medium and examine microscopically at 100–200×. Spray-dried particles are translucent and contain air bubbles and numerous small granules within particles. Shape of particles ranges from spherical to elongate to irregular with rounded outlines and essentially no sharp angles. In rehydrated powder, practically no intact tomato cells are evident. Drum-dried or similarly processed powder or flakes are characterized by irregular-shaped particles with angular outlines and practically no embedded air bubbles.

Perform mold count as described in **44.211**.

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(3) In the final action method for *Geotrichum* mold counting, **44.216**, clarify the calculation by changing **44.216** to read:

Calc. mycelial fragments/500 g product:

$$N = (S/V\{\text{slides}\}) \times (500/W) \times V\{\text{diln}\}$$

where S = total mycelial fragments counted; $V\{\text{slides}\}$ = total vol. counted (0.5 mL/slide); W = net wt of sample, g; and $V\{\text{diln}\}$ = vol. after final diln with stabilizer soln.

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(4) In the final action method for *Geotrichum* mold count in vegetables, fruits, and juices (canned), **44.219**, correct and clarify the calculation. Change **44.219(c)** to read:

(c) Transfer to g-s graduate. Dil. to ≥100 mL ($V\{\text{prepn}\}$) and mix well. Quickly pour off two 25 mL aliquots ($V\{\text{aliq.}\}$ = sum of aliquots taken) into sep. centrf. tubes and proceed as in (**b**). Keep final vols equal after dilg with stabilizer soln; $V\{\text{diln}\}$ = sum of vol. in both tubes. Proceed as in **44.214**, pipeting 1 slide from each dild aliquot. Calc. mycelial fragments/500 g product:

$$N = (S/V\{\text{slides}\}) \times (500/W) \times V\{\text{diln}\} \times (V\{\text{prepn}\}/V\{\text{aliq.}\})$$

where S = total mycelial fragments counted; $V\{\text{slides}\}$ = total vol. counted (0.5 mL/slide); W = net wt of sample, g; $V\{\text{diln}\}$ = sum of vol. in both centrf. tubes after final diln with stabilizer soln; $V\{\text{prepn}\}$ = vol. before aliquots removed; and $V\{\text{aliq.}\}$ = sum of vol. of aliquots taken.

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(5) In the final action method for *Geotrichum* mold count in comminuted fruits and vegetables, **44.220–44.222**, clarify the calculation by changing the last sentence in **44.222** to read: "Express results in mycelial fragments per 100 mL prepn: $N = S \times 100$, where S = total mycelial fragments/mL sample prepn counted (0.5 mL/slide)."

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(6) In the final action method for rot fragment count in comminuted tomato products, **44.224**, 2 statements were omitted in the revision in "Changes in Official Methods," A supplement. Correct the revision as follows:

(a) To follow ". . . (Dual Manufacturing . . . Chicago, IL 60647).", add "Rinse beaker with addnl 200 mL H_2O and pour H_2O directly from beaker over sieve as before."

(b) After the sentence "Blow out last drop if necessary.", add "Examine each slide at 40–45×, using transmitted light."

(c) Add additional references: JAOAC **68**, 278, 402, 896(1985).

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(7) The method for rot fragment count in comminuted tomato products, **44.224**, as revised and corrected, was declared surplus.

45. FORENSIC SCIENCES

No additions, deletions, or other changes.

46. MICROBIOLOGICAL METHODS

(1) The following first action methods were adopted final action:

(a) Aerobic plate count, most probable number of coliform bacteria and *Escherichia coli*, and *Staphylococcus* in frozen, chilled, cooked, or prepared foods, **46.013–46.015**.

(b) Thermophilic bacterial spores in sugars, **46.078–46.082**.

(c) Virus in ground beef, **46.188–46.190**.

(d) Sporeformers in low-acid canned foods, gas chromatographic method, **46.A01–46.A05**.

(e) Poliovirus 1 in oysters, **46.A12–46.A22**.

(f) *Salmonella* in foods, DNA hybridization method, **46.C07–46.C16**.

(g) *Salmonella* in low-moisture foods, enzyme immunoassay screening method, **46.C17–46.C25**.

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(2) In the final action optical somatic cell counting method III, **46.171–46.175**, include in **46.172** use of the Fossomatic-90 Somatic Cell Counter.

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(3) The following final action methods for somatic cell counting in milk were declared surplus:

Method I, **46.152–46.160**; method II, **46.161–46.170**; membrane filter deoxyribonucleic acid method, **46.176–46.180**.

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(4) The following interim dry-rehydratable film method for enumeration of coliforms and aerobic bacteria in dairy products was adopted first action:

Bacterial and Coliform Counts in Dairy Products
Dry Rehydratable Film Methods
First Action

46.E01 *Principle*

Method uses bacterial culture plates of dry medium and cold H_2O-sol. gel. Undild or dild samples are added to plates at rate of 1.0 mL per plate. Pressure, when applied to plastic spreader placed on overlay film, spreads sample over ca 20 sq. cm growth area. Gelling agent is allowed to solidify and plates are incubated and then counted. Pipet, plate loop continuous pipetting syringe, or automatic pipet can be used for sample addn for bacterial count analyses.

46.E02 *Apparatus and Reagent*

(a) *Aerobic count plates.*—Plates contain std methods media nutrients, **46.005(g)**, cold H_2O-sol. gelling agent coated onto film base, overlay film coated with gelling agent, and 2,3,5-triphenyltetrazolium chloride indicator. Circular growth area of single plate contains ca twenty 1 cm squares outlined on film base. Petrifilm Aerobic Count Plates™ (available from Medical-Surgical Division/3M, 225-5S 3M Center, St. Paul, MN 55144) meet these specifications.

(b) *Coliform count plates.*—Plates contain violet red bile nutrients conforming to APHA standards as given in Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., 1984 (American Public Health Association, 1015 18th St, NW, Washington, DC 20005), cold H_2O-sol. gelling agent, and 2,3,5-triphenyltetrazolium chloride indicator. Petrifilm Coliform Count Plates ™ (available from Medical-Surgical Division/3M) meet these specifications.

(c) *Plastic spreader.*—Provided with Petrifilm plates, consists of recessed side and smooth flat side, designed to spread sample evenly over plate growth area.

(d) *Pipets.*—Calibrated for bacteriological use, or plate loop continuous pipetting syringe to deliver 1.0 mL. Automatic pipet to deliver 1.0 mL may be used.

(e) *Colony counter.*—Std app., Quebec model preferred, or one providing equiv. magnification and visibility.

(f) *Dilution water.*—See **46.005(a)**.

46.E03 *Sample Preparation*

(a) For total plate counts: Aseptically prep. 1:10 diln (11 g/99 mL diln H_2O). Mix well and plate. Prep. addnl dilns as required. Ordinarily, 1:10 and 1:100 dilns are sufficient.

(b) For coliform counts:

(1) Cream, half-and-half, condensed milk, egg nog, cottage cheese, butter, margarine, and related products.—Make 1:5 diln (24.75 g/99 mL diln H_2O). Mix well and plate 1 mL on each of 2 plates. Multiply total of counts on 2 plates by 2.5 to obtain count/g.

(2) Sour cream, dips, and yogurt.—Proceed as in **(a)** except after diln, adjust pH to 6.6–7.2 with 0.1N NaOH (ca 0.1mL/g sample).

(3) Buttermilk.—Make 1:10 diln (11 g/99 mL diln H_2O). Mix well and plate 1 mL on each of 2 plates. Multiply total of counts on 2 plates by 5 to obtain count/g.

(4) Ice cream, sherbet, and mixes.—Hydrate dry-film plates with 1 mL sterile diln H_2O and allow at least 1 h for gel to solidify. Then, lift top film of prehydrated dry-film coliform count plate (gel will adhere to top film) and dispense 0.5 mL of 2:3 homogenate (10 g/5 mL diln H_2O) onto bottom film of each of 3 plates. Replace top film gently over sample. Add counts on the 3 plates to obtain count/g. Alternatively, plate 1 plate and multiply result by 3 to obtain count/g.

(5) Cheese.—Proceed as in (a). Do not use citrate buffer to homogenize sample.

(6) Chocolate milk.—Proceed as in (a).

46.E04 *Analysis*

(a) *Bacterial colony count.*—Use dry-film aerobic count plates. Place plate on flat surface. Lift top film and inoculate 1 mL sample onto center of film base. Carefully roll top film down onto inoculum. Distribute sample over prescribed growth area with downward pressure on center of plastic spreader device (recessed side down). Leave plate undisturbed 1 min to permit gel to solidify. Incubate plates 48 ± 3 h at 32 ± 1°.

In incubator, place plates in horizontal position, clear side up, in stacks not exceeding 20 units. Count plates promptly after incubation period. If impossible to count at once after required incubation, store plates at 0–4.4° for not >24 h. This should be avoided as a routine practice.

Use std colony counter for counting purposes. Magnifier-illuminator may also be used to facilitate counting. Colonies stain in various shades of red. Count all colonies in countable range (25–250 colonies).

To compute bacterial count, multiply total number of colonies per plate (or av. number of colonies per plate if counting duplicate plates of same diln) by reciprocal of diln used. When counting colonies on duplicate plates of consecutive dilns, compute mean number of colonies for each diln before detg av. bacterial count. Estd counts can be made on plates with >250 colonies and should be reported as estd counts. In making such counts, circular growth area can be considered to contain ca twenty 1 cm squares. To isolate colonies for further identification, lift top film and pick colony from gel.

(b) *Coliform count.*—Use dry-film coliform count plates. Proceed as in (a), but distribute prepd sample over plate by using plastic spreader, flat side down. Incubate plates 24 ± 2 h at 32 ± 1°. Count as in (a), but count only red colonies that have one or more gas bubbles associated (within 1 colony diam.) with them. Count all colonies in countable range (15–150 colonies). Red colonies without gas bubbles are not counted as coliform organisms.

Ref. JAOAC 72, March/April issue (1989).

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(5) The following temperature-independent pectin gel method for enumeration of coliforms in dairy products was adopted first action:

Coliforms in Dairy Products
Pectin Gel Method
First Action

46.E05 *Principle*

Method uses pretreated plates contg thin "hardener" layer and liq. medium contg nutrients with pectin as sole gelling agent. Liq. medium (10–12 mL) is poured into pretreated plate and undild or dild sample is added. Plate is rotated and rocked to mix sample and medium. Plates are then allowed to rest on level surface until medium solidifies. Then, 3–4 mL liq. medium is poured as overlay and allowed to solidify. Total process is done at ambient temp. Plates are then incubated and counted as for agar-based prepns.

46.E06 ***Materials***

Note: Pectin base medium may be formulated from individual ingredients; suitability for analysis must be demonstrated.

Pectin gel and plates.—Violet red bile (VRB) pectin gel is available as sterile liq. in individual units contg sufficient gel to pour 1 plate or in units to pour 8 plates. VRB Redigel and preteated plates (RCR Scientific, Inc., 206 W Lincoln Ave, Goshen, IN 46526), or equiv., meet specifications of method.

To prep. VRB pectin gel from individual ingredients, suspend 7.0 g pancreatic digest of gelatin, 3.0 g yeast ext, 10.0 g lactose, 1.5 g bile salts No. 3, 5.0 g NaCl, 0.03 g neutral red, and 0.002 g crystal violet in 500 mL H_2O. Suspend 15 g low methoxyl pectin in 500 mL H_2O. Heat individual mixts until all ingredients are dissolved. Autoclave solns 15 min at 121°. Combine nutrient and pectin solns and adjust pH to 7.4 ± 0.2. To prep. pretreated petri plates, prep. hardener layer mixt. of 1% agar with 0.02 $CaCl_2$ concn. Sterilize mixt. by autoclaving 15 min at 121°. Aseptically dispense 5 mL portions of mixt. into sterile petri plates.

46.E07 ***Preparation of Samples***

To prep. dilns, measure (or weigh) 11 mL (or g) sample and dil. in 99 mL Butterfield's or 2% Na citrate diluent. For solid samples, blend 2 min at 10 000 to 12 000 rpm. Prep. addnl dilns so that total colonies/plate is in 25–250 range. Incubate plates 48 ± 3 h at 32 ± 1°. Shake all dilns 25 times in 30 cm arc. Pipets must accurately deliver required vol. Do not use to deliver <10% of their total vol. For example, to deliver 1 mL, do not use pipet >10 mL; to deliver 0.1 mL, do not use pipet >1 mL.

46.E08 ***Determination***

(*1*) Lift lid of pretreated petri plate and pour ca 75% (10–12 mL) of liq. medium from tube into plate. (*Note*: Remove cap from each tube of liq. pectin gel medium as it is needed to pour plate.) Prep. number of plates, in duplicate, needed for samples being run. Replace lid and swirl plate to cover bottom with liq. medium. Plates must be used within 5 min.

(*2*) Add inoculum (sample) to liq. pectin gel in petri plate. Touch pipet tip once to dry spot on inside wall of plate (above level of liq. medium) after dispensing sample to rest point in pipet tip. *Immediately* rotate and rock plate to mix sample thoroly with pectin gel. Do not spill mixt. over sides of plate. (*Note*: This step is primary difference in procedure between pectin gel and agar-based media. *Do not* add inoculum (sample) to pretreated petri plate and pour pectin gel over it. This would lock sample in one small area of plate without sepn of individual colonies.)

(*3*) Let inoculated plates stand on level surface until pectin gel is solid, and then pour remaining medium (3–4 mL) from tube as overlay and let gel solidify. Incubate in same manner as for agar-based plates (24 ± 2 h at 32 ± 1°).

(*4*) After 24 h incubation, count all red or pink colonies. Report as coliforms/mL or g.

(*5*) Pick 5 colonies of each type present on each plate and transfer to brilliant green lactose bile broth fermentation tubes (**46.013(c)**). Incubate 48 ± 3 h at 32 ± 1° and check for gas production, which is considered pos. for coliforms.

(*6*) If any picks from step *5* are neg. for gas production, adjust counts (step *4*) accordingly.

Ref.: JAOAC **72**, March/April issue (1989).

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(6) The interim MICRO-ID system method was adopted first action as an alternative to biochemical testing for foodborne *Escherichia coli* and *Salmonella* sp., and for presumptive identification of other Enterobacteriaceae in foods:

Salmonella sp., *Escherichia coli*, and Other *Enterobacteriaceae* in Foods

Biochemical Identification Kit Method

First Action

Use of com. biochem. kit as alternative to conventional biochem. testing in **46.016(a)–(f)** (*E. coli*) and **46.121–46.124** (*Salmonella* sp.) is based on demonstration in analyst's laboratory of adequate correlation between biochem. kit intended for use and conventional biochem. tests in **46.016(a)–(f)** and **46.121–46.124**. Com. biochem. kit should not be used as substitute for serological tests for *Salmonella* as described in **46.121–46.128**. Com. biochem. kit can be used for presumptive identification of other *Enterobacteriaceae* isolated from foods.

46.E09 ***Principle***

Method uses kit in which inoculum contains preformed enzymes at levels detectable in 4 h by means of sensitive indicator system. Kit contains filter paper discs impregnated with reagents which detect presence of specific enzymes and/or metabolic products produced by certain microorganisms. These reagents include substrate to be acted on by bacterial enzyme, and detection system which reacts with metabolic end product to yield readily identifiable color change. Precise quantities of substrate and/or detection reagents are supplied to each disc so that chem. incompatible materials are sepd until tray is inoculated. Tests included are Voges-Proskauer (VP), nitrate reductase, phenylalanine deaminase, H_2S, indole, ornithine decarboxylase, lysine decarboxylase, malonate utilization, urease, esculin hydrolysis, β-galactosidase, and arabinose, adonitol, inositol, and sorbitol fermentations.

46.E10 ***Method Performance***

Results	*Percent Agreement*[1]	*95% Confidence Range (Approx.)*
Salmonella sp.	98.8	97.2–100
E. coli	97.7	94.6–100
Other enterics[2]	84.6	81.2–88.0

[1] Agreement with conventional biochem. tests (AOAC methods).

[2] *Enterobacteriaceae* correctly identified to genera other than *Salmonella* and *E. coli*.

46.E11 ***Apparatus, Culture Media, and Reagents***

Use distd or deionized H_2O.

(**a**) *Plate count agar (standard methods agar) slants.*—5.0 g tryptone, 2.5 g yeast ext, 1.0 g dextrose, and 15.0 g agar. Suspend ingredients in 1 L H_2O. Heat to boiling to dissolve medium completely. Dispense 8–10 mL portions into 16 × 150 mm test tubes. Autoclave 15 min at 121°. Before medium solidifies, place tubes in slanted position so that adequate slants are formed.

(**b**) *Physiological saline.*—Dissolve 8.5 g NaCl in 1 L H_2O. Final pH *must be* 6.0 ± 0.5. Do not use saline prepns contg preservatives such as Na azide or other bacterial growth inhibitors. Saline does not need to be sterile but should be freshly prepd.

(**c**) *20% KOH soln.*—Slowly add 20 g KOH pellets to 60 mL H_2O. Dissolve by stirring. Add sufficient H_2O to prep.

100 mL soln. Keep KOH soln in tightly closed container when not in use. **Caution**: caustic reagent. Handle with care.

(**d**) *Test tubes.*—16 × 100 mm or larger. One test tube is required for each isolate to be identified.

(**e**) *Pipets.*—1 mL and 5 mL serological, with cotton plug.

(**f**) *Pathotec cytochrome oxidase test.*—No. 34191 (Organon Teknika Corp., 100 Akzo Ave, Durham, NC 27704); or equiv.

(**g**) *MICRO-ID identification kit and manual.*—No. 34146 (Organon Teknika Corp.).

(**h**) *Support rack.*—To hold test kit units (Organon Teknika Corp., No. 34147).

46.E12 *Preparation of Inocula*

(*1*) Select isolated colony from agar medium. Transfer colony to plate count agar slant. Incubate 18–24 h at 35°. *Note*: Cultures older than 30 h may give false neg. results.

(*2*) Perform cytochrome oxidase test on portion of growth from slant. Cytochrome oxidase-neg. rods should be further tested.

(*3*) Pipet ca 3.5 mL physiological saline (**b**) into 16 × 100 mm test tube for each isolate to be identified. Transfer growth from each slant into tube of saline until density of suspension of organisms is equiv. to McFarland No. 2.0. *Note*: Sterile test tubes are not required.

46.E13 *General Instructions*

Components and procedures of test kit have been stdzd for use in MICRO-ID identification system. Components or procedures other than those supplied by Organon Teknika Corp. may yield unsatisfactory results, and should be pretested.

46.E14 *Inoculation and Reading of Unit*

(*1*) Open sealed, moisture-proof, foil package and remove test unit. Do not remove clear plastic tape that covers test wells.

(*2*) Record sample no. and other required information on area provided on right side of cover.

(*3*) Open cover and let unit lie flat on laboratory bench.

(*4*) Pipet ca 0.2 mL of organism suspension into each inoculation well at top of unit.

(*5*) Close cover and stand tray upright in support rack. (Make sure that organism suspension is in contact with all substrate discs. DO NOT moisten detection discs.)

(*6*) Incubate 4 h at 35–37°. DO NOT use CO_2 incubator.

(*7*) After 4 h incubation, place each unit flat on bench, open lid, and add 2 drops (ca 0.1 mL) of 20% KOH soln (c) to inoculation well of VP test ONLY. Do not add KOH to any other inoculation well. Close lid and hold tray upright. Be certain that KOH flows down into VP test soln.

(*8*) Rotate unit clockwise ca 90° so upper discs in first 5 wells become wet. Hold tray upright and tap gently on bench to dislodge any suspension trapped under upper disc. Be certain that each upper disc in reaction chambers 1–5 is moistened by this procedure.

(*9*) Read all reactions immediately, except VP test, as pos. or neg. according to color changes listed below. Let color develop in VP well for ca 10 min, and then read. Read color of upper disc for first 5 tests; read color of organism suspension for remaining 10 tests. Record result for each biochem. test on encoding forms supplied with system.

Test	*Positive Reaction*	*Negative Reaction*
Voges-Proskauer	pink to red	light yellow
Nitrate reductase	red	colorless to light pink
Phenylalanine deaminase	green[1]	light yellow
H_2S	brown to black[2]	white
Indole	pink to red	light yellow to orange
Ornithine decarboxylase	purple to red–purple	amber to yellow
Lysine decarboxylase	purple to red–purple	amber to yellow
Malonate utilization	green to blue	yellow
Urease	orange to red–purple	yellow
Esculin hydrolysis	brown to black	no color change or beige
β-Galactosidase	light yellow to yellow	colorless
Fermentations:		
Arabinose	yellow to amber	red–purple to purple
Adonitol	yellow to amber	red–purple to purple
Inositol	yellow to amber	red–purple to purple
Sorbitol	yellow to amber	red–purple to purple

[1] In phenylalanine deaminase test, any green color in organism suspension also indicates pos. reaction.

[2] Pos. H_2S reaction might vary from thin, dark line at bottom of detection disc to entire disc turning black. It is often advisable to read this disc before it has been wetted.

(*10*) Use MICRO-ID identification manual (**g**) to det. 5 digit octal no. for each isolate, and record identification of isolate.

46.E15 *Confirmation (Salmonella sp. Only)*

For confirmation of cultures presumptively identified as *Salmonella* sp., see **46.135**.

Ref.: JAOAC **71**, 968 (1988).

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(7) The interim 1-2 TEST system method for screening all foods for the presence of motile *Salmonella* was adopted first action:

Motile *Salmonella* in Foods
Immunodiffusion Screening Method
First Action

Method is screening procedure for presence of motile *Salmonella* in all foods. It is not a confirmatory test because polyvalent H (flagellar) antibodies used in test may cross-react with small percentage of non-*Salmonella*. *Note*: Method does not detect nonmotile salmonellae.

If test is pos., enrichment broth from inoculation chamber of test unit must be streaked onto selective/differential agar media as in **46.118**, or, if test is performed on raw or highly contaminated product, enrichment broth must be transferred to tetrathionate broth and incubated 18–24 h in 43 ± 0.5° H_2O bath, and then streaked onto selective/differential agar media. Typical or suspicious colonies must be identified as in **46.119–46.128**.

46.E16 *Principle*

Detection of *Salmonella* is based on presence and observation of *Salmonella* immobilized in motility medium by polyvalent H (flagellar) antibodies. Immobilization of motile *Salmonella* results in development of well defined band of cells (immuno-band). Fig. **46:E1** shows small disposable plastic device (1-2 TEST unit) which has 2 chambers. Smaller inoculation chamber contains selective tetrathionate broth supplemented with brilliant green and L-serine. Enriched sample is inoculated into this chamber. Central motility chamber of unit contains peptone-based, nonselective motility medium. Motility chamber is sealed with gel-former plug. Tip of this plug forms void in motility medium for addn of flagellar antibody prepn. For shipping, opening between 2 chambers is sealed with polyethylene chamber plug, which is removed and discarded prior to addn of inoculum. *Salmonella* inoculated into tetrathionate-brilliant green-serine broth move from this medium into motility medium to react with flagellar antibodies.

46.E17 *Method Performance*

For all foods:

Results	*Percent*	*95 % Confidence Range (Approx.)*
Agreement[1]	96.1	94.5–97.7
False neg. (BAM/AOAC)[2]	1.7	0.5–3.0
False neg. (immunodiffusion)[3]	3.6	1.8–5.5

[1] Rate reflects no. of samples read identically between BAM/AOAC (Bacteriological Analytical Manual (1984) 6th ed., AOAC, Arlington, VA) culture method and immunodiffusion method.

[2] Rate reflects no. of samples found to be pos. by immunodiffusion method but detd as neg. by BAM/AOAC culture method.

[3] Rate reflects no. of samples found to be pos. by BAM/AOAC culture method but detd as neg. by immunodiffusion method.

Of 17 laboratories, 8 had complete agreement between culture and immunodiffusion methods; 11 showed agreement on ≥97% of samples; 14 showed agreement on ≥95%; 16 showed agreement on ≥93%.

46.E18 *Reagents*

Items (**a**)–(**c**) are available as BioControl 1-2 TEST (BioControl Systems, Inc., 19805 North Creek Pkwy, Bothell, WA 98011). Store at refrigerator temp. (4–8°C; 39–46°F). Items are stable 3 months (90 days) from date of receipt.

(**a**) *1-2 TEST unit.*—See Fig. **46:E1**.

(**b**) *Iodine-iodide soln.*—1 vial for 12 units.

(**c**) *Antibody.*—1 vial for 12 units.

(**d**) *Diagnostic reagents.*—Necessary for cultural confirmation of presumptive pos. 1-2 TESTS. See **46.116**.

46.E19 *Preparation of Sample*

Most foods require only pre-enrichment of product in non-inhibitory broth to initiate growth of salmonellae before inoculation into unit. Exceptions are given below. Methods

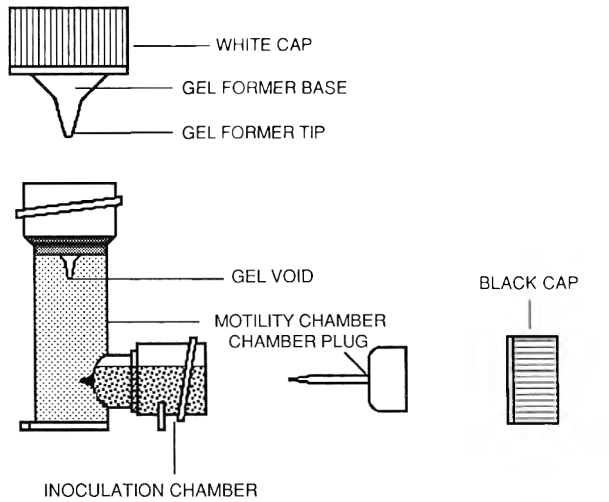

FIG. 46:E1—Test unit for immunodiffusion screening method for motile *Salmonella*

used for sample prepn may vary with product and should be performed as in **46.117** and **46.132**.

Raw flesh foods or highly contaminated products.—No pre-enrichment is required. Aseptically weigh 25 g sample into sterile blender jar. Add 225 mL tetrathionate broth without brilliant green dye. Blend 2 min. Securely cap jars and let stand 60 min at room temp. Mix well by shaking. Add 2.25 mL 0.1% soln of brilliant green dye. Aseptically transfer contents of each jar to sterile 500 mL wide-mouth, screw-cap jar. Loosen jar caps ¼ turn and incubate 24 ± 2 h at 35°.

Flour-containing products (soy flour, wheat flour, dough, pasta, cake mix).—Transfer 1 mL incubated pre-enrichment mixt. to tetrathionate broth as in **46.118(a)**. Incubate 24 ± 2 h at 35°.

46.E20 *General Instructions*

Components and procedures of test kit have been stdzd for use in 1-2 TEST procedures. Components or procedures other than those supplied by BioControl Systems, Inc., may yield unsatisfactory results, and should be pretested.

46.E21 *Immunodiffusion Detection*

(**a**) *Test unit preparation.*—Each test unit has 2 chambers: inoculation chamber and motility chamber (Fig. **46:E1**). Each step of prepn sequence can be performed on individual unit or multiple units as needed. Sample nos. can be recorded on lower portion of motility chamber but must NOT interfere with reading of results. Alternatively, sample nos. may be recorded on flat surface of white cap. When cap is replaced, it must be screwed on tightly.

(*1*) Position unit with black cap UP, and remove black cap. Add 1 drop of iodine-iodide soln to inoculation chamber, and replace black cap. Gently shake unit to mix and resuspend enrichment ingredients.

(*2*) Position unit with white cap UP, and remove white cap. Snip off tip of gel-former plug with scissors and discard tip. Cut should be made at point where tip meets base of plug. If tip of gel-former plug is not removed, antibody soln will be displaced from gel void when white cap is replaced.

(*3*) Add 1 drop of antibody prepn to gel void in motility chamber. Replace white cap. Antibody prepn should fill ca ⅔ of gel void. This can be detd by observing blue antibody soln in gel void.

(*4*) Position unit with black cap UP, and remove black cap. Remove chamber plug from inoculation chamber with sterile forceps and discard plug. Do not replace black cap

until unit is inoculated. If chamber plug is not removed, bacteria will be unable to move from inoculation chamber to motility chamber.

(b) *Inoculation.*—Prior to inoculation, be sure that enrichment broth contg sample is well mixed. Use pipet to transfer 0.1 mL enriched sample into inoculation chamber. Replace black cap.

(c) *Incubation.*—Place inoculated unit in incubator with white cap UP. Incubate unit in shipper/incubation tray at 35° for min. of 8 h.

(d) *Reading positive results.*—After 8 h incubation, unit may be inspected for pos. results: With white cap UP, hold unit next to strong light. Desktop fluorescent light is recommended for reading test results. Carefully observe motility chamber gel by rotating unit back and forth thru various angles in front of light source.

Pos. test is indicated by presence of white band that is U-shaped or meniscus-shaped. Band, which forms as motile *Salmonella* are immobilized by antibodies that have diffused into gel, is seen in upper half of motility chamber gel.

Pos. test indicates that sample contains *Salmonella.* Pos. test results should be confirmed by std culture methods outlined in *Confirmation of Positive Samples.*

Pos. unit can be stored up to 1 week at refrigerator temp. (4–8°).

(e) *Reading negative results.*—If no band is seen after initial 8 h incubation, reincubate units for min. of 6 h but not more than 12 h. After this incubation period, read units as described in (d), *Reading positive results.* Units that show no band after this second incubation indicate neg. test result. Neg. units that were incubated at least 14 h require no addnl incubation. Neg. test results indicate that sample does not contain levels of motile *Salmonella* detectable by immunodiffusion test.

Neg. units show uniform turbidity thruout motility chamber as result of movement of motile bacteria in gel. However, after initial 8 h incubation, movement of bacteria thru gel may not be complete.

46.E22 ***Confirmation of Positive Samples***

Presence of band of cells indicates that *Salmonella* may be present in sample. Perform cultural confirmation by using 3 mm loop to obtain inoculum from tetrathionate-brilliant green-serine broth in inoculation chamber and streaking HE, XLD, and BS plates.

For raw or highly contaminated products, transfer 0.1 mL of the tetrathionate-brilliant green-serine broth to test tube contg 10 mL tetrathionate-brilliant green broth. Incubate 18–24 h in 43 ± 0.5° H_2O bath, and then streak into HE, XLD, and BS plates. Identify typical or suspicious colonies from selective plates as in **46.119–46.128.**

Ref.: JAOAC 72, March/April issue (1989).

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(8) The interim TECRA *Salmonella* visual immunoassay system method for screening all foods for the presence of *Salmonella* was adopted first action:

Salmonella in Foods

Colorimetric Polyclonal EIA Screening Method

First Action

Method is screening procedure for presence of *Salmonella* in all foods; it is not a confirmatory test because polyclonal antibodies used in test may cross-react with small percentage of non-*Salmonella.*

Enrichment broths and M-broths from samples pos. by EIA method must be streaked on selective media as in **46.118** and typical or suspicious colonies must be identified as in **46.119–46.128.**

Detn of pos. result may be performed (*1*) visually by aid of color comparator card where pos. result is valid when neg. and pos. controls match those described on card or (*2*) instrumentally using filter photometer having 414 nm filter where pos. result is valid only when neg. and pos. controls possess acceptable optical density readings.

46.E23 ***Principle***

Detection of *Salmonella* antigens is based on enzyme immunoassay using highly purified antibodies prepd from antigens unique to *Salmonella.* Polyclonal antibodies to *Salmonella* antigen are adsorbed onto internal surface of 96-well microtiter tray. Sample to be assayed is placed into well of tray. If *Salmonella* antigens are present in sample, they will attach to specific antibody adsorbed on well. All other material in samples is washed away. Conjugate is added and will bind to *Salmonella* antigens if they are attached to adsorbed antibody on surface of well. Wells are washed to remove unbound conjugate, and enzyme substrate is added. Dark blue-green color indicates presence of *Salmonella* antigen in sample.

46.E24 ***Method Performance***

For all foods:

Results	*Percent*	*95% Confidence Range (Approx.)*
Agreement[1]	96.8	95.4–98.2
False neg. (BAM)[2]	1.6	0.5–2.7
False neg. (EIA)[3]	1.4	0.4–2.4

[1] This rate reflects no. of samples read identically between AOAC/BAM (Bacteriological Analytical Manual, 1984, 6th ed., AOAC, Arlington, VA) culture method and EIA.

[2] This rate reflects no. of samples found to be pos. by EIA but neg. by AOAC/BAM culture method.

[3] This rate reflects no. of samples found to be pos. by AOAC/BAM culture method but neg. by EIA.

Of 14 laboratories, 3 had complete agreement between culture method and EIA method. Excluding 1 food group, turkey, 13 of the 14 laboratories had perfect agreement between BAM/AOAC and EIA methods. Laboratory that did not have perfect agreement had difference in each of pepper, nonfat dry milk, and chocolate food groups.

46.E25 ***Reagents***

Items (a)–(m) are available as TECRA Salmonella Visual Immunoassay (Bioenterprises Pty Ltd, 28 Barcoo St, Roseville, NSW 2069, Australia). Substitutions must be pretested for equivalency.

(a) *Antibody adsorbed strips.*—Removawell® (Dynatech Laboratories, Inc.) strips. Polyclonal antibodies to *Salmonella,* 96 wells. Store wells at 2–8° when not in use.

(b) *Tray.*—Sufficient to secure individual wells or strips.

(c) *Control antigens.*—Pos. control (lyophilized). Purified *Salmonella* antigen, which reacts with antibodies to *Salmonella,* 1 vial. Neg. control (lyophilized lactose), which is

nonreactive with antibodies to *Salmonella,* 1 vial. Reconstituted control antigens are stable 28 days when stored at 2–8°.

(**d**) *Controls diluent.*—1 vial (5 mL/vial). Contains 0.006 g Tris [tris(hydroxymethyl)aminomethane], 0.044 g NaCl, 0.0025 g Tween 20 (polyoxyethylene 20 sorbitan monolaurate), and 0.005 g thimerosal in H_2O.

(**e**) *Conjugate.*—1 vial (lyophilized). Contains 147 ng anti-*Salmonella* antibodies (from sheep) conjugated to horseradish peroxidase, 0.00686 g $Na_2B_4O_7$, 0.12 g Dextran T10, 0.06 g hydrolyzed gelatin, 0.0024 g $CaCl_2$, and 120 ng thimerosal. Reconstituted conjugate is stable 28 days when stored at 2–8°.

(**f**) *Conjugate diluent.*—1 vial (22 mL/vial). Contains 0.42 g $Na_2B_4O_7$, 0.193 g NaCl, 0.22 g hydrolyzed gelatin, and 0.0022 g thimerosal in H_2O.

(**g**) *Substrate.*—1 vial (lyophilized). Contains 0.011 g 2,2′-azino-di(3-ethylbenzthiazoline sulfonate) and 0.123 g NaH_2PO_4. Reconstituted substrate is stable 28 days when stored at 2–8°.

(**h**) *Substrate diluent.*—1 vial (22 mL/vial). Contains 0.116 g citric acid, 0.0011 g H_2O_2, and 0.0185 g NaOH in H_2O.

(**i**) *"Stop" soln.*—1 vial (6 mL/vial). Vial contains 0.15 g NaF in H_2O. **Caution**: Avoid contact with skin. If contact occurs, wash area with H_2O.

(**j**) *Wash soln concentrate.*—1 vial (25 mL/vial). Contains 1.45 g Tris, 7.03 g NaCl, 0.5 g Tween 20, and 0.0025 g thimerosal in H_2O.

(**k**) *Package insert.*

(**l**) *Data record sheet.*

(**m**) *Color comparator card.*—For visual interpretation of pos. and neg. tests.

(**n**) *M-broth.*—5.0 g yeast ext, 12.5 g tryptone, 2.0 g D-mannose, 5.0 g Na citrate, 5.0 g NaCl, 5.0 g K_2HPO_4, 0.14 g $MnCl_2$, 0.8 g $MgSO_4$, 0.04 g $FeSO_4$, 0.75 g Tween 80. Suspend ingredients in 1 L H_2O and heat to boiling for 1–2 min. Dispense 10 mL portions into 16 × 125 mm screw-cap test tubes. Cap tubes loosely and autoclave 15 min at 121°. Tighten caps securely for storage. Final pH should be 7.0 ± 0.2.

(**o**) *Diagnostic reagents.*—Necessary for culture confirmation of presumptive pos. EIA tests; see **46.116**.

46.E26 *Apparatus*

(**a**) *Incubator.*—35–37°.

(**b**) *Multipipets.*—Capable of delivering accurate amts in ranges 50–250 μL and 5–50 μL.

(**c**) *Water bath.*—Capable of maintaining 100°. Autoclave set at 100° is acceptable alternative, as are generators of flowing steam.

(**d**) *Plastic squeeze bottle.*—500 mL, for dispensing wash soln. Automatic washer may be used.

(**e**) *Plastic film wrap or sealable plastic container.*—To cover wells during incubation.

(**f**) *Enzyme immunoassay reader.*—Optional. Photometer with 414 ± 10 nm screening filter which will read thru microtiter plates.

46.E27 *General Instructions*

Components of kit must be refrigerated when not in use. Kit is intended for 1-time use only; do not reuse wells containing sample, reagents, or wash solution.

Include duplicate pos. and neg. control antigens with each group of test samples. All controls must function properly for test to be valid.

Use data record sheet to identify location of each test sample.

Use sep. pipets for each sample and kit reagent to avoid cross-contamination. If plastic troughs are used to dispense conjugate and substrate, ensure that they are always kept separate.

Components in kit are intended for use as integral unit. Do not mix components of different batch numbers.

46.E28 *Preparation of Sample*

(**a**) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of salmonellae. Methods used may vary with product and should be performed as indicated in **46.117**, or in Bacteriological Analytical Manual, 6th ed., AOAC, Arlington, VA, Chap. 7, section C, with following exception:

Raw or highly contaminated products.—Aseptically weigh 25 g sample into sterile blender jar. Add 225 mL sterile lactose broth. Blend 2 min at high speed (ca 20 000 rpm). Cap jars securely and let stand 60 min at room temp. Mix well by shaking and det. pH with test paper. Adjust pH to 6.8 ± 0.2, if necessary, using sterile 1N NaOH or HCl, capping jar securely and mixing well before detg final pH. Aseptically transfer contents of each jar to sterile wide-mouth, screw-cap 500 mL jar. Loosen jar caps ¼ turn and incubate 24 ± 2 h at 35°.

(**b**) *Selective enrichment.*—Transfer 1 mL incubated pre-enrichment mixts to selenite cystine broth and 1 mL to tetrathionate broth as in **46.118(a)**. For all foods other than raw or highly contaminated products, incubate 6–8 h at 35°. Selective enrichments of raw or highly contaminated product must be incubated 16–20 h at 35°.

(**c**) *Post-enrichment.*—Remove selective broths from incubation and mix by hand or by vortex mixer. Remove 1 mL from tetrathionate tube and transfer to 10 mL tube of sterile M-broth which has been warmed to 35°. Also remove 1 mL from selenite cystine tube and transfer to sep. tube of M-broth. For all foods other than raw or highly contaminated products, incubate M-broth tubes 14–18 h and return selective enrichment broth tubes to 35° incubator and incubate for addnl 16–18 h. For raw or highly contaminated products, incubate M-broth tubes 6 h at 35° and return selective enrichment broth tubes to 35° and incubate for addnl 6 h at 35°.

(**d**) *Preparation of sample for EIA analysis.*—Remove M-broth tubes from incubation and mix tubes by hand or vortex mixer. Combine 1.0 mL from each M-broth tube in clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2–8°) remaining M-broth and tetrathionate and selenite cystine tubes from (c) for culture confirmation of any EIA pos. samples. Cool heated M-broths to 25–37° prior to analysis by EIA.

46.E29 *Enzyme Immunoassay*

(*1*) Following reagents must be prepd prior to commencing assay:

(a) Prep. working strength wash soln by dilg contents of 1 vial of wash soln conc. to 1 L with distd or deionized H_2O into plastic reagent bottle. Plastic squeeze bottle is ideal for washing trays manually.

(b) Prep. reconstituted neg. control by transferring 2 mL controls diluent to vial of lyophilized neg. control antigen; mix thoroly. Similarly prep. reconstituted pos. control by transferring 2 mL controls diluent to vial of lyophilized pos. control antigen; mix thoroly.

(c) Prep. reconstituted conjugate by adding 5 mL conju-

gate diluent to vial of lyophilized conjugate. Let conjugate rehydrate at room temp., mix, and then pour contents of vial into conjugate diluent vial. Finally, gently mix reconstituted conjugate.

(d) Prep. reconstituted substrate by adding vial of substrate diluent to lyophilized substrate. Be sure substrate has dissolved and mixt. is room temp. prior to use. Reconstituted substrate will appear pale green.

(e) Use stop soln as received. No reconstitution is required.

(*2*) Secure desired no. of test (Removawell) strips in tray, allowing 1 well per food sample plus 4 wells for controls. PRESS WELLS FIRMLY INTO PLACE. Remove sealing film from top of wells to be used. Transfer 0.2 mL of each heated M-broth sample to single well. Transfer 0.2 mL aliquots of reconstituted neg. control into 2 wells and 0.2 mL aliquots of reconstituted pos. control into 2 wells. Record sample position on sample record sheet provided. *Note*: Be sure numbered tag at end of each test strip has been removed.

(*3*) Cover tray and incubate 30 min at 35–37° in std laboratory incubator. Tray must be covered to prevent evapn. Plastic film or sealed plastic container may be used.

(*4*) After incubation, wash plate by hand using plastic squeeze bottle contg working strength wash soln or use automatic washer charged with working strength wash soln as follows:

(a) Quickly invert tray, emptying its contents into container.

(b) Remove any residual liquid by FIRMLY tapping tray face-down on paper towel several times.

(c) Completely fill each well with working strength wash soln.

(d) Repeat (a)–(c) **2** more times.

(*5*) Empty tray according to *4*(a) and (b); then add 0.2 mL reconstituted conjugate to each well. Cover tray and incubate 30 min at 35–37°.

(*6*) Empty contents of tray and wash it thoroly **4** times according to *4*(a)–(c); then empty tray according to *4*(a) and (b).

(*7*) Add 0.2 mL reconstituted substrate to each well. Incubate at room temp. (20–25°) until pos. control has reached color equiv. to pos. control on color comparator card or to $A \geq 1.0$. Because color development tends to conc. around edges of wells, it is important to tap sides of plate *gently* to mix contents prior to reading result. In this way, accurate readings will be obtained.

(*8*) Add 0.02 mL stop soln to each well. Incubation time should be ca 10–20 min. If >25 min has elapsed and A of 1.0 has not been attained, test is invalid.

46.E30 ***Reading***

Results of tests can be detd (*1*) visually or (*2*) with microtiter tray reader.

(*1*) Place tray on white background, and then compare individual test wells with color comparator. Pos. control should give strong blue-green color indicating that all reagents are functional. If pos. control is lighter than "Positive Control" on color comparator card, test is invalid; refer to "Troubleshooting Guide" in package insert (k). If neg. control is darker than "Negative" on color comparator card, it is probable that tray was inadequately washed, and assay must be repeated. Duplicate control antigens should appear equiv. by eye.

(*2*) A max. of blue-green end product occurs at 414 nm; therefore, tray can be read at 414 ± 10 nm. For single and dual wavelength readers, set reader to zero (blank) on air. For dual wavelength readers, set second ref. wavelength at 490 ± 10 nm. $A > 0.3$ indicates pos. result. $A > 0.25$ for neg. control indicates insufficient washing of tray. Pos. control should give $A \geq 1.0$.

46.E31 ***Confirmation of Positive EIA Samples***

Pos. EIA reading indicates that *Salmonella* may be present. However, since antibodies may cross-react with a few other organisms, culture confirmations should be performed by streaking HE, XLD, and BS plates from tetrathionate broth, selenite cystine broth, and M-broth tubes as described in **46.118**, and typical or suspicious colonies should be identified as in **46.119–46.128**.

Ref.: JAOAC **71**, 973(1988).

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(9) The interim Q-TROL *Salmonella* detection kit system method for screening all foods for the presence of *Salmonella* was adopted first action:

Salmonella in Foods

Fluorogenic Monoclonal EIA Screening Method

First Action

Method is screening procedure for presence of *Salmonella* in all foods; it is not a confirmatory test because monoclonal antibodies used in test may cross-react with small percentage of non-*Salmonella*.

Enrichment broths and M-broths from samples pos. by EIA method must be streaked on selective media as in **46.118** and typical or suspicious colonies must be identified as in **46.119–46.128**.

46.E32 ***Principle***

Detection of *Salmonella* antigens is based on enzyme immunoassay which measures *Salmonella* antigen in foods and feeds. Monoclonal antibodies to *Salmonella* antigen are coated on internal surface of plastic microtiter strip wells, and sample to be assayed is added to strip well. If *Salmonella* antigens are present in sample, they will be bound to antibody adsorbed onto surface of well. All other material in sample is washed away.

Salmonella antibody conjugated to alk. phosphatase is added and will bind to *Salmonella* antigens if they are attached to adsorbed antibody on surface of well. This forms antibody-antigen-antibody complex. Unbound conjugate is removed by washing, and fluorescent substrate is added. Samples with fluorescent value greater than or equal to recommended cutoff value are considered pos. for *Salmonella* antigens.

46.E33 ***Method Performance***

For all foods:

Results	*Percent*	*95% Confidence Range (Approx.)*
Agreement[1]	98.5	97.9–99.1
False neg. (BAM)[2]	1.1	0.8–1.4
False neg. (EIA)[3]	0.8	0.0–2.7

[1] This rate reflects no. of samples read identically between AOAC/BAM (Bacteriological Analytical Manual (1984) 6th ed., AOAC, Arlington, VA) culture method and EIA.

[2] This rate reflects no. of samples found to be pos. by EIA but neg. by AOAC/BAM culture method.

[3] This rate reflects no. of samples found to be pos. by AOAC/BAM culture method but neg. by EIA.

Of 11 laboratories submitting usable data, 6 (55%) had complete agreement between culture method and EIA; 10 laboratories (91%) showed agreement on ≥96% of samples; all laboratories showed agreement on ≥93% of samples.

46.E34 *Reagents*

Items (a)–(j) are available as Q-TROL Salmonella Detection Kit (Dynatech Laboratories, Inc., 14340 Sullyfield Circle, Chantilly, VA 22021).

(a) *Antibody coated microtiter wells.*—Monoclonal antibody to *Salmonella,* eight 12-well strips. Stable 28 days when stored at 2–8°.

(b) *Microtiter strip well holder.*—Sufficient for securing individual wells or strips.

(c) *Control antigens.*—Pos. control (lyophilized boiled suspension of *S. typhimurium*) purified *Salmonella* antigen, which reacts with antibodies to *Salmonella,* 1 vial; neg. control (lyophilized boiled suspension of *Proteus mirabilis*), which is nonreactive with antibodies to *Salmonella,* 1 vial. Reconstituted control antigens are stable 28 days when stored at 2–8°.

(d) *Tween 20.*—1 vial. 25% Tween 20 (polyoxyethylene (20) sorbitan monolaurate) in H_2O. After opening, soln is stable 28 days when stored at 2–8°.

(e) *Phosphate buffer-saline tablets.*—For prepn of PBS-Tween soln. Dissolve 1 tablet in 100 mL distd or deionized H_2O to prep. 0.01M phosphate-buffered 0.85% saline. Add 8 drops Tween 20. PBS-Tween soln is used to rehydrate pos. and neg. control antigens and for wash steps, and is stable 7 days when stored at 2–8°.

(f) *Enzyme conjugate.*—1 vial contg antibody of *Salmonella* conjugated to alk. phosphatase (lyophilized). Reconstituted conjugate is stable 28 days when stored at 2–8°.

(g) *Conjugate diluent.*—1 vial (10 mL/vial). Contains 0.05M tris buffer (pH 8), 0.02% NaN_3, 1 mM $MgCl_2$, and 1% bovine serum albumin.

(h) *Substrate tablets.*—0.13 mg 4-methylumbelliferyl phosphate (4-MUP) per tablet. Reconstituted substrate must be used within 2 h.

(i) *Substrate diluent.*–1 vial (21.0 mL/bottle). Aq. soln of 10% diethanolamine with 0.02% NaN_3 as preservative.

(j) *Stop soln.*—1 vial (5.5 mL/vial). Aq. soln of 2% Na_3PO_4.

(k) *M-broth.*—5.0 g yeast ext, 12.5 g tryptone, 2.0 g D-mannose, 5.0 g Na citrate, 5.0 g NaCl, 5.0 g K_2HPO_4, 0.14 g $MnCl_2$, 0.8 g $MgSO_4$, 0.04 g $FeSO_4$, 0.75 g Tween 80. Suspend ingredients in 1 L H_2O and heat to boiling for 1–2 min. Dispense 10 mL portions into 16 × 125 mm screw-cap test tubes. Cap tubes loosely and autoclave 15 min at 121°. Tighten caps securely for storage. Final pH should be 7.0 ± 0.2.

(l) *Diagnostic reagents.*—Necessary for cultural confirmation of presumptive pos. EIA tests; see **46.116**.

46.E35 *Apparatus*

(a) *Fluorometer.*—To measure relative fluorescence of contents of microtiter well (Micro FLUOR® Reader, Dynatech Laboratories, Inc.; or equiv.).

(b) *Microtiter strip well-washer/aspirator.*—With 12 channels to wash entire strip.

(c) *Pipets.*—Capable of delivering 50–200 μL.

(d) *Water bath.*—Capable of maintaining 100°. Autoclave set at 100° is acceptable alternative, as are generators of flowing steam.

46.E36 *General Instructions*

Components of kit must be refrigerated when not in use. Kit is intended for 1-time use only; do not reuse wells contg sample, reagents, or wash soln.

Include 3 neg. and 1 pos. control antigens with each group of test samples. All controls must function properly for test to be valid.

Caution: Diluents for conjugate and substrate contain NaN_3 as preservative. Flush drains with H_2O if any solns contg NaN_3 are discarded in sink. Flushing will prevent formation of lead or copper azide in plumbing, which may explode upon percussion (such as hammering). 4-MUP diluent and stop soln are basic and may cause skin irritation. If contact with skin occurs, flush area with H_2O.

Use data record sheet to identify location of each test sample.

Use sep. pipets for each sample and kit reagent to avoid cross-contamination. If plastic troughs are used to dispense conjugate and substrate, ensure that they are always kept separate.

Components in kit are intended for use as integral unit. Do not mix components of different batch numbers.

46.E37 *Preparation of Sample*

(a) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiated growth of salmonellae. Methods used may vary with product and should be performed as indicated in **46.117**, or in Bacteriological Analytical Manual, 6th ed., AOAC, Arlington, VA, Chap. 7, sec. C, with following exception:

Raw or highly contaminated products.—Aseptically weigh 25 g sample into sterile blender jar. Add 225 mL sterile lactose broth. Blend 2 min at high speed (ca 20 000 rpm). Cap jars securely and let stand 60 min at room temp. Mix well by shaking and det. pH with test paper. Adjust pH to 6.8 ± 0.2, if necessary, using sterile 1N NaOH or HCl, capping jar securely and mixing well before detg final pH. Aseptically transfer contents of each jar to sterile wide-mouth, screw-cap 500 mL jar. Loosen jar caps ¼ turn and incubate 24 ± 2 h at 35°.

(b) *Selective enrichment.*—Transfer 1 mL incubated pre-enrichment mixts to selenite cystine broth and 1 mL into tetrathionate broth as in **46.118(a)**. For all foods, incubate 6–8 h in 35° water bath.

(c) *Post-enrichment.*—Remove selective broths from incubation and mix by hand or by vortex mixer. Remove 1 mL from tetrathionate tube and transfer to 10 mL tube of sterile M-broth which has been warmed to 35°. Also remove 1 mL from selenite cystine tube and transfer to a separate tube of M-broth. For all foods, incubate M-broth tubes for 14–18 h and return selective enrichment broth tubes to 35° incubator and incubate for addnl 16–18 h.

(d) *Preparation of sample for EIA analysis.*—Remove M-broth tubes from incubation and mix tubes by hand or vortex mixer. Combine 0.5 mL from each M-broth tube in clean screw-cap tube and heat 20 min in boiling H_2O bath or flowing steam. Refrigerate (2–8°) remaining M-broth and tetrathionate and selenite cystine tubes from (c) for cultural confirmation of any EIA pos. samples. Cool heated M-broths to 20–30° prior to analysis by EIA.

46.E38 *Enzyme Immunoassay*

(1) Following reagents must be prepd prior to commencing assay:

(a) *PBS-Tween soln.*—For every two 12-well strips to be used, dissolve 1 PBS tablet in H_2O and prep. soln as in (e).

(b) *Reconstituted control antigens.*—Transfer 3 mL PBS-Tween soln to neg. control vial and mix contents thoroughly. Transfer 3 mL PBS-Tween soln to pos. control vial and mix contents thoroughly. These solns are reconstituted neg. and pos. controls, resp.

(c) *Reconstituted enzyme conjugate.*—Add 10 mL (1 vial) conjugate diluent to conjugate vial. Mix and let contents of vial rehydrate at room temp.

(d) *Stop soln.*—No reconstitution is required. Warm soln at 35° if crystals are present.

(*2*) Turn on power to reader and printer. Allow at least 2 h warm-up.

(*3*) Remove necessary number of microtiter strips from Al foil pouch, allowing 1 well per food sample plus **4** wells for controls. Secure strips in strip well holder. Transfer 100 μL neg. control antigen into each of wells A-1, A-2, and A-3. Transfer 100 μL pos. control antigen into well designated A-4. Transfer 100 μL each heated M-broth sample to single well. Record sample position on sample record sheet provided.

(*4*) Incubate tray for 60 min at 20–25°.

(*5*) After incubation, aspirate samples from wells and add 300 μL PBS-Tween soln to each well by use of washer/aspirator.

(a) Repeat this step 4 more times.

(b) Aspirate last wash. Invert tray and firmly tap it on absorbent paper several times to remove last traces of fluid.

(*6*) Add 100 μL reconstituted enzyme conjugate to bottom of each well and incubate 40 min at 20–25°.

(*7*) During this incubation period, prep. substrate by adding one 4-MUP substrate tablet to 5.2 mL substrate diluent. Dissolve 1 substrate tablet for every 2 microtiter strips to be used. Swirl soln occasionally to dissolve tablet(s).

(*8*) Repeat steps *5*(a) and (b).

(*9*) Add 200 μL 4-MUP substrate to bottom of each well. Incubate 20 min at 20–25°.

(*10*) Add 50 μL stop soln to each well.

46.E39 ***Reading***

(*1*) Place tray in reader. Read relative fluorescent units (RFU) of each control and sample well. Calc. av. RFU of the 3 neg. control wells. Individual neg. control values should be ≥0.85 av. RFU and ≤1.15 av. RFU. If 1 value is outside this range, discard that value and recalc. mean. If 2 values are outside range, test is invalid and must be repeated. Multiply av. valid neg. controls by 2.3 to det. cutoff value. Any sample with value above cutoff value is considered reactive.

If av. of neg. control values exceeds 1600 RFU, cutoff will exceed dynamic range of reader and test is invalid. Poor washing and deterioration of substrate may result in high readings of neg. control.

46.E40 ***Confirmation of Positive EIA Samples***

Pos. EIA reading indicates that *Salmonella* may be present. However, since antibodies may cross-react with a few other organisms, culture confirmations should be performed by streaking HE, XLD, and BS plates from tetrathionate broth, selenite cystine broth, and M-broth tubes as described in **46.118**, and typical or suspicious colonies should be identified as in **46.119–46.128**.

Ref.: JAOAC 72, March/April issue (1989).

47. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

48. RADIOACTIVITY

No additions, deletions, or other changes.

49. VETERINARY ANALYTICAL TOXICOLOGY

(1) The following first action methods were adopted final action:

(a) Arsenic in liver tissue, spectrophotometric method, **49.B01–49.B05**.

(b) Nitrate in forages, potentiometric method, **49.B06–49.B13**, as revised by adding Table **49:E1**.

---

(2) In the potentiometric method for nitrate in forages, **49.B06–49.B13**, make the following changes:

(a) Add a table of conversion factors for units of nitrate and nitrite concentrations.

(b) Change the last sentence of **49.B13** to read: "If desired, convert nitrate concn expressed as % KNO_3 to other units as shown in Table **49:E1**.

(c) Add an additional reference: JAOAC **69**, 283(1986).

50. STANDARD SOLUTIONS AND CERTIFIED REFERENCE MATERIALS

No additions, deletions, or other changes.

51. LABORATORY SAFETY

No additions, deletions, or other changes.

COMPLETE TEXT OF USE-DILUTION METHODS

The use-dilution methods for testing disinfectants against *Salmonella choleraesuis*, **4.007–4.009**, *Staphylococcus aureus*, **4.010**, and *Pseudomonas aeruginosa*, **4.011**, were editorially revised in this supplement. The complete text of the methods is printed here.

USE-DILUTION METHODS[1]

(Applicable to testing disinfectants miscible with H_2O to confirm phenol coefficient results and to det. max. dilns effective for practical disinfection. These microbiological methods are technique-sensitive methods in which extreme adherence to the method with identified critical control points, good microbiological techniques, and quality controls is required for proficiency and validity of results. These methods have been validated using distd H_2O only without soil challenge.)

Testing Disinfectants against *Salmonella choleraesuis*

Use-Dilution Method

First Action

4.007 ***Reagents***

(a) *Culture media.*—*See* **4.001**.

(b) *Test organism, Salmonella choleraesuis.*—(ATCC

[1] The use-dilution methods for testing disinfectants, **4.007–4.009**, **4.010**, and **4.011**, are technique-sensitive and may produce questionable results unless conducted by experienced, trained analysts under strictly controlled conditions. Users of the methods are advised to consult the following reports of recent studies for current scientific data and interpretation: JAOAC **69**, 1003(1986); **70**, 635(1987); **70**, 903(1987); **71**, 9(1988); **71**, 288(1988); **71**, 868(1988); **71**, 1187(1988). Infect. Control **8**, 501(1987).

Table 49:E1 Conversion Factors for Units of Nitrate and Nitrite Concentrations[a]

From / To	NO_3^--N (14.01)	NO_3^- (62.01)	$NaNO_3$ (85.01)	KNO_3 (101.11)	NO_2^--N (14.01)	NO_2^- (46.01)	$NaNO_2$ (69.01)	KNO_2 (85.11)
NO_3^--N (14.01)	1.000	0.2259	0.1648	0.1386	1.000	0.3045	0.2030	0.1646
NO_3^- (62.01)	4.426	1.000	0.7294	0.6133	4.426	1.348	0.8986	0.7286
$NaNO_3$ (85.01)	6.068	1.371	1.000	0.8408	6.068	1.848	1.232	0.9988
KNO_3 (101.11)	7.217	1.631	1.189	1.000	7.217	2.198	1.465	1.188
NO_2^--N (14.01)	1.000	0.2259	0.1648	0.1386	1.000	0.3045	0.2030	0.1646
NO_2^- (46.01)	3.284	0.7420	0.5412	0.4550	3.284	1.000	0.6667	0.5406
$NaNO_2$ (69.01)	4.926	1.113	0.8118	0.6825	4.926	1.500	1.000	0.8108
KNO_2 (85.11)	6.075	1.373	1.001	0.8418	6.075	1.850	1.233	1.000

[a] Find current unit of concentration on top row. Find desired unit of concentration in left column. Obtain conversion factor at intersection of row and column. Desired concentration = current concentration × conversion factor. Numbers in parentheses below units of concentration are formula masses. Atomic masses: N: 14.01; O: 16.00; Na: 23.00; K: 39.10. 1% = 10 000 ppm.

10708). Obtain annually directly from ATCC. Maintain stock culture on nutrient agar slants by monthly transfers. Incubate new stock transfer 2 days at 37°; then store at 2–5°. From stock culture inoculate tube of nutrient broth and incubate at 37°. Make 3 consecutive 24 hr transfers; then inoculate tubes of nutrient broth (2 for each 10 carriers to be tested), using one loop of inoculum with each tube; incubate 48–54 hr at 37°.

(c) *Phenol.*—*See* **4.002(f)**.

(d) *Sterile distilled water.*—Prep. stock supply of H_2O in 1 L flasks, plug with cotton, sterilize 20 min at 121°, and use to prep. dilns of medicants.

(e) *Asparagine soln.*—Make stock supply of 0.1% asparagine ("Bacto") soln in H_2O in erlenmeyer of convenient size, plug with cotton, and sterilize 20 min at 121°. Use to cover metal carriers for sterilization and storage.

(f) *Sodium hydroxide soln.*—Approx. 1*N* (4%). (For cleaning metal carriers before use.)

4.008 *Apparatus*

(a) *Glassware.*—As in **4.002(a)**. Also: straight side Pyrex test tubes, 20 × 150 mm; 15 × 110 mm petri dishes; 100 mL, 300 mL, and 1 L erlenmeyers. Sterilize petri dishes in closed metal containers. Use 25 × 150 mm straight side tubes for disinfectant soln. (Smaller tubes can give high percentage of false positives when sides are touched.)

(b) *Water bath and racks.*—*See* **4.002(b)** and (c).

(c) *Transfer loops and needles.*—(*1*) *See* **4.002(d)**. (*2*) Make 3 mm right angle bend at end of 50–75 mm nichrome wire No. 18 B&S gage. Have other end in suitable holder (glass or Al rod).

(d) *Carriers.*—Polished stainless steel cylinders (penicillin cups), 8 ± 1 mm od, 6 ± 1 mm id, length 10 ± 1 mm, of type 304 stainless steel, SS 18-8. (Obtainable from S. & L. Metal Products Corp., 58-29 57 Drive, Maspeth, NY 11378.) Discard cylinders that are visibly damaged (dull, chipped, dented, or gouged). Biologically screen remaining cylinders by performing Use-Dilution Test with *Staphylococcus aureus* ATCC 6538 and 500 ppm alkyldimethylammonium chloride with alkyl chain distribution C14, 50%; C12, 40%; C16, 10% (e.g., BTC-835, Onyx Chemical Co., Jersey City, NJ 07302). Discard those cylinders giving pos. results in screening procedure. In subsequent testing of samples, cylinders in tubes showing growth must be rescreened and may not be reused unless screen test results in no growth.

(e) *Petri dishes.*—Have available ca 6 sterile petri dishes matted with 2 layers of S&S No. 597 or Whatman No. 2, 9 cm filter paper.

(f) *Pipets.*—Use only disposable pipets. (Reusable pipets may have residues or chips.)

4.009 *Operating Technic*

Soak ring carriers overnight in 1*N* NaOH, rinse with tap H_2O until rinse H_2O is neut. to phthln, then rinse twice with distd H_2O; place cleaned ring carriers in multiples of 10 in cotton-plugged erlenmeyers or 25 × 150 mm cotton plugged Pyrex test tubes, cover with asparagine soln, **4.007(e)**, sterilize 20 min at 121°, cool, and hold at room temp. Vortex-mix nutrient broth test culture 3–4 s and let stand 10 min at room temp. before continuing. Transfer 20 sterile ring carriers, using flamed nichrome wire hook, into 20 mL 38–54 hr nutrient broth test culture in sterile 25 × 150 mm medication tube. One or 2 addnl carriers may be added at same inoculum rate to serve as reserves. Carriers that fall over in petri dishes cannot be used in test. After 15 min contact period remove cylinders, using flamed nichrome wire hook, shake carrier vigorously against side of tube to remove excess culture, and place on end in vertical position in sterile petri dish matted with filter paper, **4.008(e)**, making sure that carriers do not touch to prevent improper drying. Cover and place in incubator at 37° and let dry 40 min. Hold broth culture for detn of its resistance to phenol by phenol coefficient method, **4.003**.

From 5% stock phenol soln (1–20) make 1–90 and 1–100 dilns directly into medication tubes. Place tube for each diln in H_2O bath and let come to 20°. Make stock soln of germicide to be tested in sterile g-s cylinder. From this soln make 10

mL dilns to be tested, depending upon phenol coefficient found and/or claimed against *S. typhi* at 20°, directly into each of ten 25 × 150 mm medication tubes; place the 10 tubes in H_2O bath at 20° and let come to temp. Prep. diln of germicide to be tested by diln in sterile H_2O, **4.007(d)**. Diln of sample should be made using ≥1.0 mL of sample. Use v/v dilns for liq. products and w/v dilns for solids. Round to 2 decimal places toward a stronger product. To ensure stable product, solns should be prepd ≤3 hr prior to use. Place tubes in 20° H_2O bath ≥10 min. Det. diln to be tested by multiplying phenol coefficient number found and/or claimed by 20 to det. number of parts H_2O in which 1 part germicide is to be incorporated. This detn is not required when disinfectant under test yields phenol coefficient that cannot be converted validly to presumptive use-diln, or when analyst dets that use-diln range can be found without resort to phenol coefficient test.

Add 0.5 mL of test culture suspension to 1–90 diln of phenol control; after 30 sec interval, add 0.5 mL to 1–100 diln of control, using sterile cotton-plugged pipets. After adding culture, agitate tubes gently but thoroly to distribute bacteria evenly, and replace in bath; 5 min after seeding first medication tube, transfer 1 loopful of mixt. of culture and dild phenol from medication tube to corresponding subculture tube. After 30 sec, transfer loopful from second medication tube; 5 min after making first set of transfers begin second set of transfers for 10 min period; and finally repeat for 15 min period. Use technic of loop sampling, flaming loop and mouths of tubes, and agitating medication and subculture tubes as in phenol coefficient method, **4.003**. Incubate subcultures 48 hr at 37° and read results. Resistance in 48–54 hr culture of *S. choleraesuis* should fall within range specified for 24 hr culture of *S. typhi* in phenol coefficient method.

Without touching sides of tube with contaminated carrier or hook, either when placing carrier in tube or when withdrawing hook, add 1 contaminated dried cylinder carrier at 1 min intervals to each of the 10 tubes of use-diln of germicide to be tested. (*Note*: Proper execution of transfer step is one of the most critical, technique-sensitive areas of method. False positives will result if sides of tube are touched.) Thus, by time 10 tubes have been seeded, 9 min will have elapsed, plus 1 min interval before transfer of first carrier in series to individual tube of subculture broth. This interval is const for each tube with prescribed exposure period of 10 min. The 1 min interval between transfers allows adequate time for flaming and cooling nichrome wire hook and making transfer in manner so as to drain all excess medication from carrier by shaking carrier against side of tube. Shorter intervals may be used in adding and removing carriers if 2 alternately flamed and cooled hooks are used. Individual manipulation of carriers is required; use of semiautomated ring carrier is prohibited. (*Note*: Above step is one of the most critical, technique-sensitive areas of method. False positives can result from transfer of live organisms to sides of tube due to aerosol formation.) Flame lips of medication and subculture tubes in conventional manner. Immediately after placing carrier in medication tube, swirl tube 3 times before placing it back in bath. Thoroly shake subculture tubes, incubate 48 hr at 37°, and report results as + (growth) or − (no growth) values. Growth in tubes should be checked by gram stain to ensure that no contamination is present. Check ≥20% of pos. tubes. Confirm all pos. results by duplicate testing to assure against false pos. tests.

Where there is reason to suspect that lack of growth at conclusion of incubation period may be due to bacteriostatic action of medicant adsorbed on carrier that has not been neutzd by subculture medium used, transfer each ring to new tube of sterile medium and reincubate for addnl 48 hr at 37°. Where soln under test is such that material adsorbed on ring carriers and transferred into subculture medium makes it unsuitable for growth of test organism, as may be case with concd acids and alkalies, products carrying antibiotics, and wax emulsions, transfer each ring to new tube of sterile medium 30 min after initial transfer and incubate both primary and secondary subculture tubes 48 hr at 37°. Results showing no growth on all 10 carriers will confirm phenol coefficient number found. Results showing growth on any of the 10 carriers indicate phenol coefficient number to be unsafe guide to diln for use. In latter case, repeat test, using lower dilns (higher concns) of germicide under study. Max. diln of germicide which kills test organism on 10 carriers in 10 min interval represents presumed max. safe use-diln for practical disinfection.

Refs.: J. Bacteriol. **49**, 526(1945). Am. J. Vet. Res. **9**, 104(1948). JAOAC **36**, 466(1953); **70**, 318(1987); **71**, 117(1988); **72**, 116(1989).

Testing Disinfectants against *Staphylococcus aureus*

Use-Dilution Method

First Action

4.010 ***Determination***

Proceed as in **4.009** except change phenol dilns and test organism to those specified in **4.005**. Use 48–54 hr culture of *Staph. aureus* FDA 209, ATCC No. 6538, having at least resistance specified for 24 hr culture at 20° in phenol coefficient method, **4.005**. Obtain organism annually, directly from ATCC. Prior to beginning Use-Dilution Test, vortex-mix nutrient broth culture as in **4.009**. Results showing growth on any of 10 carriers indicate that diln is too high for use in disinfecting where pyogenic bacteria must be killed. In such cases repeat test, using lower dilns (higher concns). Max. diln of germicide which kills both this test organism and *S. choleraesuis* on 10 carriers in 10 min interval represents max. presumed safe use-diln for disinfecting in hospitals, clinics, and other places where pyogenic bacteria have special significance.

Note: While killing in 10 of 10 replicates specified provides reasonably reliable index in most cases, killing in 59 out of 60 replicates is necessary for confidence level of 95%.

Refs.: J. Bacteriol. **49**, 526(1945). Am. J. Vet. Res. **9**, 104(1948). JAOAC **36**, 466(1953); **70**, 318(1987); **71**, 117(1988); **72**, 116(1989).

Testing Disinfectants against *Pseudomonas aeruginosa*

Use-Dilution Method

First Action

4.011 ***Determination***

Proceed as in **4.009**. Use 48–54 hr nutrient broth culture *Ps. aeruginosa* PRD 10 (ATCC 15442). Carry stock culture on BBL CTA (cystine trypticase agar) in stab culture incubated 48 hr at 37° and stored at 5° with transfer every 30 days. Transfer nutrient broth test cultures daily for 30-day intervals with incubation at 37°. Make fresh transfer from stock culture every 30 days. Do not shake 48–54 hr test culture but decant liq. culture aseptically, leaving pellicle behind, to obtain 20 mL culture for inoculating 20 carriers in medication tube.

Proceed with vortex-mixing as in **4.007** prior to use of culture. Alternatively, pellicle may be carefully suctioned off, and culture can be poured into clean, sterile tube before vortex-mixing. Any disruption of pellicle resulting in dropping, breaking up, or stringing of pellicle in culture before or during its removal renders that culture unusable in Use-Dilution Test. This is extremely critical because any pellicle fragments remaining will result in uneven clumping and layering of organism on cylinder, allowing unfair exposure to disinfectant and causing false pos. results.

Refs.: J. Bacteriol. **49**, 526(1945). Am. J. Vet. Res. **9**, 104(1948). JAOAC **36**, 466(1953); **70**, 318(1987); **72**, 116(1989).

INDEX

Entries are located by section number. First action methods are designated by an asterisk, e.g., **6.A01***, which shows that the method was adopted first action at the 1984 meeting and became official on publication in "Changes in Official Methods" in the March/April 1985 issue of *J. Assoc. Off. Anal. Chem.* as part of the first or A supplement to the current (14th) edition of *Official Methods of Analysis.* Methods designated B were adopted in 1985 and published in 1986 in the second supplement. Methods designated C were adopted in 1986 and published in 1987 in the third supplement. Methods designated D were adopted in 1987 and published in 1988 in the fourth supplement. Methods designated E were adopted in 1988 and will be official on the thirtieth day after publication of this *Journal* issue.

Actions on present official methods are identified as in the following examples: first action method adopted final action, **6.296** (final 1987); revision in an official method, **2.011** (rev. 1987). Other actions such as repeals and deletions are similarly shown with the action and year of publication of the action in "Changes in Official Methods of Analysis," e.g., **30.063** (repealed 1987).

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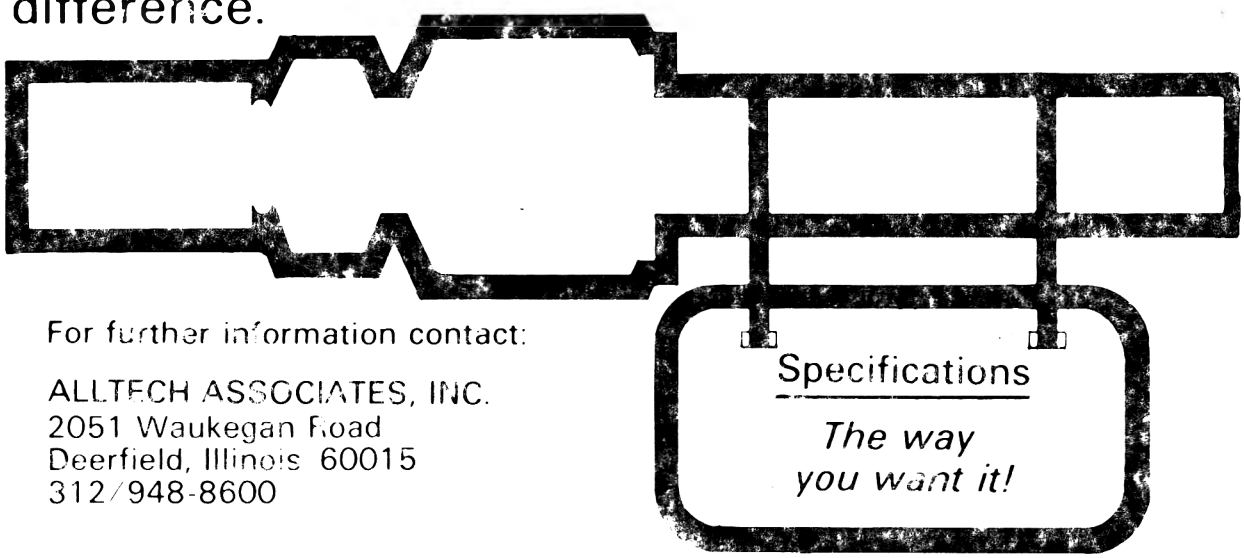

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